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^{13}C-octanoic acid breath test for measuring gastric emptying of solids

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Abstract. – Non invasive evaluation of gastric emptying is generally performed by scintigraphy which is, however, difficult to perform and not suitable to children and childbearing women. A new method based on stable isotope breath testing analysis has been introduced in clinical practice: the ^{13}C-octanoic acid breath test. In this paper, an overview of the current knowledge on this technique is given with special emphasis on the principle of the test, the mathematics used to analyse the results, and the physiological, pathological, and pharmacological aspects of gastric emptying studied with this new method.

Key Words:

Octanoic, Breath test, Gastric emptying, Scintigraphy.

Introduction

Gastric emptying is a highly coordinated physiological response to the presence of food which can be impaired in several pathological conditions (Table I). Since its introduction in clinical practice, scintigraphy has been considered the "gold standard" technique for measuring gastric emptying[1]. However, expensive equipment is needed as well as availability of qualified personnel of nuclear medicine department. Moreover, scintigraphy induces a significant radiation burden[2] which limits its application in children, fertile women, and subjects undergoing repetitive measurements of gastric emptying in a short period of time. By all these reasons, other non-invasive techniques such as ultrasonography[3], impedance epigastrography[4], applied potential tomography[5], and magnetic resonance[6] have been proposed. Recently, breath tests with stable isotopes have been developed to reliably assess gastric emptying of both solids[7] and liquids[8,9]. The main advantage of breath test technology is represented by the lack of radiation burden. Moreover, breath test are non invasive, non operator-dependent and can be performed several times in the same subject without biological hazard.

Principle of ^{13}C-octanoic Acid Breath Test

The rationale of ^{13}C-octanoic acid breath test (^{13}C-OBT) to measure gastric emptying of solids is based on (1) the firm retention of ^{13}C-octanoic acid in the solid phase of a standard test meal during its passage through the gastric environment, followed by (2) a rapid disintegration of the solid phase in the duodenum with (3) subsequent absorption of ^{13}C-octanoic acid and (4) hepatic oxidation to $^{13}CO_2$ (Figure 1). *In vitro* validation studies[7] have shown that in gastric environment ^{13}C-octanoic acid is firmly retained in the yolk of scrambled egg used as test meal. Once the meal reaches the duodenal lumen, ^{13}C-octanoic acid is rapidly absorbed through intestinal mucosa and oxidized to $^{13}CO_2$ in liver. The appearance of $^{13}CO_2$ in breath after oral administration of ^{13}C-octanoic depends mainly by the gastric emptying of the egg yolk into the duodenum (*rate limiting step*). The other metabolic steps (absorption and oxidation) do not influence the rate of breath $^{13}CO_2$ excretion as shown by studies[7] in which, after duodenal instillation of ^{13}C-octanoic acid, $^{13}CO_2$ appears in breath almost immediately with a very little inter-subject variability.

Matematical Analysis of $^{13}CO_2$ excretion curves for gastric emptying

A percentage of dose/h curve after ingestion of a ^{13}C-octanoic acid labeled solid test

Table I. Delayed and accelerated gastric emptying states

Delayed gastric emptying states
PAcid-peptic related diseases
Gastro-esophageal reflux disease
Gastric ulcer disease
Gastritis
Atrophic gastritis associated or not with pernicious anaemia
Viral gastro-enteritis (acute – chronic?)
Mechanical factors
Gastric carcinoma
Idiopathic hypertrophic pyloric stenosis
Metabolic and endocrine disorders
Diabetes (ketoacidosis, gastroparesis)
Hypothyroidism
Uraemia
Hypokaliemia, hypercalcemia
Hepatic coma
Collagen vascular diseases
Pseudo-obstruction
Chronic idiopathic intestinal pseudo-obstruction
Secundary (amyloidosis, dermatomyositis, muscular dystrophies)
Gastric surgery
Postoperative ileus
Vagotomy
Gastric resection
Neurological disorders
Central nervous system diseases, brainstem lesions, spinal cord injuries
Parkinson's disease
Drugs
Anticholinergics, opioid drugs, tricyclic antidepressants, somatostatin, etc.
Idiopathic
Gastric dysrhythmias-tachygastria
Gastroduodenal dissynchrony
Severe exercise
Accelerated gastric emptying states
Zollinger-Ellison syndrome
Vagotomy + pyloroplasty/antrectomy
Drugs
Motilin-receptor agonists, cisapride, domperidone, metoclopramide, etc.
Mild exercise

meal is characterized by an ascending slope, a peak of excretion, and a descending exponential slope (Figure 2). In their validation paper, Ghoos et al[9] developed two different formulas for analyzing the $^{13}CO_2$ excretion curves but, for simplicity, we describe only one of the two. This formula is derived by the Siegel's function[10] used to describe the scintigraphic retention curve (Figure 2), i.e. the percentage of retention of a g-emitting labeled meal in the gastric area as a function of time. The scintigraphic retention curve is expressed by:

$$y = 1- (1- e^{-kt})^b$$

where "y" is the fractional dose of radioactivity retained in the stomach at time t, and "k" and "b" are estimated regression constants. Two parameters are derived from this formula:

- the gastric half emptying time: $t_{1/2s} = (-1/k)\ 5 \ln (1-2^{(-1/b)})$ and
- the lag time: $t_{lags} = \ln (b)/k$

in which $t_{1/2s}$ is the time at which half of the γ-emitting labeled meal is retained in the stomach and t_{lags} is the time needed for the antral contractions to grind solids into particles small enough to pass through the pylorus. Both parameters are important since they properly describe the biphasic nature of gastric emptying of solids. For liquid test meals, t_{lags} is usually absent and only $t_{1/2s}$ is therefore calculated.

In the formula by Ghoos[7], the cumulative dose of $^{13}CO_2$ excreted as a function of time is given by:

$$z = m (1- e^{-kt})^b$$

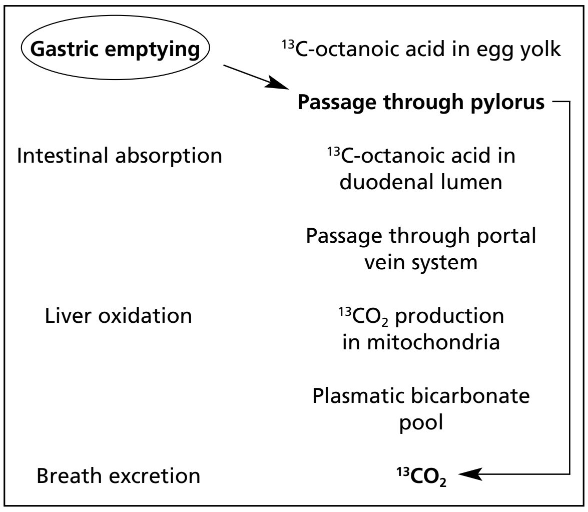

Figure 1. Sequential metabolic steps after ingestion of the ^{13}C-labeled baked egg yolk. The rate limiting step of the breath $^{13}CO_2$ excretion is represented by the gastric emptying of the yolk.

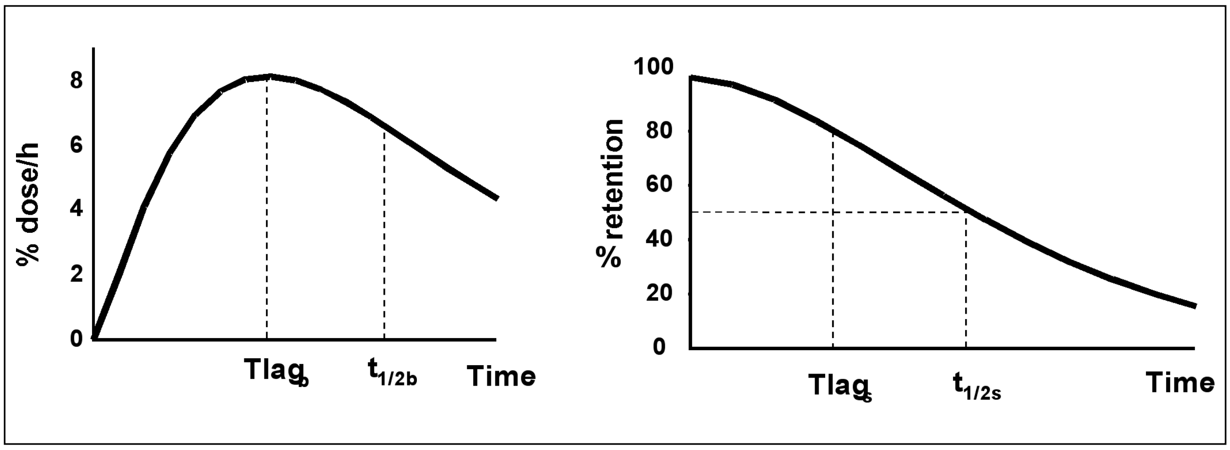

Figure 2. $^{13}CO_2$ breath excretion curve (in % dose/h) and scintigraphic curve (in % gastric radioactivity retention) after administration of an egg in which the yolk has been labeled with 100 mg of ^{13}C-octanoic acid and the white has been doped with 2 μCi of ^{99m}Tc-albumin colloid. Both curves are fitted by means of non linear regression analysis.

where z is the cumulative dose of $^{13}CO_2$ excreted in breath per hour; t is the time (in hours); m, k, and b are estimated regression constants, with m the total amount of $^{13}CO_2$ recovered when time is infinite. As the curve z (% cum-dose curve) is obtained after integration of a curve z' (% dose/h curve), the curve z' can be expressed as:

$$z' = mkbe^{-kt} (1- e^{-kt})^{b-1}$$

Non linear regression analysis is performed on the data of the curve z' to estimate m, k and b, using the least square method. This analysis can be easily performed by using the "Solver" procedure in Excel (Office, Microsoft). Two parameters can be derived from this formula:

$$t_{1/2exc} = (-1/k) \ln (1-2^{-1/b})$$

i.e. the area under the fitted curve until half of the dose of CO_2 is excreted of the cumulative $^{13}CO_2$ excretion when time is infinite, and

$$t_{lagexc} = (\ln\beta)/k$$

i.e. the time corresponding to the maximum of the curve z'.

Validation Versus Scintigraphy

In the original paper[7], Ghoos et al validated the ^{13}C-OBT against scintigraphy which is traditionally considered 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 of which the yolk was doped with 100 mg ^{13}C-octanoic acid and the egg white was labeled with 110 MBq of ^{99m}Tc albumin colloid. The egg was baked and ingested within 10 minutes with two slices of white bread and 5 g of margarine, followed immediately by 150 ml of water. Immediately after the ingestion of the standard meal, each subject underwent simultaneously scintigraphic scans and breath collection at regular intervals of time for two hours. Breath sampling lasted another two hours with 15-minute intervals. Two curves were obtained for each subject: the scintigraphic and the % dose/h curves. Using the formulas by Siegel[10] and Ghoos[7], four parameters were derived: $t_{1/2s}$ and $t_{1/2exr}$, and t_{lags} and t_{lagexc}. By means of the regression analysis, two models were obtained:

$t_{1/2exr} = 66 + 1.12\ 5\ t_{1/2s}$ (with r-value = 0.89 and p-value < 0.0001) and
$t_{lagexc} = 60 + 0.94\ 5\ t_{lags}$ (with r-value = 0.92 and p-value < 0.0001)

According to these model, $t_{1/2b}$ (i.e.: breath test determined gastric half emptying time) and t_{lagb} (i.e.: breath test determined lag phase) were calculated:

$t_{1/2b} = t_{1/2exr} - 66/1.12$ and
$t_{lagb} = t_{lagexc} - 60/0.94$

By definition, the correlation coefficients between $t_{1/2b}$ and $t_{1/2s}$ is 0.89 and between t_{lagb} and t_{lags} was 0.92. Based on data obtained from 42 normal healthy volunteers (22 women, 20 men; mean age 22 years), Ghoos[7] calculated a mean value of 72 ± 22 min for $t_{1/2b}$ and of 32 ± 20 min for t_{lagb}. Interestingly, both within-subject variability (interindividual variability) and between-subject variability (day to day variability) were small and not statistically significant.

Clinical Applications

The ^{13}C-OBT is a useful tool to evaluate gastric emptying either in physiological and in pathological states. The test has been proved to be sensitive enough to detect changes in gastric emptying induced by drugs such as motilin-receptor agonists, cisapride, anticholinergics, and octreotide. In one study[11], in which nine healthy volunteers were tested, erythromycin lactobionate (200 mg given i.v., 30 minutes before the OBT) significantly increased both $t_{1/2}$ and t_{lag}; on the contrary, propantheline, an anticholinergic drug (30 mg given p.o. 60 minutes before the OBT) significantly reduced $t_{1/2}$ but not t_{lag}. In another study[12], cisapride (10 mg p.o. the day before and 30 minutes before the OBT) was effective in decreasing significantly both $t_{1/2}$ and t_{lag} of solids in six healthy and six dyspeptic subjects with delayed gastric emptying. No influence of cisapride was seen on $t_{1/2}$ and t_{lag}, when a liquid test meal was used. Healthy subjects were also tested in two different days without prokinetic drugs and intra-individual variability was shown to be quite good (CV of $t_{1/2}$ for solids, 20%, and for liquids, 5%). In another study[13], subcutaneous injection of a single physiological dose (50 mg) of octreotide induced a marked delay in the gastric emptying of solids and liquids in healthy volunteers. This effect was not due to variation of absorption or metabolism of octanoic acid after administration of octreotide. All these studies[11-13] show that the OBT is able to assess the effect of drugs on gastric emptying parameters and represents a promising tool for clinico-pharmacological studies.

The OBT has been also used to evaluate the physiology of gastric emptying of solids and liquids in both adults and children. In one study[14], gastric emptying of a low caloric (150 kcal) test meal was similar in children and adults; however, increasing the caloric content of the test meal (up to 250 kcal) gastric emptying significantly decreased in a similar fashion in children and adults. Interestingly, when full cream milk (134 kcal) was used, a slower gastric emptying of the milk compared with the low caloric solid meal was observed in children but not in adults. Intestinal neuro-endocrine mechanisms following milk assumption could be different between children and adults. Gastric emptying of a mixed liquid-oil meal in healthy volunteers was investigated in another study[15]: the emptying of the oil phase of the meal occurred independently from the fluid phase. The increase of the amount of fat led to a decreased rate of emptying of the oil phase while the emptying of the fluid phase was unchanged. In non dyspeptic pregnant women, the OBT showed a gastric emptying of solids which was not significantly different from controls[16]. In pre-term infants, results of OBT were found to be similar to those reported using other techniques[17]. The OBT has been also employed to study the effects of gastric emptying on the rate of intraluminal lipolysis (tested with ^{13}C-"mixed trigliceride"-breath test) in patients with pancreatic insufficiency[18] and the rate of ureolysis (tested with ^{13}C-urea breath test) in *Helicobacter pylori* infected and uninfected subjects[19]. In the last study, gastric emptying was not significantly influenced by infection; on the contrary, the rate of gastric emptying had some influence on the urea breath test results modifying the time of contact of labeled urea with the bacteria.

The test has been successfully used in different pathological conditions. In patients with distal gastrectomy, an early dumping of the oil phase of the meal was observed compared to healthy controls[20]. During recovery from *hyperemesis gravidarum*, women had an accelerated gastric emptying compared to non dyspeptic pregnant women[21] which strongly correlated with thyroid hyperfunction[22]. This finding suggests that vomiting in pregnancy is not correlated with delayed gastric emptying, but rather with causes of central origin. Gastric emptying has been also evaluated by OBT in patients with non-ulcer dyspepsia[23]. About 30% of patients with non ulcer dyspepsia had a delayed gastric emptying as compared to controls. This figure is very similar to that found in a group of dyspeptic patients studied by the OBT[24]: almost one third of patients displayed a significant delay in gastric emptying which was significantly correlated with a group of symptoms such as postprandial fullness, nausea, and vomiting. In the same study, patients were also evaluated by means of the urea breath test but no correlation between delayed gastric emptying and presence of *Helicobacter pylori* infection was found. Children with celiac disease have been studied by the OBT before and after instituting a gluten-free diet[25]. A significantly delayed gastric emptying was observed in celiac patients at diagnosis compared with healthy age-matched controls. After 6 months of gluten-free diet, gastric emptying parameters returned to normal range in celiac patients. This finding suggests that gluten-related mucosal damage causes, through a neuro-hormonal mechanism, a significant alteration in gastric emptying of solids which normalizes when small intestinal mucosa restores.

Potential Drawbacks

The OBT has been validated against scintigraphy and a good correlation between the results obtained from the two techniques has been achieved. However, a report from Mayo Clinic in US found that this correlation was not so close as previously suggested. Choi et al[26] have repeated the same experience made in Leuven by performing simultaneous OBT and scintigraphy in 15 healthy subjects. They found that gastric emptying parameters from the two methods were not significantly correlated. Moreover, increasing breath test duration (up to 5 or 6 hours) yielded decreasing estimates of both $t_{1/2}$ and lag phase. A good reproducibility, however, was obtained with a mean coefficient of variation of $t_{1/2}$ of 20% between individuals and 12% within the same individual. They concluded that the OBT required further validation but it seemed useful for intra-individual comparisons. Different critiques have been made to this study[27]. First, data obtained from only 15 subjects should be regarded with caution especially when regression analysis is used. Second, US researchers used a labeled tracer which was different from that originally used by Ghoos et al[7]. Third, no data on r^2 coefficient obtained between the experimental and the fitted $^{13}CO_2$ excretion curve for each subject was given. Fourth, another study[27] performed by our group has obtained the same results of the Leuven group, confirming that the OBT is a reliable test to assess gastric emptying. Finally, in a paper published in 2000 by the same Mayo Clinic group, the Authors[28] used a new test meal incorporating a ^{13}C-labelled alga (Spirulina platensis). Breath test measurements and scintigraphic scans were performed simultaneously. US researchers found that this "new" [^{13}C] S platensis breath test was able to measure gastric emptying $t_{1/2}$ for solids with results comparable with those obtained from scintigraphy.

In conclusion, the OBT has been proposed in 1993 as a non invasive, reliable, and safe test for measuring the gastric emptying of solids. In view of the use of ^{13}C-labeled meals, the test has the potential for widespread clinical application in centers where gamma-camera facilities are not available. Moreover, pregnant women and children may be tested without biological hazard. Results from the OBT correspond to those obtained from scintigraphic method which is currently regarded as the "gold standard" for evaluating gastric emptying. The close correlation between breath test and scintigraphy has been definitely proven even by those who questioned on the validity of the test in the past. However, new studies are needed to validate a simple, "office-based" OBT with standardized ^{13}C-enriched test meals.

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