Carbon mixed triglyceride breath test and pancreatic enzyme supplementation in cystic fibrosis

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Abstract

Children with cystic fibrosis have variable degrees of exocrine pancreatic insufficiency which, if untreated, is the main cause of fat malabsorption. The impact of pancreatic enzyme supplementation on fat digestion was measured in 41 children with cystic fibrosis, 11 healthy controls, and five children with mucosal diseases by a non-invasive test of intraluminal lipolysis using $^{13}$carbon (13°C) labelled mixed triglyceride (1,3-distearyl, 2[13C] octanoyl glycerol). The children with cystic fibrosis without pancreatic supplements had a median (range) 13C cumulative percentage dose recovered over six hours (cPDR) of 3.1% (0–31.7), the controls 31.0% (21.8–41.1), and the subjects with mucosal disease 27.8% (19.7–32.5). In 23 subjects with cystic fibrosis the usual dose of pancreatic enzyme supplements increased the cPDR to a median of 23.9% (0–45.6), and twice the usual dose of enteric coated microspheres increased the cPDR to 31.1% (11.1–47.8). There was no significant difference between the median cPDR of normal controls and children with mucosal disease, but there was a highly significant difference between these groups and children with untreated cystic fibrosis. Thirteen children with cystic fibrosis had no 13C recovery in their breath without enzymes and 10 showed marked increases with regular enzymes. In eight children doubling the dose of enzymes caused no or minimal improvement. The mixed triglyceride breath test offers a simple, non-invasive way of assessing the need for pancreatic enzyme supplementation in children with cystic fibrosis and could be used to optimise treatment.

Methods

We studied 57 children with 127 breath tests; 41 children with cystic fibrosis, 11 healthy controls, and five children with gastrointestinal diseases. Patients with cystic fibrosis were selected from those attending the cystic fibrosis clinic of the Royal Hospital for Sick Children in Glasgow.

Of the 41 children with cystic fibrosis, 21 were boys and 20 were girls (mean (SD) age 8.2 (3.1) years). The genotype was known in 34 children. Half (21) were homozygous for F508, 10 were heterozygous, two subjects carried AF508/R117H, and one carried G542/R560T. All children with cystic fibrosis were studied when free of pulmonary infections. Two children had evidence of liver disease (significant increase in serum aspartate aminotransferase and ultrasound signs of portal hypertension).

The 11 healthy controls (mean (SD) age 9.8 (2.2) years), four boys and seven girls, were recruited from among the staff's children.

Five children with gastrointestinal diseases were studied: four boys with short bowel syndrome and one girl with untreated coeliac disease (mean (SD) age 8.5 (2.2) years).

The principal investigator (SA) obtained informed consent from parents for the study, which was undertaken with the approval of the hospital ethics committee.

The MTG (1,3-distearyl, 2(carboxyl-13C)octanoyl glycerol, 99 atoms % excess) was taken as a test meal containing 0.7 g/kg of fat up to a maximum of 15 g (total energy intake 6.4 kcal/kg body weight up to a maximum of 135 kcal) in the form of a mixture of a palatable long chain triglyceride emulsion (Calogen, Scientific Hospital Supplies) with olive oil (5% volume), sweetened with chocolate milk shake (5% volume). The MTG was added to the test meal...
After an overnight fast duplicate breath samples were collected for baseline measurements. Children ingested the test meal and thereafter breath samples were obtained every 30 minutes for six hours using a face mask for infants or, for children, by blowing directly via a one way valve (Ambu paediatric valve, Ambu International Copenhagen, Denmark) into a 600 ml reusable oxygen reservoir bag (Laerdal Medical Ltd, Orpington), which had been fitted with a three way tap. Samples (20 ml) of expired air were aspirated from the bag and stored in 16 ml Vacutainers (Becton Dickinson Ltd, Meylon, France) before mass spectrometry analysis. The children were not allowed to eat until at least three hours after ingesting the test meal, and then only taking foods of relatively low 13C abundance. The test was performed once on all 41 subjects and twice on 23 children with cystic fibrosis, without and with the regular dose of enzyme supplementation (all taking Creon 8000, Duphar Laboratories Ltd, Southamton), and a third time on these 23 children with cystic fibrosis, doubling the amount of enzymes.

13C enrichment was measured with an isotope ratio mass spectrometer (SIRA 10 VG Isogas, Middlewich, Cheshire). The 13C enrichment of each sample was expressed as the relative difference (δ) between the sample and the international limestone standard PeeDee Belemnite. The percentage of MTG recovered was calculated for each 30 minute interval as the percentage dose recovered (PDR) for the interval t to t+1 (minutes) using a formula described elsewhere. A cumulative PDR (cPDR) was obtained by summation of the single PDR values for each time interval.

The mean baseline δ values of the control subjects was −26.8‰, which implied avoidance of 13C enriched food before the test, whereas it was −23.7‰ for children with cystic fibrosis (p < 0.0001). There was no statistical difference between the control children and those with gastrointestinal diseases, nor between control children and children with cystic fibrosis receiving double their normal doses of pancreatic enzymes.

The mean baseline δ values on the subsequent days in the children with cystic fibrosis who underwent tests with enzymes and double enzymes were −23.1 and −24.3‰. The natural enrichments of 13C in Calogen and olive oil were −20.5 and −29.5‰.

The background variability of 13C in the breath was measured in four healthy adult volunteers and one child with cystic fibrosis by giving the test meal without the MTG. The
variability, expressed as the standard deviation of the mean $\delta^{13}$C value obtained over six hours, ranged between 0.16 and 0.41.

There was no significant relation between the cPDR and the dose of lipase ingested (IU/kg/meal/day). There was no significant relation between the cPDR and age, body weight, sex, or evidence of liver disease. Of the six children with cystic fibrosis with cPDR within the normal range (fig 1), four had genotypes other than $\Delta F 508$.

Discussion
We have shown that in children with cystic fibrosis the addition of pancreatic enzyme supplements leads to an increase in cPDR to within the normal range measured in healthy controls. Our study complements and supports that of Murphy et al.4

The labelled substrate consists of a triglyceride with two molecules of stearic acid at the 1 and 3 positions and $^{13}$C-octanoic acid in the 2 position. The rate limiting step in its digestion is hydrolysis of the two stearyl groups by pancreatic lipase. This gives it an advantage over trioctanoin,6 in which the labelled medium chain fatty acid (octanoic) is present in all three positions. The appearance rate of the tracer in the breath correlates well with lipase secretion and when compared with an invasive direct function test it was found to have a sensitivity of 89% and a specificity of 81%.3

Evidence that the MTG breath test measures intraluminal lipolysis and that mucosal uptake is not a rate limiting step is provided by the five children with small bowel disease (four of whom had had resections), whose cPDR was within the normal range, suggesting that even a marked reduction in the mucosal surface area for absorption of the octanoate does not alter the results. Reproducibility tests in the normal subjects showed that all were within 20% of the mean and all fell within the normal range.

The fact that the median cPDR of children taking a full dose of pancreatic enzymes was comparable with that of controls suggests that the test does measure fat digestion and that other variables are not major determinants of $^{13}$C recovery in breath. The MTG breath test may be used to measure the degree to which intraluminal fat hydrolysis reaches the normal range and hence whether optimum doses of pancreatic enzyme supplements are being used. In a child with steatorrhoea or poor growth, for instance, a MTG breath test can be used to determine whether an increase in enzyme supplements would improve fat digestion. Moreover, concern about the adverse effects of high dose enzyme supplements on the large bowel7 require that the lowest effective dose should be used.

The $^{13}$C MTG breath test is therefore a safe, non-invasive way to measure the efficiency of fat digestion, with advantages over faecal fat estimation for this purpose. With growing use of $^{13}$C breath tests for the non-invasive detection of Helicobacter pylori infection,1 the wider availability of isotope ratio mass spectrometry in medical centres, and the declining costs of labelled substrates,3 we believe that it should become more widely available and used to monitor enzyme supplementation in children with cystic fibrosis.

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