Importance of measuring CO₂-production rate when using ¹³C-breath tests to measure fat digestion

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Stable isotope breath tests offer a safe, repeatable non-invasive method of measuring fat digestion. They involve the ingestion of a substrate labelled with ¹³C followed by serial measurements of the ¹³C:¹²C ratio in expired CO₂, from which the percentage of the ¹³C dose recovered (PDR) can be calculated. However, PDR depends on the CO₂-production rate. Our aim was to compare results obtained using directly measured CO₂-production rates with those calculated from two predicted values. Twelve normal healthy children and twenty-four children with cystic fibrosis (CF) (without or with the normal dose of enzyme supplementation) were studied with 1,3-distearyl, 2-[carboxyl-¹³C] octanoyl glycerol. The volume of CO₂ produced (litres/min) was measured at rest for 30 min approximately 3 h after substrate ingestion, and the results were converted to mmol/min. For each subject the expected BMR was calculated from the equation of Schofield (1983, based on sex, age, weight and height), and from these values, CO₂-production rate was derived. Surface area was calculated and an estimated value of 5 mmol/m² per min (Shreeve et al. 1970) was used. Using these three CO₂-production rates, three different PDR were calculated and compared. In healthy children there was a close concordance between measured and predicted CO₂-production rates, but children with CF had a mean measured CO₂-production rate 39% higher than normal children. This use of normal data for predicted CO₂-production rates in children with CF underestimates cumulative PDR. If direct measurements of CO₂-production rate are not available or impossible to perform the VCO₂ obtained from the BMR calculated using the equations of Schofield (1985) or Shreeve et al. (1970) can be used in normal children. However, if accurate results for PDR are to be obtained, CO₂-production rates should be measured when performing breath tests in conditions where energy expenditure and/or CO₂-production rate are not expected to be normal.

¹³C-Breath tests: Stable isotopes: Cystic fibrosis

There is a growing interest in the use of stable isotopes in clinical practice, particularly for the measurement of digestion and absorption in health and disease (Amarri & Weaver, 1995). Carbon-13 (¹³C) breath tests offer a safe, repeatable method which involves the administration of a substrate labelled with ¹³C followed by serial measurements of the ¹³C:¹²C ratio in expired CO₂, from which the percentage ¹³C recovered (PDR) can be calculated.

Not all breath tests quantify the amount of tracer oxidized; for instance the results of the ¹³C-urea breath test for the diagnosis of Helicobacter pylori infection are usually expressed in units of ¹³C-enrichment of breath above baseline (Graham et al. 1987), and motility studies use time units (Ghoos et al. 1993). In paediatric medicine, measurement of ¹³C-PDR has been used to assess fat digestion (Murphy et al. 1990; McClean et al. 1993; Amarri et al. 1997) and starch digestion (Amarri et al. 1995) in children with cystic fibrosis (CF) and undernutrition (Weaver et al. 1995).

However, the results of tests in which results are expressed as PDR crucially depend upon an accurate measurement of CO₂-production rate. Many researchers...
use a standard value of 5 mmol/m² per min derived from extrapolations of measurements of adults (Shreeve et al. 1970). In investigations of children, no values for CO₂-production rate have been established as appropriate for this purpose.

The PDR is calculated using the formula:

\[
\frac{(13\delta - 13\delta_n)}{2} \times \frac{(13\delta_{t+1} - 13\delta)}{(t+1 - t) \times R_{PDB} \times \ldots}
\]

\[
\frac{mg \text{ substrate}}{mol. \text{ wt}} \times \frac{P \times n}{100} \times 10^{-3} \times CO₂ \times 100%,
\]

where \(13\delta = [(R_S/R_{PDB}) - 1] \times 10^3\) (\(R_S = 13\delta_{13}\text{C} \) in the sample, \(R_{PDB} = 13\delta_{13}\text{C} \) in PDB (international standard PeeDeeBelemnite) - 0.0112372); \(P\) is the atom % excess; \(n\) is the number of labelled carbon positions; \(13\delta_n, 13\delta_{t+1}, 13\delta\) are enrichments at times \(t, t+1\), and predose respectively; and CO₂ is the CO₂-production rate. A cumulative PDR can be obtained by summation of the single PDR value for each time interval and represents the area under the curve obtained during the duration of the test. Such a summary measure of substrate digestion is recommended as the most appropriate index for statistical analysis of differences between groups (Matthews et al. 1990).

CO₂-production rate is a multiplier in this equation and thus incorrect values will produce proportionally inaccurate estimates of PDR. For example, a 20% increase in CO₂-production rate (e.g. from 5 to 6 mmol/m² per min) will result in a 20% increase in cumulative PDR. This can be important when nutrient digestion in children with increased respiratory or metabolic rates (such as those with CF) is compared with that in healthy controls.

The aim of the present study was to compare the results obtained for \(^{13}\text{C}\)-breath tests, using directly measured CO₂-production rates, with those calculated from predicted BMR (Schofield, 1985) and predicted CO₂-production rates (Shreeve et al. 1970).

### Subjects and methods

#### Subjects

Twelve normal healthy children (aged 5–12 years; four males, eight females) and twenty-four children with CF (aged 1–12 years; twelve males and twelve females) were studied. While some children with CF were studied more than once with \(^{13}\text{C}\)-breath tests, CO₂-production rate was measured once in all children. The total number of tests performed was forty-six. Weight-for-age and height-for-age standard deviation scores, derived from Tanner-Whitehouse Standards (Tanner et al. 1966) using the Castlemead Growth Programme (1993), were respectively 0.17 (SD 1.12) and 0.53 (SD 0.9) for controls and -0.71 (SD 1.22) and -0.29 (SD 1.11) for children with CF.

#### Methods

The mixed triacylglycerol (1,3-diesteryl, 2[carboxyl-\(^{13}\text{C}\)] octanoyl glycerol, 99 atom % excess; MTG) breath test was performed as previously described (Amarri et al. 1997). CO₂-production rate was measured using a metabolic cart (Deltatrac; Datex Instrumentation Corp., Helsinki, Finland) and ventilated hood with a flow rate of 40 ml/min. The monitor was warmed to operating temperature for 30 min and calibrated using CO₂-O₂ (5:95, v/v). Measurements were made for 30 min (one measurement/min) at between 3 and 3.5 h after \(^{13}\text{C}\)-substrate ingestion.

Measurements were averaged to obtain a value for CO₂-production rate in ml/min, which was converted to mmol using the formula:

\[
V_{CO₂}(\text{mmol/min}) = \frac{V_{CO₂}(\text{ml/min}) \times \text{R} \times (273.15 + ^{\circ}\text{Celsius})}{12372}
\]

where gas constant \(R = 0.08206\) litres/atm per °Celsius per mol.

CO₂-production rate was also calculated from the equations for BMR of Schofield (1985), based on sex, age, weight and height, using the equations of Elia & Livesey (1988). The Haycock formula was used to calculate surface area (Haycock et al. 1978) and the value of 5 mmol/m² per min estimated by Shreeve et al. (1970) was used.

Using these three values of CO₂-production rates, three different measurements of PDR were calculated, using the formula above, and compared.

#### Statistical analysis and ethical approval

The data were stored and analysed using a microcomputer spreadsheet and statistical package (Minitab® Statistical Software, Minitab Inc., State College, PA, USA). Bias and limits of agreement of PDR using the measured and estimated CO₂-production rates were calculated using the methods of Bland & Altman (1986). The project was undertaken with the approval of the Yorkhill NHS Trust Ethics Committee, and performed with the informed consent of the parents of the children studied.

#### Results

Thirty-six measurements of CO₂-production rate and forty-six MTG breath tests were performed. Table 1 shows a comparison of measured CO₂-production rates, those derived from Schofield (1985) and those advocated by Shreeve et al. (1970). Data are presented as absolute rates and normalized for surface area. In healthy children (controls) there was close concordance between measured and predicted CO₂-production rates. However, children with CF had a mean measured CO₂-production rate that was 39% higher than that of control children (\(P < 0.0001\)). Thus, using these three measurements of CO₂-production rate to calculate PDR resulted in no significant differences between the mean results for normal children, but in children with CF, use of predicted CO₂-production rate underestimated the PDR by an average of 39%.

Table 2 shows the agreement between PDR derived from these data, using measured \(v\) Schofield (1985) data and measured \(v\) Shreeve et al. (1970) data.

There was a wide range in cumulative PDR and the absolute differences between PDR derived from measured
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Table 1. Comparison of measured CO₂-production rates with those calculated from Schofield (1985) and that proposed by Shreeve et al. (1970), and of cumulative ¹³C-percentage dose recovered obtained using these figures, shown as mean and sd

<table>
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<tbody>
<tr>
<td></td>
<td>n*</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>CO₂ production</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (mmol/m² per min)</td>
<td>12</td>
<td>4.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Controls (mmol/min)</td>
<td>12</td>
<td>4.4</td>
<td>0.6</td>
</tr>
<tr>
<td>CF (mmol/m² per min)</td>
<td>24</td>
<td>6.8</td>
<td>1.1</td>
</tr>
<tr>
<td>CF (mmol/min)</td>
<td></td>
<td>6.0</td>
<td>1.4</td>
</tr>
<tr>
<td>PDR (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>12</td>
<td>30.9</td>
<td>6.9</td>
</tr>
<tr>
<td>CF - PES</td>
<td>18</td>
<td>12.0</td>
<td>9.5</td>
</tr>
<tr>
<td>CF + PES</td>
<td>16</td>
<td>18.4</td>
<td>7.3</td>
</tr>
</tbody>
</table>

CF, cystic fibrosis; PDR, percentage dose recovered; PES, pancreatic enzyme supplements.

*Number of subjects for CO₂-production rates, and number of tests performed for PDR.

Table 2. Agreement between cumulative percentage dose recovered obtained using measured and estimated CO₂-production rates derived from Schofield (1985) and Shreeve et al. (1970)

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<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>CF - PES CF + PES</td>
</tr>
<tr>
<td>No. of PDR tests performed</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>Bias</td>
<td>-1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Limits of agreement</td>
<td>-7.3, 5.1</td>
<td>-2.7, 5.7</td>
</tr>
</tbody>
</table>

CF, cystic fibrosis; PDR, percentage dose recovered; PES, pancreatic enzyme supplements.

and estimated CO₂-production rate increased with increasing cumulative PDR. In children with CF with PDR > 5% there was a positive difference between measured and predicted values, and in children with high PDR there was a wider range in variation, in both positive and negative directions. The bias and limits of agreement are shown in Table 2.

**Discussion**

¹³C-breath tests are based on the principle that the rate-limiting step between ingestion of labelled substrate and its recovery in the breath is digestion (Amarri & Weaver, 1995). The MTG has been recommended as a substrate for the indirect measurement of fat digestion (Vantrappen et al. 1989) because the two stearic acid molecules at the sn-1 and sn-3 positions are cleaved by pancreatic lipase to release [¹³C]-octanoyl glycerol, which is rapidly and completely absorbed and then oxidized (Metges & Wolfman, 1991) and the ¹³C is expired in breath as CO₂.

Although there are other factors that regulate the handling of the substrate in the body between ingestion and expiration (Weaver et al. 1993; Kalivianakis et al. 1997), variations in these between patients and controls are relatively small in comparison with major differences in digestive capacity, as for instance in patients with CF (Amarri et al. 1997).

¹³C derived from the products of digestion, after absorption and oxidation, enters endogenous substrate pools or the HCO₃⁻ pool within the body. Although CO₂ in the breath is in equilibrium with the HCO₃⁻ pool, the latter is probably not a single pool, and the kinetics of ¹³C distribution are therefore complex. Pallikarakis et al. (1991) have shown that quantitative changes in the HCO₃⁻ pool may influence the rate of appearance of ¹³C-labelled CO₂ in the breath.

It is important to note that in a large number of studies, reviewed by Elia (1990), recovery of CO₂ after administration of labelled HCO₃⁻ varied from 50 to 100%. The reasons for incomplete recovery have been ascribed to CO₂ fixation, equilibration, entry of label into slowly turning over pools of CO₂ (Elia, 1990), and also to possible methodological problems in some of the studies (Leijssen & Elia 1996). The scatter of observed cumulative PDR (Amarri et al. 1997) must be in part related to HCO₃⁻ pool size, but despite these uncertainties the MTG breath test can distinguish normal from pathological findings (Amarri et al. 1997).

In the calculation of PDR a measure of CO₂-production rate is an essential component of the equation (see p. 542), and when studying children with CF and other causes of increased metabolic rate and/or hyperventilation it is obviously vital to measure CO₂-production rate rather than depend on a predicted value (Shreeve et al. 1970) or one derived from BMR (Schofield, 1985). Our findings of an increased production rate, both absolute and relative to body surface area, in children with CF agree with other published data (Buchdahl et al. 1988). However, measuring CO₂-production rate may present difficulties in children, and it is therefore important to assess the need for this measurement, which could be performed simultaneously with continuous measurements of ¹³CO₂-enrichment, as a
single procedure. In this study we have shown that some children could be mis-categorized as having normal or abnormal fat digestion, by using calculated rather than measured CO₂-production rates.

Using a cut-off of 20 % PDR to define the lower value of the normal range (Amarri et al. 1997) up to 10 % of children with CF studied when not taking enzyme supplements are 'incorrectly' designated as having abnormally low MTG digestion, when the results of their breath tests are within the normal range after calculation of results using measured CO₂ rates. However, using the same cut-off in analysis of the results obtained from children with CF who were receiving enzymes results in up to 55 % being 'incorrectly' designated as having results below the normal range (Fig. 1).

We have shown that it is desirable to measure CO₂-production rate directly in children in whom metabolic rate is increased in order to obtain accurate measurements of ¹³C-recovery in the breath, and thereby to calculate the amount of ¹³C-labelled substrate that has been digested in the small intestine. If direct measurements of CO₂-production rate are not available or possible, then VCO₂ obtained from the BMR calculated using the equations of Schofield (1985) or Shreeve et al. (1970) can be used in normal children. However, they are not appropriate when calculating the results of ¹³C-breath tests in conditions where altered energy expenditure or CO₂-production rates are expected. In this case an alternative would be to use the mean value for CO₂-production rates obtained from the children studied here (6·8 mmol/m² per min) to calculate PDR.

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References

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