Breath tests for the assessment of the orocecal transit time

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Abstract. Orocecal transit time (OCTT) is one of the main determinant of the hunger ratings and gastrointestinal sensitivity. While marked-isotopes scintigraphy is the gold standard in its determination in the clinical frame, breath tests are cheap, well-tolerated and non-invasive alternatives. In fact C-13 and C-14 stable isotopes breath tests can be used to assess gastric emptying and OCTT in the clinical and research frames. Moreover, hydrogen (H2) lactulose breath test can be used to assess OCTT in the research frame only due to its laxative action; inulin breath test, devoid of this bias, could be replacing it. However, the main limitation in the use of breath tests in the OCTT determination is their low reproducibility.

Keywords: Lactose ureyde, Octanoate, Lactulose, Inulin, OCTT.

Introduction

Marked isotopes scintigraphy is the gold standard for the assessment of gastric emptying and total OCTT. It is a pretty cheap investigation but quite time consuming and not devoid of limitations for the patient such as its use in pregnant women and children. In order to overcome these limitations they have been ruled out several stable-isotopes and hydrogen (H2) breath tests for the determination of gastric emptying, one of the main component of the upper GI tract motility involved in the control of hunger and nutrient acquisition in man, and for the total orocecal transit time (OCTT), involved in the regulation of gastrointestinal sensitivity and motility together with the migrating motor complex (MMC).

This review describes technical and experimental/clinical applications of C-13, C-14 and H2 breath tests for the assessment of gastric emptying and OCTT in man.

C-14 octanoate and C-13 glycine breath tests

General principles

14C-octanoic acid breath test (14C-OBT) is able to measure gastric emptying of solids based on the retention of 14C-octanoic acid in the solid phase of a standard test meal (egg yolk) during its passage in the stomach, followed by a rapid disruption in the duodenum with subsequent absorption of 14C-octanoic acid in the mesenteric vessels and consequent hepatic oxidation to 14CO2. Thus, the appearance of 14CO2 in breath after oral administration of 14C-octanoic depends on the gastric emptying of the egg yolk into the duodenum.

A percentage of dose/h curve after ingestion of a 14C-octanoic acid labeled solid test meal is characterized by an ascending slope, a peak of excretion, and a descending exponential slope. Ghoo et al. validated the 14C-OBT against scintigraphy, the gold standard technique to evaluate gastric emptying of solids. Both volunteers and dyspeptic subjects were given standard test meal consisting of a scrambled egg whose yolk was doped with 14C-octanoic acid and the egg white labeled with 99mTc albumin colloid.

Interestingly, both within-subject variability (inter-individual variability) and between-subject variability (day to day variability) were small and not statistically significant.

13C-glycine breath test can be used as marker of the liquid phase of gastric emptying because it is easily solubilized in water and rapidly absorbed and converted into 13CO2 after it enters the small bowel. Moreover, the use of glycine breath test allows simultaneous measurement of both the solid and liquid phase of the meal, in association with 14C-octanoic acid.

Glycine is not absorbed in the stomach; the site of absorption of glycine and other neutral amino acids is located in the proximal intestine, using mainly active transport mechanisms. After
transport across the intestinal wall, glycine is partly oxidized to CO₂ by different pathways³,¹³,¹⁴.

The dose/h curve after ingestion of a ¹³C-glycine labeled liquid test meal is similarly characterized by an ascending slope, a peak of excretion, and a descending exponential slope³.

The ¹³C-glycine breath test compares favorably with other tests of liquid gastric emptying, e.g. aspiration technique¹⁵, radioscintigraphy¹,³, paracetamol absorption test¹⁶, the echographic method¹⁷ and the recent MRI technique³,¹⁸.

The good correlation of both the breath-test-determined gastric half-emptying time and the peak excretion time with the radioscintigraphic half-emptying time depends on the fact that these two breath-test parameters are independent of the endogenous CO₂ production and on the amount of ¹³C-glycine converted to CO₂¹⁻³.

Clinical applications

The ¹⁴C-OBT and ¹³C-glycine breath test are useful tools to evaluate gastric emptying either in physiological and in pathological states; they are also able to detect changes in gastric emptying induced by drugs such as motilin-receptor agonists, cisapride, anticholinergics, and octreotide⁵,¹⁰⁻¹³. In addition OBT has been also used to evaluate differences in the physiology of gastric emptying of solids and liquids in both adults and children. The OBT has been also employed to study the effects of gastric emptying on the rate of intraluminal lipolysis (tested with ¹³C-“mixed triglyceride”-breath test) in patients with pancreatic insufficiency¹⁸ and the rate of ureolysis (tested with ¹³Curea breath test) in *Helicobacter pylori* infected and uninfected subjects¹⁻³,¹⁸.

For example in patients with distal gastrectomy, an early dumping of the oil phase of the meal was observed compared to healthy controls¹⁹.

In patients with non-ulcer dyspepsia²⁰ about 30% of them had a delayed gastric emptying as compared to controls. In another group of dyspeptic patients studied by the OBT²¹ almost one third of them displayed a significant delay in gastric emptying, significantly correlated with symptoms such as postprandial fullness, nausea, and vomiting. Children with celiac disease, studied by the OBT before and after gluten-free diet²² presented a significantly delayed gastric emptying as compared with healthy age-matched controls. After 6 months of gluten-free diet, gastric emptying parameters returned to normal range in patients.

As cited above similar clinical applications can be performed with ¹³C-glycine breath test. In fact, although there is lacking information about intragastric distribution of the different phases of the test meal, breath-test measurements of gastric emptying offer several advantages over radioscintigraphic techniques.

C-¹³ lactose ureyde breath test

General principles

Radioscintigraphy is usually considered the reference technique for measuring OCTT. However, as mentioned above, several drawbacks limit its application in routine clinical practice: relevant costs, time and specialized personnel are required, and, least but not last, the use of radioactive isotopes is associated with some irradiation risk (<3 mSv). Therefore, it is conceivable to avoid repeating the exam at short intervals especially in children while pregnant women that should avoid it at all¹.

Breath tests involving the stable carbon isotope, ¹³C have been successfully introduced with several clinical and research applications, including gastric emptying, as described above¹⁰. Glycosyl ureides have been studied extensively for their physical and chemical properties¹,²³. In fact, the enzymes of the brush border of the human intestine are notable to split the bond of sugars to urea. Because glycosyl ureides are only slightly absorbed in the small bowel without further metabolism, they can reach the large bowel unaltered. On the other hand, colonic flora is able to split the bond of sugars to urea²⁴,²⁵ and, subsequently produce, among the other products, CO₂, free in the breath of the host as ¹³CO₂. Therefore, breath sampling after oral administration of the labeled molecule, at regular time intervals for approximately ten hours, allows the time of appearance of the label in breath to be defined. This point in time indicates the time needed by the marker molecule, together with the meal in which it was integrated, to reach the cecum²⁶.

In the validation paper by Geypens et al¹ OCTT results from scintigraphy (mean OCTT= 283±53 min) and breath test (mean OCTT= 292±58 min) correlated well (r = 0.94). Altman-Bland statistics showed close agreement between scintigraphy and breath test. No significant difference between male and female subjects was observed.

Open issues in the application of this breath test are represented by a diminished response after use of antibiotics and an altered response after
laxatives. In patients with bacterial overgrowth, the substrate may be split in the small intestine and yield a falsely accelerated transit time. Wutzke et al have compared the LUBT and the lactulose H2 breath test in healthy adults. They demonstrated that the lactulose H2 breath test resulted in a statistically significant shorter OCTT (1.18 hours) in comparison with the LUBT. Finally, LUBT remains the only validated breath test for clinical and research examination in man although one limitation is its duration (approximately 10 hours) and substrates costs.

Clinical applications

To date, there are a few clinical trials using this breath test. However, the test is suitable for studying the influences of pharmaceuticals and nutrients on intestinal motility both in adults and children. In particular, children studies have highlighted technical issues of LUBT with different results according to the experimental setup. The validation study by Geypens et al and the subsequent study in dyspeptic children have showed that those used setup clearly allow a good clinical reproducibility and avoid the laxative effect of lactulose breath test in comparison with scintigraphy.

H2 breath tests for the assessment of OCTT

General principles

The appearance in the expiratory breath of hydrogen (H2) produced by colonic fermentation of an ingested liquid or solid sugar compound may be used to measure OCTT. The outcome of reproducibility test with the liquid meal is poorer than with the solid meal. The major factor determining the poor reproducibility of the liquid meal is the phase of the interdigestive migrating motor complex. Transit time with the solid meal is significantly longer than with the liquid meal because the addition of lactulose to a solid meal accelerates small bowel transit.

Ingestion of the test-meal is followed by a biphasic breath hydrogen profile: the first peak follows the meal; the second, associated with the meal entering the caecum, normally occurs hours later. Increments of 3, 5 or 10 ppm of hydrogen above baseline maintained or increased in the two following determinations are considered the incoming of the meal in the caecum.

The OCTT in healthy subjects ranges respectively between 40 and 170 min for the liquid and between 192 and 232 min for a solid meal. The 10 g lactulose dosage for the liquid test has been chosen due to its prokinetic effects eliciting at increasing doses. The current protocol employing the liquid solution includes 10 g of lactulose in 100 mL of water, and a cut-off value of hydrogen ≥10 ppm (based on barium meal studies) followed by at least two other subsequent increments.

About 5-27% of normal subjects fail to produce an increment of hydrogen breath concentration after the meal due to the absence of hydrogen-producing colonic microflora and, probably, due to the presence of a metanogenic-one. The menstrual cycle may influence the OCTT.

Inulin, a natural polysaccharide with a higher degree of polymerisation than lactulose and, consequently, less active osmotically, has been proposed as an ideal substrate to be added to a solid meal for OCTT assessment. Thus, in contrast with lactulose, inulin does not shorten OCTT (ranging between 420 and 570 min after ingestion of 5 or 10 g inulin with the solid meal).

H2 breath tests are generally safe but bloating and abdominal distension may occur after colonic fermentation of lactulose.

Potential applications to the clinical and experimental setting

Although non-invasive, safe, cheap and easy to perform, the main limitation for the clinical application of the hydrogen-breath test to estimate OCTT is due to the wide results variability in healthy volunteers over the rather low liquid test reproducibility.

A delayed oro-caecal transit assessed by hydrogen breath test has been reported in various subgroups of patients, including those with depression, chronic alcoholism, constipation, acromegaly, diabetes, irritable bowel syndrome, beta thalassemia major, pregnancy, cholecystectomy, obesity, cirrhosis, scleroderma, dyspeptic patients, and in constipated children.

By contrast, fast oro-caecal transit has been reported in patients with the irritable bowel syndrome, chronic alcoholics, partial gastrectomy, post-vagotomy diarrhea and hyperthyroidism. Despite such wide applications to the
research field, so far recent guidelines do not suggest a definite clinical indication for the test in the clinical setting.

Interestingly, the test reproducibility and variability improve in the experimental setting, in particular in clinical pharmacology studies on OCTT. Transit is accelerated by misoprostol, erythromycin, metoclopramide and paroxetine, and it was delayed by loperamide, ridoxine, codeine, dopamine, peppermint oil, n-butylscopolamine and imipramine

**General conclusions and future perspectives**

Breath tests for the assessment of OCTT are safe, cheap, well-tolerated and non-invasive tools.

C-13 glycine and C-14 octanoate breath tests are normally used to assess gastric liquid and solid emptying such as C-13 lactose-ureide to assess OCTT in the clinical and research frames.

H2 lactulose is normally used to assess OCTT in the research frame only, due to its laxative properties, able to shorten OCTT. Therefore, inulin breath test, if larger placebo-controlled studies will confirm the first promising validation findings, can be considered a new tool potentially replacing LBT in the H2 assessment of OCTT.

However, the main limitation in the use of breath tests in the OCTT determination is the wide values variation among normal subjects and the poor reproducibility.

**Competing of interest**

No competing interests for any of the authors.

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