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Non-invasive assessment of intraluminal lipolysis using a $^{13}CO_2$ breath test

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Abstract

Techniques available for the study of lipase activity in the gut are unsatisfactory. Breath tests measuring labelled carbon dioxide ($^{13}CO_2$) may provide a useful means for this assessment. Six subjects with cystic fibrosis and pancreatic insufficiency and 10 controls received a test meal containing [^{13}C] trioctanoin, and breath $^{13}CO_2$ was measured using a dual inlet, dual detector isotope ratio mass spectrometer. Comparison of postprandial breath $^{13}CO_2$ enrichment allowed complete separation between children with pancreatic insufficiency and controls. Administration of one capsule of pancreatic enzyme with the test meal resulted in an increase in $^{13}CO_2$ production in all six patients, and four capsules produced a further increase in five of the six. Serial fat balance studies on four of the patients while receiving comparable doses of oral enzyme failed to demonstrate a progressive improvement in fat absorption. The [^{13}C]trioctanoin breath test may prove a safe, non-invasive technique not only for the detection of pancreatic insufficiency, but also for the quantitative study of intraluminal lipolysis.

Fat malabsorption is a major contributing factor to the growth failure and nutritional deficiencies that occur in many patients with cystic fibrosis.[1] The diagnosis of pancreatic insufficiency may be made in these individuals by direct measurement of faecal fat excretion. This technique is time consuming, unpleasant to perform, and prone to inaccuracy.[2] The 'fat tolerance test' has failed to gain widespread acceptance and its reliability has been questioned.[3] Pancreatic exocrine function may be studied more directly by duodenal intubation methods,[4] but these are time consuming and invasive. Watkins *et al* have reported that it is possible to screen for fat malabsorption by administering a triglyceride labelled with the non-radioactive isotope of carbon, ^{13}C, and by then measuring the increase in enrichment in breath $^{13}CO_2$.[5 6] This approach is especially attractive as a test for use in children as it is both safe and non-invasive.

Our aim was to re-examine the use of a $^{13}CO_2$ breath test as a technique for the detection of pancreatic insufficiency in children with cystic fibrosis, as previously reported by Watkins *et al*.[5] In addition we performed a preliminary investigation of the test as a technique for the quantitative study of intestinal lipolytic activity.

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Patients and methods

$^{13}CO_2$ breath tests were performed on six children with cystic fibrosis (age range 4–8 years) and on 10 healthy controls of similar age distribution (5–10 years). The children with cystic fibrosis had clinical features that were compatible with the diagnosis and all had abnormally raised sweat electrolyte concentrations. The presence of fat malabsorption was confirmed in four of those with cystic fibrosis by performing 72 hour fat balance studies, and in the remaining two the presence of excess faecal fat was documented by microscopy of Sudan stained faecal samples.

In order to examine the ability of the breath test to detect changes in the level of lipase activity in the gut, the children with cystic fibrosis were each studied on three occasions. One test was performed without pancreatic enzyme supplementation, one with a single capsule, and one with four capsules of a pH sensitive enteric coated enzyme microsphere preparation (Creon, Duphar) added to the test meal. Four of the children with cystic fibrosis also underwent a series of three fat balance studies while receiving pancreatic enzyme supplementation in doses approximately equivalent, in relation to fat intake, to those administered during their three breath tests. This was done by recording the subject's normal daily pattern and quantity of fat intake, and by then prescribing enzyme supplementation for the duration of the fat balance study that provided a dose of enzyme comparable in relation to fat intake with that administered with the test meals. In practice, the children received none, three to four, and 14 to 16 capsules of enzyme daily during these studies. All fat balance studies were performed under parental supervision, careful instruction being provided. Afterwards a questionnaire was completed to confirm that the test had been satisfactorily carried out. A dietary record was maintained during the study. Carmine red was employed as a marker to ensure complete stool collection. Faecal collections were stored at −20°C, and were later analysed using the method of van de Kamer *et al*.[7]

[^{13}C]TRIOCTANOIN STUDY PROTOCOL

After an overnight fast three baseline breath samples were collected. The children then drank a liquid test meal containing the isotopically labelled triglyceride [^{13}C]trioctanoin (P and S Biochemicals) dissolved in a fat emulsion, Calogen (Scientific Hospital Supplies). This meal contained 7·5 mg/kg of [^{13}C]trioctanoin in

1·4 ml/kg of Calogen. This provided 0·7 g/kg fat, which is comparable with the fat content of a normal meal. After ingestion of the test meal the children continued to fast, and further breath samples were collected at 30 minute intervals for a total of four hours.

In order to permit the collection of breath samples the children exhaled through a 10 cm length of universal tubing (Argyl bubble tubing, Sherwood Medical Industries). End expiratory air was collected by aspirating the sample into a 20 ml syringe via a butterfly needle inserted into the lumen of the tubing close to its proximal end. This technique ensured that the samples contained an adequate quantity of carbon dioxide for analysis. Breath samples were stored in 20 ml Vacutainers (Becton Dickenson Vacutainer Systems) for subsequent analysis.

Before analysis the carbon dioxide content of the breath specimens was isolated and purified using a modification of a previously described technique in which the component gases are separated by cold trapping and all but carbon dioxide are then removed by vacuum pump.[8] The purified carbon dioxide was immediately analysed using a dual inlet, dual detector isotope ratio mass spectrometer (Isospec 44, Spectramass).

Breath $^{13}CO_2$ enrichment is calculated from the increase in the ratio of $^{13}CO_2$ to $^{12}CO_2$ as measured by the isotope ratio mass spectrometer.[9] This sample ratio is compared with that obtained from a standard carbon dioxide. The results are then expressed as the 'del per mil' (δ‰) difference between the $^{13}CO_2/^{12}CO_2$ ratios of the sample (R_u) and the standard (R_s).

$$\delta^{13}C‰=(R_u-R_s/R_s)\times 10^3$$

The samples in our studies were analysed using distiller's carbon dioxide as a reference standard. The responses to the test meal were recorded as the rise in $^{13}CO_2$ enrichment above the fasting level. The overall analytic precision of the mass spectrometric analyses was (SD) 0·5‰. Results from two fasting control subjects, and also from two children with cystic fibrosis who had been given the test meal without [^{13}C]trioctanoin but with four capsules of pancreatic enzyme added, each had a variation (SD) of less than 0·7‰, with no tendency towards a progressive alteration in baseline $^{13}CO_2$ enrichment being observed over a four hour postprandial study period. As the area under the $^{13}CO_2$ curve is directly proportional to the sum of the interval sample enrichment levels this sum (Σ) was used as an index of $^{13}CO_2$ production after administration of the test meal.

Statistical analyses were performed using the non-parametric Mann-Whitney test. Ethical committee approval was received for these studies, and informed parental consent was obtained before the child's participation.

Results

Among the control subjects an increase in breath $^{13}CO_2$ was detected in every case within 60 minutes (fig 1). In seven of the 10 a peak of

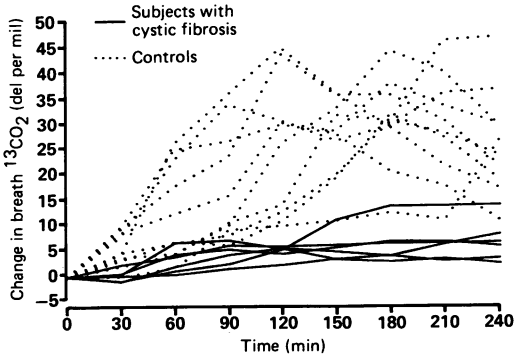

Figure 1 Changes in breath $^{13}CO_2$ in the six subjects with cystic fibrosis and 10 controls after a test meal of [^{13}C]trioctanoin.

enrichment occurred between 120 and 180 minutes, but in the remaining three the level of $^{13}CO_2$ was continuing to rise at 240 minutes. None of these subjects had returned to baseline by four hours.

Among the six with cystic fibrosis some increase in breath $^{13}CO_2$ also occurred, but the responses were much less than those seen in the control group (fig 1). By 90 minutes there was minimal overlap between patients and controls, and it was noteworthy that the lowest responder among the control subjects showed a pronounced rise in $^{13}CO_2$ enrichment at the 240 minute time point. When the results of the tests were expressed either in terms of peak $^{13}CO_2$ enrichment attained, or in terms of the increase in $^{13}CO_2$ production (Σ) (fig 2), complete separation was achieved between patients and controls. The mean value for Σ was 36·8‰ in the patients with cystic fibrosis and 176·9‰ in the control group ($p<0·02$).

Figure 3 shows an example of the breath $^{13}CO_2$ responses recorded from one of the subjects with cystic fibrosis during serial breath tests performed without enzyme supplementation, and then with one, and then four capsules of enzyme added to the test meal. In all six cases a significant increase in breath $^{13}CO_2$ production (Σ) occurred when the test meal was administered with one capsule ($p<0·05$), and in five of the six a further increase was observed with four capsules (fig 4). In one case (No 6, fig 4) the increase after four capsules was considerably less than that seen with no enzyme addition, so

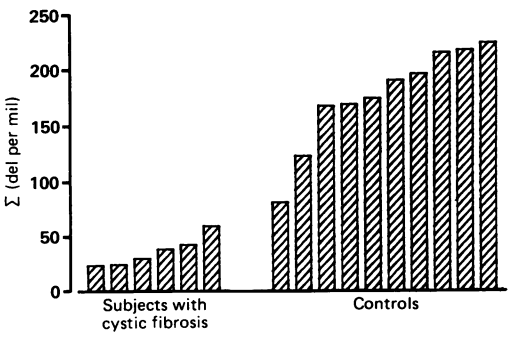

Figure 2 Breath $^{13}CO_2$ production over the four hour study period as estimated by Σ (algebraic sum of the interval sample $^{13}CO_2$ differences from the baseline levels) in the six subjects with cystic fibrosis and 10 controls after a test meal of [^{13}C]trioctanoin.

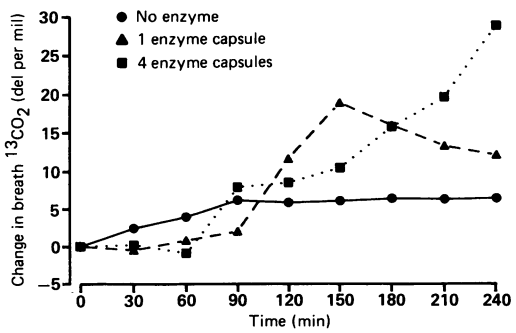

Figure 3 Breath $^{13}CO_2$ enrichment levels recorded in one of the subjects with cystic fibrosis during studies performed without pancreatic enzyme, and with one and four capsules of enzyme added to the test meals of [^{13}C]trioctanoin.

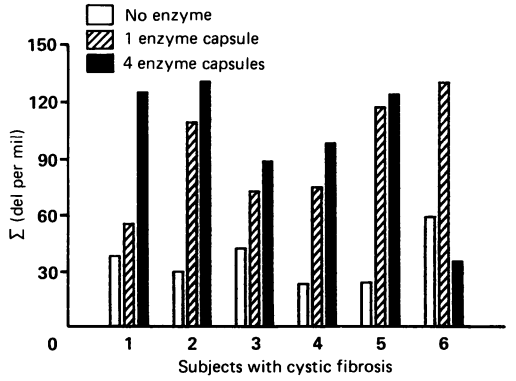

Figure 4 Breath $^{13}CO_2$ production over four hours as estimated by Σ (algebraic sum of the interval sample $^{13}CO_2$ differences from the baseline levels) in the six subjects with cystic fibrosis during studies with no enzyme supplementation, and with one and four capsules of enzyme added to the test meals of [^{13}C]trioctanoin.

that the overall incremental effect with four capsules did not reach significance.

The results of fat balance studies performed on four of the subjects with cystic fibrosis while off pancreatic enzyme supplementation, and while receiving low (three to four capsules) and finally high doses (14 to 16 capsules) of pancreatic enzyme are presented in the table. No obvious association was noted between the severity of fat malabsorption as judged by the coefficient of fat absorption and the degree of abnormality of the $^{13}CO_2$ breath test in those studies performed without the addition of enzyme to the test meal. Although an increase in the coefficient of fat absorption was observed in three of the four subjects with low dose enzyme replacement, in three no further improvement was detected with high dose supplementation, and indeed in one subject the coefficient of absorption worsened progressively with increasing enzyme supplementation.

Results of fat balance studies performed on four subjects with cystic fibrosis receiving no pancreatic enzyme replacement, on low dose replacement, and on high dose replacement. Results are given as the coefficient of absorption (%)

Subject No	*No of capsules daily*		
	0	*3–4*	*14–16*
1	55	89	78
3	83	70	58
4	56	60	79
6	85	94	93

Discussion

In recent years evidence has grown suggesting that careful attention to nutritional management in cystic fibrosis may be of great importance in influencing the course of the disease.[10] Oral enzyme replacement frequently fails to abolish steatorrhoea in cystic fibrosis, and although normal growth may be achieved, appreciable symptoms and nutritional losses often persist.[11] Attempts have been made to prevent gastric acid denaturation of ingested enzyme by administering antacids, or H_2 receptor antagonists, but the benefits have been controversial.[12–14] The use of enteric coated tablets has not found favour,[14] but newer preparations employing pH sensitive enteric coated microspheres of enzyme appear to be more successful. Consequently a range of preparations are now becoming available, some of which are claimed to have superior dissolution characteristics.[15] The availability of a safe non-invasive technique to assess lipase activity in the intestinal lumen would, therefore, be a major advance in the evaluation of enzyme treatment.

Several studies have reported the use of 'fat tolerance tests' in which the rise in plasma lipids after ingestion of a test meal is observed.[16 17] These tests appear to lack sensitivity, their value even for detection of fat malabsorption being doubtful.[3] Duodenal intubation studies have been performed to measure intestinal lipase activity after oral enzyme ingestion in patients with pancreatic insufficiency.[11 18] This approach, in addition to being invasive, merely provides information on the lipase concentration at a single point in the gut, and does not reliably estimate the overall functional level of lipolytic activity. Measurement of faecal fat excretion has been considered the gold standard for the detection of fat malabsorption, but its limitations are widely recognised, and consequently some centres have dispensed completely with this technique.[2] Although the fat balance study has, in principle, the merit of providing a direct estimate of faecal fat losses in subjects receiving pancreatic enzyme supplements, fat losses are influenced by factors other than enzyme bioavailability.

Efforts have been made in recent years to introduce breath tests for the study of intestinal absorption based on the use of ^{13}C, a non-radioactive stable isotope of carbon.[19] Watkins *et al* have reported that by utilising labelled triglycerides with differing absorption pathways it may be possible to distinguish pancreatic insufficiency from steatorrhoea of other aetiology.[6] The technique, therefore, appeared to possess considerable sensitivity, and so we considered that it might be possible to employ it for quantitative studies of fat absorption. Unlike duodenal intubation studies, a $^{13}CO_2$ breath test has the potential to provide a measure of the overall functional level of lipase activity in the intestinal lumen.

The results of this study provide further support for the ability of a $^{13}CO_2$ breath test to detect pancreatic insufficiency in children as previously reported by Watkins *et al.*[5 6] A recent study has reported a 20% increase in resting energy expenditure, and presumably there-

fore in carbon dioxide production, in individuals with cystic fibrosis.[20] Such an increase in carbon dioxide excretion could be misleading, as its dilutional effect would result in a reduction in breath $^{13}CO_2$ concentration. The subjects in that investigation ranged from 9 to 35 years, and it was noted that the resting energy expenditure correlated negatively with pulmonary function. The children in our study were significantly younger (4 to 8 years) and did not have severe pulmonary disease. Even allowing for a 20% dilutional effect in our patients, however, there was still complete separation between patients and controls. Simultaneous measurement of both total carbon dioxide production and $^{13}CO_2$ enrichment would be valuable in order to determine the exact magnitude of any dilutional effect which may exist. [^{13}C]bicarbonate kinetics are also subject to variation, and could be influenced by factors such as nutritional state[21]; it will be important to compare the 'bicarbonate recovery value' in children with cystic fibrosis and controls.

In addition to supporting the value of this technique for detecting pancreatic insufficiency, however, our study also suggests that the [^{13}C]trioctanoin breath test may prove a useful method for the quantitative study of lipolytic activity in the intestinal lumen. Only one of six cases failed to show a progressive rise in $^{13}CO_2$ production with increasing doses of enzyme. In this single case an increase in $^{13}CO_2$ production occurred with the addition of one capsule of enzyme to the test meal, but with four capsules $^{13}CO_2$ production failed to rise even to the level seen in the untreated state. This may have been because the test meal on that occasion was subject to delayed gastric emptying. It is noteworthy that the time point at which peak $^{13}CO_2$ enrichment occurred in these studies was quite variable. In several of the control subjects $^{13}CO_2$ levels were still rising and most still had breath $^{13}CO_2$ levels that were well above baseline at the end of the four hour study period. It is likely, therefore, that by prolonging the study period greater overall sensitivity would be achieved.

The [^{13}C]trioctanoin breath test provides a measure of the absorption of a medium chain triglyceride, and so the results do not directly relate to the overall complex processes involved in the absorption of dietary long chain triglycerides. Although both medium and long chain triglycerides require initial lipolysis, medium chain triglycerides, being water soluble, do not depend critically on the presence of bile salts for their digestion and absorption.[22] In cystic fibrosis bile salt deficiency is frequently present.[23] The [^{13}C]trioctanoin breath test thus focuses on the desired test parameter and a reduction in trioctanoin absorption reflects the level of lipolytic activity present in the patients digestive tract. The choice of [^{13}C]trioctanoin has an incidental advantage in that it is rapidly absorped and almost completely metabolised to carbon dioxide, thus shortening the overall study period.[5 24] This is an important practical consideration.

Although direct comparisons between the results of the $^{13}CO_2$ breath tests and fat balance studies cannot be made, it is of interest that the latter failed to detect a progressive improvement in fat absorption with increasing enzyme supplementation. The failure to improve fat absorption is not particularly surprising—complex interactions of various factors other than enzyme dosage certainly influence the degree of steatorrhoea present in individual patients. A key element in the introduction of $^{13}CO_2$ breath tests has been the development of the modern dual inlet, dual detector isotope ratio mass spectrometer, which can detect minute changes in $^{13}CO_2$ enrichment against the naturally occurring high background levels (approximately 1%). With the present growth of interest in the value of stable isotopes in biomedical research, an increasing number of medical centres are now gaining access to this technology.

Further studies with larger numbers of subjects and varied doses of pancreatic enzyme may allow optimal test conditions to be established, and may assist in further defining the sensitivity of this method for the quantitative study of intestinal lipase activity. By providing a measure of enzyme bioavailability it may potentially complement the currently available techniques for the assessment of enzyme replacement treatment.

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1 Forstner G, Gall G, Corey M, Durie P, Hill R, Gaskin K. Digestion and absorption of nutrients in cystic fibrosis. In: Sturgess JM, ed. *Perspectives in cystic fibrosis*. Toronto: Canadian Cystic Fibrosis Foundation, 1980:137–48.
2 Holmes GKT, Hill PG. Do we still need to measure faecal fat? *Br Med J* 1988;**296**:1552–3.
3 West PS, Levin GE, Griffin GE, Maxwell JD. Comparison of simple screening tests for fat malabsorption. *Br Med J* 1981;**282**:1501–4.
4 Arvanitakis C, Cook AR. Diagnostic tests of exocrine pancreatic function and disease. *Gastroenterology* 1978;**74**: 932–48.
5 Watkins JB, Schoeller DA, Klein PD, Ott DG, Newcomer AD, Hofman AF. 13C-trioctanoin: a nonradioactive breath test to detect fat malabsorption. *J Lab Clin Med* 1977;**90**: 422–30.
6 Watkins JB, Klein PD, Schoeller DA, Kirschner BS, Park R, Perman JA. Diagnosis and differentiation of fat malabsorption in children using 13C-labeled lipids: trioctanoin, triolein, and palmitic acid breath tests. *Gastroenterology* 1982;**82**:911–7.
7 Van de Kamer JH, ten Bokkel Huinink H, Weyers HA. Rapid method for the determination of fat in faeces. *J Biol Chem* 1949;**177**:347–55.
8 Schoeller DA, Klein PD. A simplified technique for collecting breath CO2 for isotope ratio mass spectrometry. *Biomedical Mass Spectrometry* 1978;**5**:29–31.
9 Schoeller DA, Schneider JF, Solomons NW, Watkins JB, Klein PD. Clinical diagnosis with the stable isotope ^{13}C in CO_2 breath tests: methodology and fundamental considerations. *J Lab Clin Med* 1977;**90**:412–21.
10 Corey M, Gaskin K, Durie P, Levison H, Forstner G. Improved prognosis in CF patients with normal fat absorption. *J Pediatr Gastroenterol Nutr* 1984;**3**(suppl 1):S99–105.
11 DiMagno EP, Malagelada J-R. Go VLW, Moertel CG. Fate of orally ingested enzymes in pancreatic insufficiency: comparison of two dosage schedules. *N Engl J Med* 1977;**296**: 1318–22.
12 Durie PR, Bell L, Linton W, Corey ML, Forstner GG. Effect of cimetidine and sodium bicarbonate on pancreatic replacement therapy in cystic fibrosis. *Gut* 1980;**21**:778–86.
13 Graham DY. Pancreatic enzyme replacement. The effect of antacids or cimetidine. *Dig Dis Sci* 1982;**27**:485–90.
14 Regan PT, Malagelada J-R, DiMango EP, Glanzman SL, Go VLW. Comparative effects of antacids cimetidine and enteric coating on the therapeutic response to oral enzymes in severe pancreatic insufficiency. *N Engl J Med* 1977;**297**: 854–8.
15 Kelleher J, Littlewood JM, Walters MP. Dissolution characteristics of microsphere pancreatic supplements. *10th International Cystic Fibrosis Congress*. Sydney, Australia. Hong Kong: Exercpta Medica, 1988:174–5.

16 Penfold WAF, Keynes WM. Use of a standard fatty meal as a test for fat absorption. *Ann Surg* 1971;**173**:157–63.
17 Bentley SJ, Eastham RD, Lane RF. Oral butter fat test meal with serum nephelometry in suspected fat malabsorption. *J Clin Pathol* 1975;**28**:80–1.
18 Dutta SK, Hubbard VS, Appler M. Critical examination of therapeutic efficacy of a pH-sensitive enteric-coated pancreatic enzyme preparation in treatment of exocrine pancreatic insufficiency secondary to cystic fibrosis. *Dig Dis Sci* 1988;**33**:1237–44.
19 Klein PD, Klein ER. Applications of stable isotopes to pediatric nutrition and gastroenterology: measurement of nutrient absorption and digestion using ^{13}C. *J Pediatr Gastroenterol Nutr* 1985;**4**:9–19.
20 Vaisman N, Pencharz PB, Corey M, Canny GJ, Hahn E. Energy expenditure of patients with cystic fibrosis. *J Pediatr* 1987;**111**:496–500.
21 Irving CS, Wong WW, Shulman RJ, O'Brien Smith E, Klein PD. [^{13}C] bicarbonate kinetics in humans: intra- vs. interindividual variations. *Am J Physiol* 1983;**245**: R190–202.
22 Greenberger NJ, Rodgers JB, Isselbacher KJ. Absorption of medium and long chain triglycerides: factors influencing their hydrolysis and transport. *J Clin Invest* 1966;**45**: 217–27.
23 Park RW, Grand RJ. Gastrointestinal manifestations of cystic fibrosis: a review. *Gastroenterology* 1981;**81**:1143–61.
24 Schwabe AD, Bennett LR, Bowman LP. Octanoic acid absorption and oxidation in humans. *J Appl Physiol* 1964; **19**:335–7.