

Recovery of $^{13}\text{CO}_2$ and $^{14}\text{CO}_2$ in human bicarbonate studies: a critical review with original data

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1. In order to establish biological and/or methodological explanations for the wide variability in recovery (50–100%) of labelled CO_2 after administration of [^{13}C]bicarbonate or [^{14}C]bicarbonate, 34 human bicarbonate studies involving 480 subjects were analysed, and potential methodological issues were investigated in the laboratory.

2. Overall, continuous infusion studies reported a higher recovery than bolus studies ($84 \pm 11\%$ versus $69 \pm 12\%$; $P < 0.001$). No significant differences in recovery were found between ^{14}C and ^{13}C studies, children and adults, obese and lean subjects, or rest and exercise (steady state). Higher recoveries were found during feeding than during fasting ($84 \pm 8\%$ versus $74 \pm 7\%$; $P < 0.001$). Different methods used to analyse the results (0–10%) and different study protocols, which include differences in the duration of infusions and background drift in ^{13}C enrichment (0–10%), contribute to the variability.

3. The laboratory studies suggest multiple sources of potential error, including loss of CO_2 from the scintillation fluid (up to $>30\%$, but only in ^{14}C studies in which the scintillation fluid is not alkalized), diffusion of CO_2 through syringes and tubing (0 to $>10\%$), non-linearity of CO_2 analysers (up to 8%), inaccuracies in the measurement of bicarbonate concentrations (^{13}C studies) or the strength of CO_2 -trapping agents (^{14}C studies; 0–8%).

4. It is concluded that much of the variability in the recovery of labelled bicarbonate is likely to be attributable to methodological differences, and that attention to these will ensure better interpretation of metabolic studies that involve oxidation of carbon-labelled substrates.

INTRODUCTION

Many human studies have assessed the oxidation of substrates, such as amino acids [1, 2], fat [3] and carbohydrates [4, 5], from the amount of labelled CO_2 exhaled after administration of ^{14}C - or ^{13}C -labelled substrates. Implicit in many of these studies is the assumption that the recovery of

labelled CO_2 is constant, at least in a given situation. However, in studies in which recovery of labelled CO_2 has been measured after administration of labelled bicarbonate, the results have been variable, with values ranging from 50% to 100% [6–37]. This variation will cause errors in the estimated rates of substrate oxidation, as well as in the rates of total energy expenditure, when this is estimated by isotopic dilution of CO_2 [11, 12].

Although the major reasons for incomplete recovery are well known (CO_2 fixation, equilibration and entry of label into slowly turning over pools of CO_2) [38], there is no universal agreement about the extent to which these processes contribute to the incomplete and variable recovery. Furthermore, as a variable recovery of labelled CO_2 implies a variable accuracy in the estimated rate of oxidation of the carbon-labelled substrate, it is important to consider both the biological and methodological factors that lead to the variable results. A sufficient number of studies involving bicarbonate kinetics has now become available to allow such an assessment to be made. This study aimed not only to do this, but also to investigate in the laboratory possible methodological reasons for the variable recovery, so that potential problems can be identified and avoided in the future.

METHODS

Database

A database was established using the results of 34 studies and 480 subjects [1–3, 6–13, 15–37], in order to assess the following issues: whether [^{14}C]bicarbonate and [^{13}C]bicarbonate are bioequivalent; the effect of priming the bicarbonate pool; the effect of the method of administration (bolus versus continuous); the effect of the route of administration (oral versus intravenous) and the period of infusion; the effect of using bicarbonate solutions of different concentrations and pH; and the adequacy of the procedures used to calculate recovery. The effect of a number of physiological factors were also assessed, e.g. age, obesity, exercise and feeding. The studies

Key words: carbon dioxide, labelled carbon dioxide, loss of label, methodology, recovery database.

Abbreviations: ANOVA, analysis of variance; APE, atoms percentage excess.

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cited in this paper represent most of the published studies on bicarbonate recovery that could be used to address the issues raised above.

The preferred method for assessing changes in recovery of labelled CO_2 in different circumstances (e.g. fed and fasted state) involved the use of information obtained on the same subjects by the same investigators. When this was not possible, the next preferred method was to compare results of different groups of subjects who were studied by the same investigators using the same techniques. When this was also not possible, results from different groups of subjects obtained by different investigators were combined and compared. For the purposes of assessing the effect of feeding, a fasted state was defined as one in which adult subjects fasted overnight and children fasted for at least 4 h.

As data from individual subjects were not always available, an overall mean and SD from a group of studies was established from (i) the mean and SD of individual studies and (ii) the number of subjects involved in each study. Among the statistics used were the Student's *t*-test, which was applied to paired data wherever possible, and two-way analysis of variance (ANOVA). Results are expressed as means \pm SD.

Laboratory studies

The laboratory experiments set out to examine the extent to which bicarbonate loses label during preparation, storage and administration, and the extent to which this loss might explain the variability in the recovery of bicarbonate reported by various workers, who employed bicarbonate solutions ranging from <0.3 to 588 mmol/l. Sodium bicarbonate, was purchased from BDH Laboratory Supplies (Poole, Dorset, U.K.; cat. no. CGS813) and $\text{NaH}^{14}\text{CO}_3$ from Amersham (Little Chalfont, Bucks, U.K.; cat. no. CFA3).

Loss during preparation. Loss of radioactivity was examined by placing 50 ml of labelled $\text{NaH}^{14}\text{CO}_3$ solutions (0.3, 1, 2, 5, 10 and >10 mmol/l) into a standard 250 ml beaker (diameter 6.7 cm) at ambient temperatures of 22°C and 30°C. Samples of 0.5 ml were taken for scintillation counting at intervals for up to 3 h. Similar procedures were used to assess the loss of radioactivity produced by blowing air (induced by a ventilator) over the surface of the bicarbonate solution and those produced by stirring the solution at three different speeds. At the low speed the surface of the solution was not visibly disturbed. The medium speed caused mild disturbance of the surface and the high speed was just sufficient to create a vortex and bubbles within the solution. No bubbles were visible at the two lowest speeds.

The effect of surface contact between the bicarbonate solution and the atmosphere on the loss of label from the bicarbonate solution was assessed

by placing 50 ml of $\text{NaH}^{14}\text{CO}_3$ solution (0.3 mmol/l) into beakers of different sizes: a 250 ml beaker (estimated cross-sectional area 35.3 cm²), a 150 ml beaker (25.5 cm²), a 100 ml beaker (17.3 cm²) and a 50 ml beaker (11.9 cm²). The effect of pH was assessed by measuring the loss of label from $\text{NaH}^{14}\text{CO}_3$ solutions (0.3 mmol/l) whose pH was adjusted with dilute hydrochloric acid and sodium hydroxide so that the final pH was 5, 6, 7 or 8. Without adjustment the pH of the solution was close to pH 6. Both the pH and temperature (approximately 22°C) were recorded during the course of the experiment (3 h).

Loss during administration (loss from syringes and tubing). Solutions of $\text{NaH}^{14}\text{CO}_3$ (0.3, 1, 2 and 10 mmol/l) were used to fill plastic syringes (Becton Dickinson, Dun Laoghaire, Co. Dublin, Republic of Ireland) of different sizes: 5 ml syringe (thickness 1 mm; estimated internal cross-sectional area 113.1 mm² and estimated internal surface area/volume ratio 3.3/cm), 10 ml syringe (1.1 mm, 161.5 mm², 2.8/cm), 20 ml syringe (1.3 mm, 291.9 mm², 2.1/cm) and 30 ml syringe (1.3 mm, 363.2 mm², 1.9/cm). The syringe tips were capped and left at room temperature (22°C). Samples (approximately 0.5 ml) were taken at 0, 40, 80 and 120 h for accurate weighing and scintillation counting. The experiments were repeated in 5 ml syringes using different concentrations of $\text{NaH}^{14}\text{CO}_3$ solutions (0.3, 1, 2 and 10 mmol/l).

An assessment was also made of the loss of label from a solution of $\text{NaH}^{14}\text{CO}_3$ (0.3 mmol/l), through the plastic tubing of different 'butterflies' (nos. 19, 21, 23 and 25; Abbott, Sligo, Republic of Ireland) with different internal diameters (0.8, 0.6, 0.4 and 0.3 mm respectively), different wall thicknesses (0.3, 0.2, 0.2 and 0.2 mm respectively), different internal cross-sectional areas (0.50, 0.28, 0.13 and 0.07 mm² respectively) and different internal surface area/volume ratios (5.0, 6.7, 10.0 and 13.3/mm respectively). Losses were also measured through Vygon tubes (Vygon 1155.6, Laboratoires Pharmaceutiques Vygon, Ecuoen, France) (internal diameter 1 mm, wall thickness 1 mm, internal cross-sectional area 0.79 mm² and surface area/volume ratio 4.0/mm) and a Vygon 220.02 tube (2.5 mm, 1.5 mm, 4.91 mm² and 1.6/mm respectively). The plastic tubes were occluded towards their ends so that the solutions within them were not exposed to the atmosphere. At intervals up to 48 h the tubes were cut and the solutions were weighed and counted for radioactivity. A separate tube was used for each time point. The experiment was repeated using different concentrations of $\text{NaH}^{14}\text{CO}_3$ solutions (0.3, 1, 2 and 10 mmol/l) and size 23 'butterflies'. Preliminary experiments had shown that placing the solution in the tubes did not result in an immediate loss of radioactivity.

Scintillation counting was performed on a Beckman scintillation counter (Beckman LS 1701) after 2.5 mmol of hyamine hydroxide (Packard

Instruments, Groningen, The Netherlands; cat. no. 6813319) and 7 ml of hyonic fluor (Packard Instruments; cat. no. 6003005) had been added to the sample. The effect of not alkalinizing the scintillation fluid by omitting the hyamine was also assessed. The percentage loss of radioactivity per day and per hour from beakers, syringes and tubes was calculated assuming that the loss was monoexponential. The assumption was consistent with the experimentally obtained results.

The linearity of four IR CO₂ analysers (Servomex, Crowborough, Sussex, U.K.) was assessed by recording the CO₂ concentrations when standard gases (usually of CO₂ concentration 0.75% or 1.00%) were accurately diluted with varying amounts of CO₂-free air at ambient pressure. Flow through three ventilated hood systems (Deltatrac, Datex Instrumentarium, Helsinki, Finland) was assessed by measuring the recovery of infused gases (mixture of 80% nitrogen and 20% CO₂). The mixture was infused at a rate of 1 l/min and measured by a wet-type flowmeter (Alexander Wright, London, U.K.), which is accurate to 0.3%. The accuracy of the assigned CO₂ concentration in commercial gases ($n=8$) (alpha and non-alpha grade; British Oxygen Supplies, Worsley, Manchester, U.K.) was assessed using a combination of methods [11]: titration of hyamine hydroxide before and after trapping CO₂; change in oxygen concentration after removal of CO₂ by soda lime; and by the Haldane manometric procedure [11].

The 'strength' of commercial preparations of hyamine hydroxide in methanol (approximately 1 mol/l) was assessed by titration with 0.2 mol/l HCl (analar grade, measured density 1.001 g/l; Merck, Lutterworth, U.K.; cat. no. 19070) in the presence of phenolphthalein [11].

The strength of the hyamine hydroxide was also assessed by titration to pH 7.

RESULTS

Database

Continuous versus bolus administration. Table 1 and Fig. 1 indicate that the recovery of labelled CO₂ is significantly greater during continuous infusion of labelled bicarbonate ($P<0.001$; two-way ANOVA) than after bolus administration. This is irrespective of whether all studies and all time points are considered together (continuous, $84.2\pm 11.1\%$; bolus, $68.5\pm 11.5\%$) or only specific studies and specific time points such as those obtained at 2.5–6.0 h. Similar differences were found when only ¹³C studies were considered, although this was not evident when only ¹⁴C studies were considered. There is significant variation in results obtained by different studies within specific categories (Table 1).

¹³C versus ¹⁴C. On average, the recovery of labelled CO₂ was found to be greater after administration of [¹⁴C]bicarbonate than after [¹³C]bicarbonate, irrespective of whether the bicarbonate was

Table 1. Comparison of recoveries of labelled CO₂ between continuous and bolus infusion studies. NS, not significant. *Studies significantly different from each other within the group ($P\leq 0.001$). †Studies significantly different from each other within the group ($P\leq 0.01$).

Data set	Bolus mean recovery (%)	No. of studies	No. of subjects	References	Continuous mean recovery (%)	No. of studies	No. of subjects	References	Residual SD (%)	P-ratio
1. All values	$68.5\pm 11.5^*$	28	187	[6, 7, 19, 21, 22, 24, 28, 31, 35, 36]	$84.2\pm 11.1^*$	39	293	[1–3, 8–13, 15–18, 20, 23, 25–27, 29, 30, 32, 34, 37]	6.9	<0.001
2. Resting, fasting, non-obese, all time points	$66.4\pm 11.0^*$	18	127	[6, 7, 19, 21, 22, 24, 28, 31, 35]	$81.7\pm 10.0^*$	13	90	[1, 9, 13, 15–18, 20, 27, 32, 34]	8.1	<0.001
3. Resting, fasting, non-obese, 2.5–6 h	$66.1\pm 12.5^*$	7	34	[19, 21, 31, 35]	$80.6\pm 6.7^*$	5	32	[1, 15, 16, 27]	6.8	<0.001
4. All ¹³ C values	$68.7\pm 11.4^*$	28	194	[6, 7, 19, 21, 22, 24, 28, 31, 35, 36]	$81.7\pm 12.7^*$	24	156	[8, 10, 13, 15, 18, 20, 25–27, 29, 30, 32, 34]	7.8	<0.001
5. All ¹⁴ C values	84.3	1	13	[33]	$87.1\pm 8.4^*$	14	131	[1–3, 9, 11, 12, 16, 17, 23]	5.1	NS
6. Resting, fasting, non-obese, all time points, ¹³ C values	$66.4\pm 11.0^*$	18	127	[6, 7, 19, 21, 22, 24, 28, 31, 35]	$76.7\pm 7.3^*$	9	53	[13, 15, 18, 20, 27, 32, 34]	8.8	<0.001
7. Resting, fasting, non-obese, 2.5–6 h, ¹³ C values	$66.1\pm 12.5^*$	7	34	[19, 21, 31, 35]	$80.0\pm 6.1^\dagger$	3	18	[15, 27]	7.7	<0.001

given continuously ($87.1 \pm 8.4\%$ versus $81.7 \pm 12.7\%$) or as a bolus (84.3% versus $68.7 \pm 11.4\%$) (see Table 1), but neither comparison was statistically significant.

Period of infusion. Several individual studies have demonstrated that the recovery of labelled CO_2 after administration of an unprimed bicarbonate infusion increases with time (e.g. see Fig. 2), but variations in 24 h recovery appear to be small. The longest infusions undertaken are those of Elia et al. [12]. Recovery was $94.8 \pm 1.6\%$ between the first and second day, gradually rising to $96.2 \pm 1.3\%$ between the fourth and fifth day. The 24 h recovery was independent of the degree of physical activity, which was undertaken in a whole-body calorimeter. The above values do not include the loss of label (approximately 2%) in urine, predominantly in the form of urea [11, 12].

When the mean recovery from all studies is compared with that obtained between 2.5 and 6.0 h (Table 1, data sets 1 and 3), no significant differ-

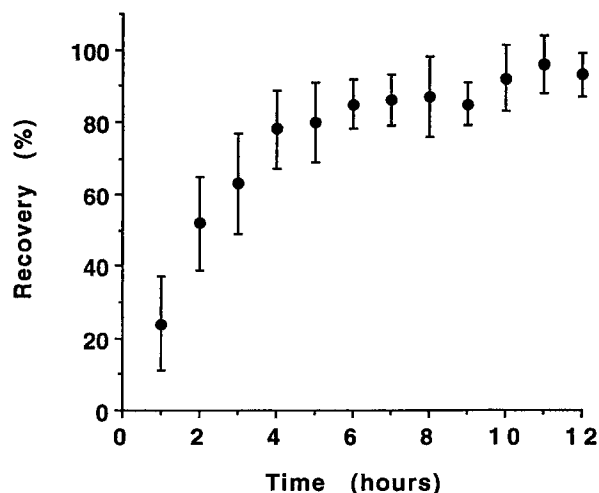


Fig. 2. Effect of the period of infusion on the recovery (%) of CO_2 after an unprimed constant infusion of ^{14}C bicarbonate ($n = 18$). The measurements of CO_2 production were made continuously.

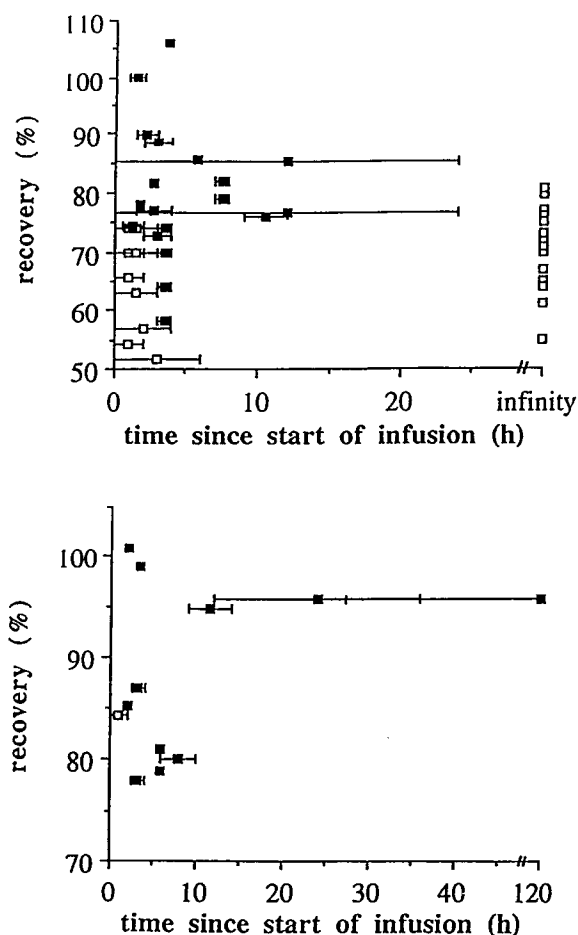


Fig. 1. Recovery (%) of ^{13}C bicarbonate (top) and ^{14}C bicarbonate (bottom) for bolus (\square) and continuous (\blacksquare) infusion versus time. Horizontal lines represent the period over which measurements were made to calculate recovery. Results at infinity were obtained by extrapolation of observed values to infinity.

ences can be demonstrated. Similar conclusions can be made when only ^{13}C bicarbonate studies are considered (Table 1, data sets 6 and 7). The results of individual studies are shown in Fig. 1.

Methods for calculating recovery. Differences in recovery of labelled CO_2 may depend on the method used to calculate recoveries. With continuous infusions, three different methods have been used. The first calculates the recovery at a given point in time (point recovery) by multiplying specific activity or change in enrichment of CO_2 at a given point in time by CO_2 production at the same time point [2, 3, 9, 16, 18, 20, 25, 29, 30, 34]. The second method calculates recovery over a specified period of time (period recovery) [11, 12, 13, 27] and the third involves calculating recovery at an apparent plateau enrichment (or an apparent plateau recovery over a variable period of time) depending on the individual subject or individual study [1, 3, 8, 9, 15, 18, 20, 23, 26, 27, 30, 34].

It is difficult to estimate the short-term differences or short-term variations in recovery obtained by using these different methods because of the frequent lack of reported data. However, some authors have reported on the coefficient of variation of 'plateau' enrichment (e.g. $<4\%$ [29] and $1.5\text{--}5\%$ [20, 30]). Others have reported on the coefficient of variation of 'plateau' recovery, which takes into account CO_2 production, e.g. $1\text{--}3\%$ [23] or 6% [9]. One study has reported on the coefficient of variation ($<10\%$) associated with the measurement of CO_2 production [2], but many studies do not give such results (e.g. [1, 8, 15]). In addition, an approximate calculation based on specific results shown graphically suggest that results taken from adjacent time points may vary by $0\text{--}6\%$ in enrichment [18, 20, 27, 30] and by as much as $0\text{--}10\%$ in recovery [2]. Furthermore, careful graphical inspection of specific

data sets suggests that a steady state is probably not reached because the enrichment of CO_2 is either rising or falling with time [18, 20, 27, 30].

Period recoveries give different results depending on the period examined (Fig. 2 and [3, 11, 12, 13, 27]). For example in one study [27] involving a primed constant infusion, recovery at 3 h was 72%, rising to 82% at 6 h (10% difference in recovery). In another study in which feeding was given hourly for 12 h, the recovery rose from 56% to 76% between 9 and 12 h [25].

With studies involving bolus administration two different models have been used to analyse the results: a one-compartment model [24, 36] and a three-compartment model [6, 7, 19, 21, 22, 28, 31, 33, 35]. A detailed comparative analysis in the results obtained using different models is lacking, although different models are expected to give different terminal exponentials, which influence the calculated area under the time-enrichment curve (an indicator of recovery).

Recovery to a given time point after bolus administration (e.g. 3 h as in some studies [6, 36]) is obviously lower than recovery to infinity. For example, using the terminal exponential of typical enrichment curves that extend to 3 h or actual curves that extend beyond 3 h, it can be calculated that approximately 10% is lost after 3 h, but only 1–2% after 6 h. However, cross-sectional comparisons between different studies show no significant differences ($68.2 \pm 10.9\%$, studies extrapolated to infinity [6, 7, 22, 24, 28, 35] versus $68.9 \pm 12.6\%$, remaining studies of 2–6 h duration [19, 21, 31, 33, 36]).

Changes in background enrichment of $^{13}\text{CO}_2$. The majority of studies (16 out of 22 studies) have assumed that the background enrichment observed before the study remains steady during the study. Others have undertaken control studies in which either the same individuals or a different group of individuals were subjected to the same protocol as the test subjects with the exception that no bicarbonate was administered [8, 13, 15, 19, 20]. Wolfe et al. [34] corrected for baseline drift using values obtained from a previous study [39]. Most bolus studies (the study of Irving et al. [19] being an exception) have assumed that the background enrichment remains steady during the study. Other studies have reported that changes in enrichment may be as large as 0.004 atoms percentage excess (APE) after ingestion of maltodextrin [40] and 0.003 APE during exercise [38].

The errors in recovery resulting from erroneous measurements in the baseline enrichment depend on the dose of bicarbonate administered. If there is an error in the measurement of background ^{13}C to the extent of 0.5 mAPE during a 3 h bolus study, the typical error in recovery is calculated to be about 1% when a $23 \mu\text{mol/kg}$ bolus dose is administered [6] and 7.3% when a $10 \mu\text{mol/kg}$ dose is given [19] (90–100% enrichment). Similarly, an error of

0.5 mAPE in the basal enrichment produces an error of about 1.5% when a constant infusion is given at a rate of $0.065 \mu\text{mol min}^{-1} \text{kg}^{-1}$ (90–100% enrichment) (e.g. [18]), but an 11-fold greater error (17%) when the dose is only $0.0055 \mu\text{mol min}^{-1} \text{kg}^{-1}$, as in the study of Garlick et al. [15].

Van Aerde et al. [30] reported graphically three baseline values before the bicarbonate infusion, which corresponded to approximately 7% of the change observed at plateau enrichment. El-Khoury et al. [13] reported a spontaneous change in background (0.001 APE) corresponding to approximately 3% of the change induced by a constant infusion of bicarbonate ($3.9 \mu\text{mol h}^{-1} \text{kg}^{-1}$). Hoerr et al. [18] reported a spontaneous change of 0.002 APE over 7 days and an additional 0.002 APE during a study in which a feeding was given for 4 h (equivalent to approximately 6% of the change induced by the constant infusion of $3.9 \mu\text{mol h}^{-1} \text{kg}^{-1}$ [^{13}C]bicarbonate).

Route of administration of labelled bicarbonate

(a) Oral versus intravenous. Three studies [6, 24, 36] have been undertaken in subjects receiving an oral bolus of [^{13}C]bicarbonate. No significant difference in recovery was demonstrated between studies involving such a method of administration ($67 \pm 5\%$; mean of three studies) and studies involving an intravenous bolus injection ($68 \pm 9\%$; mean of seven studies) [7, 19, 21, 22, 28, 31, 35].

(b) Intravenous versus subcutaneous. Elia et al. [12] compared the effects of unprimed intravenous and subcutaneous infusions of [^{14}C]bicarbonate. The recoveries over 6 h were similar to each other ($84 \pm 4\%$ versus $83 \pm 7\%$), as were those over 36 h ($95.6 \pm 1.1\%$ versus $94.8 \pm 1.6\%$) [11, 12].

Primed constant infusion. Of the constant infusion studies, 18 of 22 primed the infusion with labelled bicarbonate in order to reach an equilibrium in the bicarbonate pools in the body. The prime-infusion ratio (P/I , per minute) varied considerably in different studies: 7.6:1 [20], 10:1 [3], 357:1 [17] and 720:1 [17]. However, the mean P/I ratio (excluding these extreme studies) is $74(\pm 18):1$. Recovery of labelled CO_2 has been calculated using data obtained at variable time points after the prime, e.g. 0.5 h [20], 1.0 h [10], 1.5 h [34] (at rest) [30], 1.8 h [23], 2 h [15], 5.5 h [27], 6 h [3] and 7 h [18]. In studies in which a 'period recovery' was assessed, measurements were also made over different periods after the primed infusion: 1.5–3 h [29], 3.25–4 h [16], 9–14 h [17], 9–12 h [25], 0–24 h [13], 12–36 h [11] and 24–120 h [12]. No relationship was found between the priming dose and recovery of label, but the use of different protocols, which involved assessment of recovery over widely different periods of time, and the use of different methods to analyse the results, confounds interpretation.

Measurement of CO_2 production

(a) Continuous versus intermittent calorimetry. In nine of the bolus studies CO_2 was collected inter-

mittently using a facemask [19, 21, 22, 28], mouth-piece [6, 7, 36] or Douglas bag [31] or by an unreported technique [24]. In two bolus studies CO₂ was measured continuously by an unreported method [33, 35]. Of the 22 studies involving a continuous infusion, 10 used continuous calorimetry throughout the study [8, 9, 11, 12, 15, 16, 25, 29, 30, 32] and five of these whole-body calorimetry [9, 11, 12, 15, 25]. Eight studies used a hood or a face mask to measure the CO₂ production intermittently. The study of Wolfe et al. [34] used an anaesthetic bag to collect and measure resting CO₂ production continuously for part of the study and intermittently for the rest of the study. Three other continuous infusion studies did not indicate whether the CO₂ was measured continuously or intermittently [1, 10, 17].

(b) Period of measurement of CO₂ production. In some studies measurements of CO₂ were made for only 5 min each hour [7] or twice for an unreported period of time during a 4 h study [24]. Many studies do not give precise details about the measurement period. For example, several studies [3, 20, 26] indicate that measurements were made at hourly intervals but give no details of the measurement period.

(c) Calibration of CO₂ analysers with standard CO₂ gas. Of the 33 studies that were reviewed, 22 did not report how the CO₂ analysers were calibrated. Information on the linearity of CO₂ analysers [12] and the accuracy of the calorimeter ventilation rate [11] has been given infrequently.

Calibration of infusion pump. Of the 22 studies that gave a constant infusion of bicarbonate, 11 (six involving [¹³C]bicarbonate and five involving [¹⁴C]bicarbonate) reported on the calibration and the accuracy of the infusion pump. Three studies did this both volumetrically and gravimetrically [11, 12, 20], and one study [25] gravimetrically.

Amount of ¹³C or ¹⁴C label infused. The enrichment or specific activity of the administered isotopes may have been assumed to be the same as that given by the manufacturers, as no calibration procedures were reported in 18 of the 33 studies (12 [¹³C]bicarbonate and six [¹⁴C]bicarbonate studies). However, 15 studies reported that measurements of the enrichment or specific activity of the isotopes were made before administration to confirm or check on the values given by the manufacturers. Only three studies [11–13] reported that measurements of enrichment or specific activity of the infusate were made both before and after the study. The dose of label infused depends not only on accurate measurements of enrichment, but also on accurate measurements of bicarbonate concentration (¹³C studies only – see below) and accurate information about the volume or weight of the solution infused (see above for calibration of pumps).

Concentration of bicarbonate infused. In many studies, the concentration of bicarbonate infused is not reported (Fig. 3). The reported bicarbonate

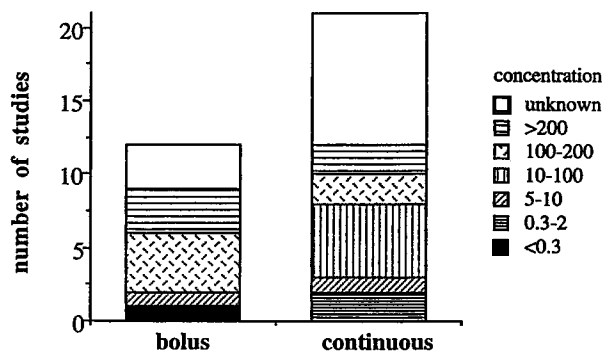


Fig. 3. Concentration of bicarbonate (mmol/l) used in different bolus and continuous infusion studies

concentrations (<0.3–588 mmol/l) are mainly above 10 mmol/l. Two studies involving bolus administration [24, 33] and three studies involving continuous infusion [17, 18, 30] used a solution with a bicarbonate concentration of <10 mmol/l.

Only seven of the 22 studies involving administration of [¹³C]bicarbonate reported on the concentration of bicarbonate, which was measured by different techniques: manometrically by the amount of CO₂ generated by addition of phosphoric acid [19, 20, 26]; enzymically [29]; by back-titration with HCl [7, 10]; or by unspecified methods [30]. Other studies give no information about either the bicarbonate concentration or the method used to measure it. Most of those that reported on the use of a specific method for measuring bicarbonate did not report on the precision of the method. However, the coefficient of variation was reported to be close to 1% in one study [18] and as high as 8% in another [19]. In a recent study, in which bicarbonate was measured using two methods, results differed between studies by 4 ± 1%, but by as much as 9% in an individual sample [37]. Using the method that gave the lower results, the recovery of ¹³CO₂ at 4 h (76%; *n* = 5) and 6 h (82% *n* = 2) is similar to that shown in Fig. 2 for ¹⁴CO₂.

Assumed versus measured strength of CO₂-trapping agent. In [¹⁴C]bicarbonate studies it is necessary to trap a known amount of CO₂, usually with an alkaline agent such as hyamine hydroxide, which can then be counted on a scintillation counter. Eight studies [2, 3, 9, 11, 12, 16, 17, 23] reported that the 'strength' of the solutions was established by titration. No titration procedures were reported in two studies [1, 33], which makes it uncertain whether the workers assumed that the normality of the solution was the same as the molarity given by the manufacturers.

pH of the solution. One study [12] reported the pH of the solution infused (pH 10.0–10.5). The study of Winchell et al. [33] used a solution of sodium bicarbonate that was alkalinized with sodium hydroxide. No information is available from other studies.

Recovery in different subjects and different situations

(a) Fed versus fasted. Four of these studies reported a significantly higher recovery in the fed state (Table 2). In two of these studies [15, 18] the feeding period followed the fasting period, so that the duration of the bicarbonate infusion was not the same in the two states. Special care was taken to avoid this problem in another study. [32] In yet another study the fasting period occurred predominantly during the night (18.00–06.00 hours), whereas the feeding period was during the day (06.00–15.00 hours) [14]. Two other preliminary reports give conflicting results: one suggests a significantly higher recovery in the fed state [32] while the other [35] suggests a lower recovery in the fed compared with the fasted state when a low-protein diet is ingested (70% versus 77%). However, the results were reversed when a high-protein diet was ingested (70% versus 55%) [35]. In another study, recovery during a euglycaemic clamp was similar to that obtained during a saline control, although in both studies there was a time-dependent increase in recovery [27]. Yet another study [31] reported no real difference in recovery between subjects studied in the fasting state and those studied during sustained oral ingestion of glucose.

Significantly higher results were obtained when all studies carried out in the fed state [1, 2, 8, 9, 11, 12,

13, 15, 18, 21, 23, 25, 26, 29–33, 35] (recovery $82.4 \pm 12.2\%$) are compared with all studies undertaken in the fasted state [1, 3, 6, 7, 9, 10, 13, 15–20, 22–24, 27, 28, 31, 32, 34–36] (recovery $75.3 \pm 13.7\%$), but this analysis does not take into account the time-dependent changes, which vary considerably between studies. Finally, two subjects who fasted for about 30 h were reported to have a $^{14}\text{CO}_2$ recovery of 99% each during a constant infusion [16].

(b) Exercise versus rest. At least three studies [7, 34, 36] have assessed the recovery of labelled CO_2 in subjects studied at both rest and exercise ($n = 32$). One study [36] reported a lower recovery in both adults and children during exercise than at rest (Table 2). The other two studies reported a higher recovery during exercise than at rest [7, 34], but in one of these [34] the exercise was started only after bicarbonate had been infused for 120 min. As a result, the recovery transiently rose to 174% and gradually returned to a lower value, which may not have entirely reached the new steady state (see also [11, 12]). Another small study involving three men and three women reported an increase in the bicarbonate retention factor during exercise [41]. Yet another study assessed the effect of exercise on different kinetic constants but did not report the changes in recovery [42].

Table 2. Effect of feeding–fasting and exercise–rest on the recovery of infused bicarbonate in the same adult subjects. NS, not significant.

Reference	No. of subjects	Fed	Fasted	P-value (paired t-test)
[18]*	6 ^a	79 ± 4.9	74 ± 4.9	< 0.001
	6 ^b	82 ± 7.3	70 ± 9.8	< 0.001
[15]*	5	89 ± 10.4	73 ± 4.0	< 0.05
[32]	5	90 ± 3.0	76 ± 5.0	< 0.02
[14]†	5	85 ± 2.5	77 ± 2.0	< 0.001
[35]	2 ^c	76 ± 8.4	55 ± 8.4	NS
	2 ^d	70 ± 8.4	77 ± 8.4	NS
All studies	31	83.2 ± 8.2	72.8 ± 7.7	< 0.001
	Rest	Exercise		
[36]‡	9 ^e	70 ± 8	63 ± 7 ^f	< 0.05
	9	74 ± 12	70 ± 7 ^f	< 0.1
[34]§	8	78 ± 6.4	106 ^f	< 0.01
[7]	6	67 ± 5.3	80 ± 11 ^f	< 0.01
			81 ± 3 ^g	< 0.01
All studies	32 (38 exercise)	72.6 ± 9.1	79.6 ± 16.7	

*Recovery in the feeding state was always assessed after the measurements in the fasting state. †Fasting was predominantly at night and feeding during the day. ‡Intermittent exercise. §Exercise was undertaken after a near steady state of enrichment was achieved at rest. When the exercise results from [34] are excluded because they were presumably obtained in a non-steady state (recovery 106%), the mean recovery value for the exercise test was 73%, which is essentially the same as the resting value. ^aIntragastrically fed. ^bIntravenously fed. ^cHigh-protein diet. ^dLow-protein diet. ^eChildren; all other studies are in adults only. ^fLight exercise. ^gModerate exercise.

(c) Obese versus non-obese. Two studies have reported results of recovery of $^{14}\text{CO}_2$ after intravenous infusion to both obese (body mass index $>30 \text{ kg/m}^2$) and non-obese subjects. No significant differences were observed with either study (Table 3).

(d) Children versus adults. Table 3 summarizes the results of individual studies that have examined recovery of labelled CO_2 in both children and adults within each study. The results are not significantly different within the studies. Two further studies have been carried out in children, but the results vary widely. In one of these [21], in which ^{13}C bicarbonate was given as a bolus injection to children who fasted for 2 h, the recovery was only $57 \pm 10\%$ ($n = 6$). In the other study [8], in which the bicarbonate as an infusion was given over a 6 h period to children receiving either a glucose diet or an isoenergetic mixed glucose-lipid diet, the recoveries were $99 \pm 4\%$ ($n = 5$) and $96.4 \pm 2.5\%$ ($n = 5$) respectively.

Laboratory studies

Loss of label during preparation of bicarbonate solutions. Typical patterns of loss of label from the bicarbonate solution (0.3 mmol/l) are shown in Fig. 4. Rapid stirring increased the loss of label from 11%/h to 58%/h. An intermediate result was obtained with the intermediate speed of stirring.

Figure 5 shows that the percentage loss of label decreased as the concentration of bicarbonate in solution rose. However, an increase in concentration was associated with an increase in pH from 6.9 (0.3 mmol/l solution) to 8.3 (10 mmol/l solution). The pH was found to have an independent effect on the loss of label as a 0.3 mmol/l solution lost 43% of label per hour at pH 5, 32% at pH 6 and 16% and 7% at pH 7 and 8 respectively.

As expected, loss of label was directly proportional to the surface area of the solution exposed to the atmosphere ($r = 0.97$). A 50 ml aliquot of

0.3 mmol/l bicarbonate solution, placed in a beaker with a cross-sectional area of 25.5 cm^2 , lost label at a rate (6%/h) that was two- to threefold greater than when placed in a beaker with a cross-sectional area of 11.9 cm^2 (loss of 2.4%/h). There was no significant extra loss of radioactivity at 30°C compared with 22°C using a solution containing 0.3 mmol/l bicarbonate. When stronger solutions of bicarbonate were used ($>10 \text{ mmol/l}$) loss of radioactivity was almost undetectable over a period of an hour. An air current over the surface of the bicarbonate solution was found to have no detectable effect on the loss of label.

Loss of label during administration (loss from syringe and tubing). The percentage loss of radioactivity per day and per hour in different syringes depends on the size of the syringe (Table 4). The ratio of internal surface area to volume (per cm) was positively related to the percentage loss of radioactivity per day ($y = -0.51 + 1.22x$; $r = 0.80$). This was also found to occur with administration tubes. The loss of radioactivity from 'butterflies' with the same wall thickness (size 21, 23, 25), was found to range from 42% to 57%/day (2.3–3.5%/h) and to be related to the internal surface area to volume ratio (Fig. 6). The loss of radioactivity from the other administration sets was 43%, 34% and 18% per day (2.3, 1.7 and 0.8%/h) for 'butterfly' size 19, Vygon 1155.60 and Vygon 220.02 respectively. The percentage loss of radioactivity from bicarbonate solutions of different concentrations decreases as the concentration of bicarbonate increases (Table 5).

This study suggests that when labelled bicarbonate is infused it is better to use a large rather than a small syringe (with the appropriate infusion rate) because the larger syringe has a smaller surface area to volume ratio, which is associated with a smaller fractional loss of label. For example, the fractional loss of label from a 30 ml syringe appears to be 2–3 times less than in a 5 ml syringe. However, a small tube has a larger fractional loss per hour than a

Table 3. Recovery of labelled CO_2 after administration of intravenous labelled bicarbonate to children/adults and obese/non-obese subjects (results shown on the same line were reported in the same study)

Reference	Children	n	Adults	n	P-value	Details
[6]	73 ± 13	10	71 ± 9	12	NS	^{13}C , oral bolus, fasted, rest, 3 h study
[36]	70 ± 8	9	74 ± 12	9	NS	^{13}C , oral bolus, fasted, rest, 3 h study
	63 ± 7	9	70 ± 7	9	NS	^{13}C oral bolus, fasted, exercise, 3 h study
All studies	68.8 ± 10.4	28	71.6 ± 18.8	30	NS	
Reference	Obese	n	Non-obese	n	P-value	Details
[9]	94 ± 6	8	94	1	NS	^{14}C , continuous intravenous infusion during 12–24 h fasting (after 0–12 h feeding)
[3]	81	20	79	14	NS	^{14}C , rest, intravenous bolus, fasted, 8 h study

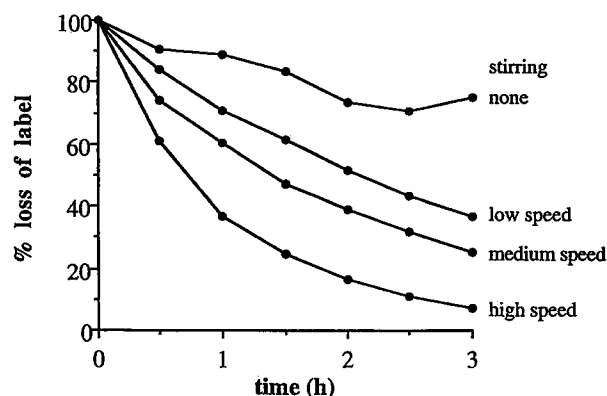


Fig. 4. Loss of radioactivity from 50 ml of a 0.3 mmol/l [^{14}C]bicarbonate solution kept at 22°C in a 250 ml beaker with either no stirring or low, medium or high stirring speed (see text for details)

large tube, but despite this small tubes are recommended because of the smaller volume involved.

Total loss from the syringe is generally of greater importance than loss from the tube, at least partly because of the larger volume of infusate in the syringe. However, when a small syringe (5 ml) and a large, long tube are used ('butterfly' size 19;

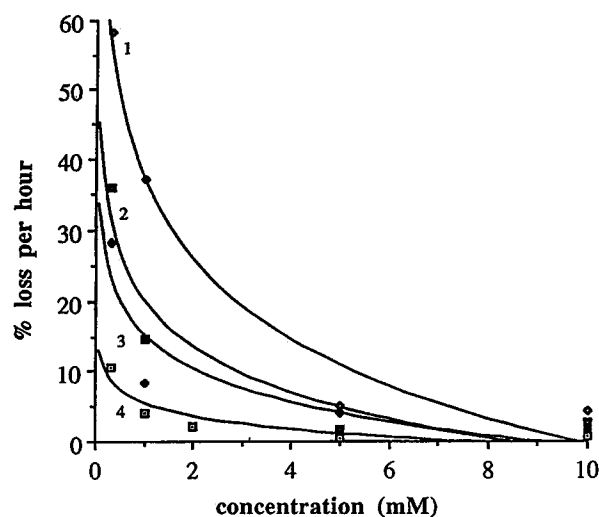


Fig. 5. Effect of the concentration of bicarbonate on the loss of radioactivity from 50 ml of a solution of 0.3 mmol/l [^{14}C]bicarbonate kept at 22°C in a 250 ml beaker. 1, no stirring; 2, low stirring; 3, medium stirring; 4, high stirring (see text for details).

Table 4. Percentage loss of radioactivity per day and per hour in a bicarbonate solution of 0.3 mmol/l kept in 5, 10, 20 and 30 ml syringes

Size of syringe (ml)	Loss/day (%)	Loss/h (%)
5	3.4	0.15
10	3.2	0.14
20	2.2	0.09
30	1.4	0.06

>30 cm), loss of label through the tube can be calculated (using data obtained in this study) to be quantitatively more important. Calculations using the results of the Vygon 1155 tube, which is long (approximately 100 cm) and has a large cross-sectional area, also suggest that the tube has more influence on the loss than a 30 ml syringe.

Other measurements. Non-linearity of commercial IR CO_2 analysers was found to give errors in CO_2 production of up to 8%. Provided that the CO_2 analysed is calibrated with the same reference gas, random errors of CO_2 readings are small (approximately 0.5%). The measurement of CO_2 concentration (0.75% or 1.00%) of test gases was found to vary by $\pm 0.7\%$ of the stated value for alpha tested gases and $\pm 3\%$ for non-alpha tested gases. The ventilation rate through commercial indirect calorimeters was found to vary from -1% to $+4\%$ of the

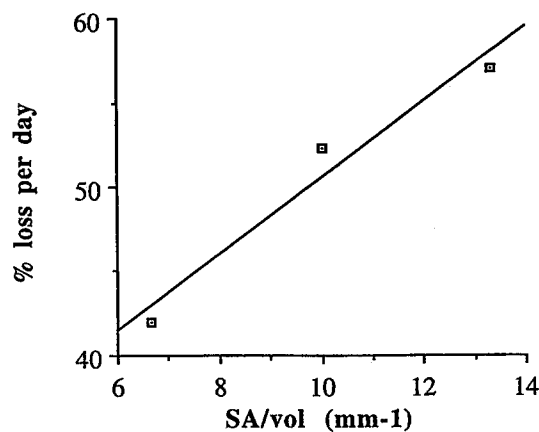


Fig. 6. Percentage loss of radioactivity per day from a bicarbonate solution (0.3 mmol/l) kept in 'butterflies' nos. 21, 23 and 25, which have the same wall thickness but different cross-sectional areas (SA) (see text for details)

Table 5. Loss of radioactivity in bicarbonate solutions of different concentrations in a 5 ml syringe and a size 23 'butterfly'

Bicarbonate (mmol/l)	Syringe		Butterfly	
	Loss/day (%)	Loss/h (%)	Loss/day (%)	Loss/h (%)
0.3	3.20	0.14	52.3	3.04
1	1.49	0.06	24.8	1.18
2	0.47	0.02	15.4	0.69
10	0.14	0.01	2.3	0.10

stated value. Repeat measurements over a period for 1 week give reproducible results for the same calorimeter (1%). The strength of hyamine hydroxide (normality of solution, assessed by titration in the presence of phenolphthalein) was found to be 3–8% higher than the molarity of the solution as stated by the manufacturers (approximately 1 mol/l). Furthermore, indicators such as phenolphthalein change colour in a hyamine-methanol mixture at an alkaline pH (approximately 9). Titration to pH 7.0 with the reagents used was found to require a small additional amount of acid (1.4%). The precision of the titration was found to be good (<0.5%).

Scintillation counting in the absence of hyamine hydroxide was found to result in a rapid early loss of label (approximately 15% in ≤ 5 min and approximately 35% by 400 min; Fig. 7). The loss from 1 mmol of hyamine hydroxide that had been neutralized with CO_2 (indicated by decoloration of phenolphthalein; Fig. 7) was similar to the loss from sodium bicarbonate solutions (1 ml of 0.3 and 150 mmol/l added to 7 ml of scintillant). The loss was slow (approximately 5%) between 100 and 400 min. However, when the cap of the scintillation vial was opened and aerated for 2 min, a further loss of up to 30% occurred. No significant loss occurred when the scintillant (7 ml) was alkalized with 2.5 mmol hyamine. The precision in ^{14}C counting was found to be 0.6%.

DISCUSSION

One of the most consistent findings of the present analysis is the large variability in recovery of labelled

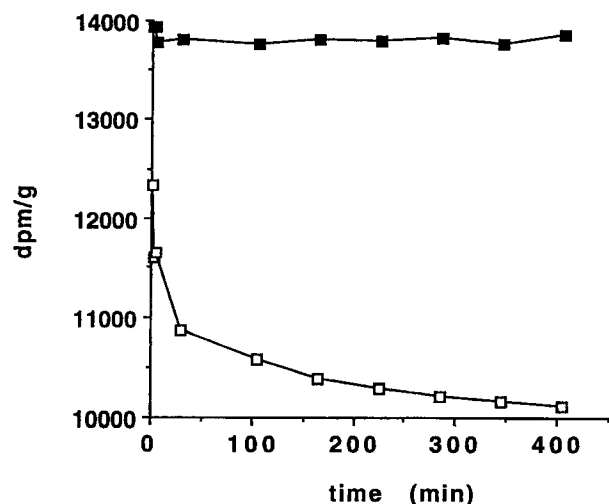


Fig. 7. Decrease in radioactivity (d.p.m./g) from a solution of hyamine hydroxide (approximately 1 mol/l)-phenolphthalein that had been decolorized with CO_2 . The measurements were made after addition of 7 ml of scintillant (hyonic fluor) in a scintillation tube that was capped (□). Loss from the same solution is prevented by addition of 2.5 mmol of alkaline unneutralized hyamine hydroxide (■).

CO_2 , irrespective of the way in which different studies are categorized (e.g. studies with $[^{14}\text{C}]$ bicarbonate or $[^{13}\text{C}]$ bicarbonate, those involving bolus or continuous administration of label and those involving short- or long-term administration of bicarbonate). This variability, which occurs even between different groups of healthy individuals of similar age, is so large (e.g. 50–100% recovery) that it is hard to attribute it entirely to biological factors. This also applies to the higher recovery of label in continuous infusion studies compared with bolus studies. The variability is likely to be influenced by differences in the methods used to analyse CO_2 production and tracer enrichment (or specific activity), differences in study design and the use of different procedures to analyse the results.

Potential methodological problems

It is not possible to provide an accurate assessment of the methodological errors associated with many of the published studies because the information provided in many of the papers is incomplete. However, our laboratory studies suggest that potentially important errors may arise from multiple sources: inadequate linearization of CO_2 IR analysers (up to 8%); inaccurate gas flowmeters (up to 4%) and, to a lesser extent, infusion pumps used to administer bicarbonate (approximately 1%); loss of label during preparation (0 to >50%) and administration of the bicarbonate solution (0–3%/h); problems associated with scintillation counting of $^{14}\text{CO}_2$ (0–30%), although this problem can be minimized or abolished by alkalization of the scintillant; errors in the assigned normality of the alkaline solution used to trap $^{14}\text{CO}_2$ (0–8%); and errors in the concentration of CO_2 assigned to the reference CO_2 gases (0–3%). Differences between methods used to measure bicarbonate concentration ($[^{13}\text{C}]$ bicarbonate studies) (4% or more [42]), can also contribute to the errors, as can changes in background enrichment (^{13}C studies; 0 to >10%; see below).

It is obvious that workers should take care to ensure that their procedures are robust, reproducible and accurate, e.g. by checking the accuracy of calibration gases, CO_2 analysers, infusion pumps and flowmeters, normality of trapping agents (^{14}C studies) and alkalization of scintillants.

Study design

Although some studies have made continuous measurements of CO_2 production to calculate recovery of labelled CO_2 [8, 9, 11, 12, 15, 16, 25, 30–33, 35], most studies have measured the CO_2 production intermittently in the resting state (sometimes infrequently over a short period of time – see Results) and assumed it to be representative

of the CO₂ production over the entire period of the study. However, it is hard to envisage subjects who remain motionless in studies that last up to 12 h. If an increase in CO₂ production due to physical activity occurred between the measurement periods, then the measured rate of CO₂ production will underestimate the overall rate of CO₂ production. Furthermore, an increase in physical activity between measurement periods will reduce the specific activity of CO₂, a change that could be carried over into the measurement period and potentially exaggerate the error in recovery. Uncertainties about differences in CO₂ production between measurement and non-measurement periods make it difficult to assess the extent of this error, although one can readily envisage differences between studies of up to 5% and possibly more. It is also possible that intermittent use of facemasks and mouthpieces may cause transient hyperventilation in anxious individuals (increased CO₂ production), but the extent to which this might occur generally is uncertain. This problem can be largely prevented by continuous measurement of CO₂ production over prolonged periods of time, but this has been undertaken in only a minority of studies.

In primed constant infusion studies, the priming dose relative to the constant infusion rate has differed considerably. Incorrect dosing and biological variability in the size and turnover rate of the bicarbonate pools means that it may take a variable time to achieve a near steady state. Thus, a carry-over effect may occur into the measurement period which in some studies began as early as 30 min after the prime (see Results). It is recommended that evidence should be provided that a steady state or a near steady state has been achieved over an extended period of time.

Procedures used to analyse results

Differences in the methods used to assess recovery of labelled CO₂ in bolus studies (e.g. the use of different models at different periods of time ranging from 3 h to infinity) can cause differences as large as 10–15% (see Results). Similarly, the different ways in which recovery is assessed in constant infusion studies can contribute to differences of up to 10%. A particular problem that concerns ¹³C studies is background drift [39]. In some studies such a drift is assumed not to occur, while in others it has been assumed that the changes are the same as those reported in previous studies. Care should be taken in the general use of the latter approach, not only because ¹³CO₂ background enrichment is different between different populations, such as Europeans and North Americans, but also because changes induced by feeding or exercise can differ severalfold between these populations [40]. This is because the traditional North American diet contains a high percentage of carbohydrate

derived from C₄ plants (e.g. maize, sugar cane) and thus has a higher natural ¹³C enrichment than the European diet, which contains a high percentage of carbohydrate derived from C₃ plants (potato, sugar beet). Both exercise (transiently) and feeding cause an increase in the proportion of energy derived from carbohydrate. Furthermore, fasting (e.g. during studies of 6–12 h duration) may cause a background drift in the opposite direction, because the contribution of carbohydrate oxidation to energy expenditure decreases with time, whereas that of fat, which has a low natural ¹³C enrichment relative to carbohydrate, increases with time [40].

A drift or an error in the measurement of basal enrichment has a greater effect on the recovery when a small dose of label is used rather than a large dose. Errors as high as 17% can occur when the value assigned to the baseline enrichment is incorrect by only 0.5 mAPE (see Results). Therefore, it is recommended that an appropriate dose of [¹³C]bicarbonate should be carefully chosen, especially during exercise, when the increment in the enrichment of CO₂ is low as a result of high CO₂ production rates. In addition, background changes should be taken into account by appropriate control studies in which no tracer is infused.

Potential biological problems

Overall, the literature does not suggest a significantly different recovery of ¹⁴CO₂ compared with ¹³CO₂. There is great variability in the reported results with either tracer, which can be explained by multiple methodological problems and some biological variability. However, to assess whether the two tracers are truly bioequivalent it is necessary to undertake infusion of [¹³C] bicarbonate and [¹⁴C]bicarbonate simultaneously under carefully controlled conditions.

Different subject characteristics (obese versus non-obese, children versus adults) and exercise do not appear to be associated with overall significant differences in recovery although individual studies with different protocols might suggest that significant differences exist. There is a time-dependent increase in recovery of label (Fig. 2), as label has more time to recycle through slowly turning pools and more time to be released from substrates that had previously fixed it (see below). The design of some studies, in which feeding [18] or exercise [34] follows measurements in the resting and fasted state, prevents a distinction being drawn between a time-dependent change in recovery and one due to the exercise or feeding. Results may also differ depending on whether a constant bicarbonate infusion is started while the subject is exercising or while in the resting state some time before exercise is started. In the latter case the enrichment of CO₂ will change from one near steady state, which is associated with a high CO₂ enrichment, to another

state that is associated with a lower enrichment and higher CO₂ production. This means that there will be a washout of labelled CO₂ from the body pool so that recovery of labelled CO₂ may transiently rise [11, 34] to values as high as 170% before slowly declining to a new near steady state.

Another interesting observation is that recovery of labelled CO₂ is lower during the night than during the day, at least partly because the enrichment or specific activity of CO₂ in the body pools increases as a result of low CO₂ production [12]. This results in storage or accumulation of labelled CO₂ in the body pool and a lower recovery of label in breath. This circadian pattern in CO₂ recovery can confound studies [24] such as those involving feeding or fasting, especially when feeding is undertaken in one part of the 24 h period and fasting in another. Short-term variations in recovery (e.g. 0–4 h) may occur not only because of changes in CO₂ production, which cause transient storage or loss of CO₂ from the body pool, but also from altered kinetics between different pools of CO₂. Such changes are induced by exercise, which produces changes in the distribution of blood flow within the body and increases oxidation of substrates that are involved in metabolic recycling of CO₂. One exercise study [36] reported lower than normal recovery of labelled CO₂. It is uncertain whether the reduction in intestinal blood flow due to the exercise, which was intermittent in this last study, is associated with a slower rate of absorption of label into the body from the gastrointestinal tract after oral dosing.

The incomplete recovery in breath of infused label can be explained by at least three processes. Firstly, there is some non-pulmonary loss of label (e.g. approximately 1% loss of CO₂ occurs transcutaneously), a small amount is lost in faeces (approximately <0.5%) and some is lost in urine (e.g. <1% to 3% is lost as urinary urea, which is formed from CO₂). Secondly, label is lost in other gases, but in man this is very small. Methane that is formed from reduction of CO₂ accounts for 0–0.2% of CO₂ production. Thirdly, CO₂ may enter bicarbonate/carbonate pools that turn over slowly, e.g. bone. Fourthly, CO₂ may be fixed in intermediary metabolites (often via the citric acid cycle) that ultimately end up as glycogen protein and fat, which also turn over slowly (see [38] for details). Although these processes operate at different rates between individuals, the recovery of label is likely to increase with time as label equilibrates and recirculates. Indeed, in whole-body calorimetry studies of 12–36 h [11] or longer [12], recovery of label as gaseous CO₂ is about 95.5% (and approximately 97.5% if urinary losses are included) with remarkably little variability between individuals (SD approximately 1.5%). This suggests that, within the time frame of the study, virtually all the label recirculates.

Theoretically a large dose of bicarbonate may cause changes in acid–base balance and changes in

ventilation (and associated changes in CO₂ excretion), but the dose given in most bicarbonate studies is probably not sufficiently high to cause important changes.

CONCLUSIONS

This paper suggests that there is plenty of scope for methodological errors in assessing bicarbonate recovery. These have important implications not only for studies that measure substrate oxidation, but also for those that measure energy expenditure from the isotopic dilution of CO₂. It is clear that strict methodology is necessary to ensure that accurate amounts of label are infused and that accurate measurements are made of CO₂ production and CO₂ enrichment (or specific activity). Furthermore, studies that aim to ensure that measurements are made in a steady state should provide evidence for the steady state. This paper has indicated the appropriate areas and procedures which require attention.

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REFERENCES

1. Beaufre B, Horber FF, Schwenk WF, et al. Glucocorticosteroids increase leucine oxidation and impair leucine balance in humans. *Am J Physiol* 1989; **257**: E712–21.
2. James WPT, Garlick PJ, Sender PM, Waterlow JC. Studies of amino acids and protein metabolism in normal man with L-[U-¹³C]tyrosine. *Clin Sci Mol Med* 1976; **50**: 525–32.
3. Issekutz Jr B, Pavle BP, Miller HI, Bortz WM. Oxidation of plasma FFA in lean and obese humans. *Metabolism* 1968; **17**: 62–73.
4. Leijssen DPC, Saris WHM, Jeukendrup AJ, Wagenmakers AJM. Oxidation of exogenous [¹³C]galactose and [¹³C]glucose during exercise. *J Appl Physiol* (In press).
5. Saris WHM, Goodpaster BH, Jeukendrup AE, Brouns F, Halliday D, Wagenmakers AJM. Exogenous carbohydrate oxidation from different carbohydrate sources during exercise. *J Appl Physiol* 1993; **75**: 2168–72.
6. Armon YDM, Cooper C, Springer TJ, et al. Oral [¹³C]bicarbonate measurement of CO₂ stores and dynamics in children and adults. *J Appl Physiol* 1990; **69**: 1754–60.
7. Barstow JT, Cooper DM, Sobel EM, Landaw EM, Epstein S. Influence of increased metabolic rate on [¹³C]bicarbonate washout kinetics. *Am J Physiol* 1990; **259**: R163–71.
8. Bresson JL, Mariotti A, Narcy P, Riour C, Sachs C, Rey J. Recovery of [¹³C]-bicarbonate as respiratory ¹³CO₂ in parenterally fed infants. *Eur J Clin Nutr* 1990; **44**: 3–9.
9. Clugston GA, Garlick PJ. Recovery of infused [¹⁴C]bicarbonate as respiratory ¹⁴CO₂ in man. *Clin Sci* 1983; **64**: 231–3.
10. Coggan AR, Habash DL, Mendenhall LA, Swanson SC, Kien CL. Isotopic estimation of CO₂ production during exercise before and after endurance training. *J Appl Physiol* 1993; **75**: 70–5.
11. Elia M, Fuller NJ, Murgatroyd PR. Measurement of bicarbonate turnover in humans: applicability to estimation of energy expenditure. *Am J Physiol* 1992; **263**: E676–87.
12. Elia M, Jones MG, Jennings G, et al. Estimation of energy expenditure from the specific activity of urinary urea during long-term subcutaneous infusion of NaH¹⁴CO₃. *Am J Physiol* 1992; **263**: E172–82.

13. El-Khoury AE, Fukagawa NK, Sánchez M, et al. Validation of the tracer-balance concept with reference to leucine: 24-h intravenous tracer studies with L-[1-¹³C]leucine and [¹⁵N-¹⁵N]urea¹⁻³. *Am J Clin Nutr* 1994; **59**: 1000-11.
14. El-Khoury, AE, Sánchez M, Fukagawa NK, Gleason RA, Young VR. Similar 24 h pattern and rate of carbon dioxide production, by indirect calorimetry vs. stable isotope dilution, in healthy adults under standardized metabolic conditions. *J Nutr* 1994; **124**: 1615-27.
15. Garlick PJ, McNurlan MA, McHardy KC, et al. Rates of nutrient utilization in man measured by combined respiratory gas analysis and stable isotopic labelling: effect of food intake. *Human nutrition: Clin Nutr* 1987; **41C**: 177-91.
16. Glamour TS, McCullough AJ, Sauer PJJ, Kalhan SC. Quantification of carbohydrate oxidation by respiratory gas exchange and isotopic tracers. *Am J Physiol* 1995; **268**: E789-96.
17. Hamel N, Divertie G, Silverberg J, Person M, Miles J. Tracer disequilibrium in CO₂ compartments during NaH¹⁴CO₃ infusion. *Metabolism* 1993; **42**: 993-7.
18. Hoerr RA, Yong-Ming Y, Wagner DA, Burke JF, Young VR. Recovery of ¹³C in breath from NaH¹³CO₃ infused by gut and vein: effect of feeding. *Am J Physiol* 1989; **257**: E426-38.
19. Irving CS, Wong WW, Shulman RJ, Smith EO, Klein PD. [¹³C]bicarbonate kinetics in humans: intra- vs. interindividual variations. *Am J Physiol* 1983; **245**: R190-202.
20. Irving CS, Wong WW, Boutton WM, et al. Rapid determination of whole-body bicarbonate kinetics by use of a digital infusion. *Am J Physiol* 1984; **247**: R709-16.
21. Irving CS, Lifschitz CH, Wong WW, Boutton TW, Nichols BL, Klein PD. Characterization of HCO₃-CO₂ pool sizes and kinetics in infants. *Pediatr Res* 1985; **19**: 358-63.
22. Irving CS, Thomas MR, Malphus EW, et al. Lysine and protein metabolism in young women. *J Clin Invest* 1986; **77**: 1321-31.
23. Jeevanandam M., Holaday NJ., Petersen SR. Nutritional influence on the recovery of ¹³CO₂ in critically ill trauma patients. *Am J Physiol* 1994; **266**: E366-71.
24. Meineke I, de Mey C, Eggers R, Bauer FE. Evaluation of the ¹³CO₂ kinetics in humans after oral application of sodium bicarbonate as a model for breath testing. *Eur J Clin Invest* 1993; **23**: 91-6.
25. Melville S, McNurlan MA, McHardy KC, et al. The role of degradation in the acute control of protein balance in adult man: failure of feeding to stimulate protein synthesis as assessed by L-[1-¹³C]leucine infusion. *Metabolism* 1989; **38**: 248-55.
26. Motil KJ, Montandon CM, Hachey DL, Boutton TW, Klein PD, Garza C. Whole-body protein metabolism in lactating and nonlactating women. *J Appl Physiol* 1989; **66**: 370-6.
27. Reaich D, Graham KA, Cooper BG, Scrimgeour CM, Goodship THJ. Recovery of ¹³C in breath from infused NaH¹³CO₃ increases during euglycaemic hyperinsulinaemia. *Clin Sci* 1994; **87**: 415-19.
28. Thomas MR, Irving CS, Reeds PJ, et al. Lysine and protein metabolism in the young lactating woman. *Eur J Clin Nutr* 1991; **45**: 227-42.
29. Tissot S, Delafosse B, Normand S, et al. Recovery of [¹³C]bicarbonate as respiratory ¹³CO₂ in mechanically ventilated patients. *Am J Clin Nutr* 1993; **57**: 202-6.
30. Van Aerde JEE, Sauer PJJ, Pencharz PB, et al. The effect of energy intake and expenditure on the recovery of ¹³CO₂ in the parenterally fed neonate during a 4-hour primed constant infusion of NaH¹³CO₃. *Pediatr Res* 1985; **19**: 806-10.
31. Waterhouse C, Baker N, Rostami H. Effect of glucose ingestion on the metabolism of free fatty acids in human subjects. *J Lipid Res* 1969; **10**: 487-94.
32. Wenham D, Pacy PJ, Halliday D. Bicarbonate recovery: feeding versus time [Abstract]. *Proc Nutr Soc* 1991; **50**: 47A.
33. Winchell HS, Stahelin H, Kusubov N, et al. Kinetics of CO₂-HCO₃⁻ in normal adult males. *J Nucl Med* 1970; **11**: 711-15.
34. Wolfe RR, Wolfe MH, Nadel ER, Shaw JHF. Isotopic determination of amino acid-urea interactions in exercise in humans. *J Appl Physiol* 1984; **56**: 221-9.
35. Yang RD, Irving CS, Wong WW, Hoffer JH, Young VR, Klein PD. The effect of diet and meal ingestion on whole body ¹³C-bicarbonate kinetics in young men [Abstract]. *Fed Proc Am Fed Am Soc Exp Biol* 1983; **42**: 825.
36. Zancanato S, Cooper DM, Barstow TJ, Landaw E. ¹³CO₂ washout dynamics during intermittent exercise in children and adults. *J Appl Physiol* 1992; **73**: 2476-82.
37. Spear LM, Darmaun D, Sager BK, Parsons WR, Haymond MW. Use of [¹³C]bicarbonate infusion for measurement of CO₂ production. *Am J Physiol* 1995; **268**: E1123-7.
38. Elia M. The estimation of short-term energy expenditure by the labelled bicarbonate method. In: Whitehead RG, Prentice A, eds. *New techniques in nutrition research*. San Diego: Academic Press, 1991: 207-27.
39. Wolfe RR, Shaw JHF, Nadel ER, Wolfe M. Effect of substrate intake and physiological state on background ¹³CO₂ enrichment. *J Appl Physiol* 1984; **56**: 230-4.
40. Wagenmakers AJM, Rehrer NJ, Brouns F, Saris WHM, Halliday D. Breath ¹³CO₂ background enrichment during exercise: diet-related differences between Europe and America. *J Appl Physiol* 1993; **74**: 2353-7.
41. Phillips SM, Atkinson SA, Tarnopolsky MA, McDougal JD. Gender differences in leucine kinetics and nitrogen balance in endurance athletes. *J Appl Physiol* 1993; **75**: 2134-41.
42. Slinger BH, Kusubov N, Winchell HS. Effect of exercise on human CO₂-HCO₃ kinetics. *J Nucl Med* 1970; **11**: 716-18.