ORIGINAL ARTICLE

A Novel Tablet-Based ^{13}C Urea Breath Test for *Helicobacter pylori* with Enhanced Performance during Acid Suppression Therapy

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Background: The urea breath test (UBT) can still be improved in terms of user-friendliness and accuracy during acid-suppression therapy. This study was designed to evaluate a novel, rapidly disintegrating ^{13}C UBT tablet, which was supplemented with citric acid to facilitate diagnosis of *Helicobacter pylori* in the hypochlorhydric stomach. **Methods:** The efficacy of a fasting ^{13}C tablet-based UBT (TUBT) was compared with that of a standard ^{13}C UBT (SUBT) during 40 min after dosing, and optimal sampling points were determined. The single-point TUBT was validated against a 'gold standard' (GS) including a TUBT, culture, histology, and a CLO test in 134 dyspeptic patients, and its optimal cut-off point was determined by means of a biometric method. In addition, 20 SUBT-positive patients were randomized to perform either the TUBT or the SUBT after 7 days of omeprazole therapy (20 mg twice daily). **Results:** Compared with a SUBT, the TUBT gave a quicker and wider separation between positive and negative results and an earlier optimal sampling point (10 versus 40 min). At 10 min the TUBT correctly classified 40 of 42 GS-positive subjects (sensitivity, 95%) and all of 92 GS-negative patients (specificity, 100%), and the optimal cut-off point was 1.8 δ per mil. Furthermore, when optimal sampling points were used, the TUBT (t = 10 min) proved to be more accurate than the SUBT (t = 40 min) during omeprazole treatment, correctly identifying all of 10 and 3 of 9 *H. pylori*-infected patients, respectively. **Conclusions:** By supplying ^{13}C urea and citric acid as a rapid-release tablet, it is possible to shorten the duration of the ^{13}C UBT to 10 min, omit the test meal, and still maintain excellent accuracy, even during acid suppression therapy.

Key words: Acid suppression therapy; *Helicobacter pylori*; tablet-based urea breath test

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The urea breath test (UBT), first described by Graham et al. in 1987 (1), is currently considered the most important non-invasive test for *Helicobacter pylori* (2). It is relatively easy to perform, highly reliable in the pretreatment setting (3–5), and has been promoted as the 'gold standard' for confirming eradication (6–8). In its present form, however, the method is afflicted with some problems limiting its usefulness in clinical practice. The test can be performed using either ^{14}C or ^{13}C. The ^{14}C UBT has recently been considerably simplified and improved by using a capsule formulation (3, 9). Enclosing the isotope in a capsule shields the urea from exposure to urease-producing oropharyngeal bacteria, thus making it possible to shorten the duration of the test to 10 min, omit the test meal, and still maintain or even enhance the performance of the test (3). However, a more widespread availability of the ^{14}C UBT is limited by the strict regulations governing the use, handling, and storage of radioactive isotopes, which in practice precludes use outside hospitals and testing in children and women of child-bearing age. ^{13}C, on the other hand, is a perfectly safe isotope that can be used on anyone, anywhere. Home-based ^{13}C UBT kits have already been described (10, 11) but only non-formulated isotope preparations with test meals have been used so far. This means waiting a relatively long time (30–60 min) before breath sampling can be performed. Therefore, to improve the ^{13}C UBT in a manner similar to that described above for the ^{14}C UBT, we have developed and tested a rapid-release ^{13}C urea tablet as part of an easy-to-use UBT kit.

Another problem with the present UBTs is that equivocal or false-negative UBT results often occur in patients taking acid-suppression therapy (12–15). For this reason it is currently recommended that antisecretory medications should be with-

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held 5–7 days before the UBT (15), which can be very inconvenient for patients with ongoing dyspeptic symptoms and complicates routine administration of clinical testing. Why acid-suppression therapy reduces the accuracy of the UBT remains unclear. Previously, it was believed that this was a property unique to proton pump inhibitors (PPIs), since in vitro studies have shown that these drugs not only inhibit *H. pylori* urease activity (16, 17) but also exert anti-*H. pylori* effects through a urease-independent mechanism (18). However, a more recent study has shown that false-negative UBT results also occur during treatment with high doses of H_2-blockers, suggesting that this effect might be the result of a high intragastric pH (15). This possibility is further supported by the fact that an acidic environment is necessary for the production of CO_2 from degraded urea. At a pH of less than 4.0 all the bicarbonate produced from degraded urea is converted to CO_2, compared with only 50% at pH 6.1 (the pKa of carbonic acid). It is possible, therefore, that addition of enough H^+ to ensure a microenviromental pH of less than 4.0 during the test period may improve the diagnostic reliability of the UBT in patients taking acid-suppression therapy.

In the present study we have tested a newly developed rapid-release tablet containing ^{13}C urea and citric acid. Citric acid was added to increase the acidity in the local microenvironment of the hypochlorhydric stomach. Our aim was thus to evaluate this new tablet formulation to facilitate the development of a user-friendly and readily available UBT kit that could be used to detect *H. pylori* infection reliably even during ongoing acid-suppression therapy. The efficacy of this novel tablet-based ^{13}C UBT was compared with that of a conventional ^{13}C UBT, validated against a 'gold standard', and its optimal cut-off point was determined by means of a biometric method.

Materials and Methods

Subjects

The subjects included in the study comprised 147 healthy volunteers without a past or present history of gastrointestinal disease and 189 patients with dyspeptic complaints attending routine upper gastrointestinal endoscopy at the endoscopy unit of Sahlgren's University Hospital. Exclusion criteria were previous gastric surgery and use of antibiotics or antisecretory or prokinetic drugs in the 2 weeks preceding the study. In addition, we studied 147 subjects 4–12 weeks after completion of eradication therapy against *H. pylori*. The study was approved by the Ethical Committee of Göteborg University, and informed consent was obtained from each subject.

^{13}C Urea breath test

Breath tests were carried out after an overnight fast and performed under the supervision of a study nurse. Breath samples were obtained by blowing through a disposable plastic straw into a 20-ml vacutainer until condensation appeared on the vacutainer wall. Thereafter the straw was removed, and the vacutainer was immediately resealed. After collection of a base-line breath sample, 100 mg ^{13}C urea was given either in tablet form or as a water solution. In accordance with the well-validated European standard protocol (ESP) (19), the ^{13}C urea water solution was drunk 10 min after intake of a fatty test meal (one sachet of Complan, 50 ml of semi-skimmed milk, and 100 ml of Calogen), given to delay gastric emptying, whereas two ^{13}C urea tablets, each containing 50 mg ^{13}C urea and 463 mg citric acid, were ingested while fasting. Both tablets and water solution were swallowed together with 200 ml of tap water. Additional breath samples were collected 5, 10, 20, and 40 min after administration of ^{13}C urea.

Breath samples were analysed in a gas isotope ratio mass spectrometer (Automated Breath 13Carbon Analyser, Europa Scientific Ltd, Crewe, UK). Values were expressed as excess δ per mil units, which is the ratio of ^{13}C to ^{12}C in the sample compared with a standard, multiplied by 1000, minus the base-line value.

Preparation of ^{13}C urea tablets

To obtain instant disintegration and subsequent dissolution of the tablets in the stomach, several formulation factors were adjusted. The urea was milled to obtain a fine particulate quality with high surface area. Further, the concept of ordered mixing (20) was applied to counteract agglomeration of the fine urea quality (21) and thus optimize both drug dissolution (22) and mixture quality (23). To obtain both a deagglomeration of the fine particulate quality of urea and also an adhesion of primary urea particles to the coarser citric acid particles, both components were admixed for 2 h. To enhance adequate compactability and disintegration, two cellulose-based excipients, Avicel Ph 101 (60 mg/tablet) and Ac-Di-Sol (24 mg/tablet) were admixed in the ordered mixture of urea. The final tablet mass was compressed by using concave punches with a diameter of 12.0 mm. Tablets for clinical trials were produced by Diabact AB, Uppsala, Sweden.

Study design

In the first phase we compared the excretion curves of $^{13}CO_2$ after ingestion of the ^{13}C urea tablets versus after drinking the ^{13}C urea water solution. Forty dyspeptic patients (32 men, 8 women; mean age, 55 years; range, 30–81 years) were assigned to perform the tablet-based UBT and the ESP UBT in random order, separated by a washout period of at least 1 week. Patients who had an ESP UBT result exceeding 5 δ per mil at 40 min were classified as *H. pylori*-positive. The optimal sampling point for each test was determined by means of a biometric method (see below).

In the second phase the single-point tablet-based UBT was validated against a 'gold standard' based on UBT results and on those obtained by biopsy-based methods (culture, histology, and CLO testing). The patient was regarded as *H. pylori*-infected if two or more tests, whatever their nature, were

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positive (24). One hundred and thirty-four consecutive dyspeptic patients (69 men, 65 women; mean age, 54 years; range, 21–85 years) agreed to perform the tablet-based UBT immediately before they underwent a routine upper gastrointestinal endoscopy at which 5 antral biopsy specimens were taken. One specimen was for CLO testing (Delta West, Bentely, Australia), two for culture, and two for histology (Giemsa stain).

In the third phase we used a biometric method (see below) to determine the optimal cut-off value for the tablet-based UBT in a mixed population comprising 147 healthy volunteers, 189 dyspeptic patients, and 147 patients after eradication therapy, and in each of the 3 different populations. Most of the dyspeptic subjects had participated in phase 1 or 2, whereas all the remaining subjects were new participants.

In the fourth phase we compared the efficacies of the tablet-based UBT and the ESP UBT in terms of detecting *H. pylori* infection during ongoing acid-suppression therapy. The comparison was made using the optimal sampling points for each test determined in phase 1. Twenty dyspeptic subjects (14 men, 6 women; mean age, 59 years; range, 40–75 years) who were positive by the ESP UBT were randomized to perform either the tablet-based UBT or the ESP UBT after treatment with omeprazole (Losec®, Astra Hässle, Göteborg, Sweden), 20 mg twice daily for 7 days.

Biometric determination of cut-off levels

The cut-off levels between positive and negative results were determined independently of other diagnostic methods by using a biometric method for evaluating gastric urease activity previously described by Berstad et al. (25). In brief, logarithmic transformation of gastric urease activity in random subjects yields two separate populations (*H. pylori*-negative and -positive subjects), each normally distributed. Adjusted for relative frequencies, their normal probability density function intercepts at one point, estimated as the log cut-off. At this point, the probability of a false-positive or false-negative decision of whether gastric urease activity is increased or not is the smallest. Hence, the test's antilogarithm was used as the optimal cut-off value.

Statistics

Results are expressed as means ± standard error of the mean. Before the statistical analysis the excess values were transformed to the logarithmic scale. The tablet-based UBT and the ESP UBT were compared for equivalence, for *H. pylori*-negative and *H. pylori*-positive subjects separately, using analysis of variance. After omeprazole treatment the UBTs were compared by using the unpaired *t* test (two-tailed). Normality was assessed with the Wilk–Shapiro W test

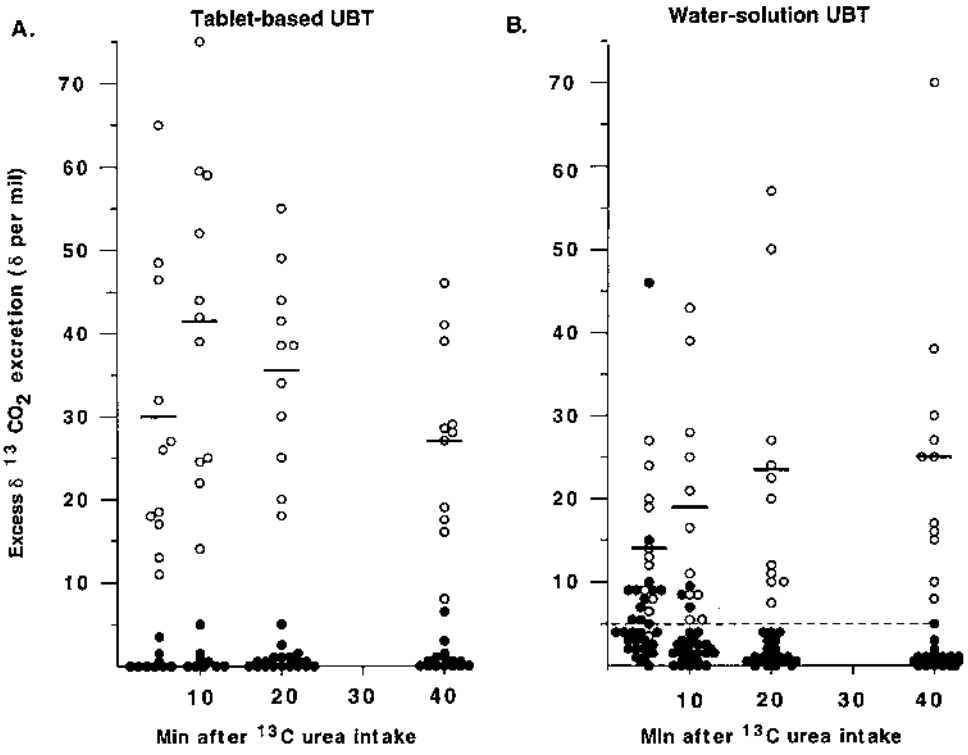

Fig. 1. Individual values of excess δ $^{13}CO_2$ excretion (δ per mil) of 12 *Helicobacter pylori*-positive (open circles) and 28 *H. pylori*-negative (filled circles) subjects after intake of 100 mg ^{13}C urea as tablets (A) and as a water solution (B). Bars represent mean values for *H. pylori*-positive subjects. Values not shown are $\leq$0.6 δ per mil. The tablet-based urea breath test (UBT) was performed while fasting, whereas the standard UBT was performed after intake of a fatty test meal, in accordance with the European Standard Protocol.

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Table I. Comparison of results of the ^{13}C tablet-based urea breath test (UBT) with those of a gold standard*

^{13}C tablet-based UBT (t = 10 min)	Gold standard		
	Positive	Negative	Total
Positive	40	0	40
Negative	2	92	94
Total	42	92	134

* The gold standard was based on the combined results of the UBT and three biopsy-based tests (culture, histology, CLO test). A patient was defined as being *Helicobacter pylori*-positive if two or more of these tests were positive. On the basic of these results, sensitivity is 40:42 = 95%, and specificity is 92:92 = 100%.

and by skewness and kurtosis. A P value of <0.05 was considered significant.

Results

Comparison of excess δ $^{13}CO_2$ excretion curves for the tablet-based UBT and the ESP UBT

In the group of 40 dyspeptic patients who underwent both the tablet-based and the ESP UBT, 12 (30%) patients had an excess δ $^{13}CO_2$ value exceeding 5 per mil at 40 min and were thus classified as *H. pylori*-positive. In these subjects the excess δ $^{13}CO_2$ values at 5, 10, and 20 min were significantly higher after intake of the ^{13}C urea tablets than after intake of the ^{13}C urea water solution (Fig. 1). Furthermore, the tablet-based UBT also gave a peak δ $^{13}CO_2$ excess value that was significantly higher (41 ± 5.6 versus 25 ± 4.9 δ per mil; $P = 0.001$) and occurred earlier (10 versus 40 min) than that obtained with the ESP UBT (Fig. 1). In the 28 *H. pylori*-negative subjects intake of the ^{13}C urea tablets resulted in an excess δ $^{13}CO_2$ excretion that barely exceeded background levels at all time points studied (Fig. 1A), whereas intake of the ^{13}C urea solution resulted in a significant increase of excess δ $^{13}CO_2$ excretion at 5, 10, and 20 min (Fig. 1B) that resulted in 12 false-positive cases at 5 min and 3 at 10 min (>5 δ per mil). No false-positive cases were observed when using the tablet-based UBT.

The biometric analysis showed that the optimal sampling point—that is, the sampling point yielding the best diagnostic reliability (in parentheses)—occurred at 10 min for the tablet-based UBT (99.7%) and at 40 min for the ESP UBT (97.7%). At 5, 10, and 20 min the statistical probability of obtaining a false diagnosis (either falsely positive or falsely negative) was lower for the tablet-based UBT than for the ESP UBT (0.8% versus 26% at 5 min, 0.3% versus 17% at 10 min, 0.4% versus 4.9% at 20 min), whereas at 40 min this factor did not differ between the two tests (2.9% versus 2.3%).

Tablet-based UBT versus a 'gold standard'

The endoscopic findings for the 134 dyspeptic patients who participated in the validation of the tablet-based UBT against a 'gold standard' included gastric ulcer (7.5%), duodenal ulcer (7.5%), gastritis (15%), normal (49%), and reflux disease (21%). *H. pylori* was detected by the 'gold standard' in 42 of the 134 (31%) patients. As compared with the 'gold standard', the tablet-based UBT gave 2 false-negative and no false-positive results, thus identifying 40 of the 42 *H. pylori*-positive patients and all of the 92 *H. pylori*-negative patients (Table I). Hence, the sensitivity and specificity of the tablet-based UBT were 95% and 100%, respectively.

Biometric determination of cut-off level

Among the mixed population comprising 147 healthy volunteers, 189 dyspeptic patients, and 147 patients after eradication therapy and in each of these subgroups the 10-min excess δ $^{13}CO_2$ values showed a bimodal log-normal distribution. Relative frequencies, logarithmic means, and standard deviations for negative and positive UBT results in the mixed population and the corresponding values for the three subgroups are given in Table II together with the estimated cut-off point and risk of errors for each group. In the mixed population the optimal cut-off point was 1.8 δ per mil. At this point, the risk of obtaining a false diagnosis was 1.1%.

Table II. Results and optimal cut-off points of the tablet-based ^{13}C urea breath test in a a mixed population and individual subgroups of healthy volunteers, dyspeptic patients, and patients after eradication therapy

	Mixed population, $n = 483$		Healthy volunteers, $n = 147$		Dyspeptic patients, $n = 189$		Patients after eradication therapy, $n = 147$	
	TUBT+	TUBT−	TUBT+	TUBT−	TUBT+	TUBT−	TUBT+	TUBT−
Relative frequency	0.25	0.75	0.22	0.78	0.31	0.69	0.20	0.80
Mean log	1.13	−0.72	1.22	−0.74	1.09	−0.72	0.95	−0.74
Standard deviation log	0.44	0.39	0.35	0.37	0.43	0.34	0.48	0.37
Optimal cut-off point (δ per mil)	1.8		2.2		1.8		1.3	
Estimated risk of errors	1.1%		0.3%		1.8%		1.8%	

TUBT+/− = increased or not increased gastric urease activity as determined with the ^{13}C tablet-based urea breath test (t = 10 min);

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The cut-off points and related risks of error (in parentheses) for healthy volunteers, dyspeptic patients, and patients after eradication therapy were 2.2 (0.3%), 1.8 (1.8%), and 1.3 (1.8%), respectively. In this study changing the cut-off from 2.2 to 1.8 δ per mil would not have affected the classification of *H. pylori*-positive and -negative subjects, since none of the healthy volunteers had excess δ $^{13}CO_2$ values in this range. On the other hand, changing the cut-off from 1.3 to 1.8 δ per mil in the posttreatment group would have meant that the three subjects with UBT values between 1.3 and 1.8 had been classified as *H. pylori*-negative instead of *H. pylori*-positive. Overall, 10 of 483 subjects (2%) had excess δ $^{13}CO_2$ values in the range between 1.3 and 2.2. Of these 10 subjects, 5 belonged to the posttreatment group, 1 to the group of healthy volunteers, and 4 to the group of dyspeptic subjects.

Tablet-based UBT versus ESP UBT during omeprazole treatment

Twenty ESP UBT-positive patients were reexamined either with the ESP UBT or with the tablet-based UBT after 7 days of treatment with 20 mg omeprazole twice daily. The comparison was made using the optimal sampling point for each test—that is, at 10 min for the ESP UBT and at 40 min for the ESP UBT. Of the 10 subjects who were randomized to perform a repeat ESP UBT, 1 was excluded owing to a missing breath sample. The other nine subjects all had a lower excess δ $^{13}CO_2$ excretion after omeprazole treatment (25 ± 4.9 versus 5.9 ± 1.5; $P = 0.008$), and in six of these the excess δ $^{13}CO_2$ value decreased to below the ESP cut-off (<5 δ per mil) (Fig. 2). Among the 10 subjects who performed the tablet-based UBT after receiving omeprazole treatment the mean excess δ $^{13}CO_2$ value was significantly higher than in the conventional UBT group (31 ± 7.6 versus 5.9 ± 1.5 δ per mil; $P = 0.0002$), and none of the 10 subjects had an excess δ $^{13}CO_2$ value of less than 5 δ per mil (Fig. 2).

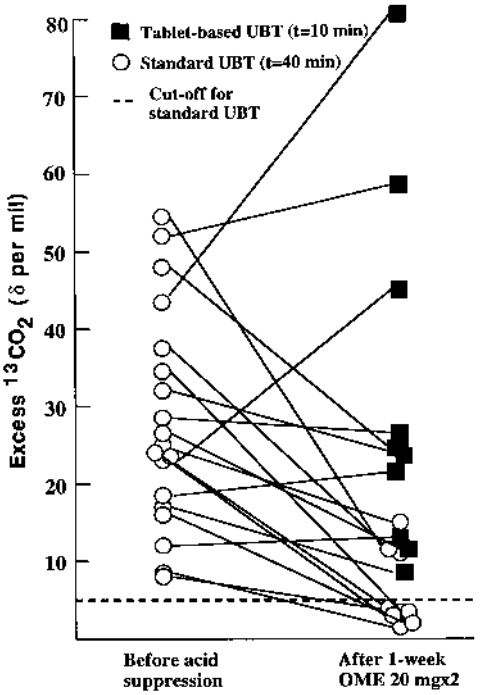

Fig. 2. Individual values of excess δ $^{13}CO_2$ excretion (δ per mil) of 19 patients who were positive by a standard urea breath test (UBT) before acid-suppressive therapy and the correspondig values after 7 days of treatment with 20 mg omeprazole twice daily, tested either with the tablet-based UBT (filled squares) or with a standard UBT (open circles). The comparison was made using the optimal sampling points determined for each test—that is, at 10 min for the tablet-based UBT and at 40 min for the standard UBT. The standard UBT was performed with a fatty test meal and a urea–water solution, in accordance with the European Standard Protocol.

Discussion

The ^{13}C UBT offers an important advantage over the ^{14}C UBT inasmuch as it can be performed outside the hospital setting in the local surgery/clinic or even at home, after which a test tube of expired air can be sent by regular mail for analysis. However, in terms of methods the ^{13}C UBT still lags behind the ^{14}C UBT, hampering a more widespread applicability of this useful test. Our previous work with the ^{14}C UBT showed the advantages of supplying the urea solution in a quick-dissolve capsule as compared with a conventional drink (3). When administered as a drink, interference from urease-producing bacteria in the oropharynx (26–28) may cause false-positive results in early breath samples (2, 3, 29). Therefore, most UBT protocols do not obtain the diagnostic breath sample until 20–30 min after dosage and usually include a test meal to prevent premature emptying of urea from the stomach (2). In contrast, when using a capsule or tablet, the problem of oropharyngeal bacteria is eliminated, and the test can be performed already after 10 min (3, 9), thereby obviating the need for a test meal. On the other hand, this makes great demands on the formulation of urea—that is, capsule or tablet; it must be resistant to disintegration in the oropharynx and at the same time guarantee disintegration and dissolution in the stomach within 2–5 min.

One major advance of the present formulation is thus that we have created a solid dosage form of ^{13}C urea (ready-to-use tablet), characterized by an almost instantaneous disintegration and dissolution of the ^{13}C urea tablet after entering the stomach (20–23). When testing this new tablet, we found that in comparison with a conventional urea drink UBT performed with a test meal, the tablet-based UBT performed while fasting gave greater separation between positive and negative results at early time points ($\leq$20 min). After drinking the urea solution, even *H. pylori*-negative subjects had an increased excess δ $^{13}CO_2$ excretion during the first 10 min, resulting in several false-positive results, whereas after intake of the tablets, the background ^{13}C urea hydrolysis was close to zero, and no false-positive results occurred. Compared with the conventional UBT, the tablet-based UBT also gave higher mean excess δ $^{13}CO_2$ values in *H. pylori*-positive subjects during the first 20 min after dosing, and the peak value

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occurred much earlier (10 versus 40 min). This suggests that the ^{13}C urea tablet confers protection against oropharyngeal urease activity, thereby leaving more urea to be hydrolysed in the stomach. It also suggests that the tablet formulation provides a more rapid and effective exposure of urea to the stomach than the urea solution. The peak values are important because they often coincide with the optimal sampling points. In accordance with this, the biometric analysis confirmed that the best diagnostic reliability for the tablet-based UBT and the ESP UBT was obtained at 10 min and 40 min, respectively. The quicker and wider separation of positive and negative results has great clinical importance not only because it enables shortening of the test time to 10 min and omission of the test meal without loss of diagnostic accuracy but also because it may enable a reduction of the dose of isotope used and hence reduced costs.

In a separate study the tablet-based UBT (10-min value) was validated against a gold standard in 134 dyspeptic subjects. The gold standard included three biopsy-based tests (culture, histology, CLO test) and the UBT result. If two or more tests were positive, the patient was defined as being *H. pylori*-positive. Compared with the gold standard, the tablet-based UBT correctly identified 40 of 42 *H. pylori*-positive patients and all of 92 *H. pylori*-negative patients, thus giving 95% sensitivity and 100% specificity, which is similar to or better than most published ^{13}C UBT protocols (2). Since both patients with false-negative UBT results were positive by all three biopsy-based tests, the most reasonable explanation of their low excess δ $^{13}CO_2$ values (0.5 and 1.5) seems to be a too rapid passage of the tablet through the stomach. This may possibly have been prevented by a test meal. However, in our view, the advantages of omitting the test meal from the protocol by far outweigh the relatively small risk of rapid emptying of the tablet, not only because the test meal increases costs and makes the test less user-friendly but also because it may reduce the UBT values (3) and thereby the separation between negative and positive results.

The most commonly used cut-off level for the ^{13}C UBT, 5 δ per mil, was originally determined for the ESP UBT (19). The optimal cut-off point for the tablet-based UBT should be lower owing to its much lower levels of background ^{13}C urea hydrolysis in uninfected subjects and greater separation between positive and negative UBT results. Furthermore, a recent study has suggested that a higher accuracy could be obtained in the UBT if two different cut-off levels are applied: a higher cut-off in a population in which a low prevalence of infection is expected—for example, healthy volunteers—and a lower cut-off in dyspeptic patients (in whom the prevalence of infection is higher than in a normal population) (30). In the present study, therefore, we used a biometric method to determine the cut-off for the tablet-based UBT with the smallest possible arbitrariness (25), both in a mixed population consisting of healthy volunteers, dyspeptic patients, and patients after eradication therapy, and in each of these subgroups separately. Ranging between 1.3 and 2.2, the cut-off points for the tablet-based UBT were, as expected, generally lower than the ones previously reported for conventional UBTs (11, 19). The optimal cut-off point in the mixed population was estimated to be 1.8 δ per mil. At this point, the statistical probability of obtaining a false-negative or -positive diagnosis was 1.1%. The same cut-off point was obtained in the subgroup of dyspeptic subjects, whereas in agreement with previous studies (19, 30), we found a slightly higher cut-off among healthy volunteers (2.2) and slightly lower one in the posttreatment group (1.3). Therefore, when using the tablet-based UBT for screening in a 'healthy' population, it might be beneficial to use 2.2 δ per mil as a cut-off point, since this decreased the risk of obtaining a false diagnosis to only 0.3%. It is more difficult to recommend an exact cut-off point to use in the posttreatment group, since some of the posttreatment results were too close to each other to be separated, and this had negative impact on the diagnostic reliability at the optimal cut-off. The best way to deal with this problem is probably to introduce a grey zone containing unreliable/uncertain results (6, 31). We therefore recommend that patients with UBT values ranging between 1.3 and 2.2 δ per mil should undergo repeat breath testing and/or a supplementary upper endoscopy with biopsies, to reliably assess their *H. pylori* status. The grey zone concept is particularly useful after eradication treament and might also help discover some incorrect diagnoses before treatment. In this study 5 of the 147 patients (3.4%) in the postreatment group and 5 of the 336 remaining subjects (1.5%) had excess δ $^{13}CO_2$ values within the grey zone.

Although several studies have shown that equivocal or false-negative UBT results often occur in patients taking acid-suppression therapy (12–15), the possibility that this effect is the result of an inherent pH-related methodologic problem has not previously been taken into consideration. In the present study we tested whether citric acid supplementation of the ^{13}C urea tablet would facilitate UBT diagnosis of *H. pylori* infection during ongoing acid-suppressive therapy (omeprazole, 20 mg twice daily) by providing an acidic microenvironment in the hypochlorhydric stomach. We found that in all nine patients who were retested with a conventional UBT (t = 40 min) after 7 days of omeprazole treatment, the excess δ $^{13}CO_2$ excretion was reduced, and six of the nine patients converted from positive to negative. By contrast, none of the 10 patients who performed the tablet-based UBT (t = 10 min) turned false negative. These results suggest that the citric acid supplementation of the ^{13}C urea tablet indeed provides H^+ ions in sufficient amounts to promote the conversion of bicarbonate to carbon dioxide in the interface between urease and ^{13}C urea. Furthemore, the notion that an acidic pH is necessary for the UBT to work properly is also in agreement with the recent finding that the urease activity of *H. pylori* is relatively low at pH 8.0 or 7.0 but increases about 10-fold at a pH below 6.5 (32). The ability to reliably diagnose *H. pylori* infection in patients taking acid-suppression therapy is a major advantage for the tablet-based UBT, since patients with

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ongoing dyspeptic symptoms often are unwilling to withhold such medication while awaiting breath testing. However, since it is unclear at present whether PPIs in therapeutic doses exert significant anti-*H. pylori* effects in vivo or just change the distribution of *H. pylori* in the stomach (12, 13, 33), further clinical trials are needed to establish whether using the tablet-based UBT will completely eliminate the problem of false-negative UBT results during acid-suppression therapy.

In conclusion, we have shown that the tablet-based ^{13}C UBT is at least as accurate as a standard ^{13}C UBT and excels in speed and simplicity. In addition, it offers the unique advantage of being able to reliably detect *H. pylori* infection during acid-suppression therapy. This extremely user-friendly method together with new, cheap, readily available on-line techniques for analysis of $^{13}CO_2$ (34–36) may herald more widespread use of the UBT both inside and outside the hospital setting.

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