Validation of the Lactose-[^{13}C]Ureide Breath Test for Determination of Orocecal Transit Time by Scintigraphy

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The breath test using oral administration of a ^{13}C-labeled substrate, lactose-ureide (LU), to measure orocecal transit time (OCTT) was validated against ^{99m}Tc-scintigraphy. Although LU is not absorbed in the human small intestine, colonic bacteria readily metabolize LU, producing ^{13}C-labeled CO_2. The time at which $^{13}CO_2$ appears in breath corresponds to the OCTT. **Methods:** Twenty-two healthy volunteers ingested a meal labeled with ^{99m}Tc and ^{13}C-LU. Scintigraphy was performed over 8 h at time intervals of 10 or 15 min. OCTT with scintigraphy was defined as the time at which at least 10% of the label had entered the colon. Breath samples were obtained every 10–15 min for 10 h and measured by isotope ratio mass spectrometry. OCTT was defined as the time of first significant increase above baseline. The results were compared using correlation and Altman-Bland statistics. **Results:** 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. **Conclusion:** The breath test using ^{13}C-LU is a valid alternative to scintigraphy techniques for measuring OCTT.

Key Words: orocecal transit; ^{13}C breath test; ^{99m}Tc-scintigraphy

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Physiological events in the small intestine are complex and difficult to study and include many intrinsic functions. The small bowel serves as its own motor, its own food absorptive capacity, its own conduit and seems to influence propulsive action of other organs (ileal brake). Despite difficulties, significant advances have been made in describing the propulsion of the intestinal contents, the contractions responsible for these movements and the regulation of motility. Numerous techniques have been used to record and describe motor activity of the small intestine including radioscintigraphy (*1–3*), manometry and electromyography (*4–6*), echoplanar MRI (*7*), metal sphere detection (*8*), tracing of ingested magnetic material (*9,10*), blood analysis (*11,12*) and breath hydrogen after ingestion of nondigestible carbohydrates (*13*). With each of these techniques different aspects of the same basic event may be recorded and at different regions of the small intestine. In the clinical context, however, it is of great value to have a technique to monitor global intestinal motor activity in a simple and reliable manner.

Accurate measurement of the orocecal transit time (OCTT) is an important step in achieving better insight in detecting dysmotility of the upper gastrointestinal (GI) tract (*14–24*). Moreover, total intestinal transit influences the functions of the colon by the supply of substrates (*25,26*). Efficiency of colonic fermentation (i.e., the metabolism of unabsorbed dietary components) is greatly influenced by the motility of the upper intestinal tract (*27,28*). Hence, it is of major importance to be able to measure transit times, OCTT in particular, using a relatively simple, reproducible test. Methods for measuring intestinal transit should not interfere with normal GI functions and should cause minimal discomfort for the patient. Radioscintigraphy is usually considered the reference technique for measuring OCTT. Several drawbacks, however, limit its application in routine practice. Expensive equipment, time and specialized personnel are required, and the use of radioactive isotopes is associated with some irradiation (<3 mSv). It is not preferable to repeat the technique at short intervals in children, and in pregnant women the use of this technique should be avoided completely. To overcome these problems, the hydrogen breath test has been advocated to measure mouth-to-cecum transit time (OCTT). Many authors, however, have shown that osmotically active carbohydrates, like the frequently used lactulose, alter transit time through the small intestine.

Breath tests involving the stable carbon isotope, ^{13}C, have been successfully introduced for many purposes, including gastric emptying (*29*). Glycosyl ureides have been studied extensively for their physical and chemical properties (*30–34*). The enzymes of the brush border of the human intestine are not able to split the bond of sugars to urea. Because glycosyl ureides are only slightly absorbed in the small bowel without further metabolism, they reach the large bowel unaltered. The colonic flora, by contrast, splits the bond of sugars to

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urea (*35,36*), after which further metabolism by colonic bacteria can take place. These properties make these ureides well suited to be used as markers for the measurement of intestinal transit time using a breath test with the urea moiety labeled with a carbon isotope.

Bacteria in the colon will split and metabolize glucosylureide and produce, among other products, CO_2. If the urea moiety of the molecule is labeled with ^{13}C, the isotope will be set free in the breath of the host as $^{13}CO_2$. Therefore, breath sampling after oral administration of the labeled molecule, at regular time intervals for an appropriate length of time, 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 cecum.

It was the aim of this study to investigate the validity of the lactose-[^{13}C]ureide (LU) breath test (LUBT) by direct comparison with a well-established method, namely scintigraphy using ^{99m}Tc-sulfur colloid.

MATERIALS AND METHODS

Subjects

Twenty-two healthy volunteers (11 women, 11 men; age range 22–58 y) were studied simultaneously with scintigraphy and breath test. They were all nonsmokers and had no history or symptoms of GI disease. Women were all studied in the first week of the menstrual cycle. Subjects who had used antibiotics in the 3 mo before the study were excluded. Also, no medication having any effect on GI functions was allowed. Written, informed consent was obtained from all subjects. The study protocol was approved by the ethics committee of the Catholic University of Leuven.

Breath Test

The methodology of the LUBT described by Heine et al. (*35*) and Wutzke et al. (*36*) was used with slight modification. The day before the test, 1 g unlabeled LU was administered in a glass of water three times (morning, noon and evening) to induce the proper enzyme activity in the colonic bacteria. On the morning of the test, after an overnight fast, the subjects were given a test meal consisting of one scrambled egg, two slices of white bread and one glass of water. Five hundred milligrams of the marker molecule LU were mixed with the total egg before baking. Two breath samples for basal ^{13}C excretion were obtained in exetainers (Europa Scientific, Crewe, UK) before ingestion of the test meal. Sampling was performed by having the subjects blow through a drinking straw into the exetainer. After ingestion of the test meal within 10 min, time was started for breath sample collection. During the first hour, a ^{13}C sample was obtained every 10 min. ^{13}C sampling continued for another 9 h every 15 min (total sampling time 10 h). Four hours after the test meal was eaten, one sandwich with cheese or ham was eaten with one glass of water. No other food or drink was allowed until 8 h after completion of the test meal, i.e., 2 h before sampling ended. ^{13}C samples were measured in the ABCA 20–20 IRMS (Europa Scientific). Results in isotope ratio mass spectrometry of ^{13}C are expressed as delta in permil. $\delta^{13} = (S/R - 1)1000$ with S and R the isotope ratios (^{13}C)/(^{12}C) in the sample and the internationally agreed reference pee dee belemnite, respectively.

To assess OCTT with the ^{13}C label, calculations were performed directly on measured delta values. OCTT was taken as the time at which, in breath, a significant increase from the background in ^{13}C was seen. For this purpose a statistical measure of significance was assumed: 2.5 times the SD of all previous points above the running average of all previous points.

Scintigraphy

In the test meal egg, 37 MBq ^{99m}Tc-sulfur colloid (Ultra Technicow; Mallinckrodt Inc., Petten, The Netherlands) were mixed in as a marker for scintigraphy. Measurement of the activity in the GI tract was performed using a dual-head, gamma camera with low-energy, parallel-hole collimators. Scintigraphy information was obtained by scanning every 10 min for the first hour after the test meal and then every 15 min for the next 7 h, bringing the total scintigraphy sampling time to 8 h. OCTT according to scintigraphy was assumed as the first sampling point in time at which 10% or more of the total activity was detected in the cecal region.

Statistics

The relationship between scintigraphy and breath test was evaluated by Spearman correlation analysis and Altman-Bland statistics. Differences between men and women in OCTT were assessed using Student *t* test.

RESULTS

LU was found to be tasteless, both as a watery solution and integrated in the egg of the test meal. The integration of the marker in the test meal did not cause any problems. The low doses of unlabeled induction substrate and of ^{13}C-labeled substrate were not found to cause any intestinal discomfort in any of the subjects.

For the breath test, unambiguous interpretation of OCTT in all subjects was possible using the criterion of significant increase above baseline. Figure 1 shows an example of a breath $^{13}CO_2$ excretion curve, with OCTT indicated.

The maximal increase (peak value) in ^{13}C abundance in breath, expressed as delta value, varied between 3.4 and 27.15 permil. The time of maximal increase in ^{13}C excreted in breath was 421 ± 99 min and showed a correlation of $r = 0.76$ (Spearman) with OCTT ($P = 0.0001$).

No significant difference was found between female and male subjects with OCTT by breath test (289 ± 58 min and 295 ± 61 min, respectively). Also, no significant sex-determined difference was observed with scintigraphy.

The mean value for OCTT assessed by the LUBT for all subjects was 292 ± 58 min (mean ± SD), whereas for scintigraphy a value of 283 ± 53 min was found. Correlation of both methods was highly significant, with $r = 0.94$ ($P = 0.0001$) (Fig. 2). The difference between methods against the mean of both methods shown in Figure 3 confirms this correlation. The equivalence of breath test and scintigraphy is also shown by the poor correlation ($r = 0.30$, $P > 0.1$) between the difference and the mean OCTTs of these methods. The linear regression of the data in Figure 3 does not display a y-axis intercept significantly different from 0 min.

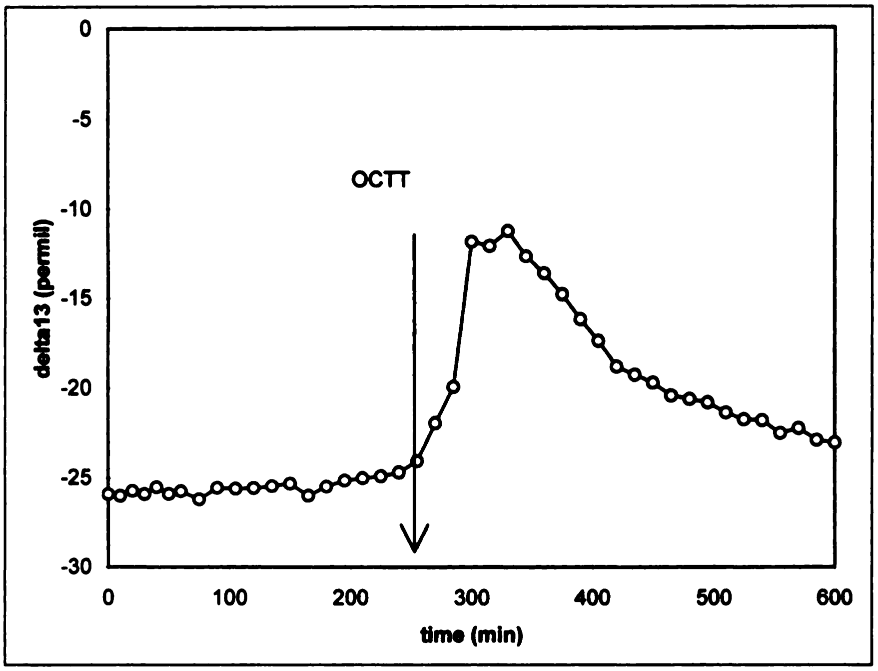

FIGURE 1. Example of ^{13}C breath excretion curve from LUBT in one healthy volunteer with indication of OCTT.

DISCUSSION

The criterion for determining OCTT using ^{13}C-LU allowed unambiguous and objective assessment. This is important, because it was found that on scintigraphy it is sometimes difficult to unambiguously interpret an image.

The mean value for OCTT found in this study is in accordance with the values found by Heine et al. (*35*) (6.0 ± 2.2 h) but is considerably longer than that found by Wutzke et al. (*36*) (3.02 ± 1.4 h) also using the LUBT. In these studies, no validation against scintigraphy was performed, and the labeled LU was (unlike in this study) not integrated in a solid meal and different meals were used. The lack of significant sex-determined difference in OCTT confirms the findings of Degen and Phillips (*37*), in which mean OCTTs for men and women were found to be 254 and 256 min, respectively. These scintigraphically determined values are in agreement with ours. Also, the values reported by Camilleri et al. (*38*), using radiolabeled fiber and pellets, and by Lartigue et al. (*39*), both using scintigraphy, are in accordance with these data.

A large range in increase in ^{13}C abundance in breath in this group of healthy volunteers might indicate a large interindi-

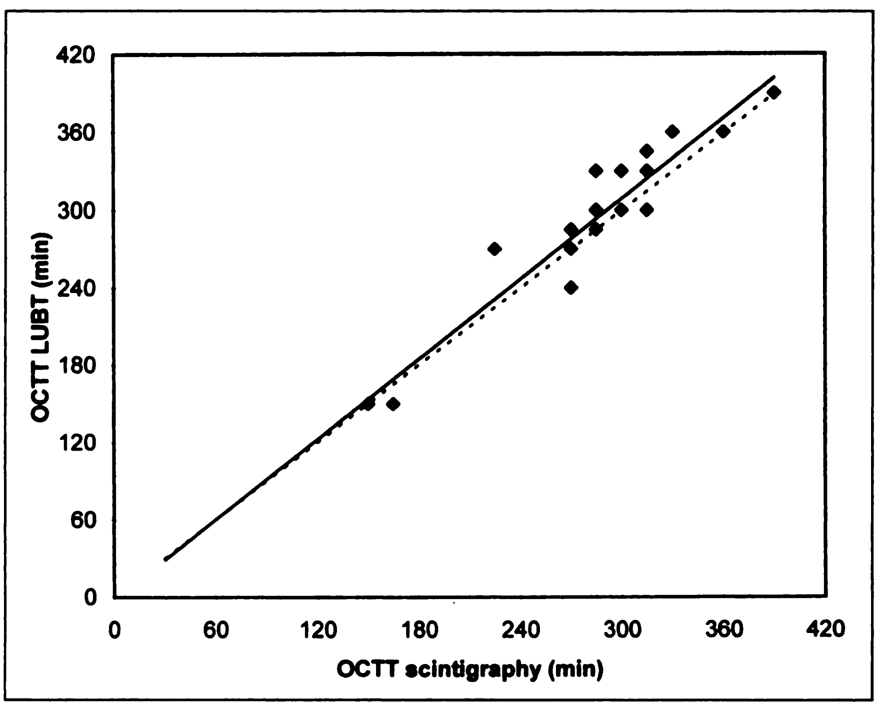

FIGURE 2. Correlation of OCTT by LUBT and OCTT by scintigraphy (broken line = line of equality; unbroken line = regression line).

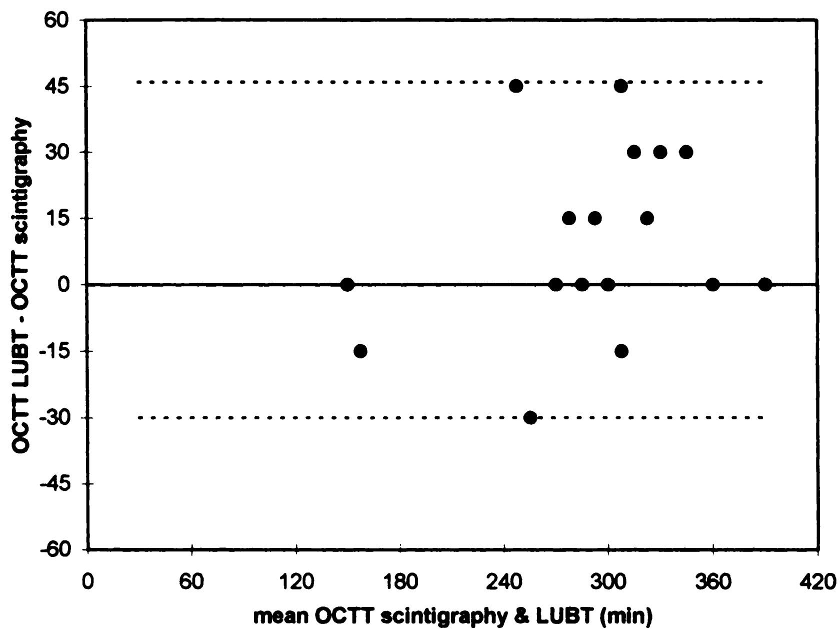

FIGURE 3. Difference in OCTT between methods against mean of methods (broken line = mean difference ± 2 SD).

vidual variability in bacterial enzyme activity, despite the identical induction regime of unlabeled LU the day before the test. This large range might also be the result of interindividual variability of transit. This certainly deserves further study, especially because the exact mechanism of bacterial metabolism of LU and glucose-ureide is unclear. The more fundamental study of the underlying biochemistry of colonic bacterial metabolism certainly may not be neglected and deserves close attention. The significant correlation between OCTT and time of peak value in the breath test, however, seems to indicate that the shape of the breath test curve, independently of its height, is a reflection of the pace at which the chyme reaches the cecum, rather than of bacterial activity. A similar correlation was observed in the earlier study with ^{13}C-LU (*35*).

The highly significant correlation (r = 0.94) of OCTT measured using LUBT and scintigraphically determined OCTT in these healthy volunteers shows that the breath test is a valid alternative to scintigraphy. The Altman-Bland statistics further confirm this and additionally show that there is neither a proportional nor a constant difference between the methods.

CONCLUSION

The LUBT is an excellent alternative to ^{99m}Tc-scintigraphy for the measurement of OCTT. Further research in pathological conditions and under pharmacological modulation of transit should be undertaken for further validation.

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