

Effect of a test meal on the intragastric distribution of urea in the ^{13}C -urea breath test for *Helicobacter pylori*

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Abstract

Test meals are invariably used in the ^{13}C -urea breath test (UBT) but their effect on the intragastric distribution and gastric residence time of urea given in the test is unknown. The site of *Helicobacter pylori* urease measured in the test is unknown and whether the test measures total or regional gastric urease is uncertain. This study reports the results of paired UBTs with simultaneous gastric distribution studies, one with and one without a fatty test meal, two weeks apart on seven *H pylori* infected subjects. The test meal did not affect UBT results at 10 minutes, but increased values at 30 minutes and thereafter. The amount of scintigraphic label in the antrum at 10 minutes was also unaffected by the meal but increased at 30 minutes and thereafter, whereas the amount in the body/fundus was greatly increased both at 10 minutes and throughout the test. There was considerable variation in intragastric distribution of urea between subjects, both with and without the test meal. This study shows that a test meal profoundly affects intragastric distribution of urea solution in the UBT, and increases UBT values at 30 minutes and later. Variability between subjects, however, means that accurate measurement of total or regional gastric urease is probably unrealistic.

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The urea breath test (UBT) is a simple, non-invasive test for *Helicobacter pylori* infection.^{1,2} Isotopically labelled urea is ingested and, if *H pylori* is present in the stomach, its urease enzyme catalyses urea hydrolysis producing labelled carbon dioxide. This is absorbed and transported to the lungs for excretion, and hence can be measured in breath samples. Two carbon isotopes are commonly used to label urea, ^{13}C and ^{14}C . ^{13}C -UBTs are conventionally performed after a test meal,^{1,3} and although this is also the case for most ^{14}C -UBTs,^{2,4} some argue that the meal can be omitted, thus simplifying and shortening the test.⁵

Urea breath tests are known to be both sensitive and specific for *H pylori* infection,³⁻⁵ but it is unclear to what extent they are quantitative. One reason for this is that the intragastric

distribution of urea given in the test is unknown, and thus the site of *H pylori* urease measured by the test is also unknown. In this study we aimed to assess the intragastric distribution of isotopically labelled urea in the ^{13}C -UBT both with and without a test meal. In particular, because of differences in inflammation between antrum and body/fundus,^{6,7} and possibly differences in bacterial load,⁸ we were interested to determine the relative distribution of urea to these two regions of the stomach. We hoped that the study would provide some insight into whether UBTs, either with or without a test meal, could potentially give an accurate measure of global or regional urease activity, and to what extent a test meal was useful or necessary.

Methods

SUBJECTS

Nine *H pylori* positive subjects, five male, median age 67 years (range 44-71) took part in the study. Exclusion criteria included past or present gastrointestinal disease, previous abdominal surgery, diabetes mellitus, the taking of drugs with an effect on gastrointestinal motility, the taking of antibiotics, bismuth compounds or omeprazole in the six weeks before the study, and a change in dose of histamine 2 receptor antagonists in the four weeks before the study.

Written informed consent was obtained from each subject. Approval was obtained from the University Hospital, Nottingham ethical committee and the study was conducted according to the guidelines of the Declaration of Helsinki (Hong Kong Amendment).

PROTOCOL

Each subject attended for two randomly ordered study days, two weeks apart. On each study day subjects received a ^{13}C -UBT, on one occasion with, and on the other without, a test meal. During each UBT a simultaneous scintigraphic gastric distribution study was performed.

Alcohol was forbidden for 48 hours before the study day, food from 8 pm the day before, and liquid from 11 pm. Subjects arrived at 8 am on the study day and had anterior and posterior reference markers attached over the right costal margin. They then brushed their teeth and washed out their mouth with antiseptic

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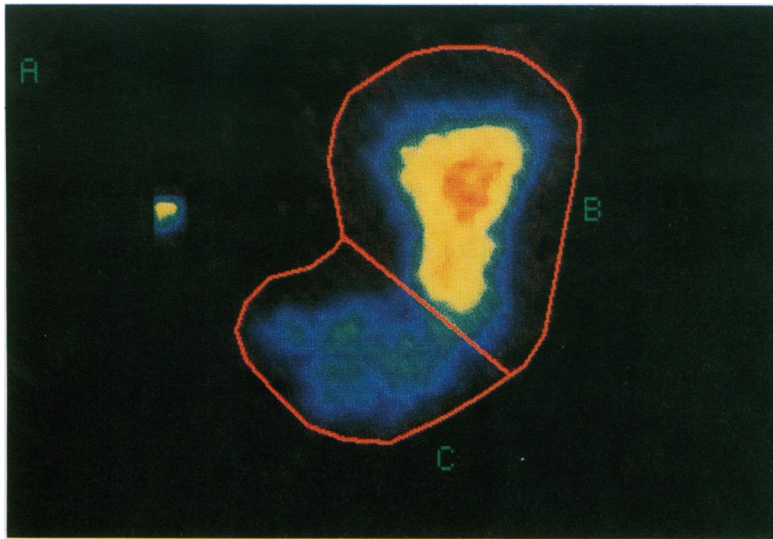


Figure 1: Scintiphotograph of the stomach taken 10 minutes after a test meal, to illustrate the regions of interest: A=whole stomach; B=body/fundus; C=antrum. The stomach is divided into body/fundus and antrum by a line drawn at 45° down from the angulus.

mouthwash. ^{13}C -urea breath testing was based on a widely used protocol.³ At $t = -10$ minutes, those subjects due to be fed consumed the test meal consisting of 50 ml Calogen mixed with 50 ml Ensure (25 g long chain triglycerides, 7 g carbohydrates, 275 kcal). At $t = -5$ minutes baseline end expiratory breath samples were obtained by breathing out through a straw into a test tube. At $t = 0$ subjects drank a solution containing 100 mg ^{13}C -urea and 3 MBq technetium-99m diethylenetriaminepentaacetic acid ($^{99\text{m}}\text{Tc}$ DTPA), a γ emitting water phase marker, dissolved together in 100 ml water. Following the described protocol,³ subjects then lay on their left then right side for two minutes each. Breath samples and scintiscans were obtained every 10 minutes for 40 minutes, then at 60 and 90 minutes.

SCINTIGRAPHIC IMAGING AND ANALYSIS

Anterior and posterior images, each of 30 second duration, were obtained by standing the subjects in front of an IGE Maxicamera II (IGE Ltd, Herts, England) fitted with a medium energy parallel hole collimator (maximum energy 300 kV).

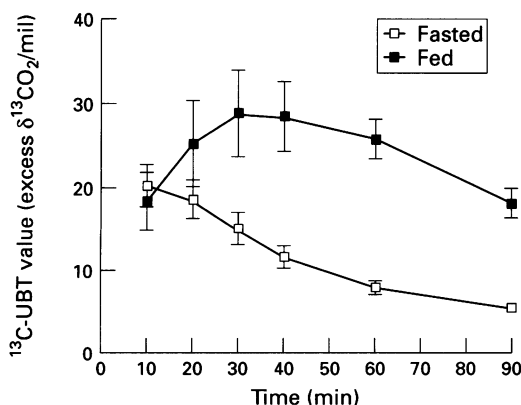


Figure 2: Mean (SEM) UBT values at various time points with and without a test meal. Values are significantly different at 30 minutes and later ($p < 0.05$).

All scintigraphic data were corrected for background radiation and radioactive decay. Tissue attenuation was corrected for by using the geometric mean of anterior and posterior counts.⁹ Corrected results for each region of interest were normalised by expression as percentage of maximum counts recorded in the stomach of that subject.

Regions of interest were the whole stomach, body/fundus, and antrum. To define these, an outline of the stomach was constructed for each scintigraphic image and subdivided into body/fundus and antrum by a line drawn at 45 degrees downwards from the angulus (Fig 1). Uniformity between images was achieved by constructing a template for each subject and using it for all his or her images.

For each region of interest time activity curves were constructed, and time to 50% emptying (T_{50}) was calculated. For the antrum, where 50% residence was often not achieved, the area under the time activity curve was used rather than a T_{50} value. Statistical comparisons between paired data were made using Wilcoxon's signed rank sum test.

BREATH SAMPLE ANALYSIS

Breath samples were analysed commercially by isotope ratio mass spectrometry (Bureau of Stable Isotope Analysis Ltd, England). Values were expressed as excess δ per mil units, which are the ratio of ^{13}C to ^{12}C in the sample compared with a standard, multiplied by 1000, minus the baseline value. UBT curves were constructed of UBT values against time. Statistical comparisons between paired data were again made using Wilcoxon's signed rank sum test.

Results

Two subjects were excluded from analysis. One had breakfast on the morning of the 'fasted' UBT and the second received antibiotics for a chest infection before the second UBT. Analysis was therefore based on paired UBTs and gastric distribution studies in seven subjects. All subjects tolerated the UBT and scintigraphy well, with no adverse events recorded.

The test meal did not affect mean UBT values at 10 minutes (excess δ per mil 20.3 fasted, 18.5 fed, $p = \text{NS}$). At 30 minutes and all subsequent time points, however, UBT values were increased by the meal ($p < 0.05$) (Fig 2).

The mean amount of urea in the antrum at 10 minutes was, like the UBT result, unaffected by the test meal (28% fasted, 26% fed, $p = \text{NS}$), but at 30 minutes and thereafter the meal increased antral residence of urea ($p < 0.05$) (Fig 3). In contrast with antral results, the mean amount of urea in the body/fundus was greatly increased at 10 minutes (12% fasted, 71% fed, $p < 0.01$) and throughout the test (Fig 3). Mean residence of urea in the whole stomach, which is the sum of antral and body/fundus residence, was therefore also greatly increased both at 10 minutes (40% fasted, 97% fed, $p < 0.01$) and at all subsequent times (Fig 4).

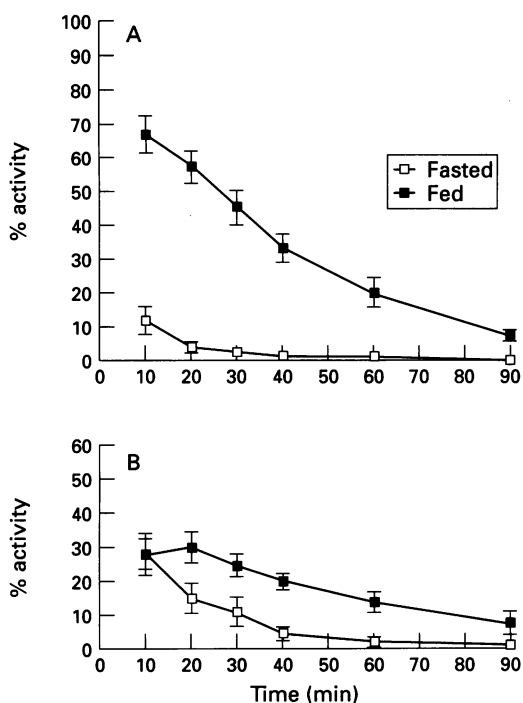


Figure 3: Mean (SEM) % of administered ^{99m}Tc in body/fundus (A) and antrum (B) at various time points with and without a test meal. Values with and without a test meal are significantly different for body/fundus at all time points ($p < 0.01$), and those for the antrum at 30 minutes and later ($p < 0.05$).

There was considerable variation between subjects in both the gastric emptying of urea solution and in the amount delivered to all areas of the stomach. For example the time to 50% emptying (T_{50}) for the whole stomach ranged from 28 to 73 minutes (Table). Distribution to body/fundus was very variable, and crucially, so was distribution to the antrum. As previously explained, T_{50} could not be used as a measure of urea residence for the antrum, but the area under the antral time activity curve varied from 830 minutes% to 2565 minutes% (Table).

Discussion

The intragastric distribution and gastric emptying of urea solution in the urea breath test are found, in this study, to be greatly affected by

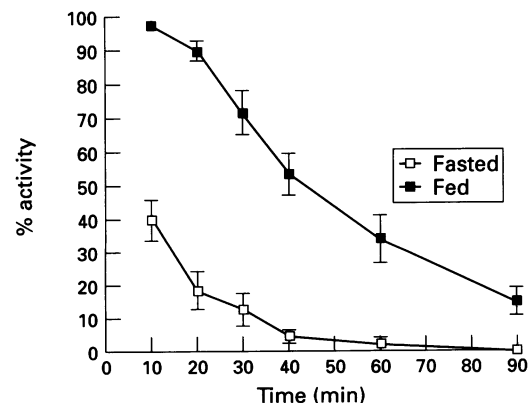


Figure 4: Mean (SEM) % of administered ^{99m}Tc in the whole stomach at various time points with and without a test meal. Values are significantly different at all time points ($p < 0.01$).

the administration of a test meal. When no test meal is given, delivery of urea solution to the body and fundus of the stomach is extremely poor, so the breath test value will reflect mainly antral urease activity. When a test meal is given, on the other hand, much better delivery of urea solution to the gastric body and fundus is achieved, so potentially urease in these areas, as well as in the antrum, can be detected. In the subjects in this study, however, the contribution of urease in the body and fundus to breath test values seems to be small. UBT values at 10 minutes are similar with or without a test meal, and the amount of urea in the gastric antrum is also similar with or without a meal at this time. In the body/fundus, on the other hand, the amount of urea present at 10 minutes is greatly increased when a test meal is given, the amount present at this time without a meal being very small. One possible interpretation of this would be that, in the subjects in this study at least, antral urease is the major contributor to UBT values.

It has been claimed that the UBT performed with a test meal can accurately quantify total urease activity, and early workers quoted values for urease activity based on UBT results.¹ We were interested to establish whether accurate quantification was a realistic aim and to assess whether regional urease activity could be assessed by manipulating intragastric distribution of urea through giving or omitting a test meal. This study shows that giving a test meal improves conditions for quantification of global urease activity, in that it improves uniformity of distribution of urea within the stomach and slows gastric emptying. Furthermore, omitting a meal potentially permits measurement of antral urease activity alone, as delivery of urea to the body/fundus in this situation is virtually nil. Variability in urea distribution between subjects, however, both with and without a test meal, is enormous, and this makes accurate quantification of both total and regional urease activity an unattainable goal.

In this study, UBT values at 10 minutes were unaffected by a test meal. Superficially it would seem therefore, that if breath sampling took place at 10 minutes the test meal may prove unnecessary. Proponents of the test meal, however, have suggested that the resultant delay in gastric emptying may avoid both false negative results, from insufficient contact between bacteria and substrate,¹⁰ and false positive results, from rapid transit of urea to the colon and hydrolysis by colonic bacteria.¹¹ In practice,

Gastric residence of ^{99m}Tc DTPA, after a test meal, for all subjects. Whole stomach and body/fundal values are expressed as time to 50% emptying (T_{50}) and antral values are expressed as area under the gastric residence curve (AUC)

Subject number	Whole stomach (T_{50} /min)	Body/fundus (T_{50} /min)	Antrum (AUC/min%)
1	60	52	1345
2	38	33	900
3	42	10	1595
4	73	32	2565
5	30	23	830
6	37	17	1710
7	28	22	1175

however, except for patients who have had gastric surgery, we are not aware of either of these problems having been reported. Potentially, a test meal may also permit later breath sampling, when UBT values are higher in *H pylori* positive patients, but whether this makes any difference to discrimination between *H pylori* positive and negative patients remains to be determined. Later breath sampling also avoids interference from the very early breath test peak caused by oral urease activity, but, again in practice, this peak is much earlier than 10 minutes,^{5 12} and oral urease activity can be reduced by mouth washing.¹² Unless these potential problems with omitting the test meal are actually encountered, there may be a case for shortening and simplifying the ¹³C-UBT by doing this, as some already do for the ¹⁴C-UBT,^{5 13} and we believe that this option deserves further assessment.

We have shown that a test meal prolongs residence time of urea in the antrum but does not increase early (10 minutes) concentrations. Likewise, early UBT values are unaffected by a test meal but later values are increased. The test meal considerably increases the amount of urea in the body/fundus throughout the test, but the contribution of this to breath test values is unclear. There is much variation in delivery of urea to all areas of the stomach between subjects, even with a test meal, and this means that accurate quantification of total or regional gastric urease activity by the UBT is probably not a realistic aim.

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