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GASTRIC EMPTYING OF A SOLID MEAL STARTS DURING MEAL INGESTION: COMBINED STUDY USING 13C-OCTANOIC ACID BREATH TEST AND DOPPLER ULTRASONOGRAPHY. ABSENCE OF A LAG PHASE IN 13C-OCTANOIC ACID BREATH TEST.

Itta M. Minderhoud
Marco W. Mundt
Jan M. Roelofs
Melvin Samsom

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ABSTRACT

Scintigraphy and the ^{13}C-octanoic acid breath test are both applied to assess gastric emptying. Using the ^{13}C-octanoic acid breath test, excretion curves show ^{13}C excretion immediately after ingestion of a solid egg meal, in contrast with scintigraphy where gastric emptying is observed after a lag phase. The aim of our study was to investigate whether transpyloric flow occurs during and directly after meal ingestion. Therefore, transpyloric flow was measured during and after ingestion of an egg meal labeled with ^{13}C-octanoic acid, using Doppler ultrasonography. The breath test was performed simultaneously, with samples taken at regular intervals. The first emptying episode was observed 6.9 (3.9–16.2) min after start of meal ingestion. A significant relation between recovery of ^{13}C and total duration of gastric emptying during the first 20 min was observed (partial correlation coefficient r = 0.80, $p < 0.001$). In conclusion, transpyloric flow starts during ingestion of a solid egg meal and results in detectable excretion of ^{13}C.

INTRODUCTION

Gastric emptying of solids is used to screen for gastric motor disorders and can be assessed using several techniques, among which gamma camera scintigraphy. Scintigraphic gastric emptying tests are used extensively in research and clinical practice and are regarded to be the gold standard in the evaluation of gastric emptying[1]. Recently, the ^{13}C-octanoic acid breath test has been introduced to assess gastric emptying of solids. This test is an indirect method, using octanoic acid enriched with ^{13}C, assuming that gastric emptying is the rate-limiting step in the ultimate delivery of $^{13}CO_2$ to the breath [1;2]. The ^{13}C-octanoic acid breath test has clear advantages when compared to scintigraphy. Patients are not exposed to radiation, the test can be performed at the bedside and in the community, and no gamma camera is required, which makes the breath test more accessible and cheaper. Gastric emptying is a pulsatile process. The most commonly used parameters to quantify gastric emptying of solids are the duration of the lag phase in minutes and the post-lag emptying rate in percentage emptied per time unit. Together these two parameters determine the t½, the time period in which 50% of the test meal has been emptied from the stomach. Since the rate of emptying is dependent on multiple factors such as meal composition, nutrient content, and particle size, it is important to use standardized meals in all techniques. The lag phase in the gastric emptying of solids is described as the time period between meal ingestion and start of gastric emptying. For the scintigraphic gastric emptying test, several mathematical models to calculate and/or express the lag phase are being applied. In 1983, Collins et al. [3] defined the lag phase as the time period between meal ingestion and start of gastric emptying when food entered the duodenum, marked by the first activity of the radiolabel in the region of interest in the duodenum. The lag phase reflected both redistribution of the meal from fundus to antrum and the time taken to grind solid food to small particles. Other definitions are used by Maes and by Camilleri [4–9], who defined the lag phase as the time period in which respectively the first 5% or the first 10% of the test meal are emptied from the stomach into the duodenum. Several mathematical models were proposed to predict gastric emptying parameters in the ^{13}C-octanoic acid breath test based on the scintigraphic emptying curve [2, 6,10-14]. The model applied to analyze the breath test is critically important. Choi et al. [5] found that mathematical analysis seems to provide the greatest error in situations associated with a prolonged lag phase and a rapid post-lag gastric emptying. These models, however, assume that the lag phase calculated using the ^{13}C-octanoic acid breath test is comparable to the lag phase seen using scintigraphy. This assumption is questionable since studies comparing both techniques show considerable ^{13}C excretion in breath samples taken 15 min after ingestion of an egg meal (no lag phase is observed in the excretion curve) while no or only small amounts of radioisotope were detected at the level of the duodenum by the gamma camera after ingestion of the same egg meal (resulting in a lag phase in the scintigraphic curve). The aim of our study was therefore to investigate whether transpyloric flow can be detected by Doppler ultrasonography, during and directly after ingestion of a standard egg meal, which results in the absorption of ^{13}C in the duodenum, explaining the absence of a distinct lag phase in the ^{13}C excretion curves.

MATERIALS AND METHODS

Subjects

Ten healthy subjects (5 male, 5 female), median age 27 (range 22–42) years, and median Quetelet index 20.9 kg/m^2 (range 18.8– 22.4), participated in this study. None had previously undergone abdominal surgery, or was receiving medication other than oral contraceptives. Screening procedures to ensure the healthy status of the subjects relied primarily on self-reported answers to standard questions about their medical condition.

Study Protocol

At 08:00 h, subjects attended the motility laboratory after an overnight fast and were placed in a sitting position leaning slightly backwards (at a 120° angle) during the experiment. A standard solid meal had to be ingested within 10 min (median 9.0 min; range 7.1-10.0 min). Every 2 min, 30 ml water was ingested by the subject. Transpyloric flow was assessed for 20 min using Doppler ultrasonography (US), during and after ingestion of the meal. The ^{13}C-octanoic breath test was performed simultaneously with the Doppler US, and after that time period up to a total of 4 h. Breath samples were taken at regular intervals and analyzed by an isotope ratio mass spectrometer (Breathmat; Finnegan, Bremen, Germany) [1].

^{13}C-Octanoic Acid Breath Test

The test meal consisted of two fried eggs, one slice of bread, 5 g margarine, and 150 ml water (total caloric value of 294 kcal and a nutrient composition of 16 g protein, 16 g carbohydrate, 18 g fat). The egg yolk of one egg was labeled with 100 mg ^{13}C-sodium-octanoic acid (598 µmol; Campro Scientific, Veenendaal, The Netherlands), dissolved in 1 ml distilled water. Breath samples were taken at baseline, before the meal and from start of ingestion of the meal every 2 min the first 30 min, every 5 min for the next 30 min and every 15 min thereafter up to 4 h.

Spit-and-Chew Test

One subject performed two breath tests using two different protocols.
For the first test, the above-described study protocol was applied. During the second breath test the chewed solid test meal enriched with ^{13}C-octanoic acid was spit out before swallowing. No Doppler US was done during this test. Breath samples were taken at baseline, before the meal and from start of ingestion of the meal every 2 min for 30 min.

Summarizing ^{13}C-Octanoic Acid Breath Test Data Values

The $^{13}CO_2$ enrichment of breath was measured by an isotope ratio mass spectrometer and was expressed as difference between the $^{13}CO_2/^{12}CO_2$ ratio of the sample and the standard. Quantity of ^{13}C appearing in breath per minute was calculated as ^{13}C (µmol/min) = delta over the baseline (DOB) x

0.0112372 x VCO_2, where 0.0112372 is the isotope abundance of the limestone standard, Pee Dee Belemnite. Basal CO_2 production rate, VCO_2, was corrected for individual basal metabolic rates according to age, gender, height and weight using the algorithms of Schofield [15]. Analysis of the total breath test was performed using the Utrecht model [10,13] in which after a non-linear curve fit procedure (m*k*b*e–kt(1– e–k.t)b–1; t =time; m, k, b being regression constants) interpolated data from the fitted curve are used to determine the lag time and the half emptying time by a regression equation. This equation was obtained by simultaneously performed breath tests and scintigraphic gastric emptying tests in a previous study [13]. The ^{13}C recovery at 10 and 20 min was calculated as area under the excretion curve of the non-fitted data.

Doppler Ultrasonography

Transpyloric flow was measured from time zero (start of meal ingestion) for a total 20 min, during and after ingestion of the meal. The applied duplex scanner (Pie Medical, with a 3.5-MHz curved array probe) enabled velocity curves of the Doppler recordings and real-time ultrasound images of antral motility and transpyloric flow to be visualized simultaneously, since the US probe was positioned at the level of the transpyloric plane with the antrum, the pylorus, and the proximal duodenum visualized. The angle between the Doppler beam and the transpyloric direction of flow was always <60°. For quantitative measurements of flow velocity and timing, pulsed Doppler was used. All ultrasound and Doppler measurements were performed by one investigator (M.W.M.). The real-time ultrasound images and velocity curves were all recorded on videotape for later analysis. An episode of gastric emptying or reflux was defined as flow across the pylorus with a mean velocity of 110 cm/s lasting 11 s. The following variables were measured: (1)

time to first gastric emptying: the first occurrence of gastric emptying after the start of ingestion of the meal; (2) number of gastric emptying or reflux episodes: the total number of gastric emptying episodes or reflux episodes occurring in the 20-min period was counted; (3) duration of the gastric emptying or reflux episodes: total duration of gastric emptying or reflux in seconds during the 20-min period was calculated. Emptying index (number of gastric emptying episodes ! mean duration) and reflux index (number of reflux episodes ! mean duration) per time interval of 2 min were calculated.

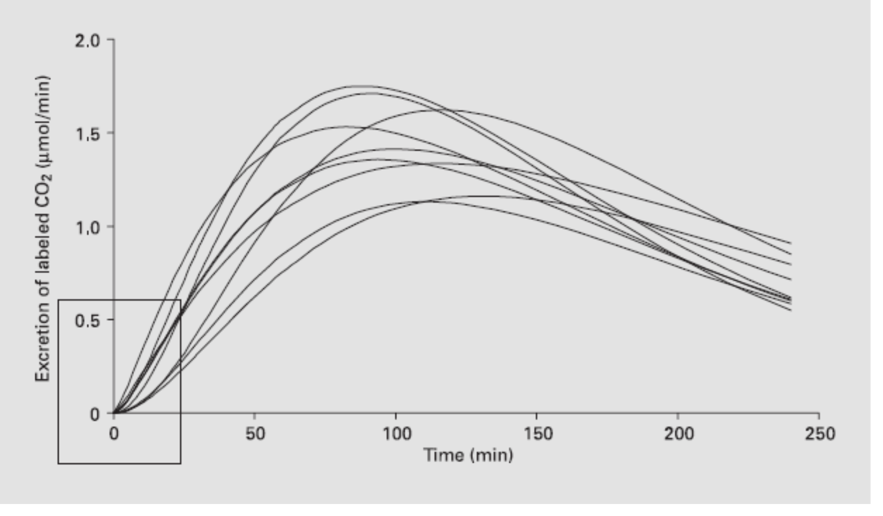

Figure 1. Fitted $^{13}CO_2$ excretion curves of 9 subjects during the total 4 h of the breath test. A frame is set around the first 20 min of the test.

Statistical Analysis

Data were presented as median and range unless stated otherwise. Partial correlations were calculated between excretion of $^{13}CO_2$ in the breath samples and the seconds of gastric empting or reflux detected by Doppler US, and for the emptying index and the reflux index. Calculation of a partial correlation is a statistical method to correct for the subjects effect, resulting in an overall residual (e.g. excretion factor) which is correlated with the dependent variable (e.g. seconds of gastric emptying). A p value of <0.05 was considered to indicate significance. Statistical analysis was performed with the SPSS Version 11.0 for Windows.

RESULTS

^{13}C-Octanoic Acid Breath Test

The median gastric emptying half-time was 100.2 (66.0–150.1) min, calculated for 9 subjects, according to the Utrecht model. In 1 subject the Utrecht model could not be applied because of the poor curve fitting of the nonlinear regression procedure. Based on the half-time, 2 subjects had a delayed gastric emptying. Figure 1 shows the fitted $^{13}CO_2$ excretion curves of the 9 subjects during the total 4 h of the breath test. Figure 2 shows the non-fitted $^{13}CO_2$ excretion curves of the 10 subjects during and 10 min after ingestion of the meal. The ^{13}C-octanoic acid test showed a median recovery of 0.5 µmol (0.0–1.5) 10 min after and 3.2 µmol (0.6–5.2) 20 min after start of meal ingestion. This was respectively 0.2% (0–0.5) and 1.4% (0.3–2.0) of the excreted ^{13}C measured after 4 h. $^{13}CO_2$ excretion was detected in most of the subjects during ingestion of the meal. The curves of 3 subjects seem to fluctuate around the baseline, suggesting they had not significantly excreted $^{13}CO_2$ during the meal. The excretion curve of the spit-and-chew experiment performed by 1 subject fluctuated around the baseline during and after the meal, which suggests absence of ^{13}C absorption by mucosal cells in the oral cavity (fig. 3).

Doppler Ultrasonography

The first gastric emptying episode was observed 6.9 (3.9–16.2) min after the start of meal ingestion. Figure 4 represents the data obtained from the Doppler US. The median emptying index and the median reflux index were respectively 3.38 (0.85–8.65) and 1.49 (0.00–7.72).

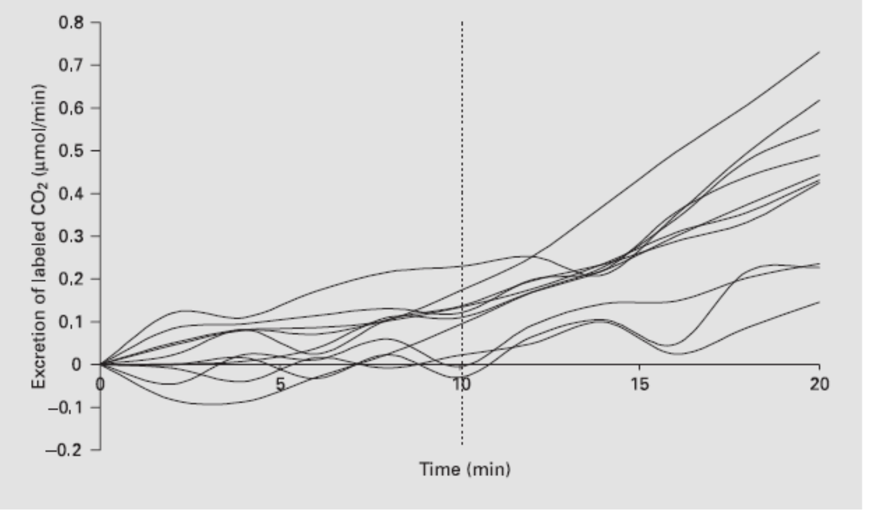

Figure 2. Non-fitted 13CO2 excretion curves of10 subjects during the first 20 min of the breath test. Vertical line at 10 min marks the end of meal ingestion.

In all subjects the emptying index was greater than or comparable to the reflux index, with the exception of subject 10 in whom the reflux index was greater than the emptying index. During the first 20 min a significant relation was observed between the excretion of ^{13}C and total number of seconds of gastric emptying (partial correlation coefficient $r = 0.80$, $p < 0.001$) (fig. 5). Moreover, the net gastric emptying index was calculated by subtraction of the reflux index from the gastric emptying index. A significant relation was found between the net gastric emptying index and excretion of ^{13}C for the total group (partial correlation coefficient $r = 0.42$, $p < 0.001$). After exclusion of subject 10, a stronger positive partial correlation of $r=0.71$ ($p=0.001$) was observed.

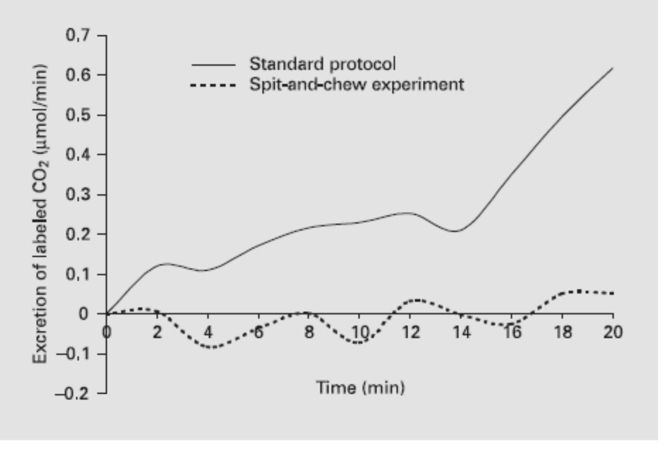

Figure 3. Non-fitted 13CO2 excretion curves of two breath tests following different protocols, during the first 20 min, performed by 1 subject.

DISCUSSION

In this study, we have shown by means of the ^{13}C-octanoic acid breath test and simultaneously performed Doppler US that gastric emptying of a fried egg meal starts during ingestion of the meal. Gastric emptying of the meal, as detected by Doppler US, results in absorption of ^{13}C in the duodenum, followed by excretion of $^{13}CO_2$ in breath, explaining the absence of a lag phase in the ^{13}C-octanoic acid breath test. Octanoic acid is a fast metabolized 8-carbon fatty acid, found in dietary fats. It is readily solubilised in egg yolk, which is lipophilic in nature.

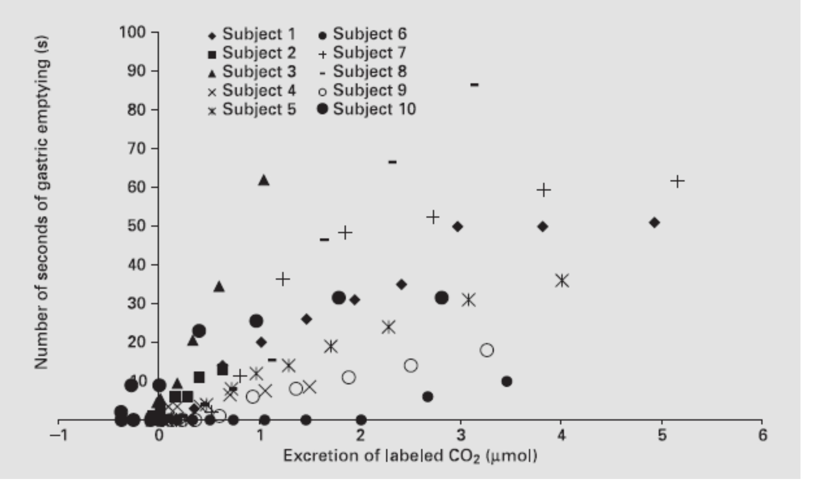

Figure 4. Number of seconds of gastric emptying or reflux detected by Doppler US, during the first 10 and the total 20 min after start of meal ingestion. GE = Gastric emptying; RF = reflux. Horizontal bars with values represent median number of seconds.

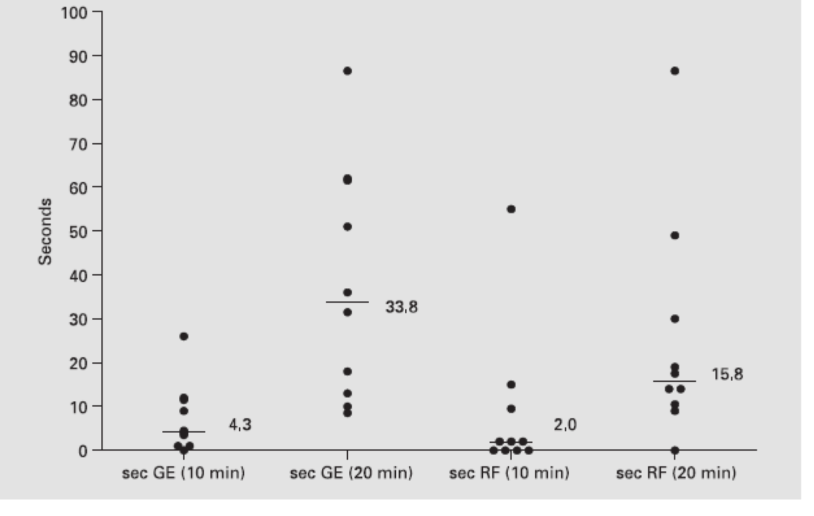

Figure 5. Partial correlation between the excretion of the 13C detected in breath samples and the total time that gastric emptying was detected with Doppler US.

After disintegration of the egg yolk in the duodenum, ^{13}C-octanoic acid is rapidly absorbed by the intestinal cells and transported to the liver, where it is preferentially oxidized to $^{13}CO_2$ [2]. The early detection of ^{13}C in the breath samples after ingestion of an egg meal can be explained in several ways; first, the stable isotope may separate from the solid phase of the meal, dissolve in the liquid phase, and empty with the 150 ml of water ingested with the meal. This may result in an erroneous assessment of the solid gastric emptying. However, Ghoos et al. [2] showed that 30 min after incubation in gastric juice at 37°C, 98.6% of the octanoic acid is still bound to the solid phase of the test meal, and more than 95% is still bound after 180 min. These findings are comparable to those obtained using radioactive isotopes incorporated in a solid meal [8]. Therefore, it is unlikely that the binding characteristics of the isotope to the substrate explains the discordance between the immediate $^{13}CO_2$ excretion in breath, minutes after meal ingestion and the lag phase seen using the scintigraphic gastric emptying test. A second hypothesis is that the octanoic acid with the stable isotope is already absorbed by the buccal mucosa, causing early $^{13}CO_2$ excretion, resulting in an inaccurate assessment of the solid emptying. To exclude this, a second breath test was done by 1 subject, using the spit-and-chew technique. The excretion curve of this experiment fluctuated around the baseline suggesting there was no absorption of ^{13}C-octanoic acid by the mucosal cells in the oral cavity. A third supposition is that gastric emptying already occurs during and directly after ingestion of the meal, as demonstrated in this study. During and directly after ingestion of a standard egg meal, $^{13}CO_2$ was excreted in breath, while transpyloric flow was visualized and confirmed by Doppler US. This is in line with the findings of Samsom et al. [16] who showed that gastric emptying already starts during ingestion of a high-caloric liquid nutrient meal, measured by Doppler US, which results in absorption of nutrients. It is conceivable that in the present study, solid particles that have been masticated to small particles, are emptied soon after ingestion. These small particles may not be detected by the scintigraphic technique, even when the measurement is done during meal ingestion. We observed a median absolute recovery of 0.5 µmol after 10 min and 3.2 µmol after 20 min in the breath test. Although the detected amount ^{13}C was small, it exceeded the lower detection limit of the isotope ratio mass spectrometer 3 times at 10 min and 10 times at 20 min after start of ingestion of the meal.

In conclusion, we have shown that gastric emptying of a fried egg meal starts during or directly after ingestion of the meal. These findings suggest that the discordance in lag phase when assessed by the ^{13}C-octanoic acid breath test or by the scintigraphic gastric emptying test are caused by the sensitivity of the technique used. These observations should be taken into consideration when statistical models are applied to calculate the lag phase using the ^{13}C-octanoic acid breath test.

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REFERENCES

(1) Kim DY, Myung SJ, Camilleri M: Novel testing of human gastric motor and sensory functions: Rationale, methods, and potential applications in clinical practice. Am J Gastroenterol 2000;95:3365–3373.

(2) Ghoos YF, Maes BD, Geypens BJ, Mys G,Hiele MI, Rutgeerts PJ, Vantrappen G: Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. Gastroenterology 1993;104:1640–1647.

(3) Collins PJ, Horowitz M, Cook DJ, Harding PE,Shearman DJ: Gastric emptying in normal subjects – A reproducible technique using a single scintillation camera and computer system. Gut 1983;24:1117–1125.

(4) Choi MG, Camilleri M, Burton DD, Zinsmeister AR, Forstrom LA, Nair KS: Reproducibility and simplification of 13C-octanoic acid breath test for gastric emptying of solids. Am J Gastroenterol 1998;93:92–98.

(5) Choi MG, Camilleri M, Burton DD, Zinsmeister AR, Forstrom LA, Nair KS: [13C]octanoic acid breath test for gastric emptying of solids: Accuracy, reproducibility, and comparison with scintigraphy. Gastroenterology 1997;112: 1155–1162.

(6) Lee JS, Camilleri M, Zinsmeister AR, Burton DD, Kost LJ, Klein PD: A valid, accurate, office-based non-radioactive test for gastric emptying of solids. Gut 2000;46:768–773.

(7) Camilleri M, Malagelada JR, Brown ML, Becker G, Zinsmeister AR: Relation between antral motility and gastric emptying of solids and liquids in humans. Am J Physiol 1985;249:G580–G585.

(8) Camilleri M, Hasler WL, Parkman HP, Quigley EM, Soffer E: Measurement of gastrointestinal motility in the GI laboratory. Gastroenterology 1998;115:747–762.

(9) Maes BD, Ghoos YF, Geypens BJ, Mys G, Hiele MI, Rutgeerts PJ, Vantrappen G: Combined carbon-13-glycine/carbon-14-octanoic acid breath test to monitor gastric emptying rates of liquids and solids. J Nucl Med 1994;35: 824–831.

(10) Samsom M, Vermeijden JR, Smout AJ, Van Doorn E, Roelofs J, van Dam PS, Martens EP, Eelkman-Rooda SJ, Berge-Henegouwen GP: Prevalence of delayed gastric emptying in diabetic patients and relationship to dyspeptic symptoms: A prospective study in unselected diabetic patients. Diabetes Care 2003;26: 3116–3122.

(11) Maes BD, Mys G, Geypens BJ, Evenepoel P, Ghoos YF, Rutgeerts PJ: Gastric emptying flow curves separated from carbon-labeled octanoic acid breath test results. Am J Physiol 1998;275:G169–G175.

(12) Lee JS, Camilleri M, Zinsmeister AR, Burton DD, Choi MG, Nair KS, Verlinden M: Accurate, simple measurement of gastric emptying by 13C-octanoic acid breath test in diabetics. Gastroenterology 1999;116:A966.

(13) Samsom M, Roelofs JM, van Rijk PP, van Doorn E: Validation of the 13C-octanoic acid breath test versus scintigraphy in healthy subjects and patients with diabetes mellitus. Gastroenterology 2001;120:A465.

(14) Viramontes BE, Kim DY, Camilleri M, Lee JS, Stephens D, Burton DD, Thomforde GM, Klein PD, Zinsmeister AR: Validation of a stable isotope gastric emptying test for normal, accelerated or delayed gastric emptying. Neurogastroenterol Motil 2001;13:567–574.

(15) Schofield WN: Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985;39(suppl 1):5–41.

(16) Samsom M, Hausken T, Renooij W: Early gastric emptying results in absorption of nutrients in healthy subjects. Gastroenterology 2000; 118:2374.