Use of [^{13}C]bicarbonate infusion for measurement of CO_2 production

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Spear, Michael L., Dominique Darmaun, Brenda K. Sager, W. Reed Parsons, and Morey W. Haymond. Use of [^{13}C]bicarbonate infusion for measurement of CO_2 production. *Am. J. Physiol.* 268 (*Endocrinol. Metab.* 31): E1123–E1127, 1995.—To determine whether infusion of ^{13}C-labeled bicarbonate can be used to measure rates of CO_2 production ($\dot{V}CO_2$), seven healthy adults received 6-h primed continuous intravenous infusions of $NaH^{13}CO_3$ and L-[1-^{14}C]leucine in the postabsorptive state while $\dot{V}CO_2$ was measured by indirect calorimetry. Indirect calorimetry and the use of specific activity and rate of $^{14}CO_2$ expired yielded identical values of $\dot{V}CO_2$: 8.97 ± 0.82 and 8.80 ± 0.83 mmol/min, respectively (P = NS). The concentration of $NaH^{13}CO_3$ in the infusates and the ^{13}C enrichment in breath CO_2 were determined using gas chromatography-isotope ratio mass spectrometry. The rate of appearance of CO_2 measured using the $NaH^{13}CO_3$ infusion rate and the steady-state breath $^{13}CO_2$ enrichments was 11.41 ± 1.56 mmol/min, which was higher ($P < 0.001$) than that determined by either of the other two methods. When corrected for the recovery of labeled CO_2 during the infusion of $NaH^{13}CO_3$ by use of published values, rate of appearance of CO_2 was 9.24 ± 0.78 mmol/min, which did not differ from $\dot{V}CO_2$ determined using the other two methods. We conclude that infusion of $NaH^{13}CO_3$ can be used to determine $\dot{V}CO_2$. This method should be useful to study the oxidation of substrates in populations such as ventilator-dependent neonates, in whom indirect calorimetry is laborious and inaccurate.

stable isotopes; substrate oxidation; metabolism; in vivo; humans

OVER THE LAST TWO DECADES, stable isotope tracers have been used extensively to investigate whole body protein metabolism in healthy adults (8, 20, 22, 31) and children (28), as well as full-term (3, 9) and premature infants (2, 23, 27). Infusions of L-[1-^{13}C]leucine have been extensively utilized to measure the rates of appearance and oxidation of leucine, from which changes in the rates of whole body protein breakdown and synthesis can be estimated (22, 26). With knowledge of the rates of leucine appearance and oxidation under near isotope and substrate steady state, the rate of nonoxidative leucine disposal, an indicator of protein synthesis, can be calculated. To determine the rate of leucine oxidation, the rate of excretion of $^{13}CO_2$ in breath must be determined; this is accomplished by multiplying the enrichment of $^{13}CO_2$ in breath by the total rate of CO_2 excreted and correcting for the recovery of ^{13}C during an infusion of $NaH^{13}CO_3$ (22).

Whereas measurement of $^{13}CO_2$ enrichment requires only intermittent sampling of breath, there are several methods to determine total CO_2 production ($\dot{V}CO_2$). Indirect calorimetry is the most commonly employed method for the determination of excretion rate of CO_2 in breath. This technique requires the patient to breathe via a mouthpiece (with the nares occluded), which requires absolute cooperation from the patient in a quiet and comfortable environment (13), or via a hood that captures all expired CO_2 (13).

Another method for measuring $\dot{V}CO_2$ involves the infusion of a ^{14}C-labeled substrate, such as [^{14}C]leucine or its α-ketoacid α-[^{14}C]ketoisocaproate (KIC), and the measurement of specific activity (SA) and the rate of excretion of $^{14}CO_2$ in expired air over a timed period at isotopic and substrate steady state. By dividing the rate of $^{14}CO_2$ excretion [disintegrations · min^{-1} (dpm) · min^{-1}] by the SA of breath CO_2 (dpm/mmol), $\dot{V}CO_2$ (mmol/min) can be measured. This method requires the capture of all expired CO_2 during several precisely timed 2-min intervals (25, 31).

To investigate protein metabolism in the premature infant with use of stable isotope tracers of amino acids, $\dot{V}CO_2$ must be measured. In this subject population, the use of radioactive isotopes is prohibited (5) and indirect calorimetry is laborious and inaccurate. During the routine care of the mechanically ventilated neonate, the clinical practice has been to utilize uncuffed endotracheal tubes because of reduced laryngeal damage, which results in significant air leaks around the tube. Therefore capture of all expired CO_2 is extremely difficult, if not impossible. Kien et al. (18, 21) described in theory the use of isotopic dilution of CO_2 during the infusion of $NaH^{13}CO_3$ to predict $^{12}CO_2$ production, which avoids the determination of total expired CO_2. Validation of such a method would provide a technique that could be utilized in a variety of clinical investigations not only in neonates and young children but in adults as well.

The purpose of this study was to determine whether the infusion of $NaH^{13}CO_3$ can be used to measure total $\dot{V}CO_2$ in healthy adult volunteers. The technical aspects of this methodology were validated, and simultaneous comparisons were made using indirect calorimetry and L-[1-^{14}C]leucine.

METHODS

Materials. Purchased lots of L-[1-^{14}C]leucine (57 mCi/mmol, Amersham, Arlington Heights, IL) and $NaH^{13}CO_3$ (98% ^{13}C, Tracer Technologies, Somerville, MA or Cambridge Isotopes Laboratories) were tested for chemical, isotopic, and optical purity. Solutions of the isotopes were passed through a 0.22-μm Millipore filter and stored in sterile sealed containers for <24 h before each infusion and kept at 4°C until used. Aliquots of the isotopic solutions were tested for sterility by use of standard microbiologic techniques and for absence of pyrogen by means of *Limulus* lysate assay.

Subjects. Seven healthy normal-weight 28.4 ± 3.2 (SE) yr old adult volunteers (5 males, 2 females) were studied. Their average weight, height, and body mass index were 83.3 ± 5.3 kg, 174 ± 4 cm, and 27.6 ± 1.9 kg/m^2, respectively. Subjects were determined to be free of metabolic, renal, hepatic, cardiac, or any major organic disease on the basis of medical history, physical exam, and routine blood chemistries.

Protocol design. The protocol was reviewed and approved by the Nemours Children's Clinic Research Committee and by the Institutional Review Committee of Baptist Medical Center (Jacksonville, FL). After written informed consent was obtained, subjects were instructed to continue their usual levels of dietary intake and physical activity for the 3 days before the study day. On the night before each study day, each subject ate dinner at 1800 and then remained fasting except for ad libitum water until completion of the infusion study at ~1300 on the day of study.

On the morning of study at ~0700, two intravenous catheters were placed: one in a forearm vein for isotope infusion and one in the dorsal vein of the contralateral hand. During the sampling period, the hand was placed in a heating pad kept at 60°C to obtain arterialized-venous blood samples (7). Before initiation of the isotope infusion, three baseline breath samples and two blood samples were collected to determine the natural background of ^{13}C and ^{14}C in the expired CO_2 and plasma KIC. At 0800, primed continuous (1, 22) intravenous infusions of L-[1-^{14}C]leucine (0.148 μCi/kg and 0.00247 μCi·kg^{-1}·min^{-1}, respectively) and $NaH^{13}CO_3$ (7.056 μmol/kg and 0.118 μmol·kg^{-1}·min^{-1}, respectively) were started. The infusions were delivered using a calibrated Razel pump and continued for 4 h in *subjects A* and *B* and for 6 h in the remaining subjects.

Arterialized blood samples were obtained at 15-min intervals during the last 2 h of tracer infusion to determine the plasma concentration and SA of plasma KIC. In all cases, regardless of the duration of the infusion, near isotopic and substrate steady state was achieved.

At 30-min intervals, starting at 1000 (*subjs A* and *B*) or 1100 (*subjs C–G*), expired air was collected in Douglas bags while the subjects wore a noseclip and a mouthpiece equipped with a one-way valve. Rates of expired $^{14}CO_2$ excretion were determined by aspiration of 2-min collection of expired air through an ethanolamine solution, as previously described (24, 30). In addition, the SA of expired CO_2 was determined at each breath sampling time by aspiration of expired air through hydroxide of hyamine in ethanol with phenolphthalein as a pH indicator, as previously described (25). Between measurements with the mouthpiece, the head of the patient was placed under the hood of a ventilated canopy for nearly continuous monitoring of $\dot{V}CO_2$ with use of a Sensormedics instrument (Yorba Linda, CA) indirect calorimeter (18).

Analytic methods. The SA of expired $^{14}CO_2$ was determined by trapping 1 mmol of CO_2 in a 2-ml aliquot of a 0.5 M solution of hyamine and counting by scintillation spectroscopy to determine the disintegrations per minute per millimole (dpm/mmol) of CO_2. The rate of $^{14}CO_2$ excretion (dpm/min) was determined by performing timed 2-min collection of expired air and subsequently trapping the entire CO_2 excretion of a 2-min collection in a solution of ethanolamine, as previously described (25, 31).

Breath $^{13}CO_2$ enrichments were measured using an automated gas chromatograph-isotope ratio mass spectrometer (GC-IRMS; VG-Optima, Northwich, UK) (15). The concentration of labeled bicarbonate in the infusates was determined by two methods: *1*) accurate weighing of the sodium bicarbonate powder on a high-precision scale at the time of preparation of the infusates and *2*) measurement of $^{13}CO_2$-to-$^{12}CO_2$ ratio in quadruplicate aliquots of the infusate by reverse isotope dilution, with natural unlabeled sodium carbonate (Na_2CO_3) as an internal standard, as follows. On the day of each infusion study, replicate aliquots of each $NaH^{13}CO_3$ solution were spiked with a known amount of unlabeled Na_2CO_3 into a 15-ml Vacutainer tube. The tube was flushed with helium, and 500 μl of 85% phosphoric acid were injected into the tube by use of an insulin syringe. The CO_2 evolved from the reacted samples was then analyzed for ^{13}C enrichment by GC-IRMS, and the $NaH^{13}CO_3$ concentration of the infusate was then calculated using standard equations for reverse isotope dilution, with the unlabeled Na_2CO_3 as an internal standard as described previously (10).

Table 1. *Effect of storage on stability of ^{13}C-labeled bicarbonate*

Day of GC-IRMS Assay	Nonreacted		Reacted	
	−70°C	Room temp	−70°C	Room temp
0	100.0	100.0	100.0	100.0
1	97.9	99.5	100.7	99.4
2	99.6	99.0	99.0	99.4
3	94.4	97.5	100.3	100.5
4	93.9	99.3	90.9	100.7
5	99.8	98.4	87.2	100.2
Mean ± SE	97.6 ± 1.1	99.0 ± 0.4	96.4 ± 2.4	100.2 ± 0.2

Replicate aliquots of all $NaH^{13}CO_3$ infusates were spiked with sodium carbonate (unlabeled Na_2CO_3) on day of infusion and reacted (with 85:15 phosphoric acid) before storage or stored nonreacted. Aliquots were assayed by gas chromatograph-isotope ratio mass spectrometry (GC-IRMS) on day of infusion (*day 0*) or on subsequent days (*days 1–5*). Results are bicarbonate concentrations measured by GC-IRMS by use of reverse isotope dilution of Na_2CO_3 internal standard and are expressed as percentage of initial infusate concentration measured on *day 0*.

Table 1 illustrates the results of a pilot study to determine the effect of storage conditions on the stability of ^{13}C-labeled bicarbonate. Replicate aliquots of all $NaH^{13}CO_3$ infusates were spiked with unlabeled Na_2CO_3 on the day of infusion and either reacted (with 85:15 phosphoric acid) before storage or stored nonreacted. Aliquots were assayed by GC-IRMS on the day of infusion (*day 0*) or on subsequent days (*days 1–5*). Results are bicarbonate concentrations measured by GC-IRMS by reverse isotope dilution of the Na_2CO_3 internal standard and expressed as percentage of the initial infusate concentration measured on *day 0*.

Table 2. *Measurement of ^{13}C-labeled bicarbonate concentration in infusates by GC-IRMS with use of reverse isotope-dilution vs. gravimetric determinations*

Subj	[$NaH^{13}CO_3$], μmol/ml		Reverse Isotope Dilution, % of Gravimetric
	Gravimetric	Reverse isotope dilution	
A	85.8	78.1	91.0
B	107.7	107.0	99.3
C	104.0	102.6	98.7
D	82.4	79.5	96.5
E	109.2	103.3	94.6
F	93.8	91.3	97.4
G	83.9	79.8	95.1
Mean ± SE	95.3 ± 4.4	91.7 ± 4.8	96.1 ± 1.1

Table 3. *^{13}C Recovery in breath during infusion of $NaH^{13}CO_3$*

Subj	Infusate $[HCO_3^-]$, μmol/ml	Pump Rate, ml/mn	$NaH^{13}CO_3$ Infusion, μmol/min	$\dot{V}CO_2$ ml/min	E_{CO_2}, mol %excess	$^{13}CO_2$ Excreted,* μmol/min	^{13}C Recovery, % of ^{13}C Infused
A	78.1	0.106	8.279	191.4	0.08096	6.904	83.6
B	107.0	0.106	11.342	287.7	0.07119	9.089	80.6
C	102.6	0.106	10.881	240.0	0.08058	8.633	79.3
D	79.5	0.106	8.428	170.0	0.09031	6.854	81.3
E	103.3	0.106	10.946	199.0	0.09133	8.114	74.1
F	91.3	0.106	9.683	177.0	0.08601	6.797	70.2
G	79.8	0.106	8.464	142.0	0.09926	6.292	74.3
Mean ± SE	91.7 ± 4.8	0.106 ± 0.000	9.718 ± 0.507	201.0 ± 18.4	0.08570 ± 0.00340	7.534 ± 0.410	77.6 ± 1.8

$\dot{V}CO_2$, CO_2 production; E_{CO_2}, ^{13}C enrichment in expired CO_2. *Calculated as $[\dot{V}CO_2 \times E_{CO_2}] / (0.0224 \text{ ml}/\mu\text{mol} \times 100)$, with assumption that 1 μmol of CO_2 = 0.0224 ml CO_2 under STPD conditions.

Aliquots of the labeled bicarbonate infusates were most stable if spiked with Na_2CO_3 internal standard immediately after the tracer infusion and reacted with phosphoric acid immediately thereafter. These remained stable at room temperature for the 5-day pilot study period.

Calculations. The bicarbonate concentration in the infusates was derived from the ^{13}C fractional abundance, measured by GC-IRMS, in a solution of pure Na_2CO_3 internal standard (f_{STD}) and in a mixed solution (f_{MIX}) prepared by spiking a known volume of the infusate with a known volume of the internal standard. The following mass balance equation was used

$$f_{MIX} \times (n_{INF} + n_{STD}) = f_{STD} \times n_{STD}) + (f_{INF} \times n_{INF}) \quad (1)$$

where n_{INF} and n_{STD} are the micromoles of carbon in the mixture contributed by the $NaH^{13}CO_3$ infusate (unknown) and the Na_2CO_3 (known) internal standard, respectively, and f_{INF} is the fractional abundance of ^{13}C in the pure $NaH^{13}CO_3$ (99%). It follows that (10)

$$n_{INF} = n_{STD} \times (f_{MIX} - f_{STD}) / (f_{INF} - f_{MIX}) \quad (2)$$

The rate of $NaH^{13}CO_3$ infusion (F, mmol/min) was calculated by multiplying the infusate concentration (mmol/l) by the output of the calibrated syringe pump (l/min).

Total $\dot{V}CO_2$ (mmol/min) was measured by indirect calorimetry using the built-in software of our Sensormedics instrument. This was compared with the $^{14}CO_2$ method and the $NaH^{13}CO_3$ method. By use of the $^{14}CO_2$ method, $\dot{V}CO_2$ was computed by dividing the $^{14}CO_2$ excretion rate ($\dot{V}_{^{14}CO_2}$, dpm/min, obtained by ethanolamine trapping) by the SA of expired CO_2 (SA_{CO_2}, dpm/mmol CO_2)

$$\dot{V}CO_2 = \dot{V}_{^{14}CO_2} / SA_{CO_2} \quad (3)$$

Third, the appearance rate of CO_2 into breath (R_{aCO_2}) was calculated using conventional isotope-dilution equations (3, 21, 22)

$$R_{aCO_2} = F[(E_i / E_{CO_2}) - 1] \quad (4)$$

where F is the $NaH^{13}CO_3$ infusion rate (mmol/min) and E_i and E_{CO_2} are the ^{13}C enrichments (atom %excess) in the bicarbonate infusate and expired CO_2 at steady state, respectively.

Statistics. Comparisons among the three measurements of $\dot{V}CO_2$ were made by repeated-measures analysis of variance followed by paired *t*-tests, with correction for the multiple comparison by use of the Bonferroni method.

RESULTS

The validity of the reverse isotope-dilution method for determining the concentration of $NaH^{13}CO_3$ was determined in samples stored in a variety of conditions, because the potential loss of labeled $^{13}CO_2$ from the $NaH^{13}CO_3$ infusate could adversely affect the results. Separate samples of a single pool of $NaH^{13}CO_3$ were analyzed in quadruplicate after the addition of Na_2CO_3 (*day 0*) with and without the immediate addition of phosphoric acid. Samples were stored at room temperature or frozen at −70°C. Aliquots were reanalyzed on each of the 5 subsequent days. Regardless of the method

Table 4. *Measurement of $\dot{V}CO_2$ by use of indirect calorimetry, [^{14}C]leucine infusion, and [^{13}C]bicarbonate infusion*

Subj	Indirect Calorimetry, mmol/min	[^{14}C]leucine Infusion		[^{13}C]bicarbonate Infusion		
		mmol/min	% of IC	mmol/min	% of IC	% of IC, corrected*
A	8.54	7.81	91.4	10.11	118.4	95.9
B	12.85	12.73	99.1	15.77	122.8	99.5
C	10.71	10.44	97.4	13.35	124.6	100.9
D	7.59	7.88	103.8	9.23	121.6	98.5
E	8.88	8.68	97.7	11.85	133.4	108.1
F	7.90	8.19	103.6	11.13	140.9	114.0
G	6.34	5.85	92.3	8.39	132.4	107.0
Mean ± SE	8.97 ± 2.169	8.80 ± 2.20	97.9 ± 4.9	11.41 ± 2.54	127.7 ± 8.0	103.4 ± 2.4

IC, indirect calorimetry. *Corrected for a recovery of 0.81 (22).

of processing or storage, no significant differences were observed in the $NaH^{13}CO_3$ concentration measured, despite a presumed significant loss of $Na^{13}HCO_3$ and Na_2CO_3 with the addition of the phosphoric acid (Table 1). The values determined on the day of clinical infusion were utilized for the calculation of the rate of $NaH^{13}CO_3$ infused.

In addition to the reverse isotope method described above, the concentration of $NaH^{13}CO_3$ in the infusate was estimated by the traditional gravimetric method. The concentration of $NaH^{13}CO_3$ determined by the reverse isotope-dilution method was 96.1 ± 1.1% of that estimated by the gravimetric method (Table 2). The small difference between the gravimetric and the reverse isotope-dilution technique remains to be explained but is most likely due to small amounts of water of hydration or other contaminants. The concentrations of the infusates determined by the reverse isotope method were used for the determination of the rate of isotope infused in the studies described below.

The data from the calculation of the total $^{13}CO_2$ recovered in expired air during the infusion of $NaH^{13}CO_3$ are provided in Table 3. The recovery of ^{13}C in breath was 77.6 ± 1.8%, which compares favorably with other published studies (4, 6, 16, 19, 22, 29, 30) for adult subjects in a resting state after an overnight fast.

The average rate of CO_2 excreted in expired air determined by indirect calorimetry was 8.97 ± 0.82 mmol/min, which was not significantly different from that determined by the use of the SA and rate of $^{14}CO_2$ expired (8.80 ± 0.83 mmol/min); the latter value was 98 ± 2% of that determined by indirect calorimetry. The rate of CO_2 expired measured using the $NaH^{13}CO_3$ infusion method was 11.41 ± 1.56 mmol/min, which was higher ($P < 0.001$) than that determined by either of the two methods. When this $\dot{V}CO_2$ was corrected for the recovery of labeled CO_2 during the infusion of $NaH^{13}CO_3$ by use of published values (22), the rate of CO_2 in expired air was 9.24 ± 0.78 mmol/min, a value that was not different from results obtained using indirect calorimetry or the $^{14}CO_2$ method (Table 4).

DISCUSSION

The present studies describe a methodology for the measurement of $\dot{V}CO_2$ in humans. Critical to this method is the accurate measurement of the rate of infusion of $NaH^{13}CO_3$. The reverse isotope method for such quantification was demonstrated to be precise and accurate, and the infusate concentration measurement was unaffected by the storage condition of the infusate sample as long as the Na_2CO_3 internal standard was added to the infusate sample on the day of study. Clearly, careless handling of the sample with prolonged exposure to the atmosphere or with acid conditions could result in loss of $NaH^{13}CO_3$ before the addition of Na_2CO_3 and should be avoided.

By use of two independent measures of the rate of CO_2 excreted in expired breath, the $NaH^{13}CO_3$ method provided a measure of $\dot{V}CO_2$ that was ~20% higher than that of the other two methods. However, when this value was corrected for CO_2 fixation, the three methods provided very similar estimates. This 20% loss of labeled C during the infusion of labeled $NaHCO_3$ is poorly understood but is believed to be the result of incorporation of labeled CO_2 into metabolic substrates as a result of CO_2 fixation reaction. Such methods have actually been utilized to measure the rate of glucose produced via gluconeogenesis (12, 14). In addition, the CO_2 enters the body bicarbonate pools and may be buffered in exchangeable pools of HCO_3^- in bone. The fractional recovery of $^{13}CO_2$ in breath is known to be affected by age, rates of energy expenditure, activity, and feeding (4, 6, 16, 19, 22, 29, 30), whereas it may not depend on the route of infusion of labeled bicarbonate (16). Moreover, with prolonged infusion (24–48 h) of labeled $NaHCO_3$, the recovery of the CO_2 increases to ~100% (11), suggesting that this pool is finite and relatively rapidly turning over.

These data collectively suggest that the $NaH^{13}CO_3$ method may provide as accurate a measure of the rate of in vivo $\dot{V}CO_2$ as indirect calorimetry or the $^{14}CO_2$ method utilized as reference methods in the present study. It is likely, however, that all methods may significantly underestimate the actual $\dot{V}CO_2$ in most cells. We and others (12, 14) demonstrated that the rate of incorporation of labeled CO_2 into glucose is significantly higher if the labeled CO_2 is generated in the intracellular space as a result of metabolism of a labeled substrate than if the label is incorporated and infused as $NaHCO_3$.

We would conclude that the presently described method for the measurement of CO_2 production by use of an infusion of $NaH^{13}CO_3$ is as good as previously described methods. In addition, the labeled bicarbonate method has the added advantage that it does not require the correction for CO_2 fixation or the quantitative collection of expired breath. This method should provide new opportunities to study the metabolism and oxidation of a number of substrates in premature neonates and children when they are ventilator dependent.

We thank Bernice Rutledge and the nursing staff at Wolfson Children's Hospital for their help in conducting the clinical studies.

This work was supported in part by a grant from the Nemours Foundation (Jacksonville, FL).

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Received 7 November 1994; accepted in final form 31 January 1995.

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