Measurement of fat digestion in early life using a stable isotope breath test

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Abstract

¹³C breath tests are a safe, non-invasive way of assessing nutrient digestion and absorption that can be used repeatedly in infancy and childhood. The aim of this study was to assess their value for measuring fat digestion in infants and young children with cystic fibrosis, and healthy controls whose pancreatic exocrine function is immature, and to monitor pancreatic enzyme supplementation.

Six infants with cystic fibrosis (aged 10–18 months) and nine healthy controls (aged 6–19 months) were studied. After an overnight fast each child ingested 7.5 mg/kg ¹³C trioctanoin (99 atom % excess) followed by a known volume of milk. Breath samples were collected before and at 30 minute intervals thereafter for five hours. The ¹³C enrichment of expired carbon dioxide was measured by gas isotope ratio mass spectrometry.

The mean (SD) percentage dose recovery of ¹³C was 13.5 (5.3) for the cystic fibrosis group and 24.2 (6.7) for the healthy controls. When those with cystic fibrosis were studied after supplementary pancreatic enzymes, the mean percentage dose recovery rose to 17.1 (6.9). Total intraluminal lipolysis was diminished by 44% in young children with cystic fibrosis. Pancreatic enzyme supplements improved digestion by 27%. The ¹³C trioctanoin breath test was effective in detecting fat maldigestion and can be used to measure the benefits of enzyme supplements in early life.

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on clinical grounds alone, because quantitative tests of fat digestion are invasive or unpleasant to perform, and it is not justified to submit children to them repeatedly.

¹⁴C (radioactively labelled) breath tests have been used extensively in adults to detect fat malabsorption and to monitor the efficacy of enzyme supplementation and H₂ antagonists on fat digestion.^{2,3} Watkins *et al* modified them for use in children by replacing the radioactive isotope (¹⁴C) with the stable isotope of carbon (¹³C).⁴ After ingestion of the substrate the ¹³C enrichment of expired carbon dioxide can be measured by gas isotope ratio mass spectrometry. To reduce the effects of gastric emptying on the rate of ¹³C recovery Watkins *et al* administered the substrate to infants and children via a duodenal feeding tube.⁴ This obviously increased the invasiveness of the test. However, in a recent study of 4–8 year old children with cystic fibrosis in which ¹³C labelled substrate was administered orally, depression of cumulative ¹³C labelled carbon dioxide production (¹³CO₂) due to delay in gastric emptying did not reduce the discriminatory value of the test.⁵

The physiology of fat digestion changes throughout the first two years of life: pancreatic lipase activity is diminished compared with older children, and preduodenal lipases assume greater importance.⁶ The aim of this study was to evaluate a modification of the ¹³C fat breath test used by Murphy *et al* to discriminate between infants with pancreatic insufficiency and healthy controls,⁵ and to measure the benefits of enzyme supplementation in this age group, when the substrate was administered orally.

Subjects and methods

SUBJECTS

Six children with cystic fibrosis (aged 10–18 months) and nine healthy controls (aged 6–19 months) were studied. The children with cystic fibrosis were diagnosed in the neonatal period by blood immunoreactive trypsin screening followed by a confirmatory sweat test. At the time of the study they were all free of respiratory symptoms but had begun enzyme supplements for failure to thrive and/or steatorrhoea. Pancreatic insufficiency was confirmed by a modified version of the pancreolauryl test.⁷

METHODS

After an overnight fast each child ingested the substrate for the modified pancreolauryl test (fluorescein dilaurate/mannitol emulsion),

The importance of maintaining optimal nutrition in children with chronic disease has been well recognised in recent years. Owing to diminished digestion, increased metabolic requirements and faecal losses the recommended energy requirements of children with cystic fibrosis are 20–50% above those of their healthy peers. There are often difficulties in ensuring that children with cystic fibrosis ingest and absorb enough energy to meet this target. Fat, the most energy dense nutrient in the diet, requires lipase for its digestion. Children with cystic fibrosis, because they have pancreatic exocrine deficiency, need extra lipase in the form of enzyme supplements. However, even with such enzyme supplementation, fat digestion may be incomplete.¹ Patients are often started on enzyme supplements empirically, and the dose is manipulated

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Patient data and dose of lipase in supplement administered to children with cystic fibrosis

	Controls (n=9)	Cystic fibrosis (n=6)
Sex (M:F)	5:4	3:3
Age range (months)	6–19	10–18
Mean (SD) weight (kg)	9.9 (1.9)	10.9 (1.9)
Median (range) dose of lipase in supplement (BP units) administered with test	–	12 000 (4000–16 000)

followed by 7.5 mg/kg of ^{13}C labelled trioctanoin (99 atom % excess) (MSD Isotopes). This was diluted in a palatable long chain triglyceride emulsion (Calogen, Scientific Hospital Supplies; 1 gm trioctanoin was made up to 20 gm of suspension with Calogen) and administered directly into the mouth with a syringe. Losses (collected in a weighed tissue) were taken into account when the dose ingested was calculated. The child then drank a known quantity of formula milk.

Breath samples were obtained before, and at 30 minute intervals after, administration of the substrate, for five hours using a facemask connected via two one way valves (Ambu Paedi valve) to a 600 ml reusable oxygen reservoir bag (Laerdal) which had been fitted with a three way tap. Samples of 20 ml of expired air were aspirated from the bag and stored in 20 ml Vacutainers (Becton Dickenson) before measurement of the $^{13}\text{C}/^{12}\text{C}$ ratio by gas isotope ratio mass spectrometry (SIRA 10 VG Isogas, Middlewich, Cheshire). The child was allowed to feed 150 minutes after ingestion of the substrate, taking foods only of low ^{13}C abundance. A spot urine sample was collected between two and four hours to measure the fluorescein/mannitol ratio and to confirm the presence of pancreatic insufficiency in the children with cystic fibrosis. The test was performed on two occasions in each child with cystic fibrosis, with and without enzyme supplementation. The dose of enzymes taken was the amount usually ingested with a snack meal (table).

MEASUREMENT OF ^{13}C ENRICHMENT

The ^{13}C enrichment of each sample was quoted as the relative difference (delta per mil ‰) between the sample and the international limestone standard (Pee Dee Belemnite, PDB)⁸ where:

$$\delta^{13} = [(R_s/R_{\text{PDB}}) - 1] \times 10^3$$

$$R_s = ^{13}\text{C}/^{12}\text{C} \text{ in the sample}$$

$$R_{\text{PDB}} = ^{13}\text{C}/^{12}\text{C} \text{ in PDB} = 0.0112372$$

In the present experiments precision of duplicate analyses of breath samples was 0.09 ‰ SD.

The background variability of ^{13}C in breath was examined in three children (one healthy child and two children with cystic fibrosis who were studied with enzyme supplements) by giving all of the 'test feed' except ^{13}C trioctanoin. The variability (SD) over five hours was less than 0.7‰ in each case.

Fat digestion was expressed as the cumulative percentage dose recovered of ^{13}C from trioctanoin over five hours.⁹ The percentage

dose recovered was calculated for each 30 minute interval as: percentage dose recovered for the interval t to t_{+1} (min) =

$$\frac{(\delta_t^{13} - \delta_0^{13}) + (\delta_{t+1}^{13} - \delta_0^{13})}{2} \times (t_{+1} - t) \times R_{\text{PDB}} \times 10^{-3} \times m^2 \times 5$$

$$\frac{\text{mg substrate}}{\text{MW}} \times \frac{P \times n}{100} \times 100\%$$

where

$$\frac{(\delta_t - \delta_0) + (\delta_{t+1} - \delta_0)}{2} \times (t_{+1} - t) \times R_{\text{PDB}} \times 10^{-3} = \text{mean mmol excess } ^{13}\text{C}/\text{mmol CO}_2 \text{ for each time interval}$$

$$\text{mmol CO}_2 \text{ excreted} = 5 \text{ mmol/m}^2/\text{min}^{10}$$

$$\text{mmol } ^{13}\text{C} \text{ administered} = \frac{\text{mg substrate}}{\text{MW}} \times \frac{P \times n}{100}$$

P = atom % excess, MW = molecular weight, n = number of labelled carbon positions, and δ_t , δ_{t+1} , δ_0 are enrichments at times t , t_{+1} , and predose respectively.

To examine the reproducibility of the percentage dose recovered over five hours as a measure of fat digestion, the test was performed on three children (two healthy children and one child with cystic fibrosis studied with enzyme supplements) on two separate days. The coefficient of variation was 2.7%.

STATISTICS

The significance of the differences in the percentage dose recovered over five hours between the untreated children with cystic fibrosis and the healthy controls was tested using the non-parametric Mann-Whitney U test. The effect of enzyme supplementation on fat digestion was tested using a paired t test.

Ethical approval for this study was obtained from the district ethics committee of Cambridge Health Authority.

Results

The ^{13}C enrichment of breath (in delta above baseline) and the cumulative percentage dose recovered from healthy control children and children with cystic fibrosis without enzyme supplements are shown in fig 1. Most control children demonstrated an early rise in ^{13}C excretion to a well defined peak at 50–150 minutes, but by the end of the test ^{13}C enrichment was close to zero compared with baseline values (fig 1A). One child (g) had a late peak (300 min) which resulted in a low percentage dose recovered over five hours (fig 1C). The flattest curve (c) in fig 1A was from the 6 month old infant. In contrast most children with cystic fibrosis had low enrichments of ^{13}C in the breath, which gradually rose over the five hours (fig 1B). The mean (SD) time to, and height of the peak values for healthy children was 143.3 (79.1) min and 30.9 (11.5) delta respectively, and 240 (86.9) min and 17.3 (6.5) delta respectively for the children with cystic fibrosis. Both of these differences were significant at $p < 0.05$.

Figure 1 (A) and (B) show the ^{13}C enrichment of breath carbon dioxide from healthy controls and children with cystic fibrosis after ingestion of ^{13}C trioctanoin. (C) and (D) represent the cumulative percentage dose recovered of ^{13}C from the same children.

Figure 2 The percentage dose recovered over five hours of ^{13}C from ^{13}C trioctanoin in healthy controls (a-i) and in children with cystic fibrosis (u-z). Mean (SD) values were 24.2 (6.7) for controls and 13.5 (5.3) for children with cystic fibrosis ($p < 0.01$).

Figure 2 shows the percentage dose recovery of ^{13}C over five hours in controls and children with cystic fibrosis. The mean (SD) values for the controls and children with cystic fibrosis were 24.2 (6.7) and 13.5 (5.3) respectively ($p < 0.01$). Even when the child with cystic fibrosis with very low recovery (z) was

excluded the differences remain significant ($p < 0.05$).

When the test was repeated on the children with cystic fibrosis with the addition of enzyme supplements, the mean time to the peak value shortened slightly from 240.0 (86.9) min to 195.0 (72.7) min (not significant), but there was no difference in the peak heights (17.3 (6.5) delta without and 17.5 (8.9) with enzymes). However, the mean percentage dose recovery increased from 13.5 (5.3) to 17.1 (6.9) – that is by 27% above untreated values ($p < 0.05$) (fig 3). The child whose percentage dose recovery did not increase with the addition of enzyme supplements (x) had the highest percentage dose recovery (without enzymes) in the cystic fibrosis group: just inside the range of the healthy control group.

Discussion

We have shown, using a ^{13}C fat breath test, that intraluminal lipolysis is diminished by 44% in young children with cystic fibrosis. Pancreatic enzyme supplements improve digestion by 27%. This study provides further

Figure 3 The percentage dose recovered over five hours in each child with cystic fibrosis when the test was performed without and with the ingestion of enzyme supplements. Mean (SD) values without enzymes were 13.5 (5.3) and with enzymes 17.1 (6.9) ($p < 0.05$).

evidence that stable isotope breath tests can be used to demonstrate fat maldigestion even in young children. The differences in cumulative ^{13}C production between the control children and those with cystic fibrosis were not as wide as those noted by Murphy *et al* in a comparable study of older children.⁵ In that study, the mean sum of ^{13}C production over four hours in patients and controls was 36.8‰ and 176.9‰ respectively, whereas in our study the values were 60.5‰ and 122.3‰ respectively. The lower cumulative $^{13}\text{CO}_2$ production in healthy infants compared with older children is probably related to small body size (which would become obvious if it were possible to compare percentage doses recovered in both studies) but may also reflect immaturity of pancreatic lipolytic activity.^{6,11} The higher production of $^{13}\text{CO}_2$ in infants compared with older children with cystic fibrosis may be due to some residual pancreatic function¹² and/or to a greater contribution of preduodenal lipase to fat digestion in this age group.^{13,14}

The rate of $^{13}\text{CO}_2$ production from children with cystic fibrosis was slow and still rising at the end of the test in three subjects (fig 1B). This has been noted in previous studies^{5,9} and may reflect ongoing fat digestion by preduodenal lipase further down the small intestine. This enzyme remains active in the more acidic environment of the gut lumen of the child with cystic fibrosis.

The failure to distinguish unequivocally between normal children and those with depressed pancreatic function should not be seen as a deficiency of the test, but rather a reflection of the interplay of physiological maturation and pathological damage of the pancreas on the results. Pancreatic lipase activity is low at birth in all children⁶ and, in those we have studied, declining pancreatic function from cystic fibrosis occurs in the face of normal maturation.

The enhanced recovery of ^{13}C in children with cystic fibrosis who were taking enzyme supplements was less than expected when compared with fat balance studies^{1,4} and with earlier studies using the ^{13}C labelled fat breath tests in adults,¹⁵ older children,⁵ and in infants when the substrate was given directly into the

duodenum.⁴ Again total intraluminal lipolysis (that is the effect of pancreatic and preduodenal lipases) was depressed less in young infants with cystic fibrosis than in older children, and the addition of pancreatic enzymes (which cannot realise their optimum activity at the low pH in the gut lumen of the child with cystic fibrosis) may not cause a large improvement in digestion. This is borne out by the lack of increase in ^{13}C recovery with the addition of enzyme supplements to the child whose percentage dose recovered was already just within the range of the healthy control group. However, further studies on the effect of increasing doses of enzyme supplements on ^{13}C recovery should be done.

We have ascribed the differences in ^{13}C recovery between normal children and those with cystic fibrosis to differences in intraluminal fat digestion. We have assumed that absorption, oxidation, and carbon dioxide production rates were similar in the two groups. Histological and functional abnormalities of the intestinal mucosa in cystic fibrosis have been well documented.¹⁶ Depletion of the bile salt pool through increased faecal losses and increased production of glycine rather than taurine conjugates contribute to fat malabsorption.¹⁷ However, as a medium chain fatty acid, the absorption of octanoic acid is dependent neither on the concentration of bile salts in the duodenum nor on mucosal integrity,¹⁸ and once absorbed it is completely oxidised.¹⁹

Because it was impractical to measure carbon dioxide production rates by indirect calorimetry in these children, we assumed a constant average value for carbon dioxide production of 5 mmol/m²/min,¹⁰ as is common practice in stable isotope studies. Carbon dioxide production is increased by significant pulmonary disease, exercise, and high dietary carbohydrate loads.²⁰ The children were studied after an overnight fast and did not take part in any strenuous activities during the test. None of the patients with cystic fibrosis had respiratory symptoms. The debate continues over whether there is a fundamental defect in energy metabolism in children with cystic fibrosis which is unrelated to pulmonary function, infection, and nutritional status.^{21,22} Such a defect might result in increased carbon dioxide production. However, in a recent study of asymptomatic infants with cystic fibrosis, total energy expenditure was the same as that measured in age matched controls.²³ Our assumption, therefore, that carbon dioxide production rates in infants with cystic fibrosis with no respiratory symptoms are not significantly different to those of healthy infants is justified. We concluded that the factor responsible for the changes in ^{13}C recovery that we described was intraluminal lipolysis.

This stable isotope breath test can detect fat maldigestion and is acceptable as a safe, repeatable, non-invasive test for assessment of enzyme supplementation and other therapeutic interventions, even in infants and young children with cystic fibrosis. In this age group, however, when pancreatic exocrine function

is immature and preduodenal lipase makes an important contribution to intraluminal lipolysis even in healthy children, the test may not discriminate completely between healthy children and those with cystic fibrosis. Cholesteryl octanoate is not hydrolysed by preduodenal lipase, and may be a more effective substrate for discriminating between the two groups of children. However, ^{13}C cholesteryl octanoate remains expensive to buy compared with ^{13}C triolein. Nevertheless, with a growing interest in the use of stable isotope labelled substrates in many fields of medical research, it is likely that they will become increasingly available for the investigation and management of patients with pancreatic insufficiency and other causes of digestive disease.

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