

¹³C mixed triglyceride breath test

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There is no easy and reliable test universally accepted in clinical practice to measure fat digestion. Fat balance studies are insensitive and unpleasant for regular clinical assessment, direct measures of pancreatic function are impractical for repeated use, the "steatocrit" method is not quantitative; tubeless tests have not proved to be effective substitutes; and clinical measures, such as stool frequency, appearance, and consistency, are subjective.

In 1981 an isotope breath test named the "mixed triglyceride (MTG) breath test" was described by Ghos *et al* to measure intraduodenal lipase activity as an index of exocrine pancreatic function in adults.¹ It was later modified by the substitution of ¹³C for ¹⁴C.² The labelled carbon, originating from the labelled fat as a result of digestion, absorption, and oxidation, can be detected in exhaled carbon dioxide and the amount recovered in the breath is an indirect measure of lipolysis within the small intestine. The MTG breath test derives its name from the presence in the test molecule of both long and medium chain fatty acids. The labelled substrate consists of a triglyceride with two molecules of stearic acid at the Sn-1 and three positions and ¹³C octanoic acid in the Sn-2 position (1,3-distearyl [¹³C octanoyl] glycerol).

The MTG has a number of advantages over other triglycerides, such as trioctanoin and triolein,³ which have been used as substrates with which to measure fat digestion in adults and children.⁴⁻⁵ Although the MTG is not representative of a naturally occurring dietary fat, the rate limiting step in its digestion is hydrolysis of the two stearyl groups by pancreatic lipase. Normal diet contains little octanoate, and the labelled tracer is not therefore diluted by unlabelled substrate. Octanoic acid, as a medium chain fatty acid, is rapidly and completely absorbed and is also speedily oxidised in the liver.⁶⁻⁷ The appearance rate of the tracer in the breath correlates well with lipase secretion and when compared with an invasive direct function test was found to have a sensitivity of 89% and a specificity of 81% in adults.² The MTG breath test has been the interest of a number of the partners in this BIOMED programme, and the abstracts summarising their work reflect current issues surrounding its use, directions for its further development, and its validation for clinical purposes. In most of the studies described, results have been expressed as "percentage dose recovered" (PDR) of the ¹³C taken by mouth as MTG over five or six hours.

In abstract 1 a comparison was made of fat digestion in normal children with that in children with cystic fibrosis (CF) and small intestinal mucosal disease. The normal range of PDR was 22-41%. Unsupplemented chil-

dren with CF had a median PDR of 3%, which increased significantly to within the normal range with pancreatic enzyme supplementation. Children with diseases of the small intestine had PDRs within the normal range. No relation was found between units of lipase taken and ¹³C recovery in the breath.

The effects of enteric coated enzyme supplements on fat digestion in children with CF were investigated (abstract 2). Equivalent amounts of lipase from conventional and high dose preparations caused comparable improvements in lipid digestion. Furthermore, a study to determine whether high strength lipase supplements might be responsible for the colonopathy of CF found no difference in intestinal transit rate or site of maximal lipolysis between children taking standard and high strength enzymes (abstract 3).

Abstract 4 describes a study of children with untreated coeliac disease to investigate whether mucosal disease might affect the results. PDR ranged from 10 to 44%, and two children whose results were in the abnormal range improved with treatment, with PDRs returning to the normal range after one year of gluten free diet, suggesting that mucosal damage may be a rate limiting factor in the MTG breath test.

A comparison was made of the MTG breath test with fat balance measurements in adults with exocrine pancreatic disease (abstract 5). There was an exponential relation between the results of the two tests, and—excluding four patients with mucosal defects—a sensitivity of 81% and specificity of 75% was found. It is argued in abstract 6 that the MTG breath test is a more sensitive method for detecting defects in lipase activity than the assessment of fat malabsorption by 72 hour faecal fat measurement in children with CF, whose PDRs ranged from 3-31%.

In abstract 7 the effects of physical exercise and composition of diet before the test were studied. Foods that were naturally enriched with ¹³C resulted in too low a PDR, but there was no substantial effect of exercise on the results. Gastric acid suppression caused some improvement in PDR in adults with CF.

The MTG breath test was compared with the secretin-pancreozymin test (abstract 8). Patients with mild, moderate, and severe pancreatic disease, and patients with non-pancreatic gastrointestinal disease and controls were studied. The sensitivity and specificity of the test was best (63% and 85% respectively) when the PDR over 150 minutes was used to discriminate between groups, and a significant relation between PDR and duodenal enzyme concentrations was found.

Abstract 9 describes the application of the MTG to study the ontogeny of fat digestion in infancy. There was an increase in the capacity

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to digest the triglyceride during the neonatal period, and by the age of 8 weeks all infants studied had PDRs within the adult range of 20–40%.

In abstract 10 the metabolic fate of medium (MCT) and long (LCT) chain triglycerides was investigated in healthy adults. After ^{13}C LCT ingestion PDR of ^{13}C in the breath was only 19% in six hours, in contrast with 61% when LCT and MCT were given together. After the LCT, ^{13}C appeared in chylomicrons and in non-esterified fatty acids and much was probably stored as adipose tissue.

Other factors affecting results of MTG breath test

Amarri and Weaver have summarised the factors that regulate the handling of ingested ^{13}C -labelled substrates and the recovery of the products of digestion in the breath.⁸ Kalivianakis *et al* studied the determinants of the $^{13}\text{CO}_2$ response in healthy adults and reported considerable intraindividual and interindividual variation, showing that different test meals and physical exercise reduce the repeatability of the MTG breath test.⁹

Evidence that the MTG breath test measures intraluminal lipolysis, and that mucosal uptake is not a major rate limiting step is provided by results from adults (abstracts 6 and 8) and children with small bowel disease (abstract 1) whose PDRs were within the normal range, suggesting that only a marked reduction in mucosal surface area for absorption of the octanoate affects the results (abstract 4).

Recovery of ^{13}C in the breath of some children with CF (abstract 1) who were taking no pancreatic enzymes was zero, and all but one showed a significant increase in PDR after ingestion of enzymes implying that preduodenal (lingual and gastric) lipases are diminished or contribute little to fat digestion in children with CF.

Other factors that may affect the results are baseline $^{13}\text{CO}_2$ enrichment (abstract 7), gastric emptying rate (abstract 2), CO_2 production rate, and energy expenditure.⁸ Although the children with CF and adults who had ingested ^{13}C rich foods (abstract 7) had slightly higher baseline δ values, the magnitude of the increase during the tests far exceeded the difference between those consuming ^{13}C enriched and non-enriched foods before the test, and could not cause false positive results.

It is unlikely that the test meal itself altered gastric emptying significantly because of the small amount of labelled substrate included in it. There is no published evidence that gastric emptying is delayed in children with CF. Octanoic acid, the fatty acid released after digestion of MTG by pancreatic lipase, has been used alone as a substrate with which to measure gastric emptying rate in adults¹⁰ and infants.¹¹ Combination of carbon labelled octanoic acid and MTG allows the contribution of gastric emptying rate to PDR to be calculated.¹²

CO_2 production rate will affect the quantity of ^{13}C detected in the breath,¹³ and ideally it should be measured directly, particularly in conditions associated with altered metabolic

rate. CO_2 production rate may be increased in children with CF, and this could lead to an increase in oxidation of absorbed ^{13}C octanoic acid. When the normal dose of pancreatic enzymes was doubled, however, the median PDR of children with CF became equal to the median PDR of control children (abstract 1).

It is unlikely that bile salt deficiency is responsible for the low PDR of the children with CF when not taking enzymes supplements, as PDR reached levels comparable with the controls with double normal doses (abstract 1), and bile salts are involved primarily in the digestion and absorption of LCTs.

Quantitation: sensitivity and specificity

There was no clear relation between the number of units of lipase ingested, nor in the “strength” of enzyme supplements, and the incremental increase in PDR in subjects with CF (abstracts 1, 2, 3, and 6). In a disease in which the degree of exocrine pancreatic hypofunction is variable it is no surprise that this is so. It is not possible therefore to predict the dose of pancreatic enzymes necessary to digest a particular quantity of ingested fat. Most children with CF, although receiving “modest” doses of pancreatic enzymes, benefited little by further increasing the ingested dose or strength (abstracts 1, 2, 3, and 6).

The fact that the median PDR of adults and children with pancreatic disease who were taking full doses of pancreatic enzymes was comparable with that of controls, suggests that the test does measure fat digestion and that lipase deficiency is the rate limiting step in ^{13}C recovery in breath. The MTG breath test may be used to compare the level of fat digestion of patients with exocrine pancreatic disease, with healthy controls and thereby the efficiency of enzyme supplements to promote fat digestion. It may also be used to measure the degree to which intraluminal fat hydrolysis reaches the normal range, and hence whether optimal doses of pancreatic enzyme supplements are being used.¹⁴ In a child, for instance with steatorrhoea or poor growth, an MTG breath test can be used to determine whether an increase in enzyme supplements would improve fat digestion.¹⁵

Sensitivity and specificity of the MTG breath test is relatively high when compared with both faecal fat measurements and with duodenal enzyme concentrations. However, the tests measure different processes in fat assimilation—intraduodenal lipolysis, fat balance, and pancreatic enzyme secretion—and 100% agreement between them should not be expected.

Conclusions

The ^{13}C MTG test is a simple, repeatable, safe, non-invasive way to measure the efficiency of fat digestion. Its simplicity, in performance and in analysis, commends it above faecal fat estimation for this purpose. With growing use of ^{13}C breath tests for the non-invasive detection of *Helicobacter pylori* infection, wider availability of isotope ratio mass spectrometry in medical centres, and declining costs of

labelled substrates it is likely that the MTG breath test will become more widely available and be used to monitor enzyme supplementation in adults and children with exocrine pancreatic insufficiency.

There remains a need for further studies aimed at standardising the way in which the test is done⁹ and at defining its sensitivity and specificity in other diseases associated with fat maldigestion in adults and children. In addition, it will be important to define the full fate of the ingested ¹³C triglyceride. In normal subjects, PDR is between 20 and 40%, suggesting that the remaining 60–80% has been either “irreversibly” deposited within the body in adipose tissues and other carbon pools, or has not been “absorbed”. Balance studies, in which the enrichment of faeces is also measured, will allow the proportion of ¹³C deposited in body pools that are not in immediate equilibrium with alveolar carbon dioxide to be calculated.

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(1) ¹³C mixed triglyceride breath test and pancreatic enzyme supplementation in children with cystic fibrosis

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Children with cystic fibrosis (CF) have variable degrees of exocrine pancreatic insufficiency which if untreated is the main cause of fat malabsorption.

Pancreatic enzyme supplements are given to promote dietary fat digestion. However, assessment of enzyme supplement requirements and effectiveness can be difficult: three day faecal fat collection is cumbersome and lacks sensitivity, the “stato-crit” is not quantitative, and clinical measures (stool frequency and consistency) are subjective. We measured the impact of pancreatic enzyme supplementation on fat digestion using a non-invasive test of intraluminal lipolysis in 41 children with CF (mean (SD) age 8.4 (2.2) years; 21 girls, 20 boys), 11 healthy controls, and five children with mucosal diseases (one with untreated coeliac disease and four with short bowel syndrome) (age 8.5 (2.2) years; eight girls, eight boys). Children ingested the ¹³C-labelled mixed triglyceride (1,3-distearyl, 2[carboxyl-¹³C]octanoyl glycerol) in a long chain triglyceride emulsion. Breath samples were analysed for ¹³C enrichment by isotope ratio mass spectrometry. CO₂ production rate was measured by indirect calorimetry. The results were expressed as ¹³C cumulative percentage dose recovered (cPDR) over six hours. The children with CF without pancreatic supplements had a median (range) cPDR of 3.1% (0–31.7), the controls 31% (21.8–41.1), and the children with mucosal disease 27.8% (19.7–32.5). In 23 subjects with CF, ingestion of usual dose of pancreatic enzyme supplements increased cPDR to a median of 23.9% (0–45.6), and twice the usual dose of enteric-coated microspheres to cPDR of 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 CF (p<0.0001). Only six children (15%) with CF had a cPDR within the normal range. All but one of the five children with mucosal disease were in the normal range. Thirteen children with CF had no ¹³C recovery in their breath without enzymes; 10 showed marked rises with regular enzymes. In eight children, doubling the dose of enzymes caused no or minimal improvement. There was considerable intersubject variation in cPDR, reflecting a wide degree of pancreatic insufficiency. There was no significant relation between units of lipase ingested and ¹³C recovery in the breath, nor between the results and age and nutritional status of the children. The mixed triglyceride breath test offers a simple, non-invasive, and repeatable way of assessing the efficiency of pancreatic enzyme supplementation in children with CF, and could be used to measure requirements and to optimise treatment.

(2) Lipid digestion in cystic fibrosis: comparison of conventional and high lipase enzyme therapy using the mixed triglyceride breath test

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Exocrine pancreatic insufficiency and lipid maldigestion occur in the majority of patients with cystic fibrosis (CF). Conventional enteric coated pancreatic enzyme replacement therapy partially corrects this defect. Newer high lipase products possibly further improve lipid digestion. In this study, we used the mixed triglyceride breath (MTG) test to evaluate whether high lipase enzymes are superior to conventional enzymes for improving fat maldigestion in children with CF. Fat digestion was studied in 13 patients, of whom 11 completed all tests. Their ages ranged from 6 to 15 years with a mean age of 10.5 years. The number of conventional pancreatic

enzyme capsules taken each day ranged from six to 45 with a mean of 19 capsules a day. Four ^{13}C MTG tests were done on separate days. One test was taken without enzyme substitution, one with three capsules of conventional enzymes (R/ Creon 3 \times 8000 FIP units lipase), and one with one capsule of high enzyme lipase (R/ Creon 25000 FIP units lipase). The fourth test was obtained with ^{13}C octanoic acid to study gastric emptying time because this variable is known to influence fat digestion. Tests were given in random order. Results were evaluated by analysis of variance and Duncan test (SAS, Proc Anova). Gastric emptying half time was below 120 minutes in nine of the 11 patients. Without enzyme intake, the mean (SD) cumulative percentage of ^{13}C dose exhaled after six hours was 7.1 (3.7) %. This significantly increased to 14.4 (4.9) % with intake of three capsules of conventional Creon and to 14.3 (5.1) % with intake of one capsule of high lipase Creon ($p=0.0008$ for both, paired t test). There was no difference between both treatments. The time course of ^{13}C exhalation measured by per cent $^{13}\text{CO}_2$ exhaled each hour did not differ between enzyme treatments. In conclusion, the ^{13}C MTG test is a practical, non-invasive test that clearly documents improved lipid digestion with pancreatic enzyme replacement therapy. Equivalent amounts of FIP lipases from conventional and high lipase preparations result in comparable improvement in lipid digestion.

(3) Comparison of the effect of standard and high strength pancreatic enzymes on fat digestion in children with cystic fibrosis

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Introduction: After reports linking fibrosing colonopathy in children with cystic fibrosis with use of high strength pancreatic enzymes (HSPEs) we decided to compare the site and pattern of lipolytic activity of HSPEs and standard strength pancreatic enzymes. **Methods:** We studied 20 children (nine boys and 11 girls) with cystic fibrosis, mean age of 10 years (range 2.7–16.6) using a stable isotope breath test. Breath samples were collected at half hour intervals for up to 12 hours after a test meal containing 10 mg/kg (<30 kg body weight) or 5 mg/kg (>30 kg). ^{13}C labelled mixed triglyceride dissolved in a long chain triglyceride emulsion, along with a standard breakfast of 140 g baked beans (as substrate for hydrogen breath test), 50 g toast, and 20 g butter. Time to peak ^{13}C recovery in breath was taken as maximal lipolytic activity and combined with gut transit times (hydrogen breath tests) to determine the site of maximum lipolysis. Each child was tested on three occasions with equivalent lipase units of one standard (Creon) and two HSPE (Creon 25000 and Pancrease HL). The percentage dose of ^{13}C lipid recovered over 7.5 hours was calculated for each test day assuming a constant average value of CO_2 production of 5 mmol/m²/minute. **Results:** The median oro-caecal transit time and time to peak ^{13}C recovery were both delayed at 6.0 hours (range 4–7.5) and 4 hours (2.5–9) respectively, and not influenced by the type of enzyme. However, for each child the relation of time to maximum lipolysis and oro-caecal transit time was consistent and independent of the type of enzyme. Time to maximum lipolysis was shorter than the oro-caecal transit time in 18/20 children. Variation of cumulative percentage dose recovery of ^{13}C was too wide to gather statistical significance. Fasting breath hydrogen was high in 14 children. **Conclusion:** To conclude, we found no

Abstract 4, Table 1 PDR in two patients retested with MTG-BT

PDR	Patient 1	Patient 2
Free diet	9.63	16.76
Six months after gluten free diet	16.27	33.82
12 months after gluten free diet	25.86	26.89

difference in the timing or pattern of lipolysis between the standard and high strength pancreatic enzymes. Maximum lipolysis occurred in the same part of the gastrointestinal tract (probably the distal small gut) with the standard and high strength enzymes.

(4) Intraluminal lipase activity in patients with coeliac disease assessed by means of ^{13}C "mixed triglyceride" breath test

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Introduction: It has been hypothesised that patients with coeliac disease (CD) have a decreased pancreatic lipase output because of the loss of gut mediated stimulatory effects of the meal on the pancreas. The ^{13}C mixed triglyceride breath test (MTG-BT) is a precise indirect test of intraluminal pancreatic lipase activity. **Aim:** To evaluate the intraduodenal lipase activity in patients with CD by means of the non-invasive and safe MTG-BT. **Methods:** Twelve subjects (two males and nine females; mean (SD) age 16.5 (11.3) years; range 2–38) with documented diagnosis of CD and on free diet were studied. Steatorrhoea was evaluated by the Van den Kamer fat measurement. A sweat test was done to exclude a concomitant cystic fibrosis. They underwent the MTG-BT in the overnight fasting state. They had breakfast consisting of two slices of white bread with 10 g of marmalade and 0.25 g/kg body weight of butter in which 4 mg/kg body weight of MTG (99% ^{13}C -enriched) had been dissolved. Breath samples were collected every 30 minutes for six hours. Measurements of $^{13}\text{CO}_2$ were done by means of ANCA-NT IRMS (Europa Scientific, Crewe, UK). CO_2 production was assumed to be 300 mmol/body surface area/hour (body surface area = $W^{0.5375} \times H^{0.3964} \times 0.024265$ with weight in kg and height in cm). Results were expressed as a percentage of ^{13}C dose recovered in six hour time (PDR). In these test conditions, normal values of PDR = 22%. **Results:** None of the patients with CD had steatorrhoea (> 7 g fat/24 hours) on free diet. All of them were negative on sweat test. The mean (SD) PDR was 28.7 (10.6) (range: 9.6–43.7). Two out of 12 (16%) patients with CD had a PDR lower than the normal value. After six and 12 months, these two patients were tested again with the MTG-BT while they were on gluten free diet with normal histology on small intestinal biopsy (table 1). Both patients with CD corrected the intraluminal pancreatic activity deficiency after starting the gluten free diet. **Discussion:** Some patients with CD have a decreased meal stimulated lipase output even in the absence of steatorrhoea. This defect is likely to be related to the intestinal mucosal damage because it is completely reversible after starting the gluten free diet. The MTG-BT could be proposed as a non-invasive test to assess the meal stimulated lipase output in diseases (such as CD) in which the main pathogenetic mechanism is represented by a serious mucosal defect.

(5) Intraluminal lipase activity and fat malabsorption: fat balance and the ^{13}C mixed triglyceride breath test compared in patients with steatorrhoea

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Introduction: Exocrine pancreatic insufficiency is characterised by a deficiency of enzyme output and intraduodenal enzyme activity after pancreatic stimulation by a meal. The ^{13}C mixed triglyceride breath test (MTG-BT) is a precise indirect test of intraluminal pancreatic lipase activity. It is therefore to be expected that it will give a good prediction of fat malabsorption in this group. The fat balance, however, is the direct test to investigate fat malabsorption. In this study we compared the fat balance and the MTG-BT in a group of patients with proved or suspected fat malabsorption. **Methods:** The MTG-BT was done in the overnight fasting state: standard breakfast, 250 mg ^{13}C -MTG, breath samples every 30 minutes, measurements of $^{13}\text{CO}_2$ in a VG SIRA-10 IRMS, CO_2 production assumed 9 mmol/kg/hour, percentage dose ^{13}C recovered (PDR) in five hours time as the test result. Fat balance: dietary counselling (fat constant diet at usual level of fat intake, two days run-in period, three days stool collection, Van den Kramer fat measurements, faecal loss as percentage of dietary intake). Forty patients participated in this study; 15 with cystic fibrosis, 10 with documented pancreatic disease, 11 patients were investigated for suspected steatorrhoea, four patients had diseases affecting gut mucosa. **Results:** An exponential relation was found between the MTG-test and the fat balance. Fat balance was below the normal value (7% loss) in 20 of the 40 patients; the MTG-BT was below the lower limit of normal (20% PDR) in 20. Excluding the four patients with mucosal defects, the MTG-BT predicted fat balance documented fat malabsorption accurately in 78% of cases. Sensitivity=81%; specificity=75%. **Discussion:** An exponential relation between the MTG-BT and the fat balance was expected because (a) such a relation is known to exist between the faecal fat excretion and post-prandial duodenal lipase activity and (b) the MTG-BT measures luminal lipase activity. Sensitivity and specificity are moderately good, taking the fat balance as ("golden") standard; however, it is well known that the fat balance is far from perfect. The MTG-BT is potentially a valuable clinical tool to detect fat malabsorption in exocrine pancreatic insufficiency and to monitor the effect of pancreatic enzyme supplementation.

(6) Evaluating fat malabsorption in children with cystic fibrosis: comparing the ^{13}C -mixed triglyceride breath test with the 72 hour faecal fat collection

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Introduction: Our purpose was to compare the results of the ^{13}C mixed triglyceride breath test (MTG-BT), assessing lipase activity, and of the 72 hour faecal fat collection in paediatric cystic fibrosis (CF) patients with failure to thrive. **Methods:** Fifteen patients (five girls, 10 boys), with proved CF and exocrine pancreatic insufficiency were selected, based on the clinical decision to re-evaluate their fat absorption. These patients, with a mean age of 12 years (range: 2–18 years), were failing to thrive and to gain weight despite nutritional advice and enzyme replacement therapy (mean: 5070 lipase units/kg/

day, range: 0–9464). The test meal for the breath test consisted of bread, margarine, ^{13}C MTG enriched chocolate paste, and whole milk (1003 kJ, 13 g fat, 250 mg ^{13}C MTG). Breath samples were collected before the test meal and every 15 minutes for six hours afterwards. The percentage of $^{13}\text{CO}_2$ in breath was analysed by isotope ratio mass spectrometry. The cumulative percentage of $^{13}\text{CO}_2$ after six hours reflects lipase activity. The mean cumulative percentage $^{13}\text{CO}_2$ was 19.9 (range: 3.4–31.0). Dietary fat intake was recorded three days before and during the 72 hour faecal fat collection. Faecal fat excretion was 6.8% (range: 1.5–39.5). On the basis of the faecal fat measurements six subjects out of a total of 15 could be considered abnormal, compared with 11 out of 15 breath tests (with respect to previously reported reference values). Both tests were abnormal in five patients and normal in three. **Conclusion:** The results indicate that the ^{13}C MTG-BT is a more sensitive method for detecting defects in lipase activity than the assessment of fat malabsorption by 72 hour faecal fat collection and suggest that it will prove helpful in treating patients with CF.

(7) Validation procedures and clinical studies on the use of the mixed triglyceride breath test in patients with exocrine pancreatic insufficiency

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Introduction: The mixed triglyceride breath test (MTG-BT) is a precise, indirect test for intraduodenal lipase activity. The MTG-BT could be used clinically to document exocrine pancreatic insufficiency or the effect of pancreatic enzyme supplementation. Potentially, the MTG-BT could replace the faecal fat balance. We evaluated the MTG-BT by studying two confounding factors: diet on the day before the test and effect of physical exercise during the test. The MTG-BT was then compared with a five day fat balance in a group of patients with suspected steatorrhoea. Subsequently, the MTG-BT was used to detect pancreatic insufficiency in adult patients with cystic fibrosis (CF) and to monitor enzyme supplementation therapy, either as such or in combination with gastric acid inhibition (omeprazole). **Methods:** The MTG-BT was done in the overnight fasting state; at 08.00 the subjects took a breakfast consisting of two slices of bread, cheese, marmalade, 10 g of butter with 250 mg of 1,3-distearyl, 2 [^{13}C -octanoyl] glycerol, supplying 1446 kJ, 42 E% as fat, 13 E% as protein and 45 E% as carbohydrate. Nothing other than tea or coffee was taken during the study. Before breakfast and subsequently every 30 minutes afterwards for five hours, duplicate samples of breath were collected in glass tubes. Breath ^{13}C enrichment in CO_2 was measured with a VG SIRA-10 IRMS. CO_2 production was assumed to be 9 mmol/kg/hour. The percentage dose recovered (PDR) in five hours time is presented as the result of the test. Faecal fat was measured according to the van den Kamer procedure. **Results:** Use of naturally ^{13}C -enriched carbohydrates (maize starch, cane sugar) in the day before the test was observed to cause an unstable (declining) baseline enrichment and hence unduly low test results. In four healthy controls, PDR was 32.5 (5.4) in resting conditions, 29.1 (3.6) PDR after exercise for five minutes at 25 watt and 29.3 (5.8) PDR after 50 watt (not significant). Healthy controls (n=42; 16 men, 26 women) showed a reference value for the MTG-BT of 33.5 (SD 6.7) PDR. Seventeen healthy subjects took the test twice: first

Abstract 9, Table 1 Results of 48 MTG-BTs in infants

	Age (days)				
	1-7	8-14	15-28	29-90	91-234
Number	23	5	5	5	10
cPDR360 (%)	16.9 (1.9)	20.7 (5.5)	19.6 (3.6)	26.3 (3.6)	30.6 (2.6)
Peak PDR (%)	2.7 (0.3)	3.3 (0.75)	3.2 (0.5)	4.1 (0.6)	4.3 (0.4)
Time to peak (minutes)	266 (22)	288 (20)	282 (31)	192 (39)	294 (28)
cPDR at peak (%)	12.0 (1.4)	13.5 (3.2)	12.1 (1.2)	11.6 (2.0)	23.8 (3.3)

series mean (SD) PDR of 34.8 (5.1), repeated tests 31.6 (6.7) PDR; mean (SD) difference between the two tests 7.1 (4.6) PDR or 20% of the mean value (not significant, Wilcoxon matched pairs signed ranking test). In a group of 40 patients (21 men, 19 women; age range=19-77 years) with suspected steatorrhoea of various aetiologies, an exponential relation was found between the MTG-BT and the fat balance. The fat balance was normal (<7% loss) in 20 patients; the MTG-BT was below the lower limit of normal (20.2 PDR) in 20 patients. Excluding four patients with mucosal defects, the MTG-BT predicted fat balance detected fat malabsorption accurately in 78% of cases (sensitivity 81%, specificity 75%). In 33 adult patients with CF (14 men, 19 women; age range 19-30), a mean of 13.4 (range 1-43) PDR was found; test results were normal in 10 patients. In a patient group with abnormal test results (n = 19) PDR increased from 5.6 (range 1-19) PDR to 11.7 (range 1-20) PDR on enzyme treatment; the normal range for PDR was not reached in any of the patients. In 10 patients, omeprazole was given in addition to pancreatic enzymes, resulting in an increase in PDR from 13.0 (range 7-20) to 16.7 (range 9-26); a PDR > 20.02 was achieved in three out of 10 patients. **Conclusions:** Food containing naturally ^{13}C enriched carbohydrates in the day before the test has to be avoided; the test diet should be eucaloric. No substantial effect of physical exercise during the test on MTG-BT results was found. The reproducibility of the MTG-BT is fair, but less than desirable. Monitoring enzyme supplementation therapy in patients with CF is feasible; an increase, but no normalisation, in PDR on enzyme supplementation could be documented. A small additional improvement was found when enzyme treatment was combined with gastric acid suppression. The MTG-BT could potentially replace the fat balance. It remains to be tested clinically whether the MTG-BT is suitable to determine optimal enzyme supplementation therapy in individual patients.

(8) Diagnostic value of the mixed triglyceride breath test for the diagnosis of exocrine pancreatic insufficiency in comparison with secretin pancreozymin test

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Introduction: A relatively new test for non-invasive assessment of exocrine pancreatic function via lipase activity is the ^{13}C mixed triglyceride breath test (MTG-BT). The clinical relevance of this test in comparison with the secretin-caerulein test as the "gold standard" of pancreatic function testing, faecal elastase concentration, ^{13}C starch breath test, and faecal chymotrypsin activity was evaluated in a controlled prospective clinical study. **Patients:** In a clinical trial of 85 patients, 30 patients with mild (n=15, mean (SD) age 44.4 (3.6)) or severe (n=15, mean (SD) age 48.1 (3.0)) exocrine pancreatic insufficiency, 23 patients with gastrointestinal diseases of non-pancreatic origin (mean (SD) age 49.6 (3.2)) and 32 healthy controls (mean (SD) age 29.3

(1.3)) were included. **Methods:** 250 mg of ^{13}C mixed triglyceride were dissolved in chocolate cream (Nutella) and given with white bread, butter, and 200 ml orange juice after a 12 hour fast. Breath samples were taken before and in 15 minute intervals for five hours after oral ingestion and the $^{13}\text{C}/^{12}\text{C}$ isotopic ratio was analysed by mass spectrometry (Isochrome μGas , Fisons Instr. UK) and expressed in δ values. The δ values (percentage dose/hour) were set in an equation system to calculate the CO_2 excretion curve. **Results:** Specificity and sensitivity for mild and severe pancreatic insufficiency has been calculated for 150 minutes, 180 minutes, and for cumulative percentage dose recovered (PDR) (five hours) with respect to the gold standard. The measurement time giving the best discrimination between mild and healthy groups was 150 minutes (sensitivity: 63% specificity; 85% for mild pancreatic insufficiency with a cut off: 4.6 percentage/dose/hour). The sensitivity after 180 minutes is lower (46%) as well as the sensitivity calculated from the cumulative curve (300 minutes). In total, the sensitivities of the MTG-BT for mild, severe, and total exocrine pancreatic insufficiency were higher (total 81%) compared with faecal chymotrypsin (total 52%) but lower compared with faecal elastase (total 93%). Significant correlations ($p < 0.001$) were found between AUC of the MTG-BT and duodenal amylase, lipase, and trypsin secretion. With regard to the moderately good sensitivity and specificity for the detection of a mild pancreatic insufficiency the MTG-BT may be a valuable diagnostic tool in clinical routine.

(9) Mixed triglyceride breath test in infants

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The mixed triglyceride breath test (MTG-BT) has been used extensively to test functional fat digestion in adults and children, particularly for the assessment of pancreatic insufficiency in cystic fibrosis (CF) and chronic pancreatitis. Recent work has suggested that the neonate has a requirement for long chain polyunsaturated fats for neural and retinal development. However, studies have shown low concentrations of pancreatic lipase in neonates compared with older children, and significant faecal fat excretion in cows' milk formula fed infants. In this study, functional fat digestive capacity was assessed in infants aged 1-234 days. A total of 48 MTG-BTs were done on 26 infants (23 at age 1-7, five at 8-14, five at 15-28, five at 29-90, and 10 at 91-234 days). CO_2 production rate was measured using indirect calorimetry in 22 studies; in the remainder it was estimated as 5 mmol/m²/minute. Results were expressed as percentage dose of ^{13}C recovered (PDR) in each 30 minute interval, and cumulative PDR (cPDR) against time after ingestion of the substrate and plotted graphically. From the graphs, cPDR at six hours, peak PDR, time to peak, and cPDR at peak were measured and expressed as means (SEM) (table 1).

Babies in the first week had cPDR 360, peak PDR, and cPDR at peak significantly lower than infants over 91 days ($p < 0.01$) and there was a trend

for these three expressions of PDR to rise with increasing age. No significant variation existed, however, in time to peak between the five age groups. PDR curves are graphical representations of the handling of ^{13}C between ingestion of substrate and excretion of CO_2 in the breath. The different expressions of PDR are all probably indices of functional fat digestion, and time to peak a measure of rate of gastrointestinal transit of substrate. In the absence of a difference in time to peak, these results suggest that the capacity of the neonate to digest triglycerides increases during early infancy, reaching normal adult levels within the first eight weeks of life.

(10) Metabolic fate of ingested long and medium triglycerides: different steps that could be investigated using ^{13}C labelled triglycerides

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Introduction: ^{13}C triglyceride breath tests are used to look at the digestion step. The appearance of ^{13}C in CO_2 after ^{13}C triglyceride ingestion, however, requires numerous metabolic pathways and is dependent on VCO_2 production. The investigation of these steps is possible using the determination of ^{13}C appearance in the lipid fraction and a precise quantification of $^{13}\text{CO}_2$ production including indirect calorimetry measurements. **Methods:** We have followed the metabolic fate of a 30 g load of long chain triglycerides (LCT) labelled with 200 mg of ^{13}C triolein and of a mixture of LCT (15 g) and

medium chain triglycerides (MCT, 15 g) labelled with 150 mg of ^{13}C trioctanoin (LCT-MCT load) in eight normal subjects. These two loads were given after an overnight fast, in random order at two week intervals. Blood and breath samples were collected every 30 minutes for six hours and continuous indirect calorimetry measurements were done. ^{13}C enrichment was determined in chylomicrons, in non-esterified fatty acids (NEFA) and in CO_2 . Exogenous lipid oxidation was calculated from $^{13}\text{CO}_2$ enrichment and CO_2 production. **Results:** After the LCT load, ^{13}C appeared rapidly in chylomicrons but also in NEFA. The ratio of enrichment between NEFA and chylomicrons was 80 (SD 3) %. ^{13}C appeared slowly in CO_2 and in six hours, only 19 (SD 2) % of the lipid load was oxidised. At the end of the study, from 300 to 360 minutes 69.7 (SD 0.1) % of lipid oxidation was from exogenous origin. After the LCT-MCT load, ^{13}C appeared rapidly in CO_2 and 61 (SD 1) % of the MCT load was oxidised in six hours. **Discussion:** These results showed that after an LCT load, an important intravascular release of TG-derived fatty acids occurred. Lipid oxidation was weakly enhanced by lipid ingestion and only 19% of the load was oxidised in six hours. Because most of the LCT are stored in adipose tissue, the breath test is not a good reflection of the digestion process. On the contrary, MCT were rapidly and more completely oxidised, thus the breath test appears to be a good index of MCT absorption. In conclusion, it appears necessary before using breath tests to investigate the process of digestion to look at the different metabolic pathways involved, and to their eventual pitfalls in pathological states.