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## Bicarbonate kinetics in Indian males

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Measurement of rates of *in vivo* substrate oxidation such as that of glucose, fatty acids and amino acids, are based on tracer ( $^{14}\text{C}$  or  $^{13}\text{C}$ ) data, and often depend on the isotopic content of expired  $\text{CO}_2$ . The recovery of tracer-labelled  $\text{CO}_2$  generated from the oxidation of  $^{13}\text{C}$  labelled substrates may not be 100% over short term. This can lead to underestimation of oxidation rate of substrates, and consequently a correction for the incomplete recovery of tracer has to be applied by the determination of the recovery of  $^{13}\text{CO}_2$  in the breath during tracer bicarbonate infusions. We have studied the recovery of tracer-labelled bicarbonate using a bolus administration model, and further characterized kinetics of bicarbonate using a three-compartment model, to assess which compartmental fluxes changed during the change from a fasted state to fed state. Recovery of bicarbonate was lower at 69% and 67% (fasted and fed state) than the value of 71% and 74% found during earlier longer term of continuous infusions. During feeding, there was a 20-fold increase in the flux of bicarbonate between the central compartment and the compartment that was equivalent to the viscera. This study shows that the difference between the fasted and fed state recovery of tracer bicarbonate similar to that obtained with continuous infusions, and that bicarbonate fluxes show large changes between different compartments in the body depending on metabolic state.

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### 1. Introduction

Measurements of the rates of oxidation of substrates, such as glucose, fatty acids and amino acids based on tracer ( $^{14}\text{C}$ - or  $^{13}\text{C}$ -labelled substrates) data often depend upon the determination of the isotopic content of  $\text{CO}_2$  in expired air (Klein 1982; Wolfe 1992). The kinetic behaviour of the bicarbonate pools and their interrelationship can affect the extent and pattern of labelling in the expired air. These factors have significance for the correct interpretation of the data used to calculate substrate oxidation in experiments involving administration of carbon labelled tracers.

When a  $^{13}\text{C}$  labelled substrate (labelled in the appropriate location on the molecule that is oxidized) is administered to a subject, the  $^{13}\text{C}$  tracer is released in the body as  $^{13}\text{CO}_2$  into the  $\text{CO}_2$  pool. This  $^{13}\text{CO}_2$  is in equilibrium with bicarbonate. This  $^{13}\text{CO}_2$  eventually is expired enabling the investigator to quantify the amount of tracer released, as the

product of the breath  $\text{CO}_2$  enrichment, and the  $\text{CO}_2$  expiration rate. However, not all the tracer-labelled  $\text{CO}_2$  is released or recovered in the breath; some of it equilibrates in slowly turning over pools such as bone, and is released over a period of time. In theory, it is possible to recover the entire released tracer in the process of oxidation if breath sampling is done over a long period (days) of time: not quite possible in human subjects. In addition, non respiratory losses of  $\text{CO}_2$  can occur. Therefore, to correct for this and to accurately determine the rate of oxidation of a carbon-labelled substrate over a shorter period of time (for example, 24 h), it is necessary to apply a correction factor to the measured rate of evolution of labelled  $\text{CO}_2$  in expired air.

This correction, or recovery factor, is estimated by the proportion of a dose of labelled bicarbonate – given intravenously or orally, as a constant infusion or as a bolus – that appears in the expired air (James *et al* 1976; Irving *et al* 1983). However, the implicit assumption (Saccomani *et al*

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1995) is that the administered labelled bicarbonate and that generated by the oxidation of the labelled substrate of interest share the same recovery factor. This assumption is probably justified, as these investigators have demonstrated that both the respiratory and nonrespiratory losses are located in the central vascular compartment, which is the pool where tracer input occurs. It has been observed (Issekutz *et al* 1968; Clugston and Garlick 1983; Hoerr *et al* 1989; El-Khoury *et al* 1994a,b) that values for recovery of CO<sub>2</sub> in expired air after administration of labelled bicarbonate, range from about 50% to greater than 90%. While Leijsser and Elia (1996) attribute much of the variability in the bicarbonate recovery to methodological differences, it does appear that bicarbonate recoveries differ between the fed and fasted states, and that continuous infusions give higher recoveries, compared to bolus doses. The duration of the experiment is also important in continuous infusions, since, as the duration of the infusion increases, different bicarbonate pools within the body would be labelled to an equilibrium state at which point it is likely that the recovery of the tracer label would be near 100%. In practice, this does not occur over 24 h, which is the usual practical duration for most captive human experiments.

Using a 24 h continuous intravenous infusion of <sup>13</sup>C-bicarbonate, the recovery factor, in healthy young adult US males, was shown to be  $76.6 \pm 2.0$  % and  $85.1 \pm 2.5$  % for fast and fed states respectively (El-Khoury *et al* 1994a,b). In contrast, we obtained recovery values of  $70.8 \pm 4.0$  % and  $73.8 \pm 4.2$  %, respectively, in healthy young adult Indian males, following essentially identical experimental conditions (Kurpad *et al* 1998).

The differences between Western and Indian subjects warrants further investigation, because of their significance in the determination of the kinetics of amino acid oxidation. We repeated the measurement of bicarbonate kinetics in Indian subjects, this time using a bolus administration of isotope along with noncompartmental methods to determine the recovery of isotope. However, the metabolic conditions in which the subjects were studied were kept similar to the earlier continuous infusion methods. The kinetics of bicarbonate were further investigated by applying the three-compartment model developed and validated by Saccomani *et al* (1995), to assess the changes in inter-compartmental fluxes of bicarbonate during the fasted and fed state.

## 2. Subjects and methods

### 2.1 Subjects

Four young Indian adult male medical students participated in this experiment. All were in good health as determined by medical history, physical examination, blood counts, and routine blood biochemical profile and urinalysis. The purpose of the study and the potential risks involved were explained to

each subject and written consent was obtained from each subject. The research protocol was approved by the Human Ethical Approval Committee of St John's Medical College.

### 2.2. Anthropometric measurements

Anthropometric and skinfold measurements were carried out on the subjects on day zero. Subjects were weighed in minimal clothing, using a digital scale (Soehnle, Germany) which had a precision of 0.1 kg. Their heights were recorded to the nearest 0.1 cm using a vertically mobile scale (Holtain, Crymych, UK). The logarithm of the sum of the four skinfolds (biceps, triceps, subscapular and suprailiac) was used, in age and gender-specific equations (Durnin and Womersley 1974), to obtain an estimate of body density, from which percentage body-fat was determined (Siri 1961).

### 2.3 Diet and experimental design

Each subject was studied after a 9-day diet period when they received a weight-maintaining diet. The subjects were encouraged to maintain their customary levels of physical activity, but were asked to refrain from excessive or competitive exercise. The diet consisted of chapattis made from cream of wheat and oil, with egg albumen as the main source of protein. Wheat was chosen as the source of carbohydrate, in order to attain a low <sup>13</sup>C content in the diet and consequently a relatively steady background in breath <sup>13</sup>CO<sub>2</sub> enrichment over the experiment period. The total daily food intake was consumed as three isoenergetic, and isonitrogenous meals (at 0800, 1300 and 2000 h). Every morning, body weight and vital signs were monitored. Meals were provided at the kitchen of the Division of Nutrition, under supervision of the dietary staff.

The subjects underwent two tracer studies; one in the fasted state, and the other, in the fed state. These studies were performed in a crossover design, with the first study (fasted or fed) being performed on day 7 and the next study (fed or fasted) on day 9.

### 2.4 Tracer protocol

The bolus-dose approach was used in this study. The tracer bolus was administered on both the tracer study days at 0900 h, and the study lasted until 1800 h of the same day. The bolus-dose of tracer was given as 1.0 mg.kg<sup>-1</sup> of <sup>13</sup>C-sodium bicarbonate (99.9 atom%; MassTrace, Woburn, MA), and prepared in physiological saline, under sterile conditions, and administered intravenously in a total volume of ~ 2ml. In all cases, the subjects consumed their last meal on the previous day at 2000 h. On the fasted day, subjects received no food during the study, but were permitted

water. On the fed-study day, the subjects received 12 isoen-ergetic and isonitrogenous small meals at hourly intervals, beginning at 0600 h and lasting until and including 1700 h (which together were equivalent to 2/3 of the dietary intake for that day). Throughout the 9 h study the subjects remained in bed, in a reclining position.

**2.4a Indirect calorimetry:** Total carbon dioxide production per minute ( $\text{VCO}_2$ ) and oxygen consumption ( $\text{VO}_2$ ) were determined using an open circuit indirect calorimeter with a ventilated hood as previously described (Shetty *et al* 1987). The rates of  $\text{O}_2$  consumption and  $\text{CO}_2$  production were expressed in liters per minute, corrected to standard temperature and pressure. The analysers were calibrated using previously calibrated standard gases (oxygen free nitrogen and span gas; Bhoruka Gas Ltd., Bangalore). Whole system calibration was verified by combustion of pure ethanol, where the observed difference between measured and predicted total  $\text{VCO}_2$  was < 3 % and the average respiratory quotient (RQ) was between 0.64 and 0.68.

### 2.5 Collection and analysis of breath samples

Three baseline breath samples were collected at 30, 15, and 5 min, before the tracer administration, following which, breath samples were collected at 0.5, 1, 2, 3, 5, 7, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420, 450, 480, 495, 510, 525 and 540 min. Breath gas was collected in a specially designed bag, with a mechanism that permitted the removal of dead space air, and was then transferred into three, 10 ml non-silicon-

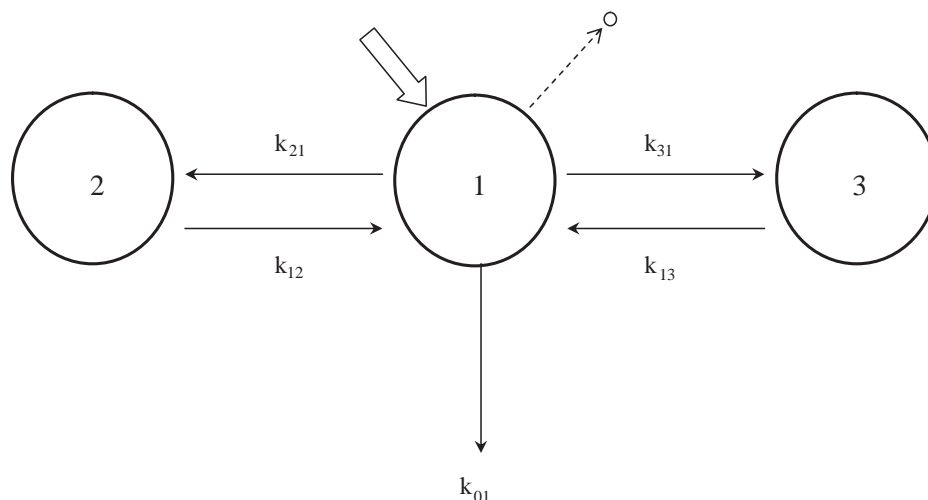
coated glass tubes (Vacutainer, Becton and Dickinson, New Jersey, USA). At time intervals where the breath sample collection coincided with hourly meals, the breath sample was collected first. The samples were stored at room temperature until analysed by isotope ratio mass spectrometry (Europa Scientific, Crewe, UK). The Atom Percent Excess (APE) was calculated by taking the arithmetic difference between enrichment of each breath sample and the pre-dose basal breath sample.

### 2.6 Calculations

A previously validated three-compartment mamillary model was used, with a modification, as described previously (Saccomani *et al* 1995). In this model (figure 1) the irreversible loss of  $\text{CO}_2$  occurs from the central compartment (compartment 1) which rapidly equilibrates with expired air representing  $\text{CO}_2$  in the circulation. The central compartment equilibrates with two peripheral compartments (compartments 2 and 3) representing tissues with slow and fast turnovers of  $\text{CO}_2$ . The injection of labelled bicarbonate is made into the central pool and sampling of  $^{13}\text{CO}_2$  is from the central compartment, and this can be represented as:

$$z(t) = \phi \text{CO}_2(t), \quad (1)$$

where  $z(t)$  is the observed flux,  $\phi \text{CO}_2(t)$  is the time course of the flux of tracer eliminated from the central compartment, and  $t$  denotes the sampling time. Note that the observed variable is the *flux* of tracer, which is the product of the enrichment and the  $\text{VCO}_2$  measurement.



**Figure 1.** Schematic representation of the three-compartment model used. The large arrow indicates the site of infusion of tracer (into the central compartment). The dotted line with the circle indicates the point of sampling (by breath) from the central compartment. Compartment 1 is the central compartment which is assumed to be the “vascular pool”; compartment 2 is the ‘fast’ compartment, assumed to be equivalent to muscle; and compartment 3 is the ‘slow’ compartment, assumed to be equivalent to the viscera. ‘k’ is the rate constant of flux.

The tracee parameters can also be obtained from this model (Saccomani *et al* 1995). That is the total CO<sub>2</sub> in the body (Q<sub>Tot</sub>), as well as the CO<sub>2</sub> content of each pool (Q<sub>1</sub>, Q<sub>2</sub>, Q<sub>3</sub>) can be obtained. Fluxes of tracee from one pool to another too were calculated as the product of the transfer coefficient and the quantity of tracee in the donor pool. Curve-fitting and model-estimation was done using SAAM II. The data analysis was done by using each individual subjects data and then computing the mean values of estimated parameters for all the subjects, or, by first calculating the mean decay of the label for all subjects; and then subjecting this mean data to curve fitting and analysis. The exponential model parameters were estimated by using the weighted least squares technique (Landaw and DiStefano 1984), and the data was weighted by the reciprocal of the square of the variance. The goodness of fit of the model to the data was checked initially by plotting the weighted residuals against time, and observing if there was a nonrandom pattern in this distribution.

Noncompartment analysis was also carried out on the data, to calculate the mean residence time (MRT), area under the curve for recovery of label, and the recovery of <sup>13</sup>CO<sub>2</sub>. MRT was calculated from the decay curve of the washout of label, as the area under the moment curve (AUMC) divided by the area under the curve (AUC), assuming a linear system (DiStefano and Landaw 1984). The respiratory fraction of dose recovered (D<sup>R</sup>), from the dose administered (D), over the entire duration of measurement, could be calculated from the integral of the product of the enrichment of <sup>13</sup>CO<sub>2</sub> in expired air and the total CO<sub>2</sub> production at each time point. Thus,

$$D^R = \int_0^{\infty} \phi^R \text{CO}_2(t) dt$$

$$= \int_0^{\infty} \text{VCO}_2(t) \cdot {}^{13}\text{CO}_2 \text{ enrichment } (t) dt, \quad (2)$$

where  $\phi^R \text{CO}_2(t)$  (equivalent to AUC) is the time course of the flux of tracer eliminated from the respiratory system, and,

$$\text{the recovery factor} = D^R/D. \quad (3)$$

Assuming a steady state, the rate of appearance of endogenous CO<sub>2</sub> could also be calculated – as the  $\phi^R \text{CO}_2(t)$  is the product of the enrichment of <sup>13</sup>CO<sub>2</sub> in the breath – and the VCO<sub>2</sub> at each time point. Then,

$$\text{CO}_2 \text{ production (mmol)} = \text{Dose (mmol)} /$$

$$\int_0^{\infty} {}^{13}\text{CO}_2 \text{ enrichment } (t) dt. \quad (4)$$

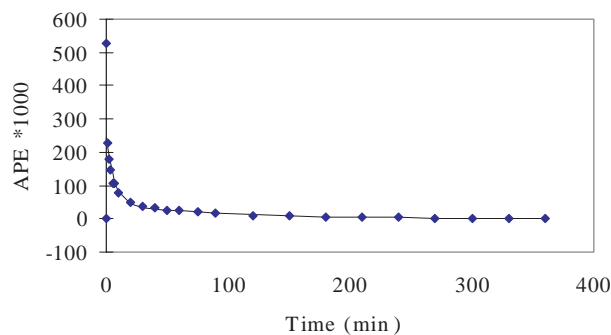
Significant differences noticed between methods and between metabolic states were assessed by the Student's

paired *t* test. Differences were considered significant if *P* < 0.05.

### 3. Results

All four subjects were between the ages of 20 and 25 years, and had a mean height and weight of  $1.7 \pm 0.1$  M and  $56.8 \pm 6.2$  kg respectively. Their mean BMI was  $19.9 \pm 0.6$  kg.M<sup>-2</sup>, and mean percent body fat was  $7.9 \pm 2.4$  %.

Figure 2 shows the appearance of the decay curve of the injected bolus of <sup>13</sup>C bicarbonate, in the breath. There was an initial increase in tracer enrichment, followed by a decline, which was fitted to a tri-exponential equation. This gave the best fit for the data in all cases. Non compartmental analyses of the MRT, gave a mean estimate of 71.4 min in the fasted state, and 53.1 min in the fed state. These estimates were significantly different from each other. The AUC method of calculating recovery (eqs 2, 3) gave a mean estimate of recovery of tracer of 68.8% and 67.4% (fasted and fed, respectively). CO<sub>2</sub> production (eq. 4) was calculated to be 12.0 mmol and 14.3 mmol in the fasted and fed state respectively. The estimated CO<sub>2</sub> production was always higher than the measured VCO<sub>2</sub>, as not all the CO<sub>2</sub> produced in the body is lost through the respiratory route. Indeed, when the compartmental estimate of <sup>13</sup>CO<sub>2</sub> recovery (see below) was used in conjunction with the non-compartmental estimate of CO<sub>2</sub> production, to estimate the respiratory loss of the CO<sub>2</sub> produced in the body (table 1), the respiratory loss of CO<sub>2</sub> was very close to the measured VCO<sub>2</sub> (8.2 and 9.7 mmol.min<sup>-1</sup> respectively for the fasted and fed state, compared to 8.3 and 9.8 mmol.min<sup>-1</sup> for fasted and fed state measured VCO<sub>2</sub>). There was a significant difference in the VCO<sub>2</sub>, MRT, and the CO<sub>2</sub> production rate, between the fasted and fed state.



**Figure 2.** Plot of the enrichment of <sup>13</sup>CO<sub>2</sub> (atom percent excess, APE) in the breath at different time points during the experiment. Note that the enrichment rises rapidly following the bolus administration of tracer, followed by a triexponential decline. Diamonds represent observed enrichment and the line joining these is the fitted tri-exponential curve.

**Table 1.** Measured values of VCO<sub>2</sub> and non-compartmental analysis.

	Fasted					Fed				
	Subject					Subject				
	1	2	3	4	Mean	1	2	3	4	Mean
VCO <sub>2</sub> mmol.min <sup>-1</sup>	8.1	8.0	8.5	8.5	8.3 ± 0.2	9.4	9.0	10.8	10.0	9.8 <sup>†</sup> ± 0.8
CO <sub>2</sub> mmol.kg <sup>-1</sup> .min <sup>-1</sup>	155.1	154.8	130.2	144.7	146.2 ± 11.7	178.0	174.7	166.4	174.0	173.3 ± 4.9
MRT (mean residence time, min <sup>-1</sup> )	73.9	74.7	70.2	66.8	71.4 ± 3.6	51.8	54.6	67.3	38.6	53.1 <sup>†</sup> ± 11.8
CO <sub>2</sub> production mmol.min <sup>-1</sup>	12.3	10.6	12.6	12.5	12.0 ± 0.9	15.6	13.4	14.3	13.9	14.3 <sup>†</sup> ± 0.9
CO <sub>2</sub> production μmol.kg <sup>-1</sup> .min <sup>-1</sup>	235.0	205.4	193.4	213.7	211.9 ± 17.5	295.3	262.0	219.1	241.1	254.3 ± 32.4
<sup>13</sup> CO <sub>2</sub> % recovery	66.7	74.5	67.3	66.6	68.8 ± 3.8	59.8	65.8	74.7	69.2	67.4 ± 6.3

*n* = 4, fasted and fed. Mean ± SD. <sup>†</sup>*P* < 0.05.

However, no significant differences were found for the recovery of <sup>13</sup>CO<sub>2</sub>.

The mean data from the compartmental analysis is presented in table 2. The absolute value of *k*<sub>01</sub> went up from 0.0613 min<sup>-1</sup> in the fasted state to 0.1121 min<sup>-1</sup> in the fed state. The VCO<sub>2</sub> also went up from 8.3 to 9.8 mmol.min<sup>-1</sup>, (or 146.2 to 173.3 μmol.kg<sup>-1</sup>.min<sup>-1</sup>) from the fasted to the fed state. Additional parameters obtained from this model were the amount of CO<sub>2</sub> (tracee, *Q*) present in each of the compartments, together with their means. There were significant differences between the fed and the fasted state, for *Q*<sub>1</sub> and *Q*<sub>2</sub>. Since the flux of tracee can be determined as the product of the transfer coefficient (e.g. *k*<sub>21</sub> or *k*<sub>12</sub>) and the amount of tracee in the pool of origin, flux rates to and from the peripheral compartments and from the central compartment were also calculated. The fasting flux rate from the central compartment to the first compartment (compartment 1 to compartment 2, flux<sub>21</sub>) was 0.6468 ± 0.0817 mmol.kg<sup>-1</sup>.min<sup>-1</sup>, in comparison to the flux rate from the central to the slow compartment (flux<sub>31</sub>) i.e. flux rate 0.0322 ± 0.0133 mmol.kg<sup>-1</sup>.min<sup>-1</sup>. The corresponding rates for the fed state were 0.9599 ± 0.3590 and 0.3399 ± 0.0636 mmol.kg<sup>-1</sup>.min<sup>-1</sup>, for flux<sub>21</sub> and flux<sub>31</sub> respectively. The ratio of the fluxes, (rapid, flux<sub>21</sub> / slow, flux<sub>31</sub>), was 22.6 in the fasted state, but this dropped significantly to 2.8 in the fed state. This was primarily because the flux to the slow compartment increased ten-fold. When the different compartmental parameters were obtained by fitting the model to the averaged individual data, there were no substantial differences between these data, and that obtained by first fitting the model to the individual data, and, then, computing the mean values of each parameter.

**Table 2.** Compartmental analysis data in the fast and fed states averaged from individuals.

	Fasted		Fed		<i>P</i>
	Mean	SD	Mean	SD	
<i>k</i> <sub>01</sub>	0.0613	0.0092	0.1121	0.0360	< 0.05
<i>k</i> <sub>12</sub> (fast)	0.0676	0.0124	0.3837	0.2333	< 0.05
<i>k</i> <sub>21</sub>	0.1840	0.0213	0.3890	0.0816	< 0.01
<i>k</i> <sub>13</sub> (slow)	0.0050	0.0017	0.0411	0.0112	< 0.01
<i>k</i> <sub>31</sub>	0.0089	0.0028	0.1482	0.0516	< 0.01
<i>Q</i> <sub>1</sub> (mmol)	199.44	21.53	138.34	37.87	< 0.05
<i>Q</i> <sub>1</sub> (mmol.kg <sup>-1</sup> )	3.56	0.70	2.51	0.89	NS
<i>Q</i> <sub>2</sub> (mmol)	553.41	111.96	161.44	74.00	< 0.01
<i>Q</i> <sub>2</sub> (mmol.kg <sup>-1</sup> )	9.67	1.06	2.91	1.49	< 0.01
<i>Q</i> <sub>3</sub> (mmol)	360.06	95.30	480.45	84.87	NS
<i>Q</i> <sub>3</sub> (mmol.kg <sup>-1</sup> )	6.44	2.12	8.45	0.91	NS
<i>Q</i> <sub>Tot</sub> (mmol)	1112.91	103.84	780.23	76.65	< 0.01
<i>Q</i> <sub>Tot</sub> (mmol.kg <sup>-1</sup> )	19.68	2.05	13.87	1.95	< 0.01

*Q*<sub>1</sub>, *Q*<sub>2</sub>, *Q*<sub>3</sub>, Amount of CO<sub>2</sub> in respective pools. *n* = 4, fasted and fed. Mean ± SD.

*Q*<sub>Tot</sub>, Total CO<sub>2</sub> in the body.

#### 4. Discussion

<sup>13</sup>CO<sub>2</sub> kinetics are important as an adjunct to studies of <sup>13</sup>C-labelled substrate oxidation studies. In these studies, the measured oxidation of the substrate is usually corrected by



a "recovery factor", for the retention of the oxidized label in the body. The reasons for the retention, or incomplete recovery of  $^{13}\text{C}$ -bicarbonate are well known and include  $\text{CO}_2$  fixation as well as equilibration and entry of the label into slowly turning over pools (Elia 1990; Leijsser and Elia 1996). In order to overcome this in an ideal situation, individual subjects would have their own recovery factor estimated on an earlier occasion, and the  $^{13}\text{C}$ -substrate oxidation study would be conducted on a different occasion (e.g. the next day). However, given the large intra-individual variability in the recovery factor estimates (Irving *et al* 1983), the preferred method is to generate a population-specific, mean recovery factor, and to apply this factor to all  $^{13}\text{C}$ -substrate oxidation studies in the particular population. The recovery factor is generated by measuring the recovery of  $^{13}\text{CO}_2$  in the breath, during a primed infusion of  $^{13}\text{C}$ -bicarbonate. The requirement of this estimate is that the subject is in a steady state (Irving *et al* 1983), which is generally ensured by conducting these studies in a resting fasted individual. When the fed-state recovery needs to be measured, the subject is fed in as near a steady-state fashion; i.e. small frequent meals to achieve a near-steady fed-state. The necessary sequel to these conditions is that the oxidation of the  $^{13}\text{C}$ -substrate needs to be measured in exactly the same conditions.

Indian estimates of bicarbonate recovery that we have generated (Kurpad *et al* 1998), are different from Western estimates (Hoerr *et al* 1989; El-Khoury *et al* 1994a,b; Leijsser and Elia 1996). Moreover, the difference appeared to be more marked for the 'fed' pattern of recovery. We have also considered, and eliminated potential methodological issues in the generation of our estimates (Kurpad *et al* 1998), such as the contribution of dietary  $^{13}\text{C}$  to the enrichment of breath  $^{13}\text{CO}_2$ , by the prior one week feeding of low  $^{13}\text{C}$  containing foods such as wheat starch and beet sugar, and the carbohydrate content of the diet (about 60%) was closer to traditional western diets than Indian diets. The preparation and delivery of the isotope solution was also considered; for example, if the diluting solution (normal saline) were to be slightly acidic, there would be loss of label on mixing the bicarbonate with the diluting solution, or, if the isotopic solution were to be frozen and stored prior to administration, there could again be loss of label from the solution. The Western (El-Khoury *et al* 1994a,b) and the Indian (Kurpad *et al* 1998) studies were exactly similar in terms of dose, duration and timings of the study, except for the different body size of the subjects. However, the body size of the individual has not been shown to influence the recovery of  $^{13}\text{CO}_2$ , as similar recoveries have been recorded in obese and non obese individuals (Issekutz *et al* 1968; Clugston and Garlick 1983; Leijsser and Elia 1996), as well as in children and adults (Van Aerde *et al* 1985; Armon *et al* 1990).

We felt that the apparent difference between the Indian and Western subjects could be investigated in greater detail by the bolus dose and compartmental analysis technique. This technique yields richer data, and the use of similar subjects allowed for an extension of the earlier studies. The literature of bicarbonate kinetics supports the three exponential decline of the enrichment of  $^{13}\text{CO}_2$  in breath (Issekutz *et al* 1968; Winchell *et al* 1970; Irving *et al* 1983; Saccomani *et al* 1995). In the present study too, we found the best solution to the decay of breath  $^{13}\text{CO}_2$  enrichment was the tri-exponential fit. The three compartmental model was chosen as it had already been validated earlier, by using model-independent approaches to compare model-dependent estimates of parameters, such as the MRT (Saccomani *et al* 1995). The physical correlates of these compartments (pools) are as follows: the central pool is equivalent to the vascular pool of bicarbonate; the fast pool (compartment 2) is equivalent to muscle; and the slow pool (compartment 3) is equivalent to the viscera (Irving *et al* 1983). This allocation of compartments is borne out by the change in the flux to the slow compartment ( $\text{flux}_{31}$ ) after perturbing the system by feeding. In this state, gut metabolism increases and the flux to and from the slow compartment (composed of viscera including the gut) would be expected to increase; and, it did, by a factor of about 10. When fed, animals and humans show an increase in metabolic rate, which is in excess of the energy cost of processing and storing food. This increase in energy expenditure is called post-prandial thermogenesis (Shetty *et al* 1981), and for example, it can be calculated that the cost of storing ingested glucose is about 12% in terms of the energy content of what is eaten (Thiebaud *et al* 1983). This is in excess of the cost of glycogen synthesis (Flatt 1978), and is likely to be due to the stimulation of the sympathetic nervous system which could stimulate heat production in the body and also in resting muscle (Kurpad *et al* 1994). Assuming that muscular activity does not occur (the subjects were in a rested state throughout the present experiment), the compartment that is presumed to represent the muscle is likely to contribute relatively less (by one order of difference) to whole body metabolic  $\text{CO}_2$  generation. Therefore, the difference between the fed and fasted state might be attributed to the actual processing of food linked to increased parasympathetic activity and insulin secretion (Nacht *et al* 1987), but might also be attributed to the actual stimulation of the sympathetic nervous system (Acheson *et al* 1984). In addition, substrate recycling may also occur, such as the triglyceride-fatty acid cycle (Elia *et al* 1987), with its own costs in energetic terms leading ultimately to the need for cellular respiration and  $\text{CO}_2$  generation.

The flux to the fast compartment (muscle) increased marginally, as the subjects remained in the resting state with minimal voluntary muscular movement. The ratio between

**Table 3.** Comparison of model parameters of the present study with literature data.

Parameter	Present study (fasted)	Irwing <i>et al</i> 1983	Saccomani <i>et al</i> 1995	Barstow <i>et al</i> 1990	Present study (fed)
<i>n</i>	4	5	9	6	4
CO <sub>2</sub> measured ( $\mu\text{mol.kg}^{-1}.\text{min}^{-1}$ )	146.2	101.1	146.9	129.8	173.3
MRT (min)	71.4		79.2		53.1
<sup>13</sup> CO <sub>2</sub> % recovery	68.8	51.8		66.8	67.4
k <sub>01</sub>	0.0613	0.0552	0.0500	0.0673	0.1121
k <sub>12</sub> (fast)	0.0676	0.1834	0.1840	0.2307	0.3837
k <sub>21</sub>	0.1840	0.1225	0.1380	0.1616	0.3890
k <sub>13</sub> (slow)	0.0050	0.0245	0.0290	0.0342	0.0411
k <sub>31</sub>	0.0089	0.0524	0.0420	0.0831	0.1482
Q <sub>1</sub> (mmol)	199.44			233.00	138.34
Q <sub>1</sub> (mmol.kg <sup>-1</sup> )	3.56	3.70	3.50	3.12	2.51
Q <sub>2</sub> (mmol)	553.41			193.00	161.44
Q <sub>2</sub> (mmol.kg <sup>-1</sup> )	9.67	4.43	3.00	2.58	2.91
Q <sub>3</sub> (mmol)	360.06			521.00	480.45
Q <sub>3</sub> (mmol.kg <sup>-1</sup> )	6.44	7.65	6.70	6.97	8.45
Q <sub>Tot</sub> (mmol)	1112.91			948.00	780.23
Q <sub>Tot</sub> (mmol.kg <sup>-1</sup> )	19.68	15.79	13.10	12.69	13.87

the fluxes (flux<sub>21</sub> / flux<sub>31</sub>) dropped from 22.6 to 2.8, indicative of the change in metabolic status after feeding. The large bone bicarbonate compartment is not represented as a separate compartment in this scheme, as the exchange to this compartment is slow, and probably un-represented over a short time frame. However, it is reasonable to suggest that a part of the nonrespiratory losses of <sup>13</sup>CO<sub>2</sub> occur to this compartment. Physiological data on the bone-pool size of bicarbonate, bone-blood flow rate and the extraction ratio of bicarbonate between bone and blood is available, to calculate that the vascular bicarbonate exchanges with bone bicarbonate at a rate of about 24  $\mu\text{mol.kg}^{-1}.\text{min}^{-1}$  (Poyart *et al* 1975; Irving *et al* 1983). This value is about one-half to one-third of the nonrespiratory losses and other exchange processes, such as biosynthetic sequestration, and exchange, must account for the remainder of the nonrespiratory losses.

A comparison of the compartmental parameters obtained in the present study, with previous studies, is shown in table 3. The fasted-state data from the present study was comparable to the data from these selected Western subject studies (Winchell *et al* 1970; Irving *et al* 1983; Barstow *et al* 1990; Saccomani *et al* 1995; Sphiris and Pallikarakis 1995). However, there were similarities between the fed-state Indian data and the fasted Western data, particularly for the recovery of <sup>13</sup>CO<sub>2</sub>. One interesting difference between the Indian and Western data was the much larger size of compartment 2 in the fasted state. That the Indian subjects could have a larger muscle mass and hence a

larger size of this particular pool is unlikely, as it is known that Asians have a lower muscle mass in comparison to Caucasians and African Americans (Song *et al* 2002). Normalization for body weight could have led to this finding, as the body size of the Indian subjects was comparatively small. However, the size of this pool dropped in the fed state, such that it became comparable to the Western studies.

In conclusion, we have validated our earlier findings of a difference between the recovery of <sup>13</sup>CO<sub>2</sub> between Indian and Western subjects. However, it is not clear why differences in the recovery of bicarbonate still exist between our studies and those in the Western literature. Large changes in flux rates between pools (particularly between the visceral and central pool) were evident when the metabolic state changed from fasted to fed.

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