Recovery of ^{13}C in breath from $NaH^{13}CO_3$ infused by gut and vein: effect of feeding

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HOERR, ROBERT A., YONG-MING YU, DAVID A. WAGNER, JOHN F. BURKE, AND VERNON R. YOUNG. *Recovery of ^{13}C in breath from $NaH^{13}CO_3$ infused by gut and vein: effect of feeding.* Am. J. Physiol. 257 (Endocrinol. Metab. 20): E426–E438, 1989.—Estimates of substrate oxidation obtained from appearance of ^{13}C or ^{14}C from tracers in breath must be corrected for retention of labeled carbon in the body. We aimed to determine the effect of a defined experimental diet and metabolic status on recovery of infused Na [^{13}C]bicarbonate in breath. Six healthy male subjects consumed an experimental diet for 7 days before receiving a continuous infusion of formula without tracer on *day 8* and received either an intragastric (ig) or intravenous (iv) infusion of Na [^{13}C]bicarbonate on *day 9* or *11* during a 4-h postabsorptive (PA), 4-h continuously fed period. A trend toward increasing PA breath enrichment during the first 7 diet days approached statistical significance ($P = 0.051$), whereas breath enrichments measured 3 h postbreakfast were consistently higher than PA values throughout and did not change over the 7-day period. Breath enrichments during a 4-h continuous ig infusion of formula without tracer on *day 8* rose 2.0 ± 0.5 atom percent excess (APE) $\cdot 10^{-3}$ above base line ($P < 0.001$, ANOVA). In the tracer studies, breath enrichments were similar for the ig and iv routes of tracer infusion. For the ig infusion the fraction of infused Na [^{13}C]bicarbonate recovered in breath as $^{13}CO_2$ was 0.74 ± 0.02 for the PA period and 0.79 ± 0.02 for the fed period. For the iv infusion the fraction recovered was 0.70 ± 0.04 for the PA period and 0.82 ± 0.03 for the fed period. Fractional recoveries were not significantly different for ig and iv routes of administration but were different for PA and fed periods ($P < 0.0001$, 2-way ANOVA). The fractional recoveries for the fed period obtained here were similar to the value 0.81 reported in a number of other studies. Recovery of tracer in breath increased linearly with O_2 uptake and CO_2 production, suggesting that factors affecting respiratory gas exchange may alter recovery. We conclude that the primary factor determining label recovery is the immediate and recent nutritional status of the host.

stable isotopes; tracer kinetics; bicarbonate; carbon dioxide

IN INVESTIGATIONS involving human subjects, the use of ^{13}C-labeled compounds to trace the kinetic aspects of substrate metabolism, including glucose, amino acids, and fatty acids, has increased during the past 10 years. When labeled carbon is given as [^{13}C]- or [^{14}C]bicarbonate, it has been well documented that a fraction of the labeled carbon liberated from the tracer is not recovered in the breath as labeled CO_2, at least during the short period of the usual tracer experiment. The fraction retained is believed to represent CO_2 fixation in metabolic pathways or loss to slowly exchanging pools (11, 17, 33, 38). This fraction has been incorporated into calculations of substrate oxidation from the dilution of label in plasma and breath to correct for carbon released from oxidation that does not appear in breath during the term of the experiment and to avoid underestimating the oxidation rate. Published estimates for this retained fraction vary widely (1, 5, 12, 17–20, 31, 37, 41) and, for the most part, the data have been obtained with subjects who are in the postabsorptive (PA) state.

An experimental model that we have used in a number of studies (e.g., Ref. 15) involves the administration of a ^{13}C-labeled substrate to subjects during a PA and continuously fed state, both periods being studied consecutively on a single day. Such a model allows the comparison of the host metabolic response to nutrient administration, often after subjects have received an experimental diet for a week or more. In view of the varying published estimates for ^{13}C retention, we considered it desirable to measure CO_2 retention under the same experimental conditions we have used for our ^{13}C-labeled amino acid studies.

This study was designed to evaluate four metabolic situations. First, we wanted to determine what change, if any, occurred in base-line PA breath $^{13}CO_2$ levels when subjects consumed our typical experimental formula diet for a week. Many formula diets include purified carbohydrate sources, most of which in the United States are derived from corn and are naturally enriched in ^{13}C (30). Second, we wanted to measure the change in breath ^{13}C enrichment that occurred during the 4-h period of continuous formula feeding when no tracer was administered. If a formula itself contributes significantly to the ^{13}C enrichment of expired air, then the enrichment of the expired CO_2 due to tracer alone could be overestimated unless the dietary contribution is actually measured under conditions similar to subsequent ^{13}C label administration. Third, we wanted to determine if administration of tracer by the intragastric route, where all tracer passes through tissues prior to reaching the lungs, resulted in a different estimate of recovery than when tracer was administered by the intravenous route. Finally, it was necessary to determine if recovery of ^{13}C in

expired air was different in subjects under the PA compared with fed conditions.

MATERIALS AND METHODS

Stable Isotope Tracers

Na [^{13}C]bicarbonate (measured to be 90 mole% excess) was obtained from Cambridge Isotope Laboratories (Woburn, MA). A stock solution of this material was prepared in 0.9% NaCl solution, membrane-filtered, dispensed in 20-ml vials, and tested for sterility and pyrogenicity prior to use. Isotopic enrichment and concentration of [^{13}C]bicarbonate in the tracer stock solution were verified using carbon magnetic resonance by a modified standard method of additions.

At the time of each tracer infusion experiment, aliquots of the tracer solution (a total volume, measured to the nearest 0.2 ml, of 0.132 ml/kg subject body wt, providing 35 μmol tracer/kg body wt) were diluted to a final volume of 94 ml with 0.9% NaCl (volume of infusate needed for an infusion lasting a total of 540 min, or 1 h longer than planned study). This dilution, when infused at a volume of 0.174 ml/min, resulted in an infusion rate of ~0.065 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. A priming dose of tracer was also prepared that consisted of 5.4 μmol [^{13}C]bicarbonate/kg body wt.

Expected rather than measured concentrations of each individual tracer solution were used in the calculations of ^{13}C recovery. We found that actual infusates, when measured after several months in freezer storage, were substantially lower in concentration than expected. Previously, when we had prepared amino acid infusates with the same technique used in these experiments, the measured concentration varied by not more than 4% from predicted. To verify the infusion concentrations that were used for these experiments, a stock solution of sodium bicarbonate (unlabeled) was prepared at 25 mg/ml and stored for 1 day in the same type of rubber-stoppered vial used for the original tracer stock solution. Replicates of the infusates were prepared for each subject using the dilution values recorded earlier and the same techniques and equipment. Infusate solutions were transferred into 60-ml syringes identical to those used in the experiments and capped until analysis on the following day. The bicarbonate concentration of each infusate was measured using a CO_2 electrode (model 446, IL Instruments, Lexington, MA) and in reference to a calibration curve. For this, standard solutions were prepared from sodium bicarbonate (unlabeled) and dissolved in saline, with concentrations ranging from 11.9 to 47.6 mmol/l. Aliquots of the calibration standards were stored under mineral oil in rubber-stoppered containers until infusates were analyzed. Measured concentrations varied from predicted concentrations by only 1.4% (range 0.1–3.8%) with this technique, which is better than the 7% figure published for an alternate procedure involving liberation of CO_2 from a tracer-spiked standard solution of Na_2CO_3 (17).

Subjects

Six healthy male Massachusetts Institute of Technology (MIT) students (Table 1) were studied at the MIT Clinical Research Center. The good health of the subjects was evaluated by medical history and physical examination as well as screening blood and urine clinical tests. Written informed consent was obtained from each subject prior to beginning the protocol, which had been approved by the MIT Committee on the Use of Humans as Experimental Subjects and the Policy and Executive Committees of the MIT Clinical Research Center.

Experimental Protocol

Diet during protocol and on infusion days. Throughout the study (see Fig. 1), subjects consumed a diet that provided 1.5 g protein $\cdot$ kg body $wt^{-1} \cdot day^{-1}$ and weight maintenance levels of total energy given in three isocaloric meals. Energy levels were estimated from a 24-h dietary recall by a research dietitian and for this group ranged from 34 to 50 kcal/kg. Protein was provided as powdered egg white prepared in an artificially flavored omelet (breakfast and lunch) and as a saccharine-sweetened, flavored formula (supper). The balance of energy needed at each meal to meet requirements was provided by a liquid formula that was based on Contra-lyte (Ross Laboratories, Columbus, OH) and flavored with Kool-Aid. Contra-lyte is comprised of 60% carbohydrate calories as partially hydrolysed corn starch and 40% fat calories as soy oil, with additional lecithin added as an emulsifying agent. Additional sodium was provided as sodium chloride (4 g), potassium as potassium phosphate, dibasic (3.2 g), and calcium and phosphorus as calcium phosphate, tribasic (1.8 g; amounts are those for a 70-kg subject and were adjusted according to body wt). Subjects also received choline (500 mg) and a multivitamin and trace mineral supplement. Each of the three fed state infusion periods provided one-twelfth of the daily protein and energy intake hourly for 4 h. The remainder of the daily formula on these days was taken as two additional meals after the infusion was completed.

Dietary constituents were measured for ^{13}C enrichment by combustion and subsequent analysis by gas isotope ratio mass spectrometry (Geochron Laboratories, Cambridge, MA). The ^{13}C natural abundance of egg white used in this study was found to be 1.09407 atom percent (AP) with reference to the PeeDee belemnite (PDB) standard, whereas the ^{13}C natural abundance of Contra-lyte was found to be 1.09186 AP. As about 15% of calories were provided by egg white and 85% by Contra-lyte, this would be equivalent to a formula ^{13}C abundance of ~1.0922 AP. Base-line PA breath values for healthy adults in Boston, measured randomly throughout the year, equal 1.08879 $\pm$ 0.0003 AP (Alfred Ajami, Tracer Technologies, personal communication); thus the diet in this study represented an enrichment of ~3.4 atom percent excess (APE) $\cdot 10^{-3}$ higher than basal breath values. Because this diet was slightly enriched with ^{13}C relative to the usual Boston diet (reflected by usual PA breath values), ingestion of this diet would be expected to raise a subject's breath ^{13}C relative to levels before starting the study.

Measurement of breath base-line $^{13}CO_2$ enrichment. For two consecutive mornings before the subjects began consuming the experimental diet, base-line PA breath sam-

TABLE 1. *Anthropometric data and daily dietary protein and energy intake for experimental subjects*

	Subject						Means ± SE
	A	*B*	*C*	*D*	*E*	*F*	
Age, yr	24	21	21	23	21	20	22±1
Weight, kg	60	73	81	68	83	99	76±6
Height, cm	171	187	180	180	175	190	181±3
Daily protein, g	90.2	109.8	121.1	101.5	125.4	148.5	115.9±9.1
Dietary energy intake, kcal	2,890	3,650	3,300	3,100	3,500	3,400	3,307±112

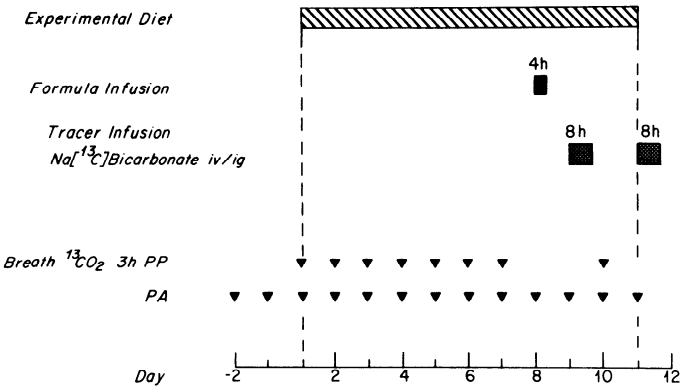

FIG. 1. Protocol outline showing schedule for experimental diet, obtaining of daily breath samples for ^{13}C enrichment, and infusion studies.

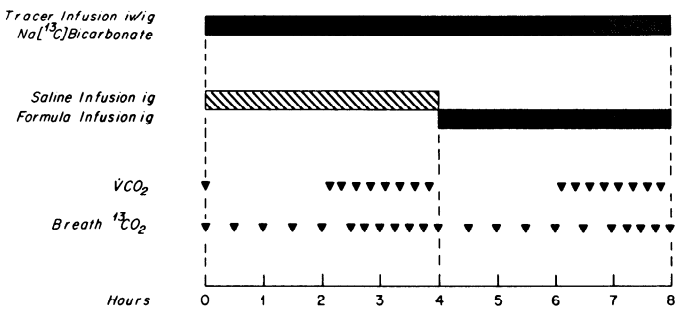

FIG. 2. Experimental outline for tracer studies on *days 9* and *11*. Priming dose of tracer was given prior to initiation of continuous intravenous or intragastric infusion.

ples were collected for $^{13}CO_2$ enrichment by having the subjects exhale into a 3-liter Rudolf anesthesia bag through a mouthpiece and two-way Rudolf valve (both from Warren E. Collins, Braintree, MA). Aliquots were transferred by syringe to 15-ml nonsterile, nonsilicon-coated evacuated glass tubes (Venoject, T-218U, Terumo Medical, Elkton, MD). On the morning the formula began and thereafter through *day 7*, base-line PA and 3-h postbreakfast breath samples were collected in the same manner. Although it would have been advantageous to have multiple time points after the meal, we were limited to the 3-h postprandial time point because our subjects, all MIT students, were attending morning classes. The 3-h time point corresponded to the time when they returned for the midday meal.

Infusion studies. Three studies were conducted in each subject; a 4-h intragastric infusion of the liquid formula, made without tracer on *day 8* and tracer infusions on *days 9* and *11* (see Fig. 1). The liquid formula used in these experiments consisted of the powdered egg white, Contra-lyte, flavoring, and minerals as noted above, but adjusted in concentration so that one-twelfth of the daily intake would be administered each hour in a volume of 125 ml. The infusions on *days 9* and *11* consisted of an initial 4-h PA period during which only saline was infused and a 4-h continuously fed period, as described below. Three of the subjects received [^{13}C]bicarbonate tracer intravenously (iv) on *day 9* and intragastrically (ig) on *day 11*. For the other three subjects the order of the tracer administration route was reversed.

All infusions began in the morning after an overnight fast (see Fig. 2). At 7 A.M., base-line breath samples for $^{13}CO_2$ enrichment were collected. If the tracer was to be infused iv, a forearm catheter was placed. A 9-French circumference feeding nasogastric tube (Entriflex, Bio-search Medical Products, Somerville, NJ), was inserted on *days 8*, *9*, and *11* for formula and/or tracer infusion. On *days 9* and *11* subjects received an iv or ig primed, continuous infusion of Na [^{13}C]bicarbonate, prepared as described above in the stated doses, that continued throughout the entire 8-h period. The tracer solution was infused by the appropriate route with a screw-driven pump (model 940, Harvard Apparatus, Harvard, MA) at a rate of 0.174 ml/min. During the first 4 h of the study, 0.9% NaCl saline solution was infused by nasogastric tube and during the second 4 h the liquid egg formula was infused, both at a rate of 125 ml/h by means of a pump (model 924, IMED, San Diego, CA). During the formula infusion on *day 8*, expired breath samples were collected into the anesthesia bags at 0, 30, 60, 120, 150, 165, 180, 195, 210, 225, and 240 min. During the tracer infusions on *days 9* and *11*, expired breath samples were collected at 0, 30, 60, 120, 150, 165, 180, 195, 210, 225, and 240 min (PA period) and at 270, 300, 360, 390, 405, 420, 435, 450, 465, and 480 min (fed period). Total O_2 consumption and CO_2 production rates were measured 8 to 10 times during the last 2 h of the formula infusion on *day 8* and during last 2 h of each period on *days 9* and *11*, as noted in Fig. 2. A face mask with two-way valve (Speak-Easy II, Respironics, Monroeville, PA) was fitted carefully to the subject's face, tested for leaks with a probe connected to the CO_2 analyzer, and connected by plastic hose to 125-liter Douglas bags (Warren E. Collins). At each collection point (spaced between the 15-min intervals of the breath $^{13}CO_2$ collection), paired 6-min expired breath collections were made. CO_2 concentration was measured with an infrared analyzer (medical gas analyzer model LB-2, Beckman Instruments, Fullerton, CA) and O_2 concentration with an electrolytic cell analyzer (model SA1, Ametek-Applied Electrochemistry, Pittsburgh, PA) after appropriate calibration of the instruments against nitrogen and reference standard gases. Total expired volume for each collection was measured

by evacuating the Douglas bags into a 120-liter gasometer and correcting these volumes to standard temperature, pressure, and dryness.

Measurement of ^{13}C enrichments. The expired breath trapped in the Vacutainer tubes was analyzed for ^{13}C isotopic enrichment as described by (39) on a dual-inlet, dual-collector isotope ratio mass spectrometer (model 3-60-RMS, Nuclide, State Farm, PA). Natural abundance CO_2 gas of known isotopic composition relative to the standard PDB was used as a reference, and the enrichment of the breath samples was reported in $AP \cdot 10^{-3}$ U.

For calculation of kinetic parameters, the breath ^{13}C enrichments at plateau during the PA and fed phases were corrected for base-line breath ^{13}C enrichments. This corrected breath ^{13}C enrichment is denoted Ec_{O_2} and is expressed in $APE \cdot 10^{-3}$ above base line. For the PA period, base-line breath ^{13}C enrichment was assumed to be the same as enrichment before the start of the tracer infusion

$$Ec_{O_2}\ (PA) = \text{breath } ^{13}C \text{ enrichment (plateau)} - \text{breath } ^{13}C \text{ enrichment (base line)}$$

For the fed period, breath ^{13}C enrichments obtained during the last hour of an individual subject's formula-only infusion were fitted to a straight line by linear regression. This procedure was carried out to smooth the data. Enrichments for each time point predicted from the regression (see data, below) were used as the assumed base-line breath ^{13}C enrichment for the respective time point of the fed study period, and 95% confidence limits for a future predicted enrichment were calculated to determine how this procedure would affect final estimates of label recovery

$$Ec_{O_2}\ (fed) = \text{breath } ^{13}C \text{ enrichment (plateau)} - \text{breath } ^{13}C \text{ enrichment (formula only)} - \text{breath } ^{13}C \text{ enrichment (PA base line)}$$

Calculation of kinetic parameters. Adopting the nomenclature of Downey et al. (9), except that we have calculated rates in min^{-1} rather than h^{-1}, the [^{13}C]bicarbonate infusion rate ($I_{^{13}C}$, $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was the product of the infusion rate (0.172 ml/min) times the concentration of [^{13}C]bicarbonate in the infusate (μmol/ml) divided by the subject's weight (W, kg).

CO_2 production ($\dot{V}co_2$; $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was calculated from the product of the ventilation rate ($\dot{V}E$ in l/min, STPD), the expired breath CO_2 concentration ($\%CO_2$, corrected for room air CO_2 and divided by 100 to convert percent to the fraction) and the conversion factor 0.0446 μmol/l, and normalized for W (kg)

$$\dot{V}co_2 = (\dot{V}E) \cdot (0.0446) \cdot (\%CO_2/100)/W$$

The rate of $^{13}CO_2$ excretion in expired breath ($F_{^{13}CO_2}$ as $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was calculated from $\dot{V}co_2$ (in $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and Ec_{O_2} (in $APE \cdot 10^{-3}$), where the divisor 100 corrects for percent

$$F_{^{13}CO_2} = (\dot{V}co_2) \cdot (Ec_{O_2}/100)$$

The fraction of infused [^{13}C]bicarbonate recovered in expired air (Xco_2) was calculated from the ^{13}C excretion rate divided by the ^{13}C infusion rate

$$Xco_2 = F_{^{13}CO_2}/I_{^{13}C}$$

We can also consider this in another way. The fraction of ^{13}C label that is retained must be proportional to the fraction of total CO_2 produced that is not exhaled in breath but participates in metabolic pathways or in acid-base balance. If the enrichment of ^{13}C in breath, Ec_{O_2}, is in equilibrium with the ^{13}C in body fluid bicarbonate/CO_2 and if we know the infusion rate of [^{13}C]bicarbonate, $I_{^{13}C}$, then we can calculate a CO_2 flux [total movement of CO_2 through the metabolic pool ($\dot{Q}co_2$)]

$$\dot{Q}co_2 = I_{^{13}C}/(Ec_{O_2}/100)$$

where the divisor 100 corrects for percent. This further assumes that a steadystate enrichment of ^{13}C in expired breath has been attained. Then, the CO_2 exhaled in breath per minute, $\dot{V}co_2$, is that portion of CO_2 flux that is not lost to metabolic pathways. Thus

$$\dot{V}co_2/\dot{Q}co_2 = F_{^{13}CO_2}/I_{^{13}C} = Xco_2$$

and this relationship is identical to the production of ^{13}C label in breath divided by the infusion rate of ^{13}C label, or the fraction of infused ^{13}C recovered in breath, Xco_2.

Statistical Analysis of Data

Descriptive statistics, linear regression analysis, and analysis of variance (ANOVA) were carried out using either the BMDP statistical software program (8) or the SAS statistical software program (29), which is available through the MIT CRC CLINFO facility. The specific application of these tests to the data are detailed below.

RESULTS

Base-Line PA and Postprandial Breath $^{13}CO_2$ During Adaptation to an Experimental Diet

Figure 3 summarizes breath ^{13}C enrichment levels above base-line value before and 3 h after completing breakfast during the first 7 days of the study. A linear regression analysis was carried out where the dependent variable was breath enrichment in $APE \cdot 10^{-3}$ (either PA, 3-h postbreakfast or the difference between PA and postbreakfast enrichments) and the independent vari-

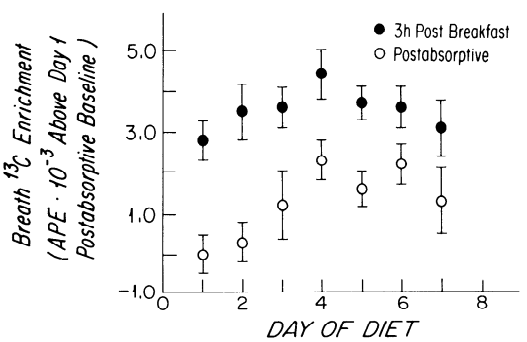

FIG. 3. Postabsorptive and 3 h postprandial breath ^{13}C enrichments during *days 1–7* of experimental diet period (mean ± SE, $n = 6$ subjects).

able was day of diet (*1–7*). For the PA basal enrichment on *day 1*, the mean of PA values on *days −2, −1,* and *1* was used. A slope different from 0 at a significance level of $P < 0.05$ was taken as an indication of adaptation to the diet in terms of breath enrichment. There was a trend toward increasing PA enrichment for which the slope was significantly positive ($x = 0.32y$, $R^2 = 0.18$, $P < 0.01$) for the group means, whereas postbreakfast enrichment did not change significantly ($x = 3.4 + 0.04y$, $R^2 = 0.003$, $P = 0.71$). The slope for difference between PA and postbreakfast enrichments was negative ($x = 3.3 - 0.25y$, $R^2 = 0.14$, $P < 0.02$).

Change in Breath ^{13}C Enrichment During Continuous Intragastric Infusion of a Formula Diet Naturally Enriched with ^{13}C

Figure 4 shows the change in breath ^{13}C enrichment (mean ± SE) that occurred during the 4-h liquid egg formula infusion on *day 8*. With linear regression analysis of enrichment vs. time, the increasing breath enrichment with time was found to be significantly positive ($x = 8.3 \cdot 10^{-3}y$, $R^2 = 0.41$, $P < 0.0001$). Even though the linear model did not provide a good fit when the earlier time points were included in the analysis, this nevertheless demonstrates that the rise in breath ^{13}C levels was significant. To use this data to correct for the contribution of diet to ^{13}C levels during the tracer infusion, enrichment values for each subject for the last hour of the formula-only infusion (from 180 to 240 min) were fitted to a straight line by linear regression (Table 2). The linear model was used because other models did not demonstrably improve the fit. As described, the predicted values from this regression were used as base-line values to correct the measured breath $^{13}CO_2$ levels during the last hour of the fed period of each tracer infusion (from 420 to 480 min) for the contribution of formula diet to the total breath ^{13}C enrichment. These corrected values for the last hour were used to compute the $F_{^{13}CO_2}$ from the tracer infusion. For each subject, measured values for breath enrichment obtained during the formula-only infusion as well as the regression equation and the predicted enrichment values from the regression are reported in Table 3. The effects of using this prediction on 95% confidence limits for corrected fed-period enrichment, $^{13}CO_2$ production, and label recovery are reported in Table 3 and 4.

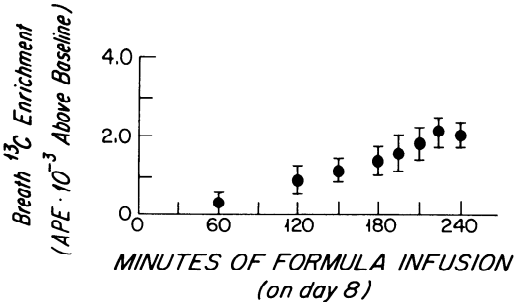

FIG. 4. Breath ^{13}C enrichments during 4 h of continuous intragastric formula infusion (without tracer) on *day 8* (mean ± SE, $n = 6$ subjects).

TABLE 2. *Breath ^{13}C enrichments ($APE \cdot 10^{-3}$) above postabsorptive base line measured for 4th h of formula-only infusion*

Subject	Time, min	Measured Enrichment	Regression Equation	Predicted Enrichment
A	180	1.7	$x = -1.1 + 0.0170y$	1.9 (1.1–2.7)
	195	2.4	$R^2 = 0.84$, $P < 0.05$	2.1 (1.4–2.9)
	210	2.3		2.4 (1.7–3.1)
	225	2.7		2.6 (1.9–3.4)
	240	2.8		2.9 (2.1–3.7)
B	180	2.2	$x = 2.0 + 0.0025y$	2.4 (0.2–4.6)
	195	2.4	$R^2 = 0.04$, $P = 0.80$	2.4 (0.5–4.4)
	210	2.7		2.5 (0.6–4.3)
	225	2.8		2.5 (0.6–4.4)
	240	2.2		2.5 (0.5–4.6)
C	180	0.7	$x = -1.2 + 0.0107y$	0.8 (−0.3–1.8)
	195	1.1	$R^2 = 0.57$, $P = 0.14$	0.9 (0.0–1.8)
	210	0.8		1.1 (0.2–2.0)
	225	1.5		1.2 (0.3–2.2)
	240	1.3		1.4 (0.4–2.4)
D	180	1.5	$x = 0.5 + 0.0060y$	1.6 (1.0–2.2)
	195	1.7	$R^2 = 0.55$, $P = 0.15$	1.7 (1.1–2.2)
	210	1.9		1.8 (1.3–2.3)
	225	2.0		1.9 (1.3–2.4)
	240	1.8		2.0 (1.4–2.6)
E	180	2.1	$x = 1.9 + 0.0027y$	2.3 (1.4–3.3)
	195	2.6	$R^2 = 0.09$, $P = 0.65$	2.4 (1.5–3.2)
	210	2.5		2.4 (1.6–3.3)
	225	2.6		2.5 (1.6–3.3)
	240	2.3		2.5 (1.5–3.5)
F	180	0.1	$x = -4.3 + 0.0240y$	0.0 (−0.6–0.6)
	195	0.3	$R^2 = 0.95$, $P < 0.005$	0.3 (−0.2–0.9)
	210	0.5		0.7 (0.2–1.2)
	225	1.1		1.1 (0.5–1.6)
	240	1.5		1.4 (0.8–2.0)

Values are the result of fitting data to a linear equation, enrichments are predicted from the regression, and 95% confidence limits (CL) for a future predicted value are given in parentheses. APE, atom percent excess.

Indirect Calorimetry During Formula Infusion and During Both Tracer Infusion Studies

Table 5 shows $\dot{V}CO_2$ and O_2 uptake ($\dot{V}O_2$) measurements and computed minute metabolic rates for each subject during the three infusion studies. The values for the formula-only infusion were slightly, although significantly, different from both tracer infusion values (e.g., metabolic rate for the formula-only infusion of 1.30 ± 0.03 kcal/min vs. 1.41 ± 0.03 for the iv tracer infusion fed period and 1.40 ± 0.03 kcal/min for the ig tracer infusion fed period, $P < 0.05$).

The reasonableness of the measurements for PA $\dot{V}CO_2$ values for individual subjects (computed as $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was assessed by calculating a theoretical range for $\dot{V}CO_2$, based on metabolic rate estimated from the Harris-Benedict (14) or Kleiber (22) equations, assuming a possible range of respiratory quotients ranging from 0.70 (theoretical minimum for a prolonged fast) to 0.84 (probable value for a brief overnight fast). Values for all subjects fell well within this range.

Effect of Metabolic State and Route of Administration on Breath $^{13}CO_2$ Enrichment During [^{13}C]bicarbonate Infusions

Figure 5 shows the measured breath ^{13}C enrichments relative to PA base line for the iv and ig tracer infusion

TABLE 3. *Breath ^{13}C enrichments for the fed portion of the intravenous tracer study, corrected enrichments for contribution of formula diet (see Table 2), and recovery of label*

Subject	Time, min	Measured Enrichment, APE·10^{-3}	Corrected Enrichment, APE·10^{-3}	$\dot{V}CO_2$, $\mu mol \cdot kg^{-1} \cdot min^{-1}$	I, $\mu mol \cdot kg^{-1} \cdot min^{-1}$	$F_{^{13}CO_2}$, $\mu mol \cdot kg^{-1} \cdot min^{-1}$	Recovery
A	420	31.6	29.7 (28.9–30.5)				
	435	33.3	31.2 (30.4–31.9)				
	450	32.3	29.9 (29.2–30.6)	184.3	0.0638	0.0556* (0.0541–0.0569)	0.87* (0.85–0.89)
	465	32.8	30.2 (29.4–30.9)				
	480	32.7	29.8 (29.0–30.6)				
B	420	35.2	32.8 (30.6–35.0)				
	435	35.6	33.2 (31.2–35.1)				
	450	36.9	34.4 (32.6–36.3)	172.3	0.0654	0.0568 (0.0534–0.0602)	0.87 (0.82–0.92)
	465	34.1	31.6 (29.7–33.5)				
	480	35.4	32.9 (30.8–34.9)				
C	420	32.6	31.8 (30.8–32.9)				
	435	33.3	32.4 (31.5–33.3)				
	450	34.1	33.0 (32.1–33.9)	168.5	0.0632	0.0533 (0.0517–0.0550)	0.84 (0.82–0.87)
	465	30.6	29.4 (28.4–30.3)				
	480	33.1	31.7 (30.7–32.7)				
D	420	37.7	36.1 (35.5–36.7)				
	435	37.8	36.1 (35.6–36.7)				
	450	37.6	35.8 (35.3–36.3)	160.0	0.0653	0.0566 (0.0557–0.0575)	0.87 (0.85–0.88)
	465	37.2	35.3 (34.8–35.9)				
	480	35.5	33.5 (32.9–34.1)				
E	420	36.7	34.4 (33.4–35.3)				
	435	37.3	34.9 (34.1–35.8)				
	450	35.9	33.5 (32.6–34.3)	140.8	0.0657	0.0488 (0.0476–0.0501)	0.74 (0.72–0.76)
	465	40.0	37.5 (36.7–38.4)				
	480	35.6	33.1 (32.1 34.1)				
F	420	38.2	38.2 (37.6–38.8)				
	435	36.7	36.4 (35.8–36.9)				
	450	37.5	36.8 (36.3–37.3)	126.6	0.0662	0.0466 (0.0459–0.0473)	0.70 (0.69–0.71)
	465	37.8	36.7 (36.2–37.3)				
	480	37.3	35.9 (35.3–36.5)				

Effect of this correction on 95% confidence limits (CL) is reported in parentheses (using CL as reported in Table 2). $\dot{V}CO_2$, CO_2 production; I, tracer infusion rate; $F_{^{13}CO_2}$, rate of CO_2 excretion in expired breath; APE, atom percent excess. * Mean.

studies. The values for individual subjects for the fed periods were corrected as described above. For the PA period mean values were 38.8 ± 0.4 APE·10^{-3} for the iv infusion and 40.0 ± 0.4 APE·10^{-3} for the ig infusion. For the continuously fed phase of the experiment, corrected enrichments were 35.4 ± 0.1 APE·10^{-3} for the iv infusion and 35.0 ± 0.2 APE·10^{-3} for the ig infusion. The values for the last hour of the PA and fed states are reported in Table 6. When these were subjected to a three-way repeated measures analysis of variance, the effect of diet (PA vs. fed) was significant ($P < 0.0001$), but the effects of route (iv vs. ig) and time of sample collection (180–240 min and 420–480 min) were not significant.

For the PA phase (180–240 min), the ratio of ig breath enrichment to iv breath enrichment was 1.03 ± 0.04. For the fed phase (420–480 min), the ratio was 0.99 ± 0.03. These differences were not significant.

Effect of Metabolic Status and Route of Tracer Administration on Recovery of Label in Breath and on Relationship Between Bicarbonate Flux and CO_2 Excretion Measured by Tracer Infusion Experiments

Recovery rates of infused ^{13}C label in expired breath were calculated for individual subjects and compared with respect to PA vs. fed status and route of tracer infusion. These values are summarized in Table 7. As expected from the analyses reported above, the fractional recoveries were not significantly different for ig and iv administration but were significantly different for PA and fed periods ($P < 0.0001$, two-way ANOVA). The values for the fed period (iv = 0.82 ± 0.03 of infused label recovered and ig = 0.79 ± 0.02 of infused label recovered) were very similar to the fraction 0.81, which has been used in a number of studies of labeled substrate oxidation (e.g., Refs. 13 and 19). However, values for the PA period were lower than fed period values for both routes of tracer administration (iv = 0.70 ± 0.04 and ig = 0.74 ± 0.02).

Values for individual subjects of bicarbonate flux, CO_2 excretion, and the difference between flux and excretion are reported in Table 8. The difference between flux and excretion was lower in the fed relative to the PA state, in absolute terms by 12.5 − 24.9 $\mu mol \cdot kg^{-1} \cdot h^{-1}$.

Relationship of Label Recovery in Breath to Respiratory Gas Exchange

Fractional recovery of label was compared with $\dot{V}O_2$ (Fig. 6) and $\dot{V}CO_2$ (Fig. 7), both normalized for body weight. In the linear regression analysis of pooled values from both PA and fed states, recovery was linearly related to $\dot{V}O_2$ ($x = 0.383 + 0.002y$, $R^2 = 0.73$, $P < 0.0001$) and to $\dot{V}CO_2$ ($x = 0.486 + 0.002y$, $R^2 = 0.67$, $P < 0.0001$).

DISCUSSION

A principal goal of many tracer kinetic experiments is to determine the oxidation rate of the traced substance

TABLE 4. *Breath ^{13}C enrichments for the fed portion of the intragastric tracer study, corrected enrichments for contribution of formula diet (see Table 3), and recovery of label*

Subject	Time, min	Measured Enrichment, APE·10^{-3}	Corrected Enrichment, APE·10^{-3}	$\dot{V}CO_2$, $\mu mol \cdot kg^{-1} \cdot min^{-1}$	I, $\mu mol \cdot kg^{-1} \cdot min^{-1}$	$F_{^{13}CO_2}$, $\mu mol \cdot kg^{-1} \cdot min^{-1}$	Recovery
A	420	31.7	29.8 (29.0–30.6)				
	435	32.3	30.2 (29.4–30.9)				
	450	30.4	28.0 (27.3–28.7)	175.4	0.0638	0.0507* (0.0493–0.0520)	0.79* (0.77–0.81)
	465	29.5	26.9 (26.1–27.6)				
	480	32.5	29.6 (28.8–30.4)				
B	420	33.6	31.2 (29.0–33.4)				
	435	32.8	30.4 (28.4–32.3)				
	450	31.6	29.1 (27.3–31.0)	175.3	0.0654	0.0520 (0.0485–0.0555)	0.80 (0.74–0.85)
	465	32.7	30.2 (28.3–32.1)				
	480	29.9	27.4 (25.3–29.4)				
C	420	32.3	31.5 (30.5–32.6)				
	435	30.6	29.7 (28.8–30.6)				
	450	28.6	27.5 (26.6–28.4)	181.7	0.0632	0.0551 (0.0534–0.0569)	0.87 (0.84–0.90)
	465	34.6	33.4 (32.4–34.3)				
	480	31.0	29.6 (28.6–30.6)				
D	420	35.8	34.2 (33.6–34.8)				
	435	36.8	35.1 (34.6–35.7)				
	450	34.7	32.9 (32.4–33.4)	150.9	0.0653	0.0505 (0.0497–0.0514)	0.77 (0.75–0.79)
	465	33.4	31.5 (31.0–32.1)				
	480	35.7	33.7 (33.1–34.3)				
E	420	36.8	34.5 (33.5–35.4)				
	435	42.1	39.7 (38.9–40.6)				
	450	41.1	38.7 (37.8–39.5)	138.1	0.0657	0.0522 (0.0510–0.0535)	0.79 (0.78–0.81)
	465	40.1	37.6 (36.8–38.5)				
	480	41.1	38.6 (37.6–39.6)				
F	420	40.0	40.0 (39.4–40.6)				
	435	39.5	39.2 (38.6–39.7)				
	450	40.7	40.0 (39.5–40.5)	122.2	0.0662	0.0478 (0.0471–0.0485)	0.72 (0.71–0.73)
	465	38.3	37.2 (36.7–37.8)				
	480	40.6	39.2 (38.6–39.8)				

Effect of this correction on 95% confidence limits (CL) is reported in parentheses (using CL as reported in Table 3). $\dot{V}CO_2$, CO_2 production; I, tracer infusion rate; $F_{^{13}CO_2}$, rate of CO_2 production in expired breath; APE, atom percent excess. * Mean.

by the appearance in breath of labeled C originating from the tracer. There are a number of assumptions that must be made to make this estimation, one of which is the amount of label released via oxidation that appears in breath. This is usually assumed to be ~80%, based on a number of studies that have measured the recovery of label from administered bicarbonate tracer in expired breath (Table 9). These studies have made the further assumption that CO_2 released from bicarbonate (a reaction catalyzed by carbonic anhydrase in blood and cytosol) and CO_2 released from oxidation of fuels (a predominantly mitochondrial process) are handled similarly by the body. Although this latter assumption is beyond the scope of this discussion, its relevance for tracer estimates of substrate oxidation may be significant (e.g., Ref. 35).

Compartmental Models of Bicarbonate Metabolism

Many of the studies that have measured recovery of labeled carbon from bicarbonate have utilized a bolus administration of tracer labeled with ^{14}C or ^{13}C. In addition to measuring total recovery, these studies have also attempted to define whole body bicarbonate metabolism in terms of pools or compartments. In general, most models that have been proposed consist of at least a rapidly turning over pool, in equilibrium with blood and well-perfused tissues, and more slowly turning over pools that are comprised of less well-perfused tissues (7, 16–18, 23, 32, 38). The more rapidly turning over pools are essentially in equilibrium with the body water pool (11), whereas the slowest turning over pool is presumed to be essentially a "sink" that is not in ready equilibrium with blood bicarbonate. Bone has been proposed as the major sink for label, based on its large carbonate and bicarbonate content (27, 28). The most obvious demonstration of this was made in autoradiographic studies in mice where residual ^{14}C was mainly found in bone (36). Label may also be recycled through the carbon skeleton of molecules such as oxaloacetate (e.g., see Ref. 21) or lost irreversibly when incorporated into a molecule such as urea, although the 550 mmol of molecular CO_2 required for urea synthesis by a 70-kg subject/day on a 1.5-g protein/kg diet (assuming that 90% of dietary N appears as urinary urea, 15.4 g N yields 550 mmol urea and requires 550 mmol CO_2) is only ~3–4% of the daily bicarbonate flux estimated in the present study to be ~14,000 mmol (based on $\dot{V}CO_2$) to 18,000 mmol (based on bicarbonate/CO_2 flux).

In the present study, we utilized an alternate mode of administration, the primed, constant infusion. This mode was chosen because it corresponded with a specific tracer experimental design, and we wanted to define what the recovery of label would be under conditions of this design. It was not our intent to define further the sites of CO_2 fixation, as this has been done in the series of studies, cited above, that have been carried out over the past 40 years with stable and radioactive tracers. Never-

TABLE 5. *Respiratory exchange measurements during the three infusion studies*

Subject	Study	Metabolic State	$\dot{V}CO_2$, ml/min	$\dot{V}O_2$, ml/min	Metabolic Rate, kcal/min
A	Formula only	Fed	216±8	266±9	1.13
	Tracer (iv)	PA	183±4	239±3	0.98
		Fed	248±7	290±7	1.29
	Tracer (ig)	PA	189±8	237±12	1.00
		Fed	236±7	276±10	1.22
B	Formula only	Fed	268±11	312±11	1.39
	Tracer (iv)	PA	194±8	259±10	1.04
		Fed	282±6	327±5	1.46
	Tracer (ig)	PA	202±3	236±2	1.05
		Fed	287±7	324±7	1.47
C	Formula only	Fed	311±7	393±10	1.64
	Tracer (iv)	PA	253±6	342±7	1.36
		Fed	306±9	379±8	1.61
	Tracer (ig)	PA	254±3	340±4	1.36
		Fed	330±5	401±6	1.73
D	Formula only	Fed	239±4	290±4	1.25
	Tracer (iv)	PA	183±6	254±7	0.99
		Fed	244±10	302±7	1.28
	Tracer (ig)	PA	196±4	264±3	1.06
		Fed	230±4	282±3	1.21
E	Formula only	Fed	227±9	295±7	1.21
	Tracer (iv)	PA	186±4	269±4	1.02
		Fed	262±6	318±3	1.37
	Tracer (ig)	PA	185±4	263±4	1.01
		Fed	257±8	313±5	1.35
F	Formula only	Fed	240±8	237±9	1.20
	Tracer (iv)	PA	195±4	289±5	1.08
		Fed	281±6	337±14	1.47
	Tracer (ig)	PA	222±8	308±5	1.21
		Fed	271±10	346±10	1.44

Values are means ± SE. $\dot{V}CO_2$, CO_2 production; $\dot{V}O_2$, O_2 uptake; PA, postabsorptive.

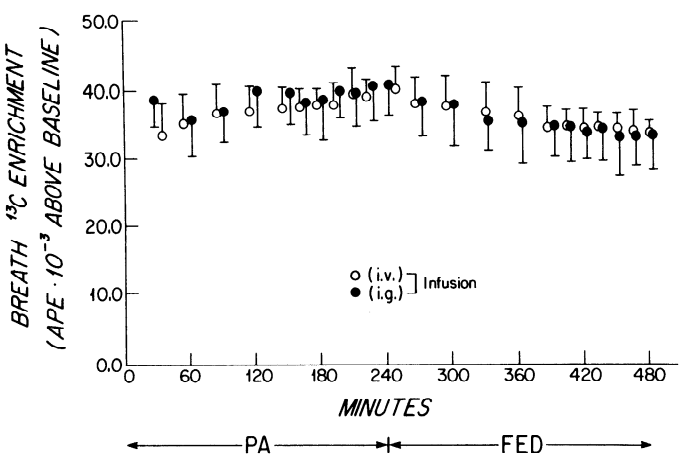

FIG. 5. Breath ^{13}C enrichments above postabsorptive base line during continuous intravenous or intragastric (ig) infusion of Na [^{13}C]-bicarbonate tracer. Saline was infused ig during 1st 4 h, and formula was infused ig during last 4 h (mean ± SE, $n = 6$ subjects).

theless, this study has made some important observations that may force reexamination of the models that have been derived from studies using tracer boluses and carried out mainly in the postabsorptive state.

Effects of Feeding on ^{13}C Recovery

The first important finding is that label recovery from bicarbonate tracer is a function of $\dot{V}O_2$ and $\dot{V}CO_2$, and as these parameters increase with feeding, label recovery also increases. The association noted in this study has been previously noted in infants (34), where recovery was significantly correlated with the level of energy intake and with $\dot{V}CO_2$, and in hypermetabolic patients with cancer (31), where recovery was significantly correlated with $\dot{V}O_2$. Interestingly, the slope of the regression of recovery against $\dot{V}O_2$ and $\dot{V}CO_2$ measured in this study is identical to the slope of the regression of recovery against $\dot{V}O_2$ noted for the patients with cancer (31). The difference between PA and fed recoveries was also recently reported by Garlick et al. (12), who with a constant intravenous infusion of [^{13}C]bicarbonate measured a PA recovery of 0.73 (where mean $\dot{V}O_2$ was 158 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and $\dot{V}CO_2$ was 125 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and a fed recovery of 0.89 (where mean $\dot{V}O_2$ was 173 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and $\dot{V}CO_2$ was 160 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). The indirect calorimetry results and change in recoveries with feeding are very similar to the present study. The relationship measured in the present study cannot necessarily be applied to other experimental and nutritional conditions (e.g., Ref. 40), but these collective findings suggest that label recovery for substrate oxidation studies may be able to be predicted on the basis of a carefully determined $\dot{V}CO_2$ or $\dot{V}O_2$, provided that recovery has been previously measured from [^{13}C]bicarbonate administration under the same experimental conditions.

Effects of Feeding on Bicarbonate Kinetics and CO_2 Production

A second important observation is that the relationship between bicarbonate-CO_2 flux, calculated from the dilution of tracer, and $\dot{V}CO_2$, measured as CO_2 appearance in breath, changes in response to feeding. Based on our initial observation that recovery increased as respiratory exchange parameters increased, we had predicted that the absolute amount of CO_2 retained per unit time would remain fairly constant, perhaps as a function of body cell or bone mass, whereas total bicarbonate flux and $\dot{V}CO_2$, as well as $\dot{V}O_2$, would increase in response to feeding. Contrary to predictions, our data show that although bicarbonate flux increased by 11%, $\dot{V}CO_2$ increased by ~37%, producing a 25–50% reduction in the absolute amount of CO_2 that was retained. This suggested that retention was affected not just by body mass but also by metabolic changes induced by feeding. It further suggested that compartmental models based on data collected during postabsorptive conditions may change during conditions of feeding or exercise, when not only fuel oxidation but also tissue perfusion is changing, as well as during longer-term fasting, when processes such as gluconeogenesis become prominent that involve molecular incorporation of CO_2. Nutritional and metabolic conditions may substantially alter the pool sizes and rate constants of these models. However, definition of multicompartmental models may not be a relevant exercise, if, as the findings of this and other studies mentioned above suggest, label recovery for substrate oxidation studies can be predicted on the basis of respiratory exchange.

TABLE 6. *Breath ^{13}C enrichments for intravenous and intragastric infusions, where values are obtained from the 4th h of the PA and continuously fed periods*

	Time, min	Subject, APE·10^{-3}					
		A	*B*	*C*	*D*	*E*	*F*
PA (iv)	180	36.7	36.3	35.3	39.0	40.2	40.2
	195	34.3	36.3	33.7	39.5	41.4	41.0
	210	34.9	37.9	36.0	42.0	44.3	41.6
	225	37.4	36.8	36.0	41.5	41.4	41.4
	240	39.1	37.7	36.0	40.1	43.4	44.0
	Mean ± SE	36.5±0.9	37.0±0.3	35.4±0.4	40.4±0.6	42.1±0.7	41.6±0.6
Fed (iv)	420	29.7	32.8	31.8	36.1	34.4	38.2
	435	31.2	33.2	32.4	36.1	34.9	36.4
	450	29.9	34.4	33.0	35.8	33.5	36.8
	465	30.2	31.6	29.4	35.3	37.5	36.7
	480	29.8	32.9	31.7	33.5	33.1	35.9
	Mean ± SE	30.2±0.3	33.0±0.4	31.7±0.6	35.4±0.5	34.7±0.8	36.8±0.4
PA (ig)	180	33.9	43.7	28.4	38.0	43.1	45.4
	195	34.4	43.9	34.9	38.0	43.5	45.4
	210	32.3	39.1	37.3	37.1	45.7	46.6
	225	34.1	41.5	39.2	35.2	45.3	48.3
	240	34.2	44.1	39.1	35.5	45.5	46.3
	Mean ± SE	33.8±0.4	42.5±1.0	35.8±2.0	36.8±0.6	44.6±0.5	46.4±0.5
Fed (ig)	420	29.8	31.2	31.5	34.2	34.5	40.0
	435	30.2	30.4	29.7	35.1	39.7	39.2
	450	28.0	29.1	27.5	32.9	38.7	40.0
	465	26.9	30.2	33.4	31.5	37.6	37.2
	480	29.6	27.4	29.6	33.7	38.6	39.2
	Mean ± SE	28.9±0.6	29.7±0.7	30.3±1.0	33.5±0.6	37.8±0.9	39.1±0.5

Fed values are corrected for diet ^{13}C background, as shown in Tables 4 and 5. APE, atom percent excess; PA, postabsorptive.

TABLE 7. *I, $F_{^{13}CO_2}$, and XCO_2 in expired breath for both intravenous and intragastric infusion routes and the PA and continuously fed periods*

	Subject, μmol·kg^{-1}·min^{-1}						Means ± SE
	A	*B*	*C*	*D*	*E*	*F*	
I	0.0638	0.0654	0.0632	0.0653	0.0657	0.0662	0.0649±0.0005
$F_{^{13}CO_2}$							
PA (iv)	0.0483	0.0452	0.0494	0.0479	0.0433	0.0367	0.0451±0.0021
PA (ig)	0.0493	0.0508	0.0501	0.0467	0.0455	0.0465	0.0482±0.0010
Fed (iv)	0.0556	0.0568	0.0533	0.0566	0.0488	0.0466	0.0529±0.0018
Fed (ig)	0.0507	0.0520	0.0551	0.0505	0.0522	0.0478	0.0513±0.0010
XCO_2							
PA (iv)	0.76	0.69	0.78	0.73	0.66	0.55	0.70±0.04
PA (ig)	0.77	0.78	0.79	0.72	0.69	0.70	0.74±0.02
Fed (iv)	0.87	0.87	0.84	0.87	0.74	0.70	0.82±0.03
Fed (ig)	0.79	0.80	0.87	0.77	0.89	0.72	0.79±0.02

I, tracer infusion rate; $F_{^{13}CO_2}$, mean rate of $^{13}CO_2$ in expired breath; XCO_2, recovery of label. Results of PA vs. fed periods significantly different at $P < 0.0001$, and results of iv vs. ig infusions not significantly different by 2-way ANOVA, where dependent measure was fraction recovered and factors were metabolic state and route of tracer infusion.

TABLE 8. *$\dot{Q}CO_2$, $\dot{V}CO_2$, and $\dot{Q}CO_2 - \dot{V}CO_2$ for intravenous and intragastric routes of tracer administration and PA and fed states*

Subject	PA (iv)			Fed (iv)			PA (ig)			Fed (ig)		
	$\dot{Q}CO_2$	$\dot{V}CO_2$	$\dot{Q}CO_2 - \dot{V}CO_2$	$\dot{Q}CO_2$	$\dot{V}CO_2$	$\dot{Q}CO_2 - \dot{V}CO_2$	$\dot{Q}CO_2$	$\dot{V}CO_2$	$\dot{Q}CO_2 - \dot{V}CO_2$	$\dot{Q}CO_2$	$\dot{V}CO_2$	$\dot{Q}CO_2 - \dot{V}CO_2$
A	178.8	136.0	38.8	193.9	184.3	9.6	188.8	140.5	48.3	217.0	175.4	41.6
B	176.8	118.5	58.3	187.4	172.3	15.1	153.9	123.4	30.5	192.4	175.3	17.1
C	178.5	139.3	39.2	193.3	168.5	24.8	176.5	139.9	36.6	201.3	181.7	19.6
D	161.6	120.0	41.6	183.9	160.0	23.9	177.4	128.6	48.8	184.0	150.9	33.1
E	156.1	99.9	56.2	176.0	140.8	35.2	146.4	99.4	47.0	162.0	138.1	23.9
F	159.1	87.8	71.3	174.1	126.6	47.5	140.7	100.0	40.7	164.0	122.1	41.9
Mean	168.4	116.8	50.9	184.8	158.8	26.0	164.0	112.1	42.0	186.8	157.3	29.5
± SE	±4.3	±8.2	±5.4	±3.4	±8.7	±5.6	±8.0	±15.6	±3.0	±8.0	±9.0	±4.5

$\dot{Q}CO_2$, bicarconate-CO_2 flux; $\dot{V}CO_2$, CO_2 production; $\dot{Q}CO_2 - \dot{V}CO_2$, retained CO_2; PA, postabsorptive.

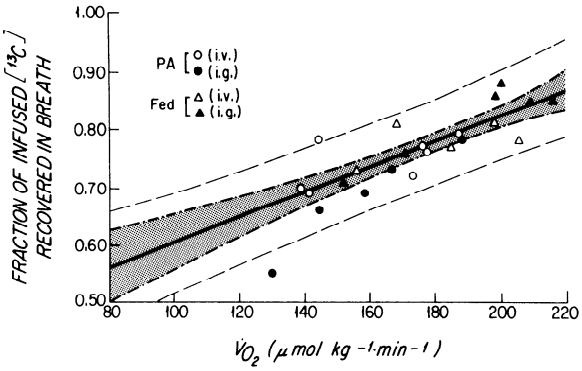

FIG. 6. Fraction of infused ^{13}C recovered in breath as a function of O_2 uptake ($\dot{V}O_2$) normalized to body weight ($x = 0.383 + 0.002y$, $R^2 = 0.73$, $P < 0.0001$).

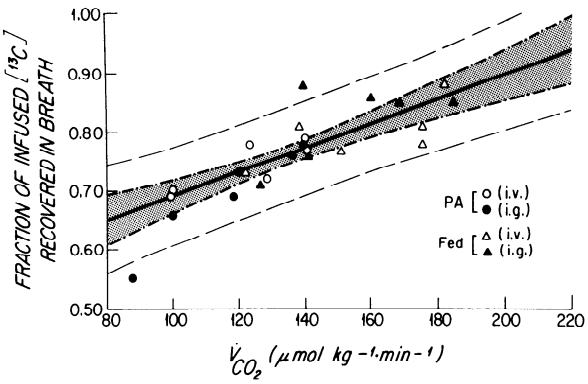

FIG. 7. Fraction of infused ^{13}C recovered in breath as a function of CO_2 production ($\dot{V}CO_2$) normalized to body weight ($x = 0.486 + 0.002y$, $R^2 = 0.67$, $P < 0.0001$).

Implications for Tracer Oxidation Studies and Estimates of Nutrient Requirements

This study has implications with respect to the estimates of amino acid requirements from ^{13}C-labeled amino acid tracer studies. In earlier studies from this laboratory (6, 25, 26, 42–44), the factor 0.81 was used to correct for retention of label during both PA and fed studies. As the mean factor for fed experiments in this paper is 0.805, previous estimates for the fed state would not be affected. However, the mean factor in the present PA experiments is ~0.72. Applying this value to the earlier studies would have the effect of raising PA oxidation estimates by ~10%. With the improved estimates of ^{13}C retention emerging from the present investigation it seems likely that our previous values for daily amino acid oxidation would need to be increased by only ~5%. Thus our previous conclusions concerning the differences between requirement estimates based on nitrogen balance data and those based on ^{13}C tracer experiments are not affected by the present findings.

Measurements of CO_2 Flux by Bicarbonate Infusion vs. CO_2 Production by Indirect Calorimetry

As an alternative to measuring respiratory exchange during tracer studies, measuring CO_2 flux by tracer kinetics would provide a better estimate for purposes of calculating oxidation. No correction factor would need to be inferred or applied. However, unless a radiotracer is used for purposes of measuring CO_2 flux, this would have to be done on another day or prior to the administration of the ^{13}C-labeled substrate. One solution that has been proposed (4, 18) is to carry out a bicarbonate infusion immediately before administering a second C-labeled substrate. The bicarbonate tracer would serve the dual purpose in that it would quantify bicarbonate-to-CO_2 flux and label recovery and also prime the bicarbonate pool for the labeled substrate study and thus shorten the infusion time required to reach steady state. Based on results of the present study, however, one should be cautious when extrapolating results from the early post-absorptive period to a fed period later in the same day, unless the relationship between recovery and respiratory exchange parameters is found to hold under both conditions for that particular study. It would also be important to measure how relationship between CO_2 flux and $\dot{V}CO_2$ changes over a daily cycle during the transitions between fasting and feeding, as it is probable that the relationship will be different at the beginning and end of each new metabolic state. This could have substantial implications with respect to our earlier estimates of amino acid requirements, cited above, where we made 12-h extrapolations based on 4-h infusion studies.

Effects of Route of Bicarbonate Tracer Administration

It is interesting that administering the tracer by nasogastric tube does not result in a lower recovery than administering the tracer by vein. Stomach acid would be expected to liberate a portion of label as CO_2, although in in vitro experiments, this quantity was far less than predicted and for the amount of tracer administered would be ~10% maximally, even in the presence of carbonic anhydrase (10). Indeed, aspirated gas from the stomach in this study had an enrichment that was more than 10 times that of expired breath (unpublished results). In addition to the potential of label being liberated by stomach acid, all tracer has to pass intracellularly through at least gut tissue prior to its first pass through the lungs and potential loss into breath. However, neither of these factors appears to have influenced label recovery to a significant extent. This suggests that calculations of substrate oxidation for a tracer that is delivered ig should be handled the same as if the tracer were delivered by the iv route. Also, intravenously administered bicarbonate does not appear to be lost significantly in the lung before it equilibrates with body tissues, as similar recoveries were noted in the dog when label was infused both iv and intra-arterially (9). Taken together, these findings suggest that route of administration is not a significant factor in determining bicarbonate recovery for purposes of calculating substrate oxidation.

Effects of Dietary ^{13}C on Bicarbonate Label Recovery or Substrate Oxidation Estimates

This study also raises a series of practical considerations with respect to the design and implementation of studies examining the effect of diet on substrate oxidation. In designing this study, we considered using a diet which was isotopically "neutral" (30) for individuals liv-

TABLE 9. *Fractional recovery of ^{13}C or ^{14}C from infused bicarbonate in breath: results from published human studies*

Investigator and Protocol Details	Protein Intake Preceding Study	Metabolic Status During Study	Fraction of Dose Recovered
	Bolus studies		
Irving et al. (17) bolus dose ^{13}C	Not controlled	Postabsorptive	0.52
Irving et al. (18) bolus dose ^{13}C	Not controlled	Postabsorptive	0.74
Issekutz et al. (19) bolus dose ^{14}C	Not controlled	Postabsorptive	0.79
Yang et al. (41) bolus dose ^{13}C	0.0 $g \cdot kg^{-1} \cdot day^{-1}$	Postabsorptive	0.72
	0.0 $g \cdot kg^{-1} \cdot day^{-1}$	Continuously fed	0.75
	1.5 $g \cdot kg^{-1} \cdot day^{-1}$	Postabsorptive	0.56
	1.5 $g \cdot kg^{-1} \cdot day^{-1}$	Continuously fed	0.77
	Constant infusion studies[1]		
Allsop et al. (1) constant infusion ^{13}C	Not controlled	Postabsorptive	0.81
Clugston and Garlick (5) constant infusion ^{14}C obese + normal subjects	75 g/day	Fed (0–12 h)	0.88
		Postabsorptive (12–24 h)	0.94
		Fed (24–36 h)	0.92
Hoerr et al. (this study) constant infusion ^{13}C	1.5 $g \cdot kg^{-1} \cdot day^{-1}$	Postabsorptive	0.72
	1.5 $g \cdot kg^{-1} \cdot day^{-1}$	Continuously fed	0.81
Issekutz et al. (19) constant infusion ^{14}C	Not controlled	Postabsorptive	0.81
Garlick et al. (12)	Not controlled	Postabsorptive	0.73
		Continuously fed	0.89
James et al. (20) constant infusion ^{14}C	Not specified	Not specified	0.80
Waterhouse et al. (37) constant infusion ^{14}C	Not controlled	Postabsorptive	0.70
		Fed glucose	0.74

[1] The recoveries reported by Shaw et al. (31) are not included because mean recoveries were not included in the reference.

ing in the Boston area. However, we chose to use a diet in which the carbohydrate source was solely derived from corn, to determine the magnitude of the effect such a diet would have on our ^{13}C tracer kinetic studies. Corn is ~1.1002 AP ^{13}C, whereas the typical Boston area expired breath is ~1.08879 AP ^{13}C, reflecting the ^{13}C of the mixed diet that is typically consumed, which has a lower ^{13}C content than corn (30). Furthermore, when tracers are used to study patients in hospital settings, practical circumstances often dictate that intravenous nutrients are used. In the United States the dextrose in these solutions is almost exclusively derived from corn. The diet in the present study was less enriched with ^{13}C than corn due to the soy oil fat source contained in the Contra-lyte, which at 1.0793 AP ^{13}C is depleted in ^{13}C relative to typical Boston area exhaled breath.

The postabsorptive breath ^{13}C enrichments during the 1st diet wk showed a gradual drift upward, although the postprandial breath enrichments remained higher than postabsorptive values and relatively constant. The postprandial breath enrichment levels were higher than postabsorptive breath enrichments at the start of the study to the same extent that study diet enrichment was higher than typical Boston breath (and presumed usual diet enrichment). A probable explanation for this was that the substrate delivered in the formula meal represented the primary fuel oxidized at the 3-h postprandial time point (30).

Although we did not carry out continuous feeding for periods longer than 4 h, the results from the experiment in which formula was infused without tracer show that over this relatively short period, the increase in base-line $^{13}CO_2$ over 4 h due to the formula infusion was ~2.0 $APE \cdot 10^{-3}$. Because the measured breath ^{13}C enrichment during the 4th h of the tracer infusion was substantially higher by 30.0 to 35.0 $APE \cdot 10^{-3}$ than the postabsorptive base-line enrichment, failure to take this diet-induced increase into account would result in an overestimate of the $^{13}CO_2$ contributed by the tracer of only 6–7%. In the present study, the effects of dietary contribution to base-line ^{13}C could have been further minimized by increasing the tracer infusion rate.

One potential problem in interpreting the rise in breath enrichment due to diet is the fact that respiratory exchange values measured during the formula-only infusion were lower than respiratory exchange values measured during the subsequent tracer infusion study. This was probably due to the effects of diurnal variation in metabolic rate on these measurements: 8 A.M. to 12 noon vs. 12 noon to 4 P.M. (2, 3). As the mixture of fuels utilized may have changed as the postabsorptive period was prolonged beyond 12 h, it is possible that the rise in enrichment might have been different if the study had been conducted between 12 noon and 4 P.M. rather than 8 A.M. and 12 noon. However, even a difference in enrichment due to the formula alone of 25% would not have changed the correction of the tracer enrichment by more than 2% in this study, so it is unlikely that an effect of this difference in respiratory exchange values noted for the morning and afternoon fed periods would have changed our interpretation significantly. Dietary contribution of ^{13}C could be significant, on the other hand, in a tracer study where the oxidation of a ^{13}C-labeled substrate like leucine was being measured. With the present formula and dietary conditions as an example and a range of CO_2 enrichments that we have measured in fed studies using leucine tracers, the error due to formula contribution of ^{13}C could result in oxidation being overestimated by 20–25%, especially if nutritional conditions favored low substrate oxidation rates and

relatively less $^{13}CO_2$ from the tracer would be appearing in breath. For this reason, we now use diets that are isotopically neutral with our subjects' basal breath enrichments for studies where oxidation measurements are critical (e.g., Ref. 6).

These results highlight the need to define the contribution of dietary ^{13}C to breath ^{13}C enrichment during a ^{13}C tracer study, in which the dietary ^{13}C content is not isotopically neutral or equivalent to that of the subject's ^{13}C enrichment in expired air at entry into a study. They also demonstrate that the time of day when the dietary contribution to ^{13}C enrichment is assessed should be the same as when the fed period of the tracer infusion is to be carried out.

Bicarbonate Tracer Infusate Measurements

Of great practical importance for the conduct of labeled bicarbonate infusions is the analysis of the infusate concentration. Many investigators now using ^{13}C-labeled tracers in metabolic studies do not have ready access to isotope ratio mass spectrometers, and isotopic measurements are made elsewhere. This means that infusate solutions are likely to require storage. Our own problems with infusate solution deterioration in freezer storage should be taken as a warning that the infusate samples should be either analyzed immediately or stored with extreme care (e.g., aliquots should be stored under mineral oil in a tube that seals with an O-ring before freezing). As the CO_2 electrode is commonly found in clinical laboratories, the technique that we used to measure infusate concentrations would have the advantage of permitting rapid and reproducible analysis of bicarbonate infusates, provided that the ^{13}C enrichment of the labeled bicarbonate is also directly verified.

Importance of Accurate Indirect Calorimetry for Determining Label Recovery or Estimating Substrate Oxidation

A final consideration is the adequacy of the indirect calorimetry in substrate oxidation studies. The technique we had available at the time this study was performed, the Douglas bag technique, has certain limitations that may favor the loss of CO_2 specifically by diffusion or leaks through plastic tubing and fittings. The mean PA respiratory quotient of 0.74 measured here may simply reflect that our subjects were 14–16 h postabsorptive at the time of the measurements but could also show that we consistently either underestimated CO_2 or overestimated $\dot{V}O_2$. The validity of our results are supported by another study (12) utilizing open-circuit indirect calorimetry. That study was carried out at a center with long experience with indirect calorimetry and obtained similar results for recovery during PA and fed states, as noted above. This emphasizes that the appropriateness of CO_2 production for a particular subject should always be evaluated during a ^{13}C tracer study, because this measurement provides the greatest source of error in estimating substrate oxidation rates (24). The values in the present study compare well against those reported in most other studies (12, 18, 19, 37, 38) that have measured recovery of ^{13}C or ^{14}C, except those reported by Irving et al. (17), where total CO_2 production appears to have been seriously underestimated. It is perhaps for this reason that the reported $^{13}CO_2$ recovery rates for their study fall well below those of other studies reported in Table 8. The subsequent study by the same authors (18) reported more reasonable CO_2 production rates and recoveries that were substantially higher than the first study.

Conclusions

We recommend that studies of substrate oxidation include an assessment of biocarbonate recovery, made by administering labeled bicarbonate. This should be carried out under conditions identical to the actual tracer study and employing the same breath collection and respiratory exchange measurement system used in the study. Because any period of experimental diet preceding the study may influence the results, the order of the bicarbonate recovery study and the tracer study should be randomized to minimize any effects of previous diet. Based on the present study, the route of bicarbonate tracer administration should not affect the recovery, but this can only be said for the precise conditions reported here and should not be presumed for other conditions such as prolonged fasting or the feeding of other fuel mixtures. Further studies of the relationship of bicarbonate flux, label recovery, and respiratory exchange are needed under a variety of conditions to answer the question as to whether label recovery can be generally predicted as a function of respiratory exchange ($\dot{V}O_2$ and $\dot{V}CO_2$).

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