«Mixed» triglyceride breath test: methodological problems and clinical applications

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SUMMARY: Laboratory assessment of pancreatic function is unpleasant for the patient and time-consuming for the investigator since it requires duodenal intubation and measurement of maximal pancreatic enzyme output by means of perfusion techniques. Non-invasive indirect tests such as bentiromide test, pancreaseauryl test and faecal fat measurement have been introduced in clinical practice but their results depend on the collaboration of the patient in collecting urine or stool. Moreover, faecal fat reflects fat malabsorption but it is neither sensitive nor specific to evaluate exocrine pancreatic function.

With the aim to determine whether steatorrhea is due to pancreatic insufficiency, several 14C- (or 13C) breath tests have been developed in which triolein, trioctanoin, tripalmitin, and cholesteryl-octanoate are used as marker substances. In 1989, G. Vantrappen and its group in Leuven developed a breath test in which a new substrate was used: the [1,3-distearyl, 2-carboxy-13C]octanoyl glycerol or 13C-triglyceride (MT). The «mixed triglyceride breath test» (MTBT) was shown to be an excellent test of exocrine pancreatic insufficiency when compared with the maximal lipase output after CCK-pancreozymin stimulation.

Aim of this paper is to review the methodology of the MTBT and its actual and future applications in clinical practice.

Palabras clave
Función pancreática exocrina, Test de aliento.

Key words
Exocrine pancreatic function, Breath test.

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METHODOLOGY

Principle of the test

The [1,3-distearyl, 2-[carboxyl-13C]octanoyl glycerol] or 13C-mixed-triglyceride (MT) is a molecule containing a 13C-labelled medium chain fatty acid (13C-octanoic acid) in the 2-position and long chain fatty acids (stearic acid) in the 1- and 3-positions (Fig. 1). Pancreatic lipase cleaves specifically the two long chain fatty acids in the 1- and 3-positions: the resulting free 13C-octanoyl monoglyceride is then rapidly absorbed by intestinal mucosa, and metabolised to 13CO2. The rate limiting step in the oxidation to 13CO2 is the hydrolysis of the fatty acids in the 1- and 3-positions of the MT (Fig. 1). By this reason, the amount of 13CO2 excreted in the breath accurately reflects the intraduodenal pancreatic lipase activity and is not merely a measure of faecal fat excretion.

Method

Patients are given a diet containing at least 90 g fat per day. After an overnight fast, they have a test meal consisting of 100 g of toast with 0.25 g of butter per kilogram of body weight, to which 16 mg of 13C-labelled MT was previously added. Breath samples are collected before the meal and every 30 minutes after ingestion of the meal up to 6 hours. 13C-enrichment in breath is determined by means of an isotope ratio mass spectrometer. All -values are expressed versus the PDB-international standard after correction for the oxygen isotope effect (1). Results are expressed as the percentage of 13C-recovery per hour and cumulative values over 6 hours. For this calculation the formula of Schoeller et al (2) is used, and the CO2 production is assumed to be constant and equal to 300 mmol/m2 body surface area per hour. Body surface area is calculated according to weight and height formula of

Fig. 1 Schematic representation of the principle of the mixed triglyceride breath test. In patients with pancreatic insufficiency, the rate limiting step of the 13CO2 excretion is represented by the intraduodenal lypolysis of the mixed triglyceride by pancreatic lipase.
Haycock et al. (3). A cumulative value of 22% over 6 hours represents the lower limit of the normal range (4).

**Correlation with lipase output and faecal fat excretion**

In their validation study (4), Vantrappen et al showed a linear correlation between the cumulative excretion of $^{13}$CO$_2$ over 6 hours and the maximal lipase output after CCK-pancreozymin stimulation (Fig. 2). By assuming cut-off values of 22% and 90 kU/h for the 6 hr-cumulative excretion of $^{13}$CO$_2$ and the stimulated lipase output, respectively, they found that the MTBT had a sensitivity of 0.89, a specificity of 0.81, a positive predictive value of 0.63, and a negative predictive value of 0.95 in the diagnosis of diseases associated with a diminished lipase output. When lipase output and $^{13}$CO$_2$ cumulative excretion were compared with daily fat excretion, they found that steatorrhea only occurred if lipase output fell below 40 kU/h and the 6 hr-cumulative excretion of $^{13}$CO$_2$ was below 7.5%. A pathological value of 6 hr-cumulative excretion of $^{13}$CO$_2$ between 7.5% and 22% denoted a lipase output between 40 and 90 kU/h, which is diagnostic for pancreatic disease even in absence of steatorrhea.

The MTBT is able to differentiate between steatorrhea associated with pancreatic disease or reduced meal-stimulated pancreatic lipase output (i.e.: untreated celiac disease) and steatorrhea of non pancreatic origin (i.e.: small intestinal bacterial overgrowth, Crohn's disease, Whipple's disease, or lymphoma). By this reason, the MTBT can take place in the diagnostic evaluation of patients with chronic unexplained diarrhoea.

**Reproducibility of the test**

In 1997, Kalivianakis et al (5) performed the MTBT in healthy adults in order to assess the intra-individual variations of the $^{13}$CO$_2$ excretion and found large coefficients of variation. This finding is not surprising since another study from the Leuven group (6) clearly showed that, at least in healthy individuals without pancreatic insufficiency, intraluminal lipolysis has a very large variance (approximately 40%) and this is due to the gastric emptying rate of fat meal. In fact, in healthy subjects, the rate of gastric emptying may be the rate limiting step of the $^{13}$CO$_2$ excretion. Consequently, a very slow gastric emptying could result in falsely pathological MTBT results. This loss of specificity may be only corrected if the gastric emptying of the test meal is simultaneously measured. An elegant solution to this problem is represented by another breath test, the $^{14}$C-octanoic acid breath test, which can be performed simultaneously to the $^{13}$C-MTBT in order to determine the gastric emptying rate of fat. So doing, Maes et al. were able to correctly evaluate the intraluminal lipolysis of MT in healthy subjects with delayed gastric emptying (6).

In patients with pancreatic insufficiency, however, gastric emptying rate of the test meal has no impact on the rate of MT digestion, with the rate limiting step in the excretion of $^{13}$CO$_2$ being represented only by the impaired hydrolysis of the MT (6).

The effect of an additional meal or physical exercise during the MTBT has been also studied by Kalivianakis et al (5) who showed that stringent prolonged fasting is unnecessary and physical exercise is undesirable for it affects the MTBT results.

**Accuracy of MTBT**

The MTBT is accurate enough to detect non invasively exocrine pancreatic insufficiency. However, falsely positive and negative results may occur due to different physiopathological conditions.

False positive tests have been reported in healthy subjects with extremely delayed gastric emptying as well as in patients suffering from extensive intestinal
mucosal damage (as in coeliac or in Crohn’s disease) (4). In these diseases, however, an impairment of the gut-mediated stimulatory effect of the meal on the pancreas has been hypothesised, as shown by the fact that the MTBT results normalise if patients are pretreated with CCK-pancreozymin (7). Moreover, when mucosal damage is repaired, like in celiac patients after gluten withdrawal, the MTBT returns to normal (8). By this reason, we believe that a pathological MTBT should not be regarded as a false positive result since a diminished meal-stimulated intraduodenal lipase activity actually exists in these conditions, and lasts until intestinal mucosa is restored.

False positive results of MTBT may occur when the contact time between the test meal and bili-pancreatic secretions is extremely short as seen in patients with gastrectomy or other surgical procedures for morbid obesity. However, in these conditions, as already discussed for the small-intestinal diseases, the term “false positive result” does not seem to be completely appropriate since lipase activity is present but unable to digest fat properly.

False positive results have been reported also in patients with diabetes or severe liver disease (4). Different factors may account for a low excretion of I3CO2 after administration of MT in these patients: delayed gastric emptying and dilution of I3C-octanoic acid in a large pool of short-chain fatty acids in diabetes and reduced I3C-octanoic acid oxidation in liver patients due to a decrease of functioning liver cell mass. Even in these diseases, however, more studies are needed to exactly evaluate the significance of these falsely positive results.

False negative results are obviously less frequent than the false positive ones and generally occur in patients with mild pancreatic disease and values of I3CO2 excretion which are a little higher than the threshold value. Consequently, patients with borderline MTBT results should be re-tested shortly, especially if the suspicion of pancreatic disease is very high.

Effects of drugs on MTBT

Since the original paper by Vantrappen et al, the MTBT was shown to be sensitive enough to monitor the efficacy of the replacement enzymatic therapy in patients with exocrine pancreatic insufficiency. Of interest is, however, the fact that the I3CO2 excretion, even improving in all pancreatic patients under replacement therapy, rarely returns to normal values. Sequential MTBT could be performed under different therapeutic regimens in order to optimise the therapy. In this respect, the MTBT seems to be more sensitive and less time-consuming than faecal fat determination in evaluating the most appropriate timing and dosage of replacement therapy.

CLINICAL APPLICATIONS

The MTBT, like other indirect pancreatic function tests, has been used in clinical practice mainly for three applications:

1. to detect decreased intraduodenal lipase activity;
2. to follow the evolution of pancreatic disease;
3. to monitor the effect of replacement enzymatic therapy in patients with pancreatic insufficiency.

A reduced intraluminal pancreatic lipase may be due to either pancreatic damage or decreased meal-stimulated pancreatic secretion. Pancreatic disease is characterised by a progressive decrease of pancreatic lipase output which can be easily assessed or monitored by means of MTBT. Pathological changes of breath test may be observed even before steatorrhea occurs. It has been shown that steatorrhea only occurs if pancreatic lipase output is reduced to ≤10% (9). By this reason, the MTBT is generally more sensitive than other steatorrhea-detecting I3C-breath tests (10-12) and may be proposed to follow the clinical evolution of patients with pancreatic diseases or diseases associated with a decreased meal-stimulated lipase output.

We have recently studied 17 untreated coeliac patients and found that the MTBT was abnormal in 3 of them and at the lower limit of the normal range in another subject. These patients were re-tested at 6 and 12 months after instituting a gluten-free diet and all of them normalised their fat digestion. This study shows that gluten enteropathy may affect the complex stimulatory neuro-hormonal network existing between the intestinal mucosa and the exocrine pancreas. That the exocrine pancreatic function is normal in these patients is shown by the «normalisation» of the MTBT after parenteral administration of CCK-pancreozymin (7). I3C-octanoic acid oxidation seems not to be the rate limiting step as shown in another study where children with small bowel resection had normal values on the MTBT (13). We also instilled I3C-octanoic acid into the duodenum of three patients with untreated coeliac disease and found no delay or decrease in I3C-octanoic acid oxidation (unpublished observations).

Another interesting application of the MTBT is to monitor the effect of replacement therapy. Vantrappen et al. showed a progressive increase in I3CO2 excretion...
after MTBT in patients with pancreatic steatorrhea under different schedule treatments (4). Amarri et al. used the MTBT to optimise the enzymatic treatment in children suffering from cystic fibrosis (13). This is particularly useful in these patients in view of the fact that high dose enzyme supplements have been reported to cause serious adverse effects on large bowel (14).

Future clinical applications of this test in patients with pancreatic insufficiency could be:
- the choice of the best enzyme preparation;
- the choice of the best administration schedule;
- the evaluation of possible causes of failures to treatment.

Future clinical applications of the MTBT in patients with diminished meal-stimulated lipase output in absence of any organic pancreatic disease could be:
- the timing of the replacement therapy (when and how long enzymes should be administered);
- the choice of the best enzyme preparation and administration schedule.

In conclusion, the MTBT is a safe, accurate non-invasive test to measure the intraduodenal activity of pancreatic lipase, with obvious advantages over faecal fat estimation. With increasing use of $^{13}$C-urea breath test for $H. pylori$ infection (15), the wider availability of mass spectrometers in several medical centres and the declining cost of $^{13}$C-labelled substrates, the MTBT will be more widely available as non-invasive test of pancreatic function.

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**BIBLIOGRAFÍA**


