Technical Note

Importance of measuring CO_2-production rate when using ^{13}C-breath tests to measure fat digestion

S. Amarri[1]*, W. A. Coward[2], M. Harding[2] and L. T. Weaver[3]

[1]*Department of Paediatrics, University of Modena, Italy*

[2]*MRC Dunn Nutrition Unit, Cambridge, UK*

[3]*Department of Child Health, University of Glasgow, Yorkhill Hospitals, Glasgow, UK*

(Received 18 June 1997 – Revised 20 January 1998 – Accepted 26 January 1998)

Stable isotope breath tests offer a safe, repeatable non-invasive method of measuring fat digestion. They involve the ingestion of a substrate labelled with ^{13}C followed by serial measurements of the $^{13}C:^{12}C$ ratio in expired CO_2, from which the percentage of the ^{13}C dose recovered (PDR) can be calculated. However PDR depends on the CO_2-production rate. Our aim was to compare results obtained using directly measured CO_2-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-^{13}C] octanoyl glycerol. The volume of CO_2 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 (1985), based on sex, age, weight and height, and from these values, CO_2-production rate was derived. Surface area was calculated and an estimated value of 5 mmol/m^2 per min (Shreeve *et al.* 1970) was used. Using these three CO_2-production rates, three different PDR were calculated and compared. In healthy children there was a close concordance between measured and predicted CO_2-production rates, but children with CF had a mean measured CO_2-production rate 39% higher than normal children. This use of normal data for predicted CO_2-production rates in children with CF underestimates cumulative PDR. If direct measurements of CO_2-production rate are not available or impossible to perform the $\dot{V}_{CO_2}$ 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, CO_2-production rates should be measured when performing breath tests in conditions where energy expenditure and/or CO_2-production rate are not expected to be normal.

^{13}C-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 (^{13}C) breath tests offer a safe, repeatable method which involves the administration of a substrate labelled with ^{13}C followed by serial measurements of the $^{13}C:^{12}C$ ratio in expired CO_2, from which the percentage ^{13}C recovered (PDR) can be calculated.

Not all breath tests quantify the amount of tracer oxidized; for instance the results of the ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection are usually expressed in units of ^{13}C-enrichment of breath above baseline (Graham *et al.* 1987), and motility studies use time units (Ghoos *et al.* 1993). In paediatric medicine, measurement of ^{13}C-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 CO_2-production rate. Many researchers

Abbreviations: CF, cystic fibrosis; MTG, mixed triacylglycerols; PDR, percentage dose recovered.

***Corresponding author:** Dr S. Amarri, fax +39 59 424583, email amarri@unimo.it

use a standard value of 5 mmol/m^2 per min derived from extrapolations of measurements of adults (Shreeve *et al.* 1970). In investigations of children, no values for CO_2-production rate have been established as appropriate for this purpose.

The PDR is calculated using the formula:

$$\frac{\frac{(^{13}\delta_t - ^{13}\delta_o) + (^{13}\delta_{t+1} - ^{13}\delta_o)}{2} \times (t_{+1} - t) \times R_{PDB} \times \dots}{\frac{\text{mg substrate}}{\text{mol. wt}} \times \frac{P \times n}{100}}$$

$$\dots \frac{10^{-3} \times CO_2}{} \times 100\%,$$

where $^{13}\delta = [(R_S/R_{PDB}) - 1] \times 10^3$ ($R_S = {}^{13}C : {}^{12}C$ in the sample, $R_{PDB} = {}^{13}C : {}^{12}C$ in PDB (international standard PeeDeeBelemnite) − 0·0112372); P is the atom % excess; n is the number of labelled carbon positions; δ_t, δ_{t+1}, δ_o are enrichments at times t, t_{+1} and predose respectively; and CO_2 is the CO_2-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).

CO_2-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 CO_2-production rate (e.g. from 5 to 6 mmol/m^2 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}C-breath tests, using directly measured CO_2-production rates, with those calculated from predicted BMR (Schofield, 1985) and predicted CO_2-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}C-breath tests, CO_2-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-distearyl, 2[carboxyl-^{13}C] octanoyl glycerol, 99 atom % excess; MTG) breath test was performed as previously described (Amarri *et al.* 1997). CO_2-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 CO_2–O_2 (5 : 95, v/v). Measurements were made for 30 min (one measurement/min) at between 3 and 3·5 h after ^{13}C-substrate ingestion.

Measurements were averaged to obtain a value for CO_2-production rate in ml/min, which was converted to mmol using the formula:

$$\dot{V}_{CO_2}(\text{mmol/min}) = \frac{\dot{V}_{CO_2}(\text{ml/min})}{R \times (273{\cdot}15 + {}^{\circ}\text{Celsius})},$$

where gas constant R = 0·08206 litres/atm per °Celsius per mol.

CO_2-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/m^2 per min estimated by Shreeve *et al.* (1970) was used.

Using these three values of CO_2-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 CO_2-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 CO_2-production rate and forty-six MTG breath tests were performed. Table 1 shows a comparison of measured CO_2-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 CO_2-production rates. However, children with CF had a mean measured CO_2-production rate that was 39 % higher than that of control children ($P < 0{\cdot}0001$). Thus, using these three measurements of CO_2-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 CO_2-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

Table 1. Comparison of measured CO_2-production rates with those calculated from Schofield (1985) and that proposed by Shreeve *et al.* (1970), and of cumulative ^{13}C-percentage dose recovered obtained using these figures, shown as mean and SD

		Measured		Schofield (1985)		Shreeve *et al.* (1970)	
	*n**	Mean	SD	Mean	SD	Mean	SD
CO_2 production							
Controls (mmol/m^2 per min)	12	4·9	0·6	5·1	0·6	5·0	
Controls (mmol/min)		5·4	0·6	5·3	1·1	5·3	1·0
CF (mmol/m^2 per min)	24	6·8	1·1	5·6	0·5	5·0	
CF (mmol/min)		6·0	1·4	5·0	1·3	4·5	1·3
PDR (%)							
Controls	12	30·9	6·9	32·0	6·3	31·6	6·0
CF − PES	18	12·0	9·5	10·5	8·2	9·2	7·3
CF + PES	16	18·4	7·3	14·5	5·8	13·4	5·5

CF, cystic fibrosis; PDR, percentage dose recovered; PES, pancreatic enzyme supplements.
*Number of subjects for CO_2-production rates, and number of tests performed for PDR.

Table 2. Agreement between cumulative percentage dose recovered obtained using measured and estimated CO_2-production rates derived from Schofield (1985) and Shreeve *et al.* (1970)

	Schofield (1985)			Shreeve *et al.* (1970)		
	Controls	CF − PES	CF + PES	Controls	CF − PES	CF + PES
No. of PDR tests performed	12	18	16	12	18	16
Bias	− 1·1	1·5	3·9	− 0·7	2·8	5·0
Limits of agreement	− 7·3, 5·1	− 2·7, 5·7	− 0·5, 8·2	− 9·1, 7·7	− 3·9, 9·4	− 0·3, 10·3

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

and estimated CO_2-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

^{13}C-breath tests are based on the principle that the rate-limiting step between ingestion of labelled substrate and 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 [^{13}C]-octanoyl glycerol, which is rapidly and completely absorbed and then oxidized (Metges & Wolfram, 1991) and the ^{13}C is expired in breath as CO_2.

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).

^{13}C derived from the products of digestion, after absorption and oxidation, enters endogenous substrate pools or the HCO_3^- pool within the body. Although CO_2 in the breath is in equilibrium with the HCO_3^- pool, the latter is probably not a single pool, and the kinetics of ^{13}C distribution are therefore complex. Pallikarakis *et al.* (1991) have shown that quantitative changes in the HCO_3^- pool may influence the rate of appearance of ^{13}C-labelled CO_2 in the breath.

It is important to note that in a large number of studies, reviewed by Elia (1990), recovery of CO_2 after administration of labelled HCO_3^- varied from 50 to 100 %. The reasons for incomplete recovery have been ascribed to CO_2 fixation, equilibration, entry of label into slowly turning over pools of CO_2 (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 HCO_3^- 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 CO_2-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 CO_2-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 CO_2-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 $^{13}CO_2$-enrichment, as a

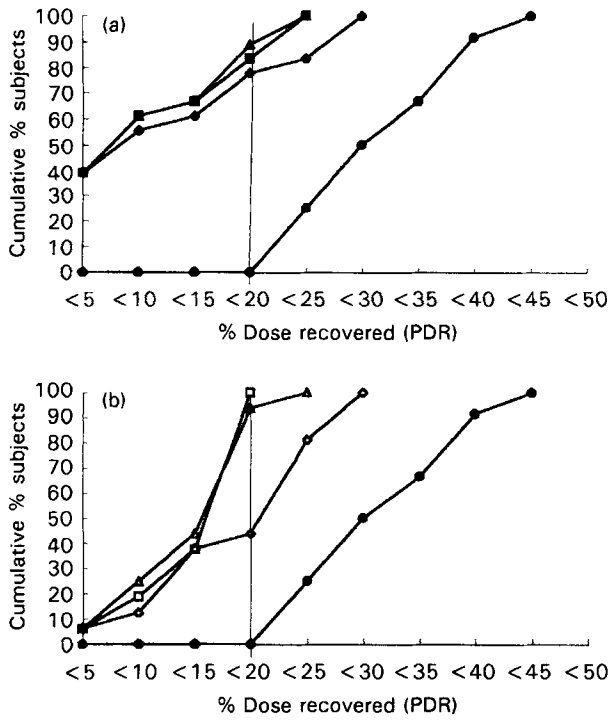

Fig. 1. Cumulative percentage of subjects for each percentage dose recovered (PDR) category. Vertical line represents lower limit of PDR in normal children. (a) CF children without pancreatic enzymes (*n* 18) and normal children (*n* 12). Controls (●); CF children with measured CO_2-production rate (◆); CF children with production rate estimated from predicted BMR (Schofield, 1985; (■)); CF children with predicted CO_2-production rate (Shreeve *et al.* 1970; (▲)). (b) CF children with pancreatic enzyme supplements (*n* 16) and normal children (*n* 12). Controls (●); CF children with measured CO_2-production rate, (◇); CF children with CO_2-production rate estimated from BMR (Schofield 1985; (□)), CF children with predicted CO_2-production rate (Shreeve *et al.* 1970; (△)).

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 CO_2-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 CO_2 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 CO_2-production rate directly in children in whom metabolic rate is increased in order to obtain accurate measurements of ^{13}C-recovery in the breath, and thereby to calculate the amount of ^{13}C-labelled substrate that has been digested in the small intestine. If direct measurements of CO_2-production rate are not available or possible, then $\dot{V}_{CO_2}$ 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 ^{13}C-breath tests in conditions where altered energy expenditure or CO_2-production rates are expected. In this case an alternative would be to use the mean value for CO_2-production rates obtained from the children studied here (6·8 mmol/m^2 per min) to calculate PDR.

Acknowledgements

We thank Dr John Evans and Dr Jim Paton for letting us study their patients and Miss Elaine Buchanan, Mr Angus Arthur, Mr Ian Brown, Mr David Carey, Dr John Reilly, Mr William Manson and Dr Tom Preston for their help and advice. We also thank the children and their parents. Dr Amarri is grateful to the European Commission for his Human Capital and Mobility Fellowship. The project was undertaken with support from the European Commission Biomed Programme.

References

Amarri S & Weaver LT (1995) ^{13}C-breath tests to measure fat and carbohydrate digestion in clinical practice. *Clinical Nutrition* **14**, 149–154.

Amarri S, Harding M, Coward WA, Evans TJ, Paton JY & Weaver LT (1995) ^{13}C and H_2 breath tests to study extent and site of starch digestion in cystic fibrosis. *Journal of Pediatric Gastroenterology and Nutrition* **20**, 448.

Amarri S, Harding M, Coward AW, Evans TJ & Weaver LT (1997) ^{13}C mixed triglyceride breath test and pancreatic enzyme supplementation in children with cystic fibrosis. *Archives of Disease in Childhood* **76**, 349–351.

Bland JM & Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **i**, 307–310.

Buchdahl RM, Cox M, Fulleylove C, Marchant JL, Tomkins AM, Brueton MJ & Warner JO (1988) Increased energy expenditure in cystic fibrosis. *Journal of Applied Physiology* **64**, 1810–1816.

Castlemead Growth Program (1993) Welwyn Garden City, UK: Castlemead Publications.

Elia M (1990) The estimation of short-term energy expenditure by the labelled bicarbonate method. In *New Techniques in Nutrition Research*, pp. 207–232 [RG Whitehead and AM Prentice, editors]. London: Academic Press.

Elia M & Livesey G (1988) Theory and validity of indirect calorimetry during net lipid synthesis. *American Journal of Clinical Nutrition* **47**, 591–607.

Ghoos YF, Maes BD, Geypens BJ, Mys G, Hiele MI, Rutgeerts PJ & Vantrappen G (1993) Measurement of gastric emptying rate of solids by means of a carbon-labelled octanoic acid breath test. *Gastroenterology* **104**, 1640–1647.

Graham DY, Klein PD, Evans DJ, Evans DG, Alpert LC, Opekun AR & Boutton TW (1987) *Campylobacter pyloridis* detected noninvasively by the ^{13}C-urea breath test. *Lancet* **i**, 1174–1177.

Haycock GB, Schwartz GJ & Wisotsky DH (1978) Geometric method for measuring body surface area: a height : weight formula validated in infants, children and adults. *Journal of Pediatrics* **93**, 62–66.

Kalivianakis M, Verkade HJ, Stellard F, Van der Werf M, Elzinga H & Vonk RJ (1997) The ^{13}C-mixed triglyceride breath test in healthy adults: determinants of the CO_2 response. *European Journal of Clinical Investigation* **27**, 434–442.

Leijssen DPC & Elia M (1996) Recovery of ^{13}C and $^{14}CO_2$ in human bicarbonate studies: a critical review with original data. *Clinical Science* **91**, 665–677.

Matthews JNS, Altman DG, Campbell MJ & Royston P (1990) Analysis of serial measurements in medical research. *British Medical Journal* **300**, 230–235.

McClean P, Harding M, Coward WA, Green MR & Weaver LT (1993) Measurement of fat digestion in early life using a stable isotope breath test. *Archives of Disease in Childhood* **69**, 366–370.

Metges CC & Wolfram G (1991) Medium and long chain triglycerides labelled with ^{13}C: a comparison of oxidation after oral and parenteral administration in humans. *Journal of Nutrition* **121**, 31–36.

Murphy MS, Eastham EJ, Nelson R & Aynsley-Green A (1990) Non-invasive assessment of intraluminal lipolysis using a $^{13}CO_2$ breath test. *Archives of Disease in Childhood* **65**, 574–578.

Pallikarakis N, Sphiris N & Lefebvre P (1991) Influence of the bicarbonate pool and on the occurrence of $^{13}CO_2$ in exhaled air. *European Journal of Applied Physiology* **63**, 179–183.

Schofield WN (1985) Predicting basal metabolic rate, new standards and review of previous work. *Human Nutrition: Clinical Nutrition* **39C**, 5–41.

Shreeve VW, Cerasi E & Luft R (1970) Metabolism of (2-^{14}C) pyruvate in normal, acromegalic and HGH-treated human subjects. *Acta Endocrinologica* **65**, 155–169.

Tanner JM, Whitehouse RH & Takaishi M (1966) Standards from birth to maturity for height, weight, height velocity, and weight velocity: British children, 1965. *Archives of Disease in Childhood* **41**, 454–471.

Vantrappen GR, Rutgeerts P, Ghoos YF & Hiele MI (1989) Mixed triglyceride breath test: a noninvasive test of pancreatic lipase activity in the duodenum. *Gastroenterology* **96**, 1126–1134.

Weaver LT, Thomas JE, McClean P, Harding M & Coward WA (1993) Stable isotope breath tests: their use in paediatric practice. In *Progress in Understanding and Management of Gastro-intestinal Motility Disorders*, pp. 155–168 [J Janssens, editor]. University of Leuven: Department of Medicine.

Weaver LT, Dibba B, Sonko B, Bohane TD & Hoare S (1995) Measurements of starch digestion of naturally ^{13}C-enriched weaning foods, before and after partial digestion with amylase-rich flour, using a ^{13}C breath test. *British Journal of Nutrition* **74**, 531–537.