Measurement of carbon dioxide production in very low birth weight babies

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Abstract

Background—CO₂ production is most commonly measured by using indirect calorimetry to quantify elimination of CO₂ in breath (VCO₂). An alternative is to measure the rate at which CO₂ appears in the body pool (RaCO₂) by infusing a ¹³C labelled bicarbonate tracer. VCO₂ and RaCO₂ generally differ but are related by c, a factor that adjusts for the incomplete recovery of infused tracer in the breath. The literature relating to human studies cites a wide range of values for c but the only neonatal study to determine c empirically estimated a mean value of 0.77.

Aim—To estimate fractional recovery rate, c, in very low birthweight babies, and assess the feasibility of using the isotopic technique to measure CO₂ production during mechanical ventilation.

Method—Eleven spontaneously breathing, continuously fed, very low birthweight infants (median birth weight 1060 g, median gestational age 29 weeks) were studied.

Results—Mean (SD) VCO₂ was 9.0 (2.0) ml/min (standard temperature and pressure dry, STPD) and mean (SD) RaCO₂ was 9.6 (2.1) ml/min (STPD). The mean (SD) value of c was estimated as 0.95 (0.13). The 95% confidence intervals of the mean were 0.87–1.03.

Conclusions—The results emphasise the importance of measuring c for a given study population rather than assuming a value based on adult studies. The close approximation of RaCO₂ and VCO₂ in this group of babies implies that the labelled bicarbonate infusion technique could be used to measure simply CO₂ production during mechanical ventilation.

Keywords: carbon dioxide; carbon isotopes; calorimetry; very low birthweight babies

The measurement of CO₂ production has important clinical applications in the management of very small babies. If O₂ consumption is measured simultaneously, respiratory quotient can be calculated, allowing total energy expenditure and nature of fuel oxidised to be deduced. Potentially this could help in the choice of more appropriate nutritional interventions for infants who show constrained capacity to eliminate CO₂ because of lung disease. Measuring CO₂ production is especially problematical in ventilated very low birthweight (VLBW) infants. Conventionally, breath CO₂ elimination (VCO₂) is calculated by measuring gas flow and the CO₂ content of inspired and expired breath. This technique, indirect calorimetry, is subject to both technical errors, such as leakage of expired gas, and confounding by changes in minute ventilation. An alternative approach is measurement of the rate at which CO₂ produced by metabolism appears in the body bicarbonate pool (RaCO₂, rate of appearance). This can be accomplished by measuring the isotopic enrichment (ratio of ¹³C to ¹²C) of CO₂ in expired breath while continuously infusing a ¹³C labelled sodium bicarbonate tracer at a known rate.

Studies of animals and adult humans have consistently shown that VCO₂ and RaCO₂ differ. The discrepancy is principally attributable to incomplete recovery of ¹³C labelled CO₂ in breath when there is insufficient time for infused label to equilibrate between body bicarbonate pools. A correction factor (c), given by the quotient of VCO₂ and RaCO₂, can be used to adjust for the fractional recovery of tracer. The literature cites mean values of c ranging between 0.5 and 1.06 in different patient groups and a value of 0.80 is often assumed in studies of substrate oxidation. A single published study of neonates yielded a mean (SD) estimate of 0.77 (0.05) but included only three babies weighing < 1500 g.

In view of the potential importance of accurately quantifying CO₂ production in VLBW babies and uncertainty about the magnitude of c in this group, we have simultaneously measured VCO₂ and RaCO₂ in spontaneously breathing babies and demonstrated the feasibility of applying the isotopic technique during mechanical ventilation.

Methods

Patients

Eleven spontaneously breathing VLBW (birth weight < 1500 g) babies were studied. All had clinically indicated venous access and weighed < 1500 g at the time of the study. Four were breathing ambient oxygen, seven were breathing air. Feeds were either expressed breast milk (n = 8) or a standard preterm formula (n = 1) administered by hourly intragastric bolus. One infant received total parenteral nutrition, and one both total parenteral nutrition and expressed breast milk. Table 1 summarises clinical details. A single ventilated baby was later studied to address problems of sampling from the endotracheal tube and to examine within patient variation in RaCO₂. St George’s Healthcare NHS Trust research ethics committee approved the study; informed written consent was obtained from the parents.
**Table 1 Clinical characteristics of the patients studied**

<table>
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<tr>
<th>Patient no</th>
<th>Birth weight (g)</th>
<th>Gestational age (weeks)</th>
<th>Study start weight (g)</th>
<th>Postnatal age (days)</th>
<th>Feeding regimen*</th>
<th>Estimated energy intake† (kcal/kg/day)</th>
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<td>31</td>
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<td>12</td>
<td>p</td>
<td>110</td>
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<td>940</td>
<td>4</td>
<td>p+e</td>
<td>58</td>
</tr>
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</table>

*e, enteral; p, parenteral.
†Estimated on the basis that breast milk had 0.67 kcal/ml, preterm formula used had 0.7 kcal/ml, and the total parenteral nutrition used had 0.4 kcal/ml.

**Design of the study**

Figure 1 summarises the protocol. Intragastrically fed babies were changed from hourly bolus feeds to continuous feeding at least one hour before the start of the study. A bolus dose of tracer (NaH13CO3) was given intravenously, followed by continuous tracer infusion. Simultaneously a Deltrac II metabolic monitor (Datex, Helsinki, Finland) was used to measure VCO2 throughout the first hour, after which breath was sampled intermittently to measure isotopic enrichment. Babies were routinely nursed in incubators, and temperature, heart rate, respiratory rate, SaO2, continuously monitored.

**Isotope samples**

Intragastrically fed babies were sampled manually every 30 minutes to measure the 13C enrichment of expired breath at steady state (in APE). The rate of appearance of CO2 (RaCO2) was then calculated using the standard single pool calculation (equation 2). To compensate for incomplete recovery of tracer in the breath, this will cause an overestimation of RaCO2, as breath isotopic enrichment was plotted against time, and the plateau defined according to the convention of taking four or more consecutive points with a coefficient of variation of < 5%.

**Model of HC13O3− kinetics**

The single pool model assumes that all bicarbonate administered and CO2 produced by metabolism enters and leaves the body from a central rapid turnover pool. This eventually attains equilibrium with slow turnover pools. The factor c corrects for overestimation of breath 13C enrichment when a study is too short for this state to be attained.

**Figure 2 Model of CO2/HC13O3− kinetics. All infused tracer and HC13O3−/CO2 generated by metabolism enter and leave the body from a central rapid turnover pool. This eventually attains equilibrium with slow turnover pools. The factor c corrects for overestimation of breath 13C enrichment when a study is too short for this state to be attained.**

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**Model of HC13O3− kinetics**

The single pool model assumes that all bicarbonate administered and CO2 produced by metabolism enters and leaves the body from a single, rapid turnover pool (fig 2). In practice, this probably communicates with slow turnover pools, representing relatively inert tissues—for example, bone. During short studies, insufficient time may elapse for tracer equilibration between pools, causing incomplete recovery of tracer in the breath. This will cause overestimation of RaCO2, as breath isotopic enrichment (Ee) forms the denominator in the calculation (equation 2). To compensate for this, a correction factor (term c, also known as the fractional recovery rate) can be derived as follows. It is first assumed that all CO2 administered as tracer and produced by metabolism is eliminated only in breath, that body CO2/bicarbonate pool size is unchanged, and that full equilibration occurs. Under such conditions:

\[ V_{CO2} = RaCO2 \]

The factor c, correcting for incomplete recovery and consequent overestimation of...
RaCO₂ is then given by:
\[ V_{CO₂} = c \cdot RaCO₂ \]  
which can be rearranged as:
\[ c = \frac{V_{CO₂}}{RaCO₂} \]  

MEASUREMENT OF VCO₂
Respiratory elimination of CO₂ (VCO₂) was measured using a commercially available open circuit indirect calorimeter (Deltatrac II metabolic monitor). This device offers a choice of four preset canopy flow rates: “baby” (3.1 litres/min), “child” (10.3 litres/min), “adult”, and “obese adult”. In accordance with manufacturer’s recommendations, the instrument was warmed up for at least 30 minutes before two point calibration with room air (CO₂ content assumed to be 0.04%) and Datex calibration gas (4.99% CO₂ later verified by BOC Analytical Division, Crawley, Sussex, UK). The baby's head and shoulders were placed under the transparent perspex canopy, and a partial seal created by tucking the integral flexible skirt beneath the body and mattress. The child flow range was chosen because Bauer et al have concluded that a flow rate of at least 4.5 litres/min is required for accurate measurement of VCO₂ using the Deltatrac in canopy mode. We also formally compared child and baby range canopy flow rates by studying four VLBW continuously fed babies over four consecutive one hour periods in a randomised 4 × 4 latin square design (see Results).

ADDITIONAL IN VITRO CALIBRATION OF THE DELTATRAC
The manufacturer recommends that the canopy flow is calibrated by burning alcohol at rates approximating adult O₂ consumption and CO₂ production. It must then be assumed that canopy flow changes proportionately when the device is switched between baby, child, and adult ranges. We chose in addition to calibrate the instrument directly in child and baby settings by infusing medical grade CO₂ (confirmed as 100% CO₂ by BOC Analytical Division) at rates similar to those we encountered clinically.

A mannequin was placed under the canopy to simulate a baby, and CO₂ injected at constant rate using a Harvard rotating screw syringe driver with four parallel mounted gas tight 50 ml polypropylene syringes. Connections were made with gas tight three way taps and PVC lines (Datex). Syringes were flushed four times with CO₂ to remove air before use. Timed collections of water delivered in the range 4–12 ml/min showed that 2.777 ml was reproducibly displaced by each revolution of the syringe driver screw. The CO₂ injection rate was therefore established by counting rotations of the screw using a vane and slotted optical switch connected to a microcomputer. Gas temperature was measured using an ELAB type CTD thermocouple (ELAB, Copenhagen, Denmark) and volumes corrected to STPD.

Results
MEASUREMENT OF RaCO₂
Analysis of variability in background ¹³C enrichment in the 11 pairs of breath samples collected at baseline of each study showed that there was a small but statistically significant variation between studies (one way analysis of variance, \( F = 766; 10, 11 \) d.f; \( p < 0.001 \)). This is allowed for in the calculation of APE and \( \delta \).

Isotopic steady state was achieved in eight subjects by 120 minutes and in all 11 by 200 minutes. Figure 3 shows individual plots of breath enrichment (atom percent excess (APE)) vs time. Patient identification numbers correspond to those in tables 1 and 2. Weight at time of study is given.

\begin{figure}
\centering
\includegraphics{plot}
\caption{Individual plots of breath enrichment (atom percent excess (APE)) vs time. Patient identification numbers correspond to those in tables 1 and 2. Weight at time of study is given.}
\end{figure}
Table 2  \( RaCO_2 \) and \( VCO_2 \) measurements by patient with individual estimates of \( c \)

<table>
<thead>
<tr>
<th>Patient</th>
<th>( RaCO_2 ) (ml/min STPD)</th>
<th>( VCO_2 ) (ml/min STPD)</th>
<th>( c )</th>
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<tbody>
<tr>
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<td>7.5</td>
<td>8.1</td>
<td>1.08</td>
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<tr>
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</tr>
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</tr>
<tr>
<td>95% CI mean</td>
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</table>

**Figure 4** Prediction of true \( VCO_2 \) from Deltatrac measurement.

**Figure 5** Study of ventilated 658 g infant. Plot of breath enrichment (atom percent excess (APE)) against time. See text for estimates of \( RaCO_2 \).

**Discussion**

Arterial \( P_{CO_2} \) reflects a balance between the rate at which \( CO_2 \) is eliminated through the lungs and the rate at which it is produced by metabolism. Much attention in neonatal intensive care has been focused on controlling elimination by mechanical ventilation, but less has been paid to minimising \( CO_2 \) production by varying the amount and type of dietary fuel. Inherent difficulties in measuring the rate of \( CO_2 \) production in small ventilated babies may be one reason why nutritional interventions have been largely overlooked.

We and others have found it impossible to measure \( VCO_2 \) using the Deltatrac in such circumstances. She was continuously fed with human milk through a nasogastric tube and ventilated with a Babylino 8000 plus ventilator (Dräger, Hemel Hempstead, Herts, UK) at constant pressure, rate, and inspired time settings throughout the 24 hour study period. \( FIO_2 \) varied between 0.4 and 0.7, and \( P_{CO_2} \) between 5.0 and 6.5 kPa. A primed continuous intravenous infusion of NaH\(^13\)CO\(_3\) was administered at a constant rate for 24 hours using doses given above (see Methods). Breath samples were collected using a syringe connected to a side port (designed for surfactant administration) on the endotracheal tube connector.

**STUDY OF A VENTILATED BABY**

To assess the feasibility of measuring \( RaCO_2 \) in small ventilated babies, we studied a 20 day old 658 g infant with chronic lung disease (gestational age 25 weeks, birth weight 587 g). We and others have found it impossible to measure \( VCO_2 \) using the Deltatrac in such circumstances. She was continuously fed with human milk through a nasogastric tube and ventilated with a Babylino 8000 plus ventilator (Dräger, Hemel Hempstead, Herts, UK) at constant pressure, rate, and inspired time settings throughout the 24 hour study period. \( FIO_2 \) varied between 0.4 and 0.7, and \( P_{CO_2} \) between 5.0 and 6.5 kPa. A primed continuous intravenous infusion of NaH\(^13\)CO\(_3\) was administered at a constant rate for 24 hours using doses given above (see Methods). Breath samples were collected using a syringe connected to a side port (designed for surfactant administration) on the endotracheal tube connector.
This is substantially higher than values previously reported in short term studies. Consideration of the model would suggest that this reflects rapid equilibration of infused isotope between body pools, with minimal trapping in slow turnover pools such as bone. This seems physiologically plausible, as VLBW babies have a low bone and fat mass, high extracellular fluid volume, and high resting metabolic expenditure relative to older infants. The only other neonatal study we have identified estimated the mean value for c as 0.77 but recruited more mature babies (mean birth weight 2120 g, mean study weight 2100 g), only three of whom weighed <1500 g. Estimates in older children and adults have varied from 0.5 to 1.06, although a value of 0.8 is commonly assumed in substrate oxidation studies. In a comparative study, RaCO₂ was found to be much faster in children than in adults, which might further support our hypothesis.

The derivation of equation 4 requires an assumption that CO₂ is eliminated only through the lungs. This may be questionable, particularly in immature babies. In adults, about 1% of CO₂ is lost across the skin and less than 5% is excreted in the urine, although the exact amount is dependent on urine pH. Although we have found no estimates of skin CO₂ loss in VLBW infants, a study of infants weighing <1000 g (gestational age 23–29 weeks) confirmed that cumulative bicarbonate loss over the first four days of life was only 1.9 (0.5) mmol/kg (mean (SD)). This is equivalent to an equimolar non-respiratory loss of 7.4 μl CO₂/min/kg body weight. These errors can therefore be considered insignificant.

A further potential source of error in the measurement of RaCO₂ is fluctuating background ¹³C enrichment during the course of a study. Control studies have been undertaken in which subjects underwent the experimental protocol without bicarbonate administration. Most, however, have regarded this as an extremely small potential error and assumed in substrate oxidation studies. In a comparative study, RaCO₂ was found to be much faster in children than in adults, which might further support our hypothesis.

As we have shown, potential errors of different sorts apply to the measurement of both RaCO₂ and VCO₂. This is not surprising, as they measure different aspects of CO₂ metabolism. One (VCO₂) measures the elimination of CO₂; the other (RaCO₂) measures the turnover (or flux, Q) of bicarbonate within the body pool. In steady state, the latter is equivalent to both the rate of appearance (Ra) and rate of disappearance (Rd). Measurement of arterial blood gas status at the beginning and end of each study may have helped to confirm that the bicarbonate/CO₂ pool was in steady state during each study, but we did not feel blood sampling ethically justifiable. Participants showed stable cardiorespiratory measurements throughout and most were breathing air. Moreover the satisfactory enrichment plateaux (fig 3) observed in the 11 studies themselves constitute evidence that the bicarbonate pool was in steady state.

The 11 studies we describe were conducted only for two to four hours, and we have not implied measurements to be representative of longer periods. In deriving c, we have made comparisons only between quantities simultaneously measured in the same baby. We performed only one 24 hour study, principally to assess the feasibility of applying the technique in a ventilated baby (fig 5). Although we attempted to measure VCO₂ by connecting the Deltatrac monitor to the expiratory port of the ventilator (Dräger Babylog 8000 plus), we obtained no satisfactory measurements, probably because a blow off mechanism in the ventilator allows inspiratory and expiratory gases to mix under certain circumstances. The many problems associated with the measurement of VCO₂ during mechanical ventilation have been well described previously. In contrast, measurement of RaCO₂ was easily accomplished using the labelled bicarbonate technique and yielded comparable values at the three plateau periods studied.

In summary, we have shown that the labelled bicarbonate infusion technique is easily applicable to the measurement of CO₂ production in VLBW babies. In contrast with experience with older children and adults, the adjustment required for retention of infused isotope in short term studies is negligible in this group of patients. This confirms the value of determining the fractional recovery rate, c, for individual patient groups. The simplicity of this technique offers new opportunities to study the interaction between fuel metabolism and respiratory function even in the smallest ventilated babies.


