Scand J Clin Lab Invest 2005; 65: 491–498

ORIGINAL ARTICLE

Comparison of different protocols for ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection in healthy volunteers

M. KOPÁČOVÁ[1], J. BUREŠ[1], V. VOŘÍŠEK[2], M. KONŠTACKÝ[1], S. REJCHRT[1], P. ŽIVNÝ[2], T. DOUDA[1] & V. PALIČKA[2]

[1]*Second Department of Internal Medicine and* [2]*Institute of Clinical Biochemistry and Diagnostics, Faculty of Medicine at Hradec Králové, Charles University in Prague, University Teaching Hospital, Hradec Králové, Czech Republic*

Abstract

Objective. The ^{13}C-urea breath test (^{13}C-UBT) is the most accurate non-invasive method for diagnosis of *Helicobacter pylori* infection. However, several methodological issues have not been resolved yet. The aim of this study was to test different protocols of ^{13}C-UBT to find the optimal test drink and sampling interval. ***Material and methods***. ^{13}C-UBT was performed at 3-day intervals in 27 healthy volunteers using citric acid (test A), orange juice (B) and still water (C) as test drinks. Breath samples were collected from time 5 to 60 min. A total number of 2106 breath samples were analysed by isotope ratio mass spectrometry (cut-off value 3.5). ***Results***. Differences in delta values were greater than would be expected by chance (A versus B and A versus C at times 20, 25, 30, 35 and 40 min, $p < 0.05$, Dunnett's method). There were no grey zone- or false-negative results among *H. pylori*-positive persons in test A at any time, but some were found in tests B and C. Optimal intervals for breath sampling are at times 20 or 25 min after ^{13}C-urea ingestion. ***Conclusions***. Citric acid solution as a test drink and 20- or 25-min breath sampling intervals are optimal for the ^{13}C-UBT in healthy volunteers.

Key Words: *Citric acid, Helicobacter pylori, isotope ratio mass spectrometry, test drink, ^{13}C-urea breath test*

Introduction

The ^{13}C-urea breath test (^{13}C-UBT) is the most accurate non-invasive method to diagnose *Helicobacter pylori* infection [1]. The test is based on the gastric urease activity of H. pylori which splits non-radioactive stable ^{13}C-labelled urea ingested by investigated persons into

Correspondence: Marcela Kopáčová, MD, PhD, Second Department of Medicine, Faculty of Medicine, Charles University, Teaching Hospital, Sokolská 581, CZ-500 05 Hradec Králové, Czech Republic. Fax: +420 49 5514 022. E-mail: kopacmar@fnhk.cz

(Received 28 December 2004; accepted 24 May 2005)

ISSN 0036-5513 print/ISSN 1502-7686 online
DOI: 10.1080/00365510500209199

NH_4^+ and ^{13}C-labelled HCO_3^-, which is expired as $^{13}CO_2$ in the exhaled breath. Expired ^{13}C-labelled carbon dioxide is measured as a $^{13}CO_2/^{12}CO_2$ ratio and results are expressed as the excess delta (difference between δ t_i and δ t_0).

Although the original ^{13}C-UBT was published as early as in 1987 [2], the test has not been unified yet. A European standard protocol [3] and US protocols [4,5] were proposed but not generally accepted. There are more than 20 commercial producers of diagnostic kits of ^{13}C-UBT with different settings. Several protocol modifications have been published. Last but not least, breath samples of ^{13}C-UBT can be measured by different methods, by means of isotope ratio mass spectrometry (gold standard), isotope-selective non-dispersive infrared spectrometry or by optogalvanic laser spectroscopy. Several issues have not yet been definitely resolved, e.g. different amount of ^{13}C-urea used, type of test drink (meal), optimal interval(s) for collecting breath samples or cut-off value. The aim of our study was to test different protocols of ^{13}C-UBT to find the optimal test drink and sampling interval.

Material and methods

Subjects

Twenty-seven healthy volunteers entered the study (12 M, 15 F, age 22–52 years, median 29). The ^{13}C-UBT was performed at 3-day intervals in all persons using three different protocols. Written informed consent for participation in the study was obtained from all individuals.

Urea breath test

Urea breath tests were performed in the morning after overnight fasting. Two baseline breath samples were taken before the test, followed by test drink ingestion: 150 ml still water with 3 g citric acid (test A) or 150 ml natural orange juice (test B) or 150 ml still water only (test C). After 5 min (at time 0) all persons ingested 100 mg ^{13}C-urea dissolved in 50 ml still water with 1 g citric acid (test A) or 50 ml natural orange juice (test B) or 50 ml still water only (test C).

Breath samples were collected in duplicate using a straw in 20-ml vacutainers at 5-min intervals from time 5 to 60 min, with the subjects lying on their left side.

A total of 2106 breath samples were analysed by means of isotope ratio mass spectrometry (AP 2003, Analytical Precision, UK). Cut-off was set at 3.5, based on our previous study of 1476 analyses [6].

Statistics

Data were analysed statistically with the Mann-Whitney rank sum test, the Kruskal-Wallis analysis of variance on ranks (ANOVA on ranks) and Dunnett's multiple comparisons using statistical software (SigmaStat, Jandel Corporation, Germany).

Results

Six persons (22%) were *H. pylori* positive and 21 subjects (78%) were *H. pylori* negative, clearly distinguished in all three tests (see Figures 1–3). Delta values were significantly

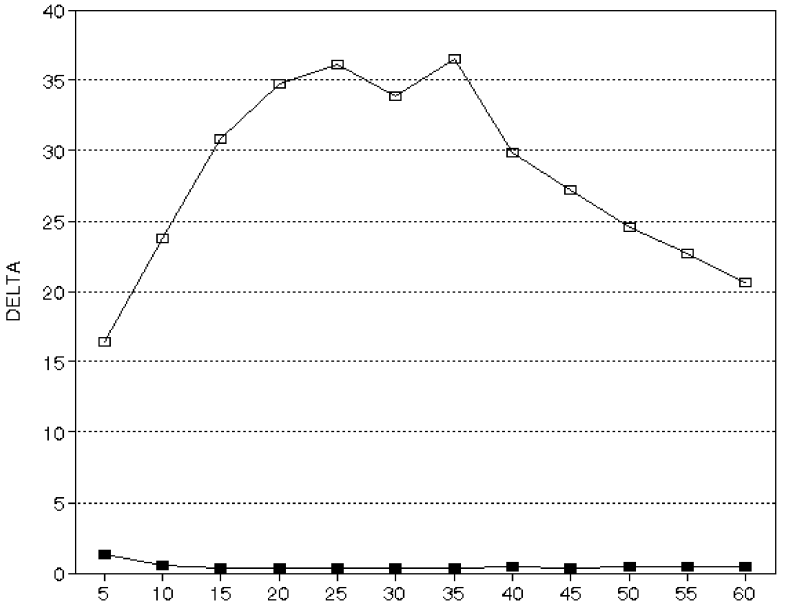

Figure 1. Test A. Average delta values of ^{13}C-urea breath test at 5-min intervals (from 5 to 60 min). Citric acid solution was used as a test drink. ■: *H. pylori*-negative persons (21 subjects); □: *H. pylori*-positive persons (6 subjects).

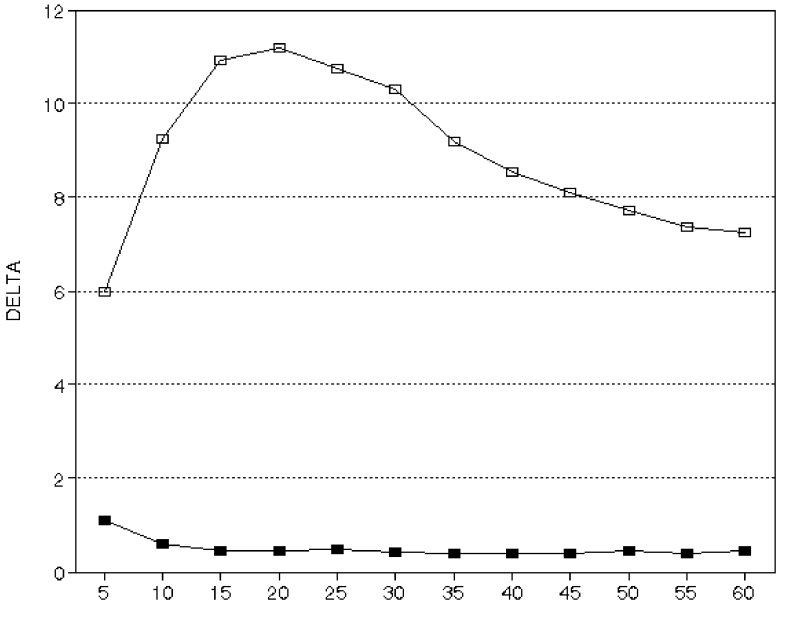

Figure 2. Test B. Average delta values of ^{13}C-urea breath test at 5-min intervals (from 5 to 60 min). Natural orange juice was used as a test drink. ■: *H. pylori*-negative persons (21 subjects); □: *H. pylori*-positive persons (6 subjects).

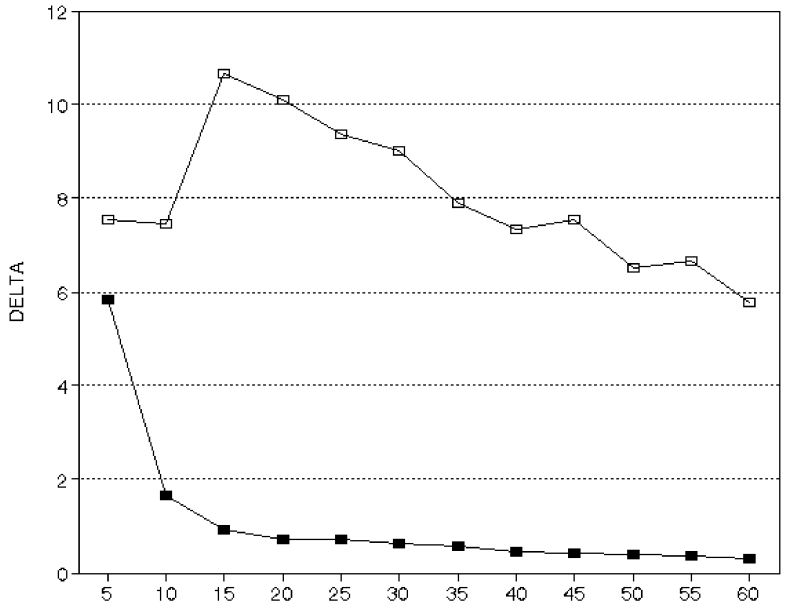

Figure 3. Test C. Average delta values of ^{13}C-urea breath test at 5-min intervals (from 5 to 60 min). Still water was used as a test drink. ■: H. pylori-negative persons (21 subjects); □: *H. pylori*-positive persons (6 subjects).

higher in test A (citric acid) compared with test B (orange juice) and test C (water) (Table I). Differences in the median values among the group were greater than would be expected by chance (A versus B and A versus C at 20, 25, 30, 35 and 40 min, $p<0.05$, Dunnett's method). There were no grey zone- or false-positive results among *H. pylori*-negative persons in any test at any time (specificity 100%). There were no grey zone- or false-negative results among *H. pylori*-positive persons in the test A (using citric acid as a test drink) at any time, but some were found in tests B and C (see Table II). Using citric

Table I. Delta values of ^{13}C-UBT in *H. pylori*-positive persons given as median and interquartile range. Different test drinks were used: citric acid (test A), orange juice (test B) or still water (test C).

Delta	Test A	Test B	Test C	Significance
Time 20	19.618	6.510	6.774	A versus B: $p=0.004$
	12.956–52.248	5.911–15.352	3.507–7.326	A versus C: $p=0.002$
				B versus C: NS
Time 25	21.041	7.587	5.819	A versus B: $p=0.003$
	13.791–50.933	6.266–15.353	3.576–8.646	A versus C: $p=0.001$
				B versus C: NS
Time 30	19.618	6.510	6.774	A versus B: $p=0.004$
	12.956–52.248	5.911–15.352	3.507–7.326	A versus C: $p=0.002$
				B versus C: NS
Time 35	22.371	5.721	4.992	A versus B: $p=0.002$
	13.289–52.878	5.153–12.479	2.822–6.912	A versus C: $p=0.002$
				B versus C: NS
Time 40	16.573	5.726	4.495	A versus B: $p=0.002$
	11.666–50.228	4.635–11.326	2.448–7.001	A versus C: $p=0.001$
				B versus C: NS

Abbreviations: ^{13}C-UBT=^{13}C-urea breath test; NS=not significant.

Table II. Grey zone and false-negative results in *H. pylori*-positive persons in test B (orange juice) and C (water). Two breath samples were analysed in each person at each time. Delta values are given as mean ± SD.

Time	Test C grey zone results (3.3–3.7)	Test B grey zone results (3.3–3.7)	Test C false-negative results (<3.3)	Test B false-negative results (<3.3)
5	3.599 $n=1$	None	None	2.516 $n=1$
10	None	None	None	None
15	None	None	None	None
20	None	None	None	None
25	3.577 $n=1$	None	3.039 $n=1$	None
30	None	None	3.051 ± 0.307 $n=2$	None
35	3.531 $n=1$	None	2.515 ± 0.383 $n=2$	None
40	None	3.693 $n=1$	2.435 ± 0.452 $n=3$	None
45	None	3.557 $n=1$	2.141 ± 0.570 $n=3$	None
50	None	3.650 $n=1$	1.815 ± 0.213 $n=3$	None
55	None	3.650 $n=1$	1.614 ± 0.119 $n=3$	2.728 $n=1$
60	None	None	1.396 ± 0.229 $n=3$	2.245 ± 0.263 $n=2$

acid solution as a test drink, the optimal time intervals for breath sampling are at 20 or 25 min after ^{13}C-urea ingestion.

Discussion

The ^{13}C-UBT is a simple, non-invasive and reliable test for the diagnosis of *H. pylori* infection. In our previous studies using citric acid solution as a test drink, we found excellent reproducibility of both analytical [6] and clinical [7] results of ^{13}C-UBT. In this study we used citric acid solution, still water or orange juice as test drinks in ^{13}C-UBT performed three times in the same group subjects at 3-day intervals. The best results were reached with citric acid; optimal intervals for breath sampling were at 20 or 25 min after ^{13}C-urea ingestion. In agreement with other studies [8], citric acid was superior to both orange juice and still water.

Since the first description of ^{13}C-UBT by Graham et al. [2] several modifications have been published to simplify and optimize the test, including the amount of ^{13}C-urea used, type of test meal, number of samples and collecting time. Comparison of different protocols and the analytical methods used must always be taken into consideration, too (mass spectrometry or infrared spectrometry or optogalvanic laser spectroscopy).

Originally, different test nutrient meals (solid, semi-solid or liquid) were used to slow gastric emptying and to maximize distribution of the substrate within the stomach. Recommended test doses of ^{13}C-urea have successively decreased from an initial 5 mg/kg body-weight [2] to 75 mg [9–11], 50 mg [12–15], 38 mg [16] or even 25 mg [17], and time of the breath test has shortened from an initial 180 min, collecting samples every 10 min [2], to baseline one and another one test breath sample at 20 min [5,14,18–20], 15

[15,21,22], 10 [12,23] or even 5 min [17]. Different test drinks (instead of test meal) were introduced, e.g. citric acid [5,8–12,24–28], orange juice [29], apple juice [30], fresh milk [31], water [20,32], or even no test meal/drink [5,33]. Even fasting before a ^{13}C-UBT may not be necessary according to some investigators [34,35].

Several papers have been published suggesting that citric acid solution is the optimal test drink [5,8–12,24–28]. Intragastric urease activity is pH-dependent, low at neutral pH, but as the external pH decreases to 6.5–5.5 there is a 10–20-fold increase in urease activity which remains high through approximately pH 2.5 [36,37]. Proton pump inhibitors (PPIs) may cause false-negative results of ^{13}C-UBT by unknown mechanism [38–42]. PPI-induced false negative results can be associated with a direct anti-*H. pylori* effect of the PPI (inhibition of the viability and growth of *H. pylori* resulting in decreased bacterial load) or associated with increased intragastric pH. According to some investigators [43], ^{13}C-UBT can be and according to others [44] cannot be prevented by acidification of the stomach. H_2-receptor antagonists differ from PPIs, as high intragastric pH may cause a reduction in urease activity, unrelated to a reduced bacterial load, and can be reversed by citric acid [44].

Citric acid slows gastric emptying in a dose-dependent manner [11,45] and some investigators have hypothesized that prolonged contact of ^{13}C-urea with bacteria results in increased amounts of hydrolyzed urea [23,24]. Later papers showed that *H. pylori* urease activity enhanced by citric acid is unrelated to rate of gastric emptying [30,45]. Activation of the cytoplasmic urease by urea entry into the cytoplasm of *H. pylori* may explain the increased $^{13}CO_2$ excretion when an acid test meal is given rather than delayed gastric emptying [30].

To summarize our results, citric acid solution as a test drink and 20- or 25-min breath sampling intervals are optimal for the ^{13}C-UBT in healthy volunteers.

Acknowledgement

This study was supported by a grant from the Ministry of Health of the Czech Republic MZ VZ 000179906.

References

[1] Malfertheiner P, Mégraud F, O'Morain C, Hungin APS, Jones R, Axon A, et al. Current concepts in tile management of *Helicobacter* infection: the Maastricht 2-2000 Consensus Report. Aliment Pharmacol Ther 2002;16:167–80.

[2] Graham DY, Klein PD, Evans DJ Jr, Evans DG, Alpert LC, Opekun AR, et al. *Campylobacter pylori* detected noninvasively by the ^{13}C-urea breath test. Lancet 1987;1:1174–7.

[3] Logan RPH, Dill S, Bauer FE, Walker MM, Hirschl AM, Gummett PA, et al. The European ^{13}C-urea breath test for the detection of *Helicobacter pylori*. Eur J Gastroenterol Hepatol 1991;3:915–21.

[4] Klein PD, Malaty HM, Martin RF, Graham KS, Genta RM, Graham DY. Noninvasive detection of *Helicobacter pylori* infection in clinical practice: the ^{13}C-urea breath test. Am J Gastroenterol 1996;91:690–4.

[5] Malaty HM, el-Zimaity HM, Genta RM, Klein PD, Graham DY. Twenty-minute fasting version of the US ^{13}C-urea breath test for the diagnosis of *H. pylori* infection. Helicobacter 1996;1:165–7.

[6] Voříšek V, Bureš J, Kopáčová M, Palička V. Isotope ratio mass spectrometry in non-invasive gastroenterological diagnostics. Klin Biochem Metab 1999;7:217–22.

[7] Kopáčová M, Bureš J, Voříšek V, Konštacký M, Rejchrt S, Živný P, et al. The diagnostics of *Helicobacter pylori* by means of the ^{13}C-urea breath test: clinical reproducibility of the test. Klin Biochem Metab 1999;7:213–6.

[8] Leodolter A, Dominguez-Munoz JE, von Arnim U, Malfertheiner P. Citric acid or orange juice for the ^{13}C-urea breath test: the impact of pH and gastric emptying. Aliment Pharmacol Ther 1999;13:1057–62.

[9] Dominguez-Munoz JE, Leodolter A, Sauerbruch T, Malfertheiner P. A citric acid solution is an optimal test drink in the ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection. Gut 1997;40:459–62.
[10] Graham DY, Malaty HM, Cole RA, Martin RF, Klein PD. Simplified ^{13}C-urea breath test for detection of *Helicobacter pylori* infection. Am J Gastroenterol 2001;96:1741–5.
[11] Leodolter A, Dominguez-Munoz JE, von Arnim U, Kahl S, Peitz U, Malfertheiner P. Validity of a modified ^{13}C-urea breath test for pre- and post-treatment diagnosis of *Helicobacter pylori* infection in the routine clinical setting. Am J Gastroenterol 1999;94:2100–4.
[12] Gatta L, Vakil N, Ricci C, Osborn JF, Tampieri A, Perna F, et al. A rapid, low dose, ^{13}C-urea tablet for the detection of *Helicobacter pylori* infection before and after treatment. Aliment Pharmacol Ther 2003;17:793–8.
[13] Bazolli F, Cecchini L, Corvaglia L, Dall'Antonia M, De Giacomo C, Fossi S, et al. Validation of the ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection in children: a multicenter study. Am J Gastroenterol 2000;95:646–50.
[14] Wong WM, Lam SK, Lai KC, Chu KM, Xia HHX, Wong KL, et al. A rapid-release 50-mg tablet based ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection. Aliment Pharmacol Ther 2003;17:253–7.
[15] Liao CC, Lee CL, Chiang TC, Lee SC, Huang SH, Tu TC, et al. The ^{13}C-urea breath test to detect *Helicobacter pylori* infection: a validated simple methodology with 50 mg ^{13}C-urea. Aliment Pharmacol Ther 2002;16:787–92.
[16] Bielanski W, Konturek SJ. New approach to ^{13}C-urea breath test: capsule-based modification with low-dose of ^{13}C-urea in the diagnosis of *Helicobacter pylori* infection. J Physiol Pharmacol 1996;47:545–53.
[17] Isomoto H, Inoue K, Shikuwa S, Furusu H, Nishiyama T, Omagari K, et al. Five minute endoscopic urea breath test with 25 mg of ^{13}C-urea in the management of *Helicobacter pylori* infection. Eur J Gastroenterol Hepatol 2002;14:1093–100.
[18] Cadranel S, Corvaglia L, Bontems P, Deprez C, Glupczynski Y, van Riet A, et al. Detection of *Helicobacter pylori* infection in children with a standardized and simplified ^{13}C-urea breath test. J Pediatr Gastroenterol Nutr 1998;27:275–80.
[19] Miwa H, Murai T, Ohkura R, Kawabe M, Tanaka H, Ogihara T, et al. Effect of fasting subjects' posture on ^{13}C-urea breath test for detection of *Helicobacter pylori* infection. Helicobacter 1997;2:82–5.
[20] Ohara S, Kato M, Asaka M, Toyota T. Studies of ^{13}C-urea breath test for diagnosis of *Helicobacter pylori* in Japan. J Gastroenterol 1998;33:6–13.
[21] Peng NJ, Hsu PI, Lee SC, Tseng HH, Huang WK, Tsay DG, et al. A 15-minute ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection in patients with non-ulcer dyspepsia. J Gastroenterol Hepatol 2000;15:284–9.
[22] Sheu BS, Lee SC, Yang HB, Lin XZ. Quantitative result of ^{13}C-urea breath test at 15(minutes may correlate with the bacterial density of *H. pylori* in the stomach. Hepatogastroenterology 1999;46:2057–62.
[23] Hamlet A, Stage L, Lonroth H, Cahlin C, Nyström C, Pettersson A. A novel tablet-based ^{13}C-urea breath test for *Helicobacter pylori* with enhanced performance during acid suppression therapy. Scand J Gastroenterol 1999;34:367–74.
[24] Graham DY, Runke D, Anderson S-Y, Malaty HM, Klein PD. Citric acid as the test meal for the ^{13}C-urea breath. Am J Gastroenterol 1999;94:1214–7.
[25] Gisbert JP, Benito LM, Lara S, Vazquez A, Jimenez I, Pajares JM. ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection: are basal samples necessary? Eur J Gastroenterol Hepatol 2000;12:1201–5.
[26] Delvin EE, Brazier JL, Deslandres C, Alvarez F, Russo P, Seidman E. Accuracy of the ^{13}C-urea breath test in diagnosing *Helicobacter pylori* gastritis in pediatric patients. J Pediatr Gastroenterol Nutr 1999;28:59–62.
[27] Leodolter A, Dominguez-Munoz JE, von Arnim U, Manes G, Malfertheiner P. ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection. A further simplification for clinical practice. Scand J Gastroenterol 1998;33:267–70.
[28] Epple HJ, Kirstein FW, Bojarski C, Frege J, Fromm M, Riecken EO, et al. ^{13}C-urea breath test in *Helicobacter pylori* diagnosis and eradication. Correlation to histology, origin of "false" results, influence of food intake. Scand J Gastroenterol 1997;32:308–14.
[29] Labenz J, Barsch G, Peitz U, Aygen S, Hennemann O, Tillenburg B, et al. Validity of a novel biopsy urease test (HUT) and a simplified ^{13}C-urea breath test for diagnosis of *Helicobacter pylori* infection and estimation of the severity of gastritis. Digestion 1996;57:391–7.
[30] Pantoflickova D, Scott DR, Sachs G, Dorta G, Blum AL. ^{13}C urea breath test (UBT) in the diagnosis of *Helicobacter pylori*: why does it work better with acid test meals? Gut 2003;52:933–7.
[31] Wang WM, Lee SC, Ding HJ, Jan CM, Chen LT, Wu DC, et al. Quantification of *Helicobacter pylori* infection: simple and rapid ^{13}C-urea breath test in Taiwan. J Gastroenterol 1998;33:330–5.
[32] Oksanen A, Bergström M, Sjöstedt S, Gad A, Hammarlund B, Seensalu R. Accurate detection of *Helicobacter pylori* infection with a simplified ^{13}C-urea breath test. Scand J Clin Lab Invest 1997;57:689–94.

[33] Wong WM, Wong BC, Wong KW, Fung FM, Lai KC, Hu WM, et al. ^{13}C-urea breath test without a test meal is highly accurate for the detection of *Helicobacter pylori* infection in Chinese. Aliment Pharmacol Ther 2000;14:1353–8.

[34] Braden B, Duan LP, Caspary WF, Lembcke B. More convenient ^{13}C-urea breath test modification still meets the criteria for valid diagnosis of *Helicobacter pylori* infection. Z Gastroenterol 1994;32:198–202.

[35] Moayyedi P, Braunholtz D, Heminbrough E, Clough M, Tompkins D, Mapstone NP, et al. Do patients need to fast for a ^{13}C-urea breath test? Eur J Gastroenterol Hepatol 1997;9:275–7.

[36] Rektorschek M, Weeks D, Sachs G, Melchers K. Influence of pH on metabolism and urease activity of *Helicobacter pylori*. Gastroenterology 1998;115:628–41.

[37] Scott DR, Weeks D, Hong C, Postins S, Melchers K, Sachs G. The role of internal urease in acid resistance of *Helicobacter pylori*. Gastroenterology 1998;114:58–70.

[38] Parente F, Sainaghi M, Sangaletti O, Imbesi V, Maconi G, Anderloni A, et al. Different effects of short-term omeprazole, lansoprazole or pantoprazole on the accuracy of the ^{13}C-urea breath test. Aliment Pharmacol Ther 2002;16:553–7.

[39] Savarino V, Bisso G, Pivari M, Zentilin P, Bilardi C, Dulbecco P, et al. Effect of gastric acid supression on ^{13}C-urea breath: comparison of ranitidine with omeprazole. Aliment Pharmacol Ther 2000;14:291–7.

[40] Stoschus B, Domingez-Munoz JE, Kalhori N, Sauerbruch T, Malfertheiner P. Effect of omeprazole on *Helicobacter pylori* urease activity *in vivo*. Eur J Gastroenterol Hepatol 1996;8:811–3.

[41] Graham DY, Opekun AR, Hammoud F, Yamaoka Y, Reddy R, Osato MS, et al. Studies regarding the mechanism of false negative urea tests with proton pump inhibitors. Am J Gastroenterol 2003;98:1005–9.

[42] Levine A, Shevah O, Shabat-Sehayek V, Aeed H, Boaz M, Moss SF, et al. Masking of ^{13}C urea breath test by proton pump inhibitors is dependent on type of medication: comparison between omeprazole, pantoprazole, lansoprazole and esomeprazole. Aliment Pharmacol Ther 2004;20:117–22.

[43] Shirin H, Frenkel D, Shevah O, Levine A, Bruck R, Moss SF, et al. Effect of proton pump inhibitors on the continuous real time ^{13}C-urea breath test. Am J Gastroenterol 2003;98:46–50.

[44] Graham DY, Opekun AR, Jogi M, Yamaoka Y, Lu H, Reddy R, el-Zimaity HM. False negative urea breath tests with H2-receptor antagonists: interactions between *Helicobacter pylori* density and pH. Helicobacter 2004;9:17–27.

[45] Shiotani A, Saeed A, Yamaoka Y, Osato MS, Klein PD, Graham DY. Citric acid-enhanced *Helicobacter pylori* urease activity *in vivo* is unrelated to gastric emptying. Aliment Pharmacol Ther 2001;15:1763–7.