body to *Helicobacter pylori* in dyspeptic patients. J Clin Pathol 1993;46:826–8.
68. Webb PM, Crabtree JE, Forman D, and The Eurogast Study Group. Gastric cancer, cytotoxin-associated gene A-positive *Helicobacter pylori*, and serum pepsinogens: An international study. Gastroenterology 1999;116:269–76.
69. Schlemper RJ, van der Werf SD, Vandenbroucke JP, Biemond I, Lamers CB. Seroepidemiology of gastritis in Japanese and Dutch working populations: evidence for the development of atrophic gastritis that is not related to *Helicobacter pylori*. Gut 1995;37: 199–204.
70. Goedhard JG, Biemond I, Gilliams JP, Pals G, Kreuning J. Serum pepsinogen I levels: assessment of gastric acid secretion? Pepsinogens in man: clinical and genetic advances, Prog Clin Biol Res 1985;173:139–46.
71. Kato I, Miki K, Muñoz N, Vivas JH, Lopez G, Peraza S, et al. Determinants of plasma pepsinogen levels in population at high risk for stomach cancer in Venezuela. Int J Cancer 1995;62:512–8.
72. Yoshihara M, Sumii K, Haruma K, Kiyohira K, Hattori N, Kitadai Y, et al. Correlation of ratio of serum pepsinogen I and II with prevalence of gastric cancer and adenoma in Japanese subjects. Am J Gastroenterol 1998;93:1090–6.
73. Varis K, Taylor PR, Sipponen P, Samloff IM, Heinonen OP, Albanes D, et al. Gastric cancer and premalignant lesions in atrophic gastritis: a controlled trial on the effect of supplementation with alpha-tocopherol and beta-carotene. Scand J Gastroenterol 1998;33:294–300.
74. Wu M-S, Lee W-C, Lin J-T, Wang H-P, Wang T-H, Chen C-J. A novel tree-structured analysis for non-invasive diagnosis of gastric adenocarcinoma. Anticancer Res 1995;15:2739–43.
75. Farinati F, Di Mario F, Plebani M, Cielo R, Fanton MC, Valiante F, et al. Pepsinogen A/pepsinogen C or pepsinogen A multiplied by gastrin in the diagnosis of gastric cancer? Ital J Gastroenterol 1991;223:194–6.
76. Goldschmiedt M, Barnett CC, Schwarz BE, Karnes WE, Redfern JS, Feldman M. Effect of age on gastric acid secretion and serum gastrin concentrations in healthy men and women. Gastroenterology 1991;101:977–90.
77. Serrono MT, Lanas Al, Lorente S, Sainz R. Cytokine effects on pepsinogen secretion from human peptic cells. Gut 1997;40:42–8.
78. Lorente S, Doiz O, Trinidad Serrano M, Castillo J, Lanas A. *Helicobacter pylori* stimulates pepsinogen secretion from isolated human peptic cells. Gut 2002;50:13–8.
79. Fukuda H, Saito D, Hayashi S, Hisai H, Ono H, Yoshida S, et al. *Helicobacter pylori* infection, serum pepsinogen level and gastric cancer: A case-control study in Japan. Jpn J Cancer Res 1995; 86:64–71.
80. Varis K, Sipponen P, Laxén F, Samloff IM, Huttunen JK, Taylor PR, et al. Implications of serum pepsinogen I in early endoscopic diagnosis of gastric cancer and dyplasia. Scand J Gastroenterol 2000;35:950–6.
81. Kikuchi S, Kurosawa M, Sakiyama T, Tenjin H, Miki K, Wada O, et al. Long-term effect of *Helicobacter pylori* infection on serum pepsinogens. Jpn J Cancer Res 2000;91:471–6.
82. Plummer M, Vivas J, Fauchere JL, Giudice GD, Pēa AS, Ponzetto A, et al. *Helicobacter pylori* and stomach cancer: A case-control study in Venezuela. Cancer Epidem Biomar Prev 2000;9:961–5.
83. Ohata H, Kitauchi S, Yoshimura N, Mugitani K, Iwane M, Nakamura H, et al. Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer. Int J Cancer 2004;109:138–43.
84. Watabe H, Mitsushima T, Yamaji Y, Okamoto M, Wada R, Kokubo T, et al. Predicting the development of gastric cancer from combining *Helicobacter pylori* antibodies and serum pepsinogen status: a prospective endoscopic cohort study. Gut 2005; 54:764–8.
85. Fujishiro M, Yahagi N, Kakushima N, Kodashima S, Nakamura M, Kobayashi K, et al. Early detection of asymptomatic gastric cancers using serum pepsinogen levels to indicate endoscopic submucosal dissection for better quality of life. Proceeding of 6th International Gastric Cancer Congress. Yokohama 2005;145–50.
86. Sasajima M, Hoshina R, Miki K. Annual reports (1997–2000) of the Research Committee for the Study of Gastric Cancer Screening System Using the Serum Pepsinogen. Clin Gastroenterol 2002;17:1555–68 (in Japanese with English abstract).
87. Tsubono Y, Hisamichi S. Screening for gastric cancer in Japan. Gastric Cancer 2000;3:9–18.
88. Miki K. Annual report 2004 of the Research Committee of the Study of High Risk Strategy of Gastric Cancer Screening, supported by a Grant-in-Aid for the Research from the Ministry of Health, Labour and Welfare, Japan. Tokyo; 2005(April). p. 1–288 (in Japanese).
89. Muto M, Saito Y, Koike T, Miki K. A case of gastric cancer with high pepsinogen II levels in both serum and ascites. Dig Endosc 2000;12:42–5.
90. Ozasa K, Kurata JH, Higashi A, Hayashi K, Inokuchi H, Miki K, et al. *Helicobacter pylori* infection and atrophic gastritis. A nested case-control study in a rural town in Japan. Dig Dis Sci 1999; 44:253–6.
91. Watanabe Y, Kurata JH, Mizuno S, Mukai M, Inokuchi H, Miki K, et al. *Helicobacter pylori* infection and gastric cancer. A nested case-control study in a rural area of Japan. Dig Dis Sci 1997;42:1383–7.
92. Asaka M, Kimura T, Kato M, Kudo M, Miki K, Ogoshi K,et al. Possible role of *Helicobacter pylori* infection in early gastric cancer development. Cancer 1994;73:2691–4.
93. Asaka M, Kato M, Kudo M, Katagiri M, Nishikawa K, Yoshida J, et al. Relationship between *Helicobacter pylori* infection, atrophic gastritis and gastric carcinoma in a Japanese population. Eur J Gastroenterol Hepatol 1995;s7–10.
94. Nomura A M Y,Klonel LN, Miki K, Stemmermann GN, Wilkens LR, Goodman MT, et al. *Helicobacter pylori*, pepsinogen and gastric adenocarcinoma in Hawaii. J Infect Dis 2005;191:2075–81.
95. Miki K, Urita Y, Nomura AMY, Kolonel LN, Stemmermann GN, Wilkens LR, et al. The relationship between *Helicobacter pylori*, pepsinogen, and gastric adenocarcinoma. Gut 2005;54 (Suppl. VII):A 70.
96. Hatakeyama M. Oncogenic mechanisms of the *Helicobacter pylori* CagA protein. Nature Rev Cancer 2004;4:688–94.

Correlation of Serum Pepsinogens and Gross Appearances Combined with Histology in Early Gastric Cancer

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The correlation between serum pepsinogen (PG) levels and the gross types was investigated in 128 consecutive patients with early gastric cancer. Although there was no significant difference in age, gender, cancer location, or cancer depth among gross appearances, the distribution of histological type was significantly different between polypoid and depressed cancers: all polypoid cancers except one were intestinal type, whereas nearly a third of depressed cancers were diffuse type. All the patients in whom *Helicobacter pylori* status was investigated had *Helicobacter pylori* infection. Combination of gross appearances and histology (polypoid cancer with intestinal type, depressed cancer with intestinal type and depressed cancer with diffuse type) showed a clear difference in distribution of serum PG levels and a ratio between levels of PG I and PG II (I/II ratio). In polypoid cancer with intestinal type, a PG I level and a I/II ratio were significantly lower than those of the others. In depressed cancer with diffuse type, PG I and PG II levels were significantly higher. These findings revealed that backgrounds such as intragastric acidity and extent of gastric atrophy might differ among early gastric cancers with different morphology and histology.

Key Words: Pepsinogen, Early gastric cancer, Gross type, Histological type

Although decreasing in incidence worldwide, gastric cancer remains a relevant cause of cancer death in many countries (1). An intense effort has been made for the early detection of the cancer, in order to improve its poor prognosis, and various mass screening methods including photofluorography (2) and serum pepsinogen (PG) assay (3,4), have been developed especially in Japan. The latter method was initially introduced as a marker of intragastric acidity and mucosal atrophy of the stomach (5,6), but its usefulness for gastric cancer screening has been established in recent years (3,4), assuming that progression of chronic gastritis causes intestinal metaplasia, which is followed by dysplasia, and finally results in gastric cancer (7). Although gastric cancer screening by serum PG assay detects approximately 80% of all gastric cancers (3, 8), this model of gastric carcinogenesis is mainly proposed for development of intestinal type cancer. Diffuse type cancer may be developed from another carcinogenetic pathway, because the different genetic and epigenetic changes are reported between the two histological types (9).

It is assumed that the gross appearances (polypoid and non-polypoid) show different genetic alterations in colorectal cancer: polypoid cancers are originated in carcinogenesis of adenoma-carcinoma sequence, whereas non-polypoid cancers in *de novo* pathway (10,11). However, such investigation has not been specifically performed for gastric cancer. Among intestinal type cancers, some are either polypoid or depressed or ulcerated. What is the determinant of gross appearances in the intestinal type cancers? Among depressed or ulcerated cancers, some are of the intestinal type while the others of the diffuse type. What is the reason why the genetically and epigenetically different cancers show the same appearances? In order to find out the answers, we investigate the correlation between serum PG levels and gross appearances of early gastric cancer.

Materials and Methods

We retrospectively analyzed 128 consecutive patients with early gastric cancer (7 with 0 I (protruded) type, 30 with 0 IIa (superficial elevated) type, 7 with 0 IIa+IIc / 0 IIc+IIa (combined superficial elevated and depressed) type, 79 with 0 IIc (superficial depressed) type, 5 with 0 IIc+III / 0 III+IIc (combined superficial depressed and excavated) type) between September 1999 and August 2001, in whom serum PG assay, using PG I and PG II RIA-bead Kits (Dinabot Co Ltd, Tokyo, Japan), had been performed before cancer removal. The patients with a previous gastrectomy, renal dysfunction (12) or proton pump inhibitor prescription (13), which interfered with the results of serum PG levels were excluded. Serum *Helicobacter pylori* (*H.plyori*) IgG antibodies were also investigated some patients using a commercial ELIZA kit (HEL-pTEST II, SILENUS Labs, Pty, Ltd. Australia) in order to find out the presence of *H.pylori*. All the cancers were removed by endoscopic mucosal resection or gastrectomy, and histopathologically investigated in detail at University Hospital, University of Tokyo, Tokyo, Japan, or its related hospitals. Endoscopic and histopathological diagnoses are made according to the Japanese classification of gastric carcinoma (14). Well and moderately differentiated tubular adenocarcinoma and papillary adenocarcinoma were grouped together as "intestinal type" and poorly differentiated adenocarcinoma and signet-ring cell carcinoma as "diffuse type", according to Laurens criteria (15). Distributions of a PG I level, a PG II level and a ratio between the levels of PG I and PG II (I/II ratio) were investigated according to each of different histological types and different gross appearances, those were polypoid cancer (0 I type and 0 IIa type) and depressed cancer (0 IIc type and 0 IIc+III / 0 III+IIc type).

Statistical analysis was carried out using the Stat View® software (Abacus Concepts, Inc., California, USA). Differences in clinicopathological features among gross appearances were analyzed by Student t-test (age distribution) or χ square test with the Yates correction (distributions of gender, cancer location, cancer depth and histological type). Differences in distributions of PG levels and their ratio among groups were analyzed by Student *t*-test. A probability (p) level of less than 0.05 was considered statistically significant.

Results

The clinicopathological features in each gross appearance of early gastric cancers are shown in Table I. There was no significant difference in age, gender, cancer location, and cancer depth among gross appearances. In histological type, a significant difference was observed between polypoid cancer and depressed cancer. All polypoid cancers except

Table I - Clinopathological features in early gastric cancers classified with gross appearances and histology

	polypoid		depressed	
	intestinal	diffuse	intestinal	diffuse
mean age	65	51	60	59
(range)	(42-87)		(42-81)	(40-72)
gender				
male	28	1	45	20
female	8	0	13	6
tumor location				
U: M: L	7: 15: 14	0: 1: 0	11: 28: 19	5: 16: 5
tumor depth				
mucosa	29	0	38	16
submucosa	7	1	20	10

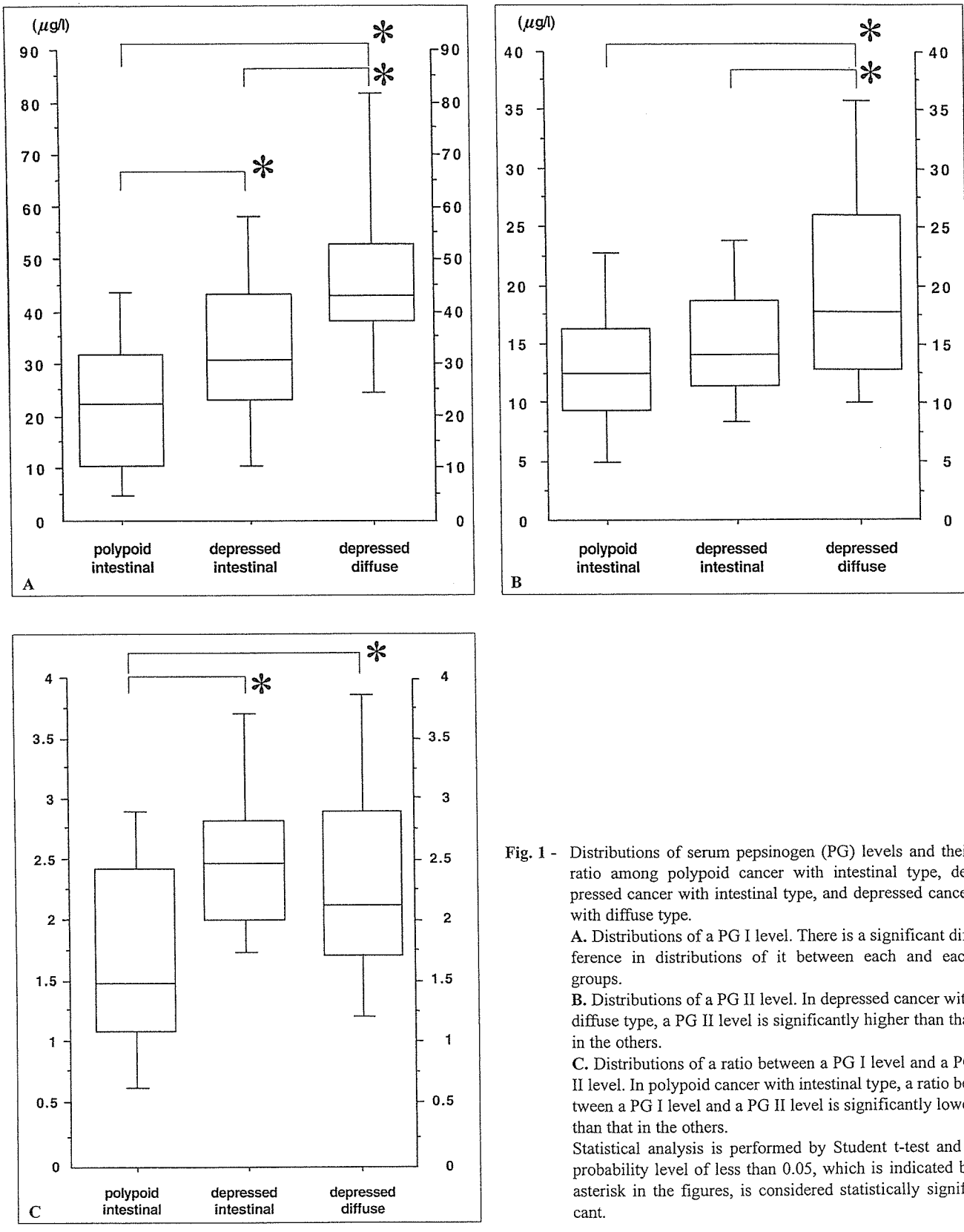

Fig. 1 - Distributions of serum pepsinogen (PG) levels and their ratio among polypoid cancer with intestinal type, depressed cancer with intestinal type, and depressed cancer with diffuse type.

A. Distributions of a PG I level. There is a significant difference in distributions of it between each and each groups.

B. Distributions of a PG II level. In depressed cancer with diffuse type, a PG II level is significantly higher than that in the others.

C. Distributions of a ratio between a PG I level and a PG II level. In polypoid cancer with intestinal type, a ratio between a PG I level and a PG II level is significantly lower than that in the others.

Statistical analysis is performed by Student t-test and a probability level of less than 0.05, which is indicated by asterisk in the figures, is considered statistically significant.

one were intestinal type, whereas nearly a third of depressed cancers were diffuse type. All the patients in whom *H. pylori* status was investigated (16 polypoid cancers, 21 depressed cancers with intestinal type and 10 depressed cancers with diffuse type) had positive results of *H. pylori* infection irrespective of gross appearances and histological types.

The distributions of a PG I level, a PG II level and a I/II ratio of each group, except for polypoid cancer with diffuse type, shows in Figures 1A, 1B and 1C, respectively. In polypoid cancer with intestinal type, a PG I level was significantly lower than that in depressed cancer with intestinal or diffuse type. Moreover, a PG I level in depressed cancer with diffuse type was significantly higher than that in depressed cancer with intestinal type. A PG II level in depressed cancer with diffuse type was significantly higher than that in the other two groups. A I/II ratio in polypoid cancer with intestinal type was significantly lower than that in the other two groups. These results summarized in Figure 2.

Discussion

Many recent studies have indicated that *H. pylori* infection is a major risk factor for the development of gastric cancer (16-19). A follow up study by Uemura et al showed that gastric cancer developed only in patients with *H. pylori* when using a full set of diagnostic tests for *H.pylori* and histological findings of severe gastric atrophy, corpus predominant gastritis,

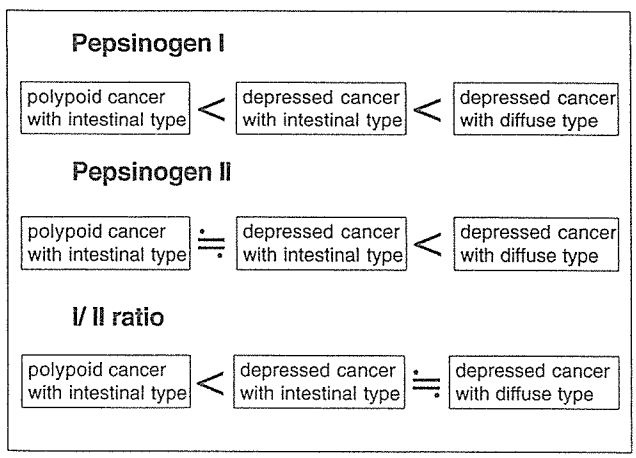

Fig. 2 - Schema showing the differences in distributions of pepsinogen levels and their ratio among early gastric cancers categorized with gross appearance and histology.

or intestinal metaplasia caused by *H.pylori* infection are at increased risk for gastric cancer (16). Our study also supported the former result that implied the necessity of *H. pylori* infection for the majority of gastric cancer patients, although *H. pylori* infection was incompletely tested among our subjects due to retrospective setting. As for as gastric cancer risk is concerned, a close relationship has been found between the presence of hypo- or achlorhydria caused by gastric atrophy and the development of gastric cancer in many studies (20-23) carried out over decades when the presence of *H. pylori* was not known. Pepsinogen, the precursor of pepsin, exists as two biochemically distinct groups of isozymes, PG I and PG II. Both are produced by the chief and mucous neck cells of the gastric fundus and corpus, and only PG II, but not PG I, is produced by the pyloric glands in the antrum and Brunner's glands in the proximal duodenum. As gastritis progresses, mild inflammation leads to elevated serum levels of PG I and PG II. As the severity of atrophy advances, chief cells are replaced by pyloric glands and the PG II level remains elevated, while the PG I level decreases. Consequently, the I/II ratio is greatly reduced (5). Therefore, both low serum PG I and a low I/II ratio, those are a PG I level $\leq$ 70 μg/l and a I/II ratio $\leq$ 3, are recognized as serological markers of gastric atrophy which may develop gastric cancer. The merits to know gastric atrophy by serological markers are; first, gastric cancer can be detected without invasive examination; second, sampling errors are minimized because the data may represent the status of atrophy of the whole stomach; third, the extent of atrophy can be easily quantified. Using this cut off point, the sensitivity and specificity of screening for gastric cancer was reported to be 84.6% and 73.5%, respectively, in the study field where 13 gastric cancers (0.25%) were detected among 5113 subjects (8). With respect to our subjects, 91.7% (33/36) of intestinal type polypoid cancers, 79.3% (46/58) depressed cancer with diffuse type, and 73.1% (19/26) of the diffuse type depressed cancers fulfilled the criteria. Yoshihara M. et al. also revealed that acid output was significantly reduced in gastric cancer patients in comparison with healthy controls, even though the histological type was diffuse type and gross type was ulcerative type (23). These findings may support that gastric mucosal atrophy may be common pre-cancerous process regardless of histology or gross appearances in most of patients with gastric cancer. We are now investigating whether it acts just as a by-stander or as an important step for gastric cancer.

In this study, we clearly distinguish the categorized groups of early gastric cancers, using serum PG levels and their ratio. The previous studies showed the difference in intragastric acidity between intestinal type and diffuse type, or polypoid type and non-polypoid type, using the endoscopic Congo red test (24) or measuring gastric acid secretion and serum PG I plus gastrin levels (19). They concluded that non-polypoid type or diffuse type gastric cancer develops in the stomach thus preserving large acid-secreting areas and acid output function to some degree, whereas polypoid type or intestinal type develops in the stomach with little or no acid-secreting area or markedly reduced acid output function. However, no combined analysis of gross appearances and histological types, especially when dealing with depressed cancers with intestinal versus diffuse type, has been performed in those studies. In our subjects, a PG I level and a I/II ratio in polypoid cancer with intestinal type were significantly lower than those in the others, whereas PG I and PG II levels in depressed cancer with diffuse type were significantly higher. From a serological point of view of PG levels, these findings indicate that polypoid cancer with intestinal type may develop from less inflammatory and more atrophic gastric mucosa than depressed cancer with intestinal type, whereas depressed cancer with diffuse type may develop from inflammatory gastric mucosa than depressed cancer with intestinal type but similar atrophy. Such a difference may suggest a diversity, not only in the intragastric acidity and the extent of gastric atrophy, but also in the carcinogenesis among those groups, although further studies using molecular markers are necessary to shed light on the underlying mechanisms.

References

1. Webb P.M., Hengels K.J., Moller H. et al.: The epidemiology of low serum pepsinogen A levels and an international association with gastric cancer rates. EUROGAST Study Group. Gastroenterology. 107: 1335-1344, 1994.
2. Fukao A., Tsubono Y., Tsuji I., Hisamichi S., Sugahara N., Takano A.: The evaluation of screening for gastric cancer in Miyagi Prefecture, Japan: a population-based case-control study. Int. J. Cancer. 60: 45-48, 1995.
3. Miki K., Ichinose M., Ishikawa K.B. et al.: Clinical application of serum pepsinogen I and II levels for mass screening to detect gastric cancer. Jpn. J. Cancer. Res. 84: 1086-1090, 1993.
4. Kodoi A., Yoshihara M., Sumii K., Haruma K., Kajiyama G.: Serum pepsinogen in screening for gastric cancer. J. Gastroenterol. 30: 452-460, 1995.
5. Samloff I.M., Varis K., Ihamaki T., Siurala M., Rotter JI.: Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. Gastroenterology. 83: 204-209, 1982.
6. Miki K., Ichinose M., Shimizu A. et al.: Serum pepsinogens as a screening test of extensive chronic gastritis. Gastroenterol. Jpn. 22: 133-141, 1987.
7. Correa P.: A human model of gastric carcinogenesis. Cancer Res. 48: 3554-3560, 1988.
8. Kitahara F., Kobayashi K., Sato T., Kojima Y., Araki T., Fujino M.A.: Accuracy of screening for gastric cancer using serum pepsinogen levels. Gut. 44: 693-697, 1999.
9. Chan A.O., Luk J.M., Hui W.M., Lam S.K.: Molecular biology of gastric carcinoma: from laboratory to bedside. J. Gastroenterol. Hepatol. 14: 1150-1160, 1999.
10. Shimoda T., Ikegami M., Fujisaki J., Matsui T., Aizawa S., Ishikawa E.: Early colorectal carcinoma with special reference to its development de novo. Cancer 64: 1138-1146, 1989.
11. Chiang J.M., Chou Y.H., Chou T.B.: K-ras codon 12 mutation determines the polypoid growth of colorectral cancer. Cancer Res. 58: 3289-3293, 1998.
12. Samloff I.M., Townes P.L.: Electrophoretic heterogeneity and relationships of pepsinogens in human urine, serum, and gastric mucosa. Gastroenterology 58: 462-469, 1970.
13. Festen H.P., Thijs J.C., Lamers C.B. et al.: Effect of oral omeprazole on serum gastrin and serum pepsinogen I levels. Gastroenterology 87:1030-1034, 1984.
14. Japanese gastric cancer association.: Japanese classification of gastric carcinoma - 2nd English edition-. Gastric Cancer 1: 10-24, 1998.
15. Lauren P.: The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. Acta Pathol. Microbiol. Scand. 64:31-49, 1965.
16. Uemura N., Okamoto S., Yamamoto S. et al: Helicobacter pylori infection and the development of gastric cancer. N. Engl. J. Med. 345:784-789, 2001.
17. Ohata H., Kitauchi S., Yoshimura N. et al: Progression of chronic atrophic gastritis associated with Helicobacter pylori infection increases risk of gastric cancer. Int. J. Cancer 109:138-143, 2004.
18. Wong B.C., Lam S.K., Wong W.M. et al: Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. JAMA. 291:187-194, 2004.
19. Watabe H., Mitsushima T., Yamaji Y. et al: Predicting the development of gastric cancer from combining Helicobacter pylori antibodies and serum pepsinogen status: a prospective endoscopic cohort study. Gut. 54:764-768, 2005.
20. Hitchcock CR., Sullivan WA., Wangensteen OH.: The value of achlorhydria as a screening testfor gastric cancer. A 10-year report. Gastroenterology 29:621-628, 1955.
21. Grossman MI., Kirsner JB., Gillespie IE.: Basal and histalog-stimulated gastric secretion in control subjects and in patients with peptic ulcer or gastric cancer. Gastroenterology

45:14-26, 1963.
22. Svendsen J.H., Dahl C., Svendsen L.B., Christiansen P.M.: Gastric cancer risk in achlorhydric patients. A long-term follow-up study. Scand. J. Gastroenterol. 21:16-21, 1986.
23. Yoshihara M., Haruma K., Sumii K. et al.: The relationship between gastric secretion and type of early gastric carcinoma. Hiroshima. J. Med. Sci. 44:79-82, 1995.
24. Tatsuta M., Okuda S,. Taniguchi H., Tamura H.: Gross and histological types of early gastric carcinomas in relation to the acid-secreting area. Cancer 43:317-321, 1979.

Received: December 13, 2005

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World J Gastroenterol 2006 May 21; 12(19): 3088-3091
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Hydrogen and methane gases are frequently detected in the stomach

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Received: 2005-12-12 Accepted: 2006-01-14

Abstract

AIM: To investigate the incidence of bacterial overgrowth in the stomach by using a new endoscopic method in which intragastric hydrogen and methane gases are collected and analyzed.

METHODS: Studies were performed in 490 consecutive patients undergoing esophagogastroscopy. At endoscopy, we intubated the stomach without inflation by air, and 20 mL of intragastric gas was collected through the biopsy channel using a 30 mL syringe. Intragastric hydrogen and methane concentrations were immediately measured by gaschromatography. *H pylori* infection was also determined by serology.

RESULTS: Most of intragastric hydrogen and methane levels were less than 15 ppm (parts per million). The median hydrogen and methane values (interquartile range) were 3 (1-8) ppm and 2 (1-5) ppm, respectively. The high hydrogen and methane levels for indication of fermentation were decided if the patient had the values more than 90 percentile range in each sample. When a patient had a high level of hydrogen or methane in one or more samples, the patient was considered to have fermentation. The overall incidence of intragastric fermentation was 15.4% (73/473). Intragastric methane levels were higher in the postoperative group than in other groups. None of the mean hydrogen or methane values was related to *H pylori* infection.

CONCLUSION: Hydrogen and methane gases are more frequently detected in the stomach than expected, regardless of the presence of abdominal symptoms. Previous gastric surgery influences on the growth of methane-producing bacteria in the fasting stomach.

Key words: Intragastric gases; Hydrogen; Methane; *Helicobacter pylori*

Urita Y, Ishihara S, Akimoto T, Kato H, Hara N, Honda Y, Nagai Y, Nakanishi K, Shimada N, Sugimoto M, Miki K. Hydrogen and methane gases are frequently detected in the stomach. *World J Gastroenterol* 2006; 12(19): 3088-3091

http://www.wjgnet.com/1007-9327/12/3088.asp

INTRODUCTION

Hydrogen breath tests have been used to evaluate intestinal transit, bacterial overgrowth, and disaccharidase deficiency[1-8]. As hydrogen production increases when a small amount of carbohydrate is supplied to colonic bacteria, the measurement of breath hydrogen concentration has been proposed as an indicator of carbohydrate malabsorption[2]. Similarly, breath methane excretion, which reflects an indirect measurement of the metabolism of the anaerobic colonic flora, has been measured[9,10]. Methanogenic bacteria utilize hydrogen, carbon dioxide, and then synthesize methane[11]. All methane absorbed from the colon reaches the lung and excretes into the breath[12]. If the fermentation occurs in the stomach, we can detect intragastric hydrogen and/or methane gas. We therefore attempted to collect intragastric gas endoscopically and measure the intragastric hydrogen and methane levels in order to determine the bacterial overgrowth in the stomach.

MATERIALS AND METHODS

Patients

Studies were performed in 490 consecutive patients (160 men and 315 women, 19-85 years old) undergoing upper endoscopy. None of the patients had a history of use of proton pump inhibitor (PPI), H_2-receptor antagonist (H_2-RA), antibiotics, steroids, or nonsteroidal anti-inflammatory drugs for a period of at least six month before the investigation. Twelve patients had a previous Billroth I

partial gastrectomy.

Collection of intragastric gas samples

Endoscopy was performed after a topical anesthesia gargle. At the time of endoscopic examination, we intubated the stomach without inflation by air, and 20 mL of intragastric gas was collected through the biopsy channel using a 30-mL syringe. The first 5 mL was discarded for reduction of dead-space error. Intragastric hydrogen and methane concentrations were immediately measured by gaschromatography using Breath Analyzer TGA-2000 (TERAMECS Co. Ltd. Kyoto) and expressed in parts per million (ppm). Linear accuracy response range was 2 to 150 ppm. After collecting an intragastric gas sample, the endoscopist inflated the stomach by air and observed the gastric mucosa.

Determination of H pylori status

At endoscopy, serum *H pylori* IgG antibody titers were measured with an ELISA method (HM-CAP). A value of >2.2 was considered seropositive and a value of <1.9 was considered seronegative. Patients with a value of neither less than 1.9 nor more than 2.2 were excluded from this study.

Statistical analysis

Data of intragastric hydrogen and methane were presented as medians, with interquartile ranges because they were not normally distributed. Comparisons of groups were made using the Mann-Whitney *U* test. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Incidence of high hydrogen and methane value in the stomach

Seventeen patients were dropped from this study because their serum *H pylori* IgG antibody titers were indeterminate. Figures 1 and 2 show the distribution of intragastric hydrogen and methane levels in the remaining 473 subjects, respectively. Most of the levels were less than 15 ppm. Overall, the median hydrogen and methane values (interquartile range) were 3 (1-8) ppm and 2 (1-5) ppm, respectively. The high hydrogen and methane levels for indication of fermentation were decided if the patient had the values more than 90 percentile range in each sample. Based on this definition, high hydrogen levels were defined as ≥21 ppm and high methane values as ≥8 ppm. In this study, when a patient had a high level of hydrogen or methane in one or more samples, the patient was considered to have fermentation. The overall incidence of intragastric fermentation was 15.4% (73/473). The incidence of intragastric fermentation determined by the intragastric hydrogen level was 11.0% (52/473), whereas those determined by the intragastric methane level was 10.8% (51/473). Of 73 patients with intragastric fermentation, gastric cancer was found in 2 (2/4, 50%), gastric ulcer in 4 (4/38, 11%), duodenal ulcer in 3 (3/15, 20%), previous gastric surgery in 3 (3/12, 25%), and others in 61 patients (61/404, 15%).

Peptic ulcer and intragastric gas concentrations

Intragastric hydrogen and methane values in relation to endoscopic diagnosis are summarized in Table 1. Intragastric methane levels were significantly higher in the postoperative group than in the gastric ulcer group and in the other groups. Intragastric hydrogen levels were lower in the gastric ulcer group than in other groups but this did not reach statistical significance.

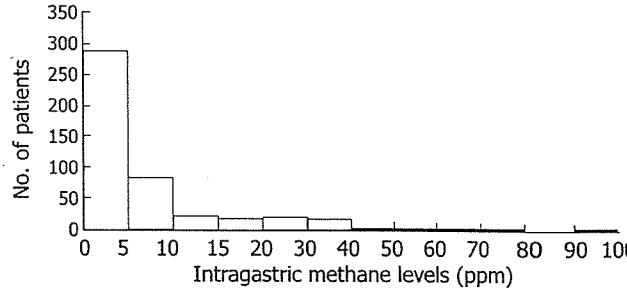

Figure 1 Distribution of intragastric hydrogen levels in all 473 subjects.

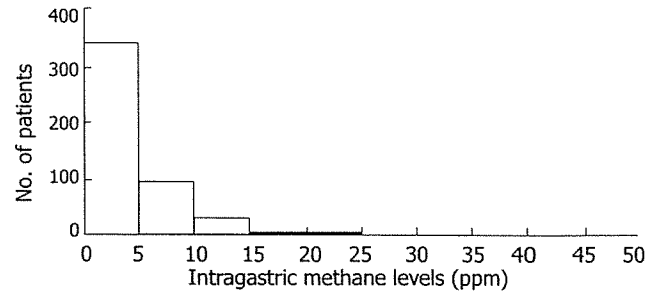

Figure 2 Distribution of intragastric methane levels in all 473 subjects.

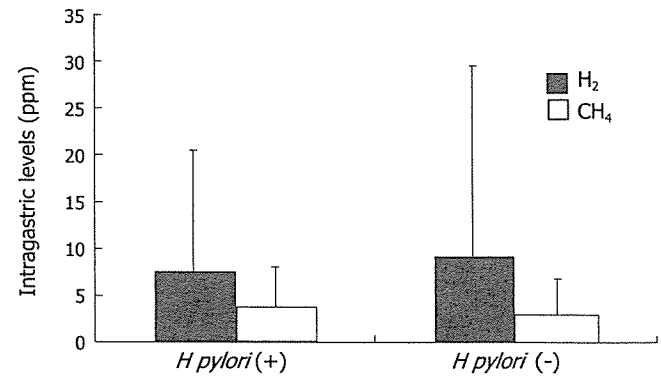

Figure 3 Intragastric hydrogen and methane levels in relation to *H pylori* status.

Table 1 Intragastric hydrogen and methane levels in relation to endoscopic findings

	Gastric ulcer	Duodenal ulcer	Postoperative stomach	Others
No.of patients	38	15	12	404
H2 (ppm)	6.8 ± 10.7*	10.9 ± 17.2	11.0 ± 13.9	8.2 ± 16.9
P values *vs**	*	0.15	0.14	0.31
CH4 (ppm)	3.7 ± 5.6	3.7 ± 3.6	8.1 ± 12.6**	3.4 ± 4.1
P values *vs***	0.047	0.1	**	0.0002

H pylori infection and intragastric gas concentrations

Figure 3 shows the means of intragastric hydrogen and methane concentrations by *H pylori* status. None of the mean values were related to *H pylori* infection.

DISCUSSION

Before the discovery of *H pylori* infection in 1983[13], it was demonstrated by many investigators that an increased number of bacteria had been found in the stomach in patients with achlorhydria or hypochlorhydria[14]. The type and numbers of microbial flora present in the stomach are affected by gastric pH[15-17], and a rise in intragastric pH has often been associated with an increased number of bacteria in gastric juice[18-20]. Atrophic gastritis is the most common cause of reduced gastric acid secretion, and it often results in bacterial overgrowth[21-23]. It is presently possible to reduce gastric acid secretion with H_2-RA or PPI. Treatment with PPI[24-26] or H_2-RA[27] induces a clinical state similar to atrophic gastritis with hypochlorhydria and frequently associated with bacterial overgrowth. Recently, there is considerable information on the efficacy of maintenance treatment with reflux esophagitis for up to 1 year[28-30]. As gastric acid plays an important part in the prevention of bacterial colonization of the stomach and the small intestine, reduction of gastric acid secretion by PPI or H_2-RA results in gastric and intestinal bacterial overgrowth[24-27,31,32]. In fact, it has been reported a marked increase in bacterial titers in fasting gastric aspirates from patients receiving H_2-RA[31,33,34]. On the other hand, the results of identification and quantification of microbes in samples from the gastrointestinal tract are significantly influenced by the culture technique[35].

The most direct method for diagnosing bacterial overgrowth is to perform microbiological cultures after obtaining gastric aspirates. Actually, the microbial flora, which is dominated by *Viridans streptococci, coaglase negative Staphylococci, Haemophilus sp.*[36], *Neisseria spp., Lactobacillus spp., Candida spp.*, and *Aspergillus spp.*[31], has been demonstrated. However, the study of gastrointestinal flora by direct methods is cumbersome, primary due to its inaccessible location. In addition, the results of identification and quantification of microbes in samples from the gastrointestinal tract are significantly influenced by difficulties in accurate tube placement, contamination during insertion, delay between sampling and inoculation of culture media, and inadequate anaerobic isolation techniques. In addition, intubation methods are time-consuming, and uncomfortable. Therefore, breath tests were devised as simple alternatives to these invasive tests.

Breath hydrogen measurement is now used in clinical practice to investigate several disorders, including small intestinal disaccharidase deficiencies, intestinal bacterial overgrowth, and orocecal transit time[1-8]. It is based on the ability of the anaerobic microflora of the colon to ferment carbohydrate that has traveled unabsorbed through the small intestine, and to produce hydrogen. This hydrogen is transported to the lungs and exhaled in the expired breath. Although breath tests, such as measuring fasting or postprandial hydrogen concentrations, are a noninvasive method, avoiding the risk of sampling error, it is unable to identify the site of overgrowth. Then, we attempted to measure intragastric hydrogen and methane concentrations so as to determine the site of bacterial overgrowth and the incidence of fermentation in the stomach.

We previously reported the endoscopic ^{13}C-urea breath test (e-UBT)[37] in which intragastric gas was collected and analyzed. Using the same sample collection method as e-UBT, hydrogen and methane gases, which are produced by hydrogen-producing bacteria, could be detected in the stomach. These values were considered to reflect directly the intragastric fermentation.

To the best of our knowledge, our study is the first investigation to measure directly intragastric hydrogen and methane concentrations. The values of intragastric hydrogen concentrations above 21 ppm and methane above 8 ppm were considered abnormal in this study. When a patient had a high level of hydrogen or methane in one or more samples, the patient was considered to have fermentation. The overall incidence of intragastric fermentation was 15.4% (73/473). There was no difference in the incidence of intragastric fermentation determined by hydrogen or methane concentrations. These results reveal that intragastric fermentation is found in more than 15% of patients without medication even after overnight fasting.

In this study, intragastric methane levels were higher in the postoperative group than in other groups, whereas there was a negligible difference of intragastric hydrogen levels between the postoperative stomach group and other groups, thereby suggesting that previous gastric surgery is more closely correlated to methane-producing bacterial overgrowth in the stomach, compared to hydrogen-producing bacterial overgrowth although the exact mechanism is unknown.

On the other hand, Fried *et al*[26] reported that most of the bacteria identified from the duodenal aspirates belonged to species colonizing the oral cavity and pharynx, suggesting a descending route of colonization. Also, Thompson *et al*[38] indicated that fermentation of ingested carbohydrate by oropharyngeal bacteria could contribute to measure breath hydrogen values soon after meal ingestion. In addition, many investigators have reported that treatment with PPI[23-25] or H_2-RA[26] induces a clinical state similar to atrophic gastritis with hypochlorhydria and a marked increase in bacterial titers in fasting gastric aspirates from patients receiving H_2-RA[18,20,21]. Although Husebye *et al*[36] have reported that fasting hypochlorhydria associated with gastric colonization of microbes belonging to the oro- and nasopharyngeal flora is highly prevalent in healthy old people, atrophic gastritis is the most common cause of reduced gastric acid secretion, and it often results in bacterial overgrowth[21,39]. We previously reported that intestinal metaplasia was detected in 65.4% (358/547) patients with serum IgG antibody to *H pylori*. Therefore, we expected that a discriminative value might be detected between *H pylori*-positive and *H pylori*-negative patients. As shown in Figure 3, none of the mean values were related to *H pylori* infection.

In summary, we measured directly intragastric hydrogen and methane concentrations using endoscopy and found the incidences of hydrogen and methane production in the stomach. In this new method, the intragastric

gas can be easily collected endoscopically and it does not take much time. Hydrogen and methane gases are more frequently detected in the stomach than expected, regardless of the presence of abdominal symptoms. Moreover, previous gastric surgery influences on the production of methane in the fasting stomach and probably the growth of methane-producing bacteria in the upper digestive tract.

REFERENCES

1 **Hoekstra JH.** Fructose breath hydrogen tests in infants with chronic non-specific diarrhoea. *Eur J Pediatr* 1995; **154:** 362-364
2 **Levitt MD,** Donaldson RM. Use of respiratory hydrogen (H2) excretion to detect carbohydrate malabsorption. *J Lab Clin Med* 1970; **75:** 937-945
3 **Bond JH Jr,** Levitt MD. Use of pulmonary hydrogen (H2) measurements to quantitate carbohydrate absorption. Study of partially gastrectomized patients. *J Clin Invest* 1972; **51:** 1219-1225
4 **Urita Y,** Hike K, Torii N, Kikuchi Y, Sasajima M, Miki K. Efficacy of lactulose plus ^{13}C-acetate breath test in the diagnosis of gastrointestinal motility disorders. *J Gastroenterol* 2002; **37:** 442-448
5 **Bond JH Jr,** Levitt MD, Prentiss R. Investigation of small bowel transit time in man utilizing pulmonary hydrogen (H2) measurements. *J Lab Clin Med* 1975; **85:** 546-555
6 **La Brooy SJ,** Male PJ, Beavis AK, Misiewicz JJ. Assessment of the reproducibility of the lactulose H2 breath test as a measure of mouth to caecum transit time. *Gut* 1983; **24:** 893-896
7 **Gotze H,** Ptok A. Orocaecal transit time in patients with Crohn disease. *Eur J Pediatr* 1993; **152:** 193-196
8 **Rhodes JM,** Middleton P, Jewell DP. The lactulose hydrogen breath test as a diagnostic test for small-bowel bacterial overgrowth. *Scand J Gastroenterol* 1979; **14:** 333-336
9 **McKay LF,** Eastwood MA, Brydon WG. Methane excretion in man--a study of breath, flatus, and faeces. *Gut* 1985; **26:** 69-74
10 **Bjorneklett A,** Jenssen E. Relationships between hydrogen (H2) and methane (CH4) production in man. *Scand J Gastroenterol* 1982; **17:** 985-992
11 **Stadtman TC.** Methane fermentation. *Annu Rev Microbiol* 1967; **21:** 121-142
12 **Bond JH Jr,** Engel RR, Levitt MD. Factors influencing pulmonary methane excretion in man. An indirect method of studying the in situ metabolism of the methane-producing colonic bacteria. *J Exp Med* 1971; **133:** 572-88
13 **Marshall BJ,** Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; **1:** 1311-1315
14 **Garrod LP.** A study of the bactericidal power of hydrochloric acid and of gastric juice. *St Barth Hosp Rep* 1939; **72:** 145-167
15 **Gray JD,** Shiner M. Influence of gastric pH on gastric and jejunal flora. *Gut* 1967; **8:** 574-581
16 **Drasar BS,** Shiner M, McLeod GM. Studies on the intestinal flora. I. The bacterial flora of the gastrointestinal tract in healthy and achlorhydric persons. *Gastroenterology* 1969; **56:** 71-79
17 **Gilman RH,** Partanen R, Brown KH, Spira WM, Khanam S, Greenberg B, Bloom SR, Ali A. Decreased gastric acid secretion and bacterial colonization of the stomach in severely malnourished Bangladeshi children. *Gastroenterology* 1988; **94:** 1308-1314
18 **Giannella RA,** Broitman SA, Zamcheck N. Gastric acid barrier to ingested microorganisms in man: studies in vivo and in vitro. *Gut* 1972; **13:** 251-256
19 **Ruddell WS,** Bone ES, Hill MJ, Walters CL. Pathogenesis of gastric cancer in pernicious anaemia. *Lancet* 1978; **1:** 521-523
20 **Reed PI,** Smith PL, Haines K, House FR, Walters CL. Gastric juice N-nitrosamines in health and gastroduodenal disease. *Lancet* 1981; **2:** 550-552
21 **Villako K,** Tamm A, Savisaar E, Ruttas M. Prevalence of antral and fundic gastritis in a randomly selected group of an Estonian rural population. *Scand J Gastroenterol* 1976; **11:** 817-822
22 **Siurala M,** Isokoski M, Varis K, Kekki M. Prevalence of gastritis in a rural population. Bioptic study of subjects selected at random. *Scand J Gastroenterol* 1968; **3:** 211-223
23 **Kreuning J,** Bosman FT, Kuiper G, Wal AM, Lindeman J. Gastric and duodenal mucosa in 'healthy' individuals. An endoscopic and histopathological study of 50 volunteers. *J Clin Pathol* 1978; **31:** 69-77
24 **Sharma BK,** Santana IA, Wood EC, Walt RP, Pereira M, Noone P, Smith PL, Walters CL, Pounder RE. Intragastric bacterial activity and nitrosation before, during, and after treatment with omeprazole. *Br Med J* (Clin Res Ed) 1984; **289:** 717-719
25 **Saltzman JR,** Kowdley KV, Pedrosa MC, Sepe T, Golner B, Perrone G, Russell RM. Bacterial overgrowth without clinical malabsorption in elderly hypochlorhydric subjects. *Gastroenterology* 1994; **106:** 615-623
26 **Fried M,** Siegrist H, Frei R, Froehlich F, Duroux P, Thorens J, Blum A, Bille J, Gonvers JJ, Gyr K. Duodenal bacterial overgrowth during treatment in outpatients with omeprazole. *Gut* 1994; **35:** 23-26
27 **Stockbrugger RW,** Cotton PB, Eugenides N, Bartholomew BA, Hill MJ, Walters CL. Intragastric nitrites, nitrosamines, and bacterial overgrowth during cimetidine treatment. *Gut* 1982; **23:** 1048-1054
28 **Klinkenberg-Knol EC,** Festen HP, Jansen JB, Lamers CB, Nelis F, Snel P, Luckers A, Dekkers CP, Havu N, Meuwissen SG. Long-term treatment with omeprazole for refractory reflux esophagitis: efficacy and safety. *Ann Intern Med* 1994; **121:** 161-167
29 **Klinkenberg-Knol EC,** Nelis F, Dent J, Snel P, Mitchell B, Prichard P, Lloyd D, Havu N, Frame MH, Roman J, Walan A. Long-term omeprazole treatment in resistant gastroesophageal reflux disease: efficacy, safety, and influence on gastric mucosa. *Gastroenterology* 2000; **118:** 661-669
30 **Hallerback B,** Unge P, Carling L, Edwin B, Glise H, Havu N, Lyrenas E, Lundberg K. Omeprazole or ranitidine in long-term treatment of reflux esophagitis. The Scandinavian Clinics for United Research Group. *Gastroenterology* 1994; **107:** 1305-1311
31 **Snepar R,** Poporad GA, Romano JM, Kobasa WD, Kaye D. Effect of cimetidine and antacid on gastric microbial flora. *Infect Immun* 1982; **36:** 518-524
32 **Ruddell WS,** Axon AT, Findlay JM, Bartholomew BA, Hill MJ. Effect of cimetidine on the gastric bacterial flora. *Lancet* 1980; **1:** 672-674
33 **Muscroft TJ,** Youngs D, Burdon DW, Keighley MR. Cimetidine and the potential risk of postoperative sepsis. *Br J Surg* 1981; **68:** 557-559
34 **Mowat C,** Williams C, Gillen D, Hossack M, Gilmour D, Carswell A, Wirz A, Preston T, McColl KE. Omeprazole, Helicobacter pylori status, and alterations in the intragastric milieu facilitating bacterial N-nitrosation. *Gastroenterology* 2000; **119:** 339-347
35 **Clarke RT,** Beauchop T. Methods for studying gut microbes. In: Clarke RTJ, Beauchop T, eds. Microbial ecology of the gut. London: Acaddemic Press, 1977: 33
36 **Husebye E,** Skar V, Hoverstad T, Melby K. Fasting hypochlorhydria with gram positive gastric flora is highly prevalent in healthy old people. *Gut* 1992; **33:** 1331-1337
37 **Urita Y,** Miki K. Endoscopic ^{13}C-urea breath test. *Dig Endosc* 2000; **12:** 29-32
38 **Thompson DG,** O'Brien JD, Hardie JM. Influence of the oropharyngeal microflora on the measurement of exhaled breath hydrogen. *Gastroenterology* 1986; **91:** 853-860
39 **Siurala M,** Isokoski M, Varis K, Kekki M. Prevalence of gastritis in a rural population. Bioptic study of subjects selected at random. *Scand J Gastroenterol* 1968; **3:** 211-223

S-Editor Wang J **L-Editor** Kumar M **E- Editor** Zhang Y

High incidence of fermentation in the digestive tract in patients with reflux oesophagitis

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Objectives **Because bacteria represent the sole source of gut hydrogen (H_2) and methane (CH_4), fasting breath H_2 and CH_4 gases have been used as markers of colonic fermentation. The presence of carbohydrates in the colonic lumen inhibits gastric and pancreatic secretions, and also influences lower oesophageal sphincter function in gastro-oesophageal reflux disease.**

Materials and methods **Studies were performed in 793 consecutive patients undergoing oesophagogastroscopy (270 men and 523 women, aged 19–85 years). A fasting breath sample (20 ml) was collected before endoscopy. At endoscopy, we intubated the stomach without inflation by air, and 20 ml of intragastric gas was collected through the biopsy channel. Next, the tip of the endoscope was inserted into the second portion of the duodenum without inflation by air, and 20 ml of intraduodenal gas was collected. H_2 and CH_4 concentrations of each sample were measured by gas chromatography.**

Results **Reflux oesophagitis was found in 147 of the 793 patients. The mean values of the H_2 and/or CH_4 levels of samples taken from the stomach, duodenum and exhaled air were higher in patients with reflux oesophagitis than those without reflux oesophagitis. High H_2 and/or CH_4 levels were more frequently found in patients with reflux oesophagitis.**

Conclusions **We concluded that the presence of fermentation in the digestive tract was considered to be a risk factor for developing reflux oesophagitis.** ***Eur J Gastroenterol Hepatol*** **18:531–535 © 2006 Lippincott Williams & Wilkins.**

European Journal of Gastroenterology & Hepatology 2006, 18:531–535

Keywords: reflux oesophagitis, fermentation, hydrogen gas, methane gas

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This work was supported in part by a grant-in-aid for research from the Ministry of Health, Labour and Welfare, Japan (16271201).

Received 11 February 2005 Accepted 11 January 2006

Introduction

Hydrogen breath tests have been used to evaluate intestinal transit, bacterial overgrowth and disaccharidase deficiency [1–8]. Because bacteria represent the sole source of gut hydrogen (H_2) and methane (CH_4), fasting breath H_2 and CH_4 gases have been used as markers of colonic fermentation [9,10]. As H_2 production increases when a small amount of carbohydrate is supplied to colonic bacteria, the measurement of breath H_2 concentration has been proposed as an indicator of carbohydrate malabsorption [2]. Similarly, breath CH_4 excretion, which reflects an indirect measurement of the metabolism of the anaerobic colonic flora, has been measured [11,12]. Methanogenic bacteria utilize H_2 and carbon dioxide (CO_2), and then synthesize CH_4 [13]. CH_4 absorbed from the colon reaches the lung and is excreted into the breath [14].

The presence of carbohydrates in the colonic lumen inhibits gastric [15] and pancreatic [16] secretions, and also influences lower oesophageal sphincter function in gastro-oesophageal reflux disease [17]. Based on the fact that 2–20% of carbohydrates escape absorption in the small intestine [18], colonic fermentation is considered to be present more frequently than expected and may result in gastro-oesophageal reflux diseases, including reflux oesophagitis. It is still unclear whether patients with reflux oesophagitis have colonic fermentation more frequently in clinical practice.

On the other hand, Ropert *et al.* [19] showed that H_2 production and gastric relaxation were greater after oral ingestion of lactulose than those after colonic infusion. This suggests that the fermentation in the digestive tract other than the colon may influence gastrointestinal motility. If fermentation occurs in the stomach or proximal small intestine, we can detect intragastric or intraduodenal H_2 and/or CH_4 gas. We attempted to collect intragastric and intraduodenal gas at endoscopy and measure the H_2 and CH_4 concentrations in gaseous samples taken from the stomach and the duodenum to

determine the presence of fermentation in the stomach and the proximal small intestine.

The aim of the present study was to investigate the incidence of fermentation in the digestive tract in patients with reflux oesophagitis and to determine whether fermentation may be associated with developing reflux oesophagitis.

Material and methods

Patients

Studies were performed in 793 consecutive patients undergoing oesophagogastroscopy (270 men and 523 women, aged 19–85 years). These patients were referred for upper endoscopy with various abdominal symptoms. The main symptom was heartburn in 138 patients, epigastric pain in 295, nausea in 54 and dysphagia in 296. They all gave informed consent. None of the patients had a history of use of a proton pump inhibitor (PPI), an H_2-receptor antagonist, antibiotics, steroids, or non-steroidal anti-inflammatory drugs for a period of at least 6 months before the investigation. Patients who had a previous history of partial gastrectomy were also excluded from the study. The study was approved by our local ethics committee.

Diagnosis of reflux oesophagitis

Reflux oesophagitis was diagnosed by the presence of mucosal breaks in the oesophageal mucosa according to the Los Angeles classification [20].

Collection of gas samples

A fasting breath sample (20 ml) was collected before endoscopy, which was performed after a topical anaesthesia gargle. At the time of endoscopic examination, we intubated the stomach without inflation by air, and 20 ml of intragastric gas was collected through the biopsy channel using a 30 ml syringe. The first 5 ml was discarded for reduction of dead-space error. Next, the tip of the endoscope was inserted into the second portion of the duodenum without inflation by air, and 20 ml of intraduodenal gas was collected in the same way. H_2 and CH_4 concentrations of each sample were immediately measured by gas chromatography using a Breath Analyzer TGA-2000 (TERAMECS Co. Ltd, Kyoto) and expressed in parts per million (ppm). The response range for linear accuracy was 2–150 ppm. After collecting intragastric and intraduodenal gas samples, the endoscopist inflated the stomach with air and observed the gastric and oesophageal mucosa. Reflux oesophagitis was endoscopically defined when the mucosal break was found in the lower oesophagus.

Statistical analysis

Data of intragastric, intraduodenal and breath H_2 and CH_4 were presented as medians, with interquartile ranges because they were not normally distributed. Comparisons of groups were made using the Mann–Whitney U-test. A P value of < 0.05 was accepted as indicating statistical significance. Odds ratios with 95% confidence intervals (CIs) were calculated to measure the strength of associations between reflux oesophagitis and fermentation in the digestive tract.

Results

Reflux oesophagitis was found in 147 of 793 patients. They were 75 men and 72 women, with a mean age of 57.9 years (range, 28–75 years). The number of patients with grade A was 128, with grade B was 18, and with grade C was one, according to the Los Angeles classification [20]. Of these patients, 83 complained of heartburn or chest pain, whereas 64 had no abdominal symptoms. Among 626 patients without reflux oesophagitis, 55 had heartburn.

Overall medians with interquartile ranges are shown in Table 1. The median H_2 value (interquartile range) is 3.0 (0–219) ppm in the stomach, 2.0 (0–56) ppm in the breath, and 3.0 (0–828) ppm in the duodenum. The median CH_4 values (interquartile range) are 2.0 (0–53) ppm, 2.0 (0–47) ppm, and 2.0 (0–91) ppm, respectively.

Hydrogen

The median value (interquartile range) of intragastric H_2 gas in patients with and without reflux oesophagitis was 4.0 (1–12) ppm and 3.0 (1–8) ppm, respectively (Table 2). The median value of fasting breath H_2 gas in patients with and without reflux oesophagitis was 2.0 (1–5) ppm and 1.5 (0.75–4) ppm, respectively. These differences were not significant. Intraduodenal H_2 concentration was significantly higher in patients with reflux oesophagitis than in those without reflux oesophagitis (4.0 (1–11) ppm vs. 3.0 (1–8) ppm, $P = 0.035$).

Methane

The median value (interquartile range) of intragastric CH_4 gas in patients with and without reflux oesophagitis was 3.0 (1–5) ppm and 2.0 (1–5) ppm, respectively (Table 2). The median value of fasting breath CH_4 gas in patients with and without reflux oesophagitis was 2.0 (0–5) ppm and 2.0 (0–3) ppm, respectively. The median value of intraduodenal CH_4 gas in patients with and without reflux

Table 1 Overall results of H_2 and CH_4 values of gas samples taken from the stomach, duodenum and exhaled air in 793 subjects

Sampling site	Gas	Median (range)	Interquartile range	90 percentile range
Stomach	H_2	3.0 (0–219)	1–9	0–22
	CH_4	2.0 (0–53)	1–5	0–8
Breath	H_2	2.0 (0–56)	1–4	0–10
	CH_4	2.0 (0–47)	0–4	0–8
Duodenum	H_2	3.0 (0–828)	1–8	0–21.8
	CH_4	2.0 (0–91)	0.5–5	0–8.8

Table 2 Comparison of H_2 and CH_4 values of gas samples taken from the stomach, duodenum and exhaled air between patients with and without reflux oesophagitis (RO)

Sampling site	Gas	Median (interquartile range)		*P*-value
		RO (+)	RO (−)	
Stomach	H_2	4.0 (1–12)	3.0 (1–8)	0.18
	CH_4	3.0 (1–5)	2.0 (1–5)	0.54
Breath	H_2	2.0 (1–5)	1.5 (0.75–4)	0.57
	CH_4	2.0 (0–5)	2.0 (0–3)	0.20
Duodenum	H_2	4.0 (1–11)	3.0 (1–8)	0.035
	CH_4	2.0 (1–5)	2.0 (0–4)	0.29

oesophagitis was 2.0 (1–5) ppm and 2.0 (0–4) ppm, respectively. There were no significant differences of gaseous levels of samples taken from the three sites in patients with and without reflux oesophagitis.

Incidence of fermentation

The high H_2 and CH_4 levels for indication of fermentation were decided if the patient had the values more than 90 percentile range in each sample. Based on this definition, high H_2 levels were defined as intragastric values ≥ 22 ppm, breath values ≥ 10 ppm, and intraduodenal values ≥ 21.8 ppm. Similarly, high CH_4 levels were defined as intragastric and breath values ≥ 8 ppm and intraduodenal values ≥ 8.8 ppm. In this study, when a patient had a high level of H_2 or CH_4 in one or more samples, the patient was considered to have fermentation.

The overall incidences of high H_2 and/or CH_4 in the stomach, the exhaled breath, and the duodenum were 15.0% (119/793), 19.4% (154/793) and 13.6% (108/793), respectively. Of these, as shown in Table 3, incidences of high breath H_2 and/or CH_4 levels were significantly higher in patients with reflux oesophagitis (39/147) than those without reflux oesophagitis (115/626) ($P = 0.027$). The incidences of fermentation in the stomach and the duodenum tend to be higher in patients with reflux oesophagitis, although the differences are not significant. The odds ratio was highest in breath sample followed by intraduodenal sample and intragastric sample. On the other hand, high H_2 and/or CH_4 levels for at least one sampling site were found in 11 of 55 patients with heartburn and normal endoscopy, and in 13 of 60 patients with heartburn and mucosal break of the distal oesophagus. There was no difference in the incidence of fermentation between the two groups.

Discussion

Before the discovery of *Helicobacter pylori* infection in 1983 [21], it was demonstrated by many investigators that an increased number of bacteria had been found in the stomach in patients with achlorhydria or hypochlorhydria [22]. A rise in intragastric pH has often been associated with an increased number of bacteria in gastric juice [23–25]. Atrophic gastritis is the most common cause of

Table 3 Incidences of high H_2 and/or CH_4 concentrations of samples taken from the stomach, duodenum and exhaled air in patients with and without reflux oesophagitis (RO)

Patients with fermentation sampling site	RO (+)	RO (−)	Odds ratio	95% CI	*P*-value
Stomach	25/147	94/626	1.16	0.70–1.85	0.55
Breath	39/147	115/626	1.60	1.05–2.42	0.027
Duodenum	24/147	84/626	1.21	0.72–1.97	0.45

CI, confidence interval.

reduced gastric acid secretion, and it often results in bacterial overgrowth [26–28]. As gastric acid plays an important part in the prevention of bacterial colonization of the stomach and the small intestine, reduction of gastric acid secretion by a PPI or H_2-receptor antagonist results in gastric and intestinal bacterial overgrowth [29–32].

On the other hand, the results of identification and quantification of microbes in samples from the gastrointestinal tract are significantly influenced by the culturing technique [32]. In addition, intubation methods are time consuming, and uncomfortable. Therefore, breath tests were devised as simple alternatives to these invasive tests. Breath H_2 measurement is now used in clinical practice to investigate several disorders, including small intestinal disaccharidase deficiencies, intestinal bacterial overgrowth and orocaecal transit time [1–8]. It is based on the ability of the anaerobic microflora of the colon to ferment carbohydrate that has travelled unabsorbed through the small intestine, and to produce H_2. This H_2 is transported to the lungs and exhaled in the breath. Although breath tests such as measuring fasting or postprandial H_2 concentrations are non-invasive, avoiding the risk of sampling error, the site of overgrowth cannot be identified. Therefore, we attempted to measure intragastric and intraduodenal H_2 and CH_4 concentrations to determine the site of fermentation.

Our study is the first investigation to take a direct measurement of intragastric H_2 and CH_4 concentrations. When the high H_2 and CH_4 levels for indication of fermentation were decided if the patient had the values more than 90 percentile range in each sample, the overall incidences of high H_2 and/or CH_4 production in the stomach and the duodenum were 15.0% (119/793) and 13.6% (108/793), respectively. These results reveal that intraluminal fermentation in the upper digestive tract is found in more than 13% of patients even after overnight fasting. Fermentation in the upper gastrointestinal tract was considered to be present more frequently than expected.

Fermentations in the stomach and the proximal small intestine, reflected by high H_2 or CH_4 concentrations, were detected more frequently in reflux oesophagitis patients. It has been unclear whether intragastric and

intraduodenal fermentation contributes to the regulation of gastric motility and lower oesophageal sphincter function, as well as colonic fermentation. First, we hypothesize that H_2 or CH_4 gas produced in the stomach may enhance gastro-oesophageal reflux because it is possible that gas production in the digestive tract might have stimulated mechano-receptors sensitive to distension. Unexpectedly, there was no significant difference in the value of intragastric H_2 gas in patients with and without reflux oesophagitis, whereas intraduodenal H_2 concentration was significantly higher in patients with reflux oesophagitis than in those without reflux oesophagitis. These results indicate that H_2 and CH_4 produced in the stomach do not result in the development of gastro-oesophageal reflux. Piche *et al.* [17] have also described that H_2 is unlikely to be the direct stimulus of motility changes. Instead of direct reaction of H_2 and CH_4, the exposure of the proximal colon to short-chain fatty acids contributes to the regulation of lower oesophageal sphincter function. Although the mechanisms by which the distal bowel affects upper digestive tract motility are unknown, several neuroendocrine peptides, such as peptide YY and glucagon-like peptide 1, have been proposed as ileocolic mediators of upper digestive tract inhibition [33,34]. Although both peptide YY and glucagon-like peptide 1 are present in the ileocolic mucosa, Piche *et al.* [17] reveal that an oral meal is a more physiological stimulus than colonic infusion of nutrients to release glucagon-like peptide 1 from the gut. This suggests that fermentation in the upper digestive tract may affect the gastric motility and lower oesophageal sphincter function. We have to carry out a further study on the relationship between release of neuroendocrine peptides and fermentation elsewhere in the distal ileum and the colon.

Although reflux oesophagitis is multifactorial in aetiology with potentially important modifying roles played by mucosal defensive factors, the effectiveness of oesophageal acid clearance, and differences in the potency of refluxate, the key event in the pathogenesis of reflux oesophagitis is movement of acid and other noxious substances from the stomach into the oesophagus. Therefore, neutralization of gastric contents is essential in the management of reflux oesophagitis. Next, the phenomenon of transient lower oesophageal sphincter relaxation has been considered an important risk factor in the development of reflux oesophagitis [35,36]. After gastro-oesophageal reflux, the oesophageal mucosa remains acidified. Because pepsin is the most aggressive factor in the development of mucosal injury and a pH below 3 is optimal for pepsin activation, prolongation of oesophageal acid clearance results in reflux oesophagitis.

It is well known that gastric acid plays an important part in the prevention of bacterial colonization of the stomach and the small intestine and that decreased acid secretion often induces bacterial overgrowth in the upper digestive tract [22,23]. However, bacterial overgrowth is not essential for bacterial fermentation because transient colonization can occur with bacteria from the oral cavity in healthy individuals. These organisms may be isolated in the stomach, particularly a few hours after a meal, but are thought to represent transient passage through the stomach. Similarly, a small number of microbes (generally fewer than 10^5 organisms per ml fluid) are isolated in the upper portion of the small intestine [37]. As oral bacteria are not much influenced by gastric acid secretion, it is possible that bacterial fermentation in the upper digestive tract occurs in patients with high acid secretion as well as those with *H. pylori*-induced atrophic gastritis.

According to the results of the present study, patients with reflux oesophagitis had high H_2 and CH_4 concentrations of samples taken from the stomach, duodenum and the exhaled air even after overnight fasting. This suggests that fermentation in the digestive tract continues for a long time and that transient lower oesophageal sphincter relaxation may probably occur during the overnight period. Recently, PPIs have failed to control night-time symptoms of gastro-oesophageal reflux disease in some patients and its causes have been unknown [38–40]. Some of patients with nocturnal acid breakthrough may have continuous fermentation in the digestive tract.

In summary, we took a direct measurement of H_2 and CH_4 concentrations in the stomach, duodenum and exhaled air, and showed high incidences of fermentation in the digestive tract in patients with reflux oesophagitis. We concluded that the presence of fermentation in the digestive tract could be considered a risk factor for developing reflux oesophagitis.

Conflict of interest
None declared.

Authors' contributions
Yoshihisa Urita wrote the paper. Motonobu Sugimoto contributed to the final draft of the manuscript. Kazuo Hike, Naotaka Torii, Yoshinori Kikuchi, Hidenori Kurakata and Eiko Kanda designed the study and reviewed the patients' records. Masahiko Sasajima and Kazumasa Miki contributed to the interpretation of the data. All authors approved the final form.

References

1 Hoekstra JH. Fructose breath hydrogen tests in infants with chronic non-specific diarrhoea. *Eur J Pediatr* 1995; **154**:362–364.
2 Levitt MD, Donaldson RM. Use of respiratory hydrogen (H_2) excretion to detect carbohydrate malabsorption. *J Lab Clin Med* 1970; **75**:937–945.
3 Bond JH, Levitt MD. Use of pulmonary hydrogen (H_2) measurements to quantitate carbohydrate absorption. *J Clin Invest* 1972; **51**:1219–1225.
4 Maffei HVL, Etz GL, Jenkins DJA. Hydrogen breath test: adaptation of a simple technique to infants and children. *Lancet* 1976; **22**:110–111.
5 Bond JH, Vevitt MD, Prentiss R. Investigation of small bowel transit time in man utilizing pulmonary hydrogen (H_2) measurements. *J Lab Clin Med* 1975; **85**:546–555.
6 La Brooy SJ, Male P-J, Beavis AK, Misiewicz JJ. Assessment of the reproducibility of the lactulose H_2 breath test as a measure of mouth to caecum transit time. *Gut* 1983; **24**:893–896.

7 Gotze H, Ptok A. Orocaecal transit time in patients with Crohn disease. *Eur J Pediatr* 1993; **152**:193–196.
8 Rhodes JM, Middleton P, Jewell DP. The lactulose hydrogen breath test as a diagnostic test for small-bowel bacterial overgrowth. *Scand J Gastroenterol* 1979; **14**:333–336.
9 Le Marchand L, Wilkens LR, Harwood P, Cooney RV. Use of breath hydrogen and methane as markers of colonic fermentation in epidemiologic studies; circadian patterns of excretion. *Environ Health Perspect* 1992; **98**:199–202.
10 Perman JA, Modler S, Barr RG, Rosenthal P. Fasting breath hydrogen concentration: normal values and clinical application. *Gastroenterology* 1984; **87**:1358–1363.
11 McKay LF, Eastwood MA, Brydon WG. Methane excretion in man – a study of breath, flatus, and faeces. *Gut* 1985; **26**:69–74.
12 Bjorneklett A, Jenssen E. Relationships between hydrogen (H_2) and methane (CH_4) production in man. *Scand J Gastroenterol* 1982; **17**: 985–992.
13 Stadtman TC. Methane fermentation. *Ann Rev Microbiol* 1967; **21**: 121–125.
14 Bond JH, Engel RR, Levitt MD. Factors influencing pulmonary methane excretion in man. *J Exp Med* 1971; **133**:572–588.
15 Jian R, Besterman HS, Sarson DL, Aymes C, Hostein J, Bloom SR, Rambaud JC. Colonic inhibition of gastric secretion in man. *Dig Dis Sci* 1981; **26**:195–201.
16 Owyang C, Green L, Rader D. Colonic inhibition of pancreatic and biliary secretion. *Gastroenterology* 1983; **84**:470–475.
17 Piche T, des Varannes SB, Sacher-Huvelin S, Holst JJ, Cuber JC, Galmiche JP. Colonic fermentation influences lower esophageal sphincter function in gastroesophageal reflux disease. *Gastroenterology* 2003; **124**:894–902.
18 Stephen AM, Haddad AC, Philipps SF. Passage of carbohydrate into the colon. Direct measurements in humans. *Gastroenterology* 1983; **85**: 589–595.
19 Ropert A, Cherbut C, Roze C, Le Quellec A, Holst JJ, Fu-Cheng X, *et al.* Colonic fermentation and proximal gastric tone in humans. *Gastroenterology* 1996; **111**:289–296.
20 Lundell LR, Dent J, Bennett JR, Blum AL, Armstrong D, Galmiche JP, *et al.* Endoscopic assessment for esophagitis: clinical and functional correlates and further validation of the Los Angeles classification. *Gut* 1999; **45**: 172–180.
21 Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active gastritis. *Lancet* 1983; **i**:1273–1275.
22 Garrod LP. A study of the bactericidal power of hydrochloric acid and of gastric juice. *St Barth Hosp Rep* 1939; **72**:145–167.
23 Giannela RA, Broitman SA, Zamcheck N. Gastric barrier to ingested microorganisms in man: studies *in vivo* and *in vitro*. *Gut* 1972; **13**:251–256.
24 Ruddell WSJ, Bone ES, Hill MJ, Walters CL. Pathogenesis of gastric cancer in pernicious anemia. *Lancet* 1978; **i**:521–523.
25 Reed PI, Haines K, Smith PLR, House FR, Walters CL. Gastric juice N-nitrosamines in health and gastroduodenal disease. *Lancet* 1981; **ii**: 550–552.
26 Villako K, Tamm A, Savisaar E, Ruttas M. Prevalence of antral and fundic gastritis in a randomly selected group of an Estonian rural population. *Scand J Gastroenterol* 1977; **11**:817–827.
27 Siurala M, Isokoski M, Varis K, Kekki M. Prevalence of gastritis in a rural population. *Scand J Gastroenterol* 1968; **3**:211–223.
28 Kreuning J, Bosman F, Kuiper G, Van der Wal A, Lindeman J. Gastric and duodenal mucosa in 'healthy' individuals. *J Clin Pathol* 1978; **31**:69–77.
29 Sharma B, Santana I, Wood E, Walt R, Pereira M, Noone P, *et al.* Intragastric bacterial activity and nitrosation before, during, and after treatment with omeprazole. *Br Med J* 1984; **289**:717–719.
30 Saltzman JR, Kowdley KV, Pedrosa MC, Sepe T, Golner B, Perrone G, *et al.* Bacterial overgrowth without clinical malabsorption in elderly hypochlorhydric subjects. *Gastroenterology* 1994; **106**:615–623.
31 Fried M, Siegrist H, Frei R, Froehlich F, Duroux P, Thorens J, *et al.* Duodenal bacterial overgrowth during treatment in outpatients with omeprazole. *Gut* 1994; **35**:23–26.
32 Clarke RT, Beauchop T. Methods for studying gut microbes. In: Clarke RTJ, Beauchop T (editors): *Microbial Ecology of the Gut*. London: Academic Press; 1977, pp. 1–33.
33 Layer P, Holst JJ, Grandt D, Goebell H. Ileal release of glucagon like peptide-1 (GLP-1). Association with inhibition of gastric acid secretion in humans. *Dig Dis Sci* 1994; **40**:501–505.
34 Allen JM, Fitzpatric MK, Yeats JC, Darcy K, Adrian TE, Bloom SR. Effects of PYY and neuropeptide Y on gastric emptying in man. *Digestion* 1984; **30**:255–262.
35 Schoeman MN, Tippett MD, Akkermans LMA, Dent J, Holloway RH. Mechanisms of gastroesophageal reflux in ambulant healthy subjects. *Gastroenterology* 1995; **108**:83–91.
36 Mittal RK, Stewart WR, Schirmer BD. Effect of a catheter in the pharynx on the frequency of transient lower esophageal sphincter relaxations. *Gastroenterology* 1992; **103**:1236–1240.
37 Murray PR. Human microbiota: systemic bacteriology. In: Balows A, Duerden BI (editors): *Topley's and Wilson's Microbiology and Microbial Infection*, 9th edition. New York: Oxford University Press; 1998, pp. 295–306.
38 Tutuian R, Katz PO, Castell DO. Nocturnal acid breakthrough: pH, drugs, and bugs. *Eur J Gastroenterol Hepatol* 2004; **16**:441–443.
39 Martinek J, Pantoflickova D, Hucl T, Benes M, Dorta G, Lukas M, Spicak J. Absence of nocturnal acid breakthrough in *Helicobacter pylori*-positive subjects treated with twice-daily omeprazole. *Eur J Gastroenterol Hepatol* 2004; **16**:445–450.
40 Katz PO, Hatlebakk JG, Castell DO. Gastric acidity and acid breakthrough with twice-daily omeprazole or lansoprazole. *Aliment Pharmacol Ther* 2000; **14**:709–714.

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World J Gastroenterol 2006 May 21; 12(19): 3092-3095
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Seventy-five gram glucose tolerance test to assess carbohydrate malabsorption and small bowel bacterial overgrowth

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Received: 2005-12-19 Accepted: 2006-01-14

Abstract

AIM: To investigate non-invasively the incidence of absorption of carbohydrates in diabetic patients during an oral glucose tolerance test (OGTT) and to determine whether malabsorption may be associated with insulin secretion and insulin resistance.

METHODS: A standard 75-g OGTT was performed in 82 diabetic patients. The patients received 75 g of anhydrous glucose in 225 mL of water after an overnight fasting and breath samples were collected at baseline and up to 120 min after ingestion. Breath hydrogen and methane concentrations were measured. Blood glucose and serum insulin concentrations were measured before ingestion and at 30, 60, 90, 120 min post-ingestion.

RESULTS: When carbohydrate malabsorption was defined as subjects with an increase of at least 10 ppm (parts per million) in hydrogen or methane excretion within a 2-h period, 28 (34%) had carbohydrate malabsorption. According to the result of increased breath test, 21 (75%) patients were classified as small bowel bacterial overgrowth and 7 (25%) as glucose malabsorption. Patients with carbohydrate malabsorption were older and had poor glycemic control as compared with those without carbohydrate malabsorption. The HOMA value, the sum of serum insulin during the test and the Δinsulin/Δglucose ratio were greater in patients with carbohydrate malabsorption.

CONCLUSION: Insulin resistance may be overestimated by using these markers if the patient has carbohydrate malabsorption, or that carbohydrate malabsorption may be present prior to the development of insulin resistance. Hence carbohydrate malabsorption should be taken into account for estimating insulin resistance and β-cell function.

Key words: 75-g OGTT; Carbohydrate malabsorption; Bacterial overgrowth; Breath test; Insulin resistance

Urita Y, Ishihara S, Akimoto T, Kato H, Hara N, Honda Y, Nagai Y, Nakanishi K, Shimada N, Sugimoto M, Miki K. Seventy-five gram glucose tolerance test to assess carbohydrate malabsorption and small bowel bacterial overgrowth. *World J Gastroenterol* 2006; 12(19): 3092-3095

http://www.wjgnet.com/1007-9327/12/3092.asp

INTRODUCTION

The oral glucose tolerance test (OGTT) is a widely used procedure in the diagnoses of diabetes and intermediate stages of hyperglycemia. Plasma glucose and insulin responses during this test reflect the ability of pancreatic β-cells to secrete insulin and the sensitivity of tissues to insulin[1]. However, it was reported that 2%-20% of carbohydrates escape small intestinal absorption[2]. Based on this fact, digestion and absorption of carbohydrates may affect the results of OGTT. If the patient had carbohydrate malabsorption, plasma glucose and insulin responses during OGTT should be lower. Furthermore, an interesting observation was the outcome of OGTT yielding a remarkable intra-individual variability[3] and this was explained by the hypothesis that the velocity of the initial phase of glucose emptying from the stomach may depend on the grade of interdigestive antral motor activity at the time of glucose ingestion[4]. Although a many investigators have reported a close association between gastric emptying and insulin secretion, the prevalence of absorption of carbohydrates in diabetic patients has been unknown.

H_2 breath tests have been used to evaluate intestinal transit, bacterial overgrowth, and disaccharidase

deficiency[5-12]. Because bacteria represent the sole source of gut H_2 and CH_4, fasting breath H_2 and CH_4 gases have been used as markers of colonic fermentation[13,14]. As H_2 production increases when a small amount of carbohydrate is supplied to colonic bacteria, the measurement of breath H_2 concentration has been proposed as an indicator of carbohydrate malabsorption[6]. Similarly, breath CH_4 excretion, which reflects an indirect measurement of the metabolism of the anaerobic colonic flora, has been measured[15,16]. Methanogenic bacteria utilize H_2, carbon dioxide (CO_2), and then synthesize CH_4[17]. CH_4 absorbed from the colon reaches the lung and excretes into the breath[18].

The aim of the present study was to investigate non-invasively the incidence of malabsorption of carbohydrates in diabetic patients during OGTT and to determine whether malabsorption may be associated with insulin secretion and insulin resistance.

MATERIALS AND METHODS

Patients

A standard 75-g OGTT was performed in 82 diet-controlled diabetic patients (42 women and 40 men; age range 30-84 years, average 62 years) without abdominal symptoms. Patients treated with alpha-glucosidase inhibitors were excluded from this study. None of the patients had a history of use of PPI, H_2-receptor antagonist, antibiotics, steroids, or nonsteroidal anti-inflammatory drugs for a period of at least six month before the investigation. Patients who had a previous history of partial gastrectomy were also excluded from the study. The study was approved by our Local Ethical Committee.

Procedures

The patients received 75 g of anhydrous glucose in 225 mL of water in the sitting position after an overnight fasting. Breath samples were collected at baseline and at 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120 min after ingestion. Breath H_2 and CH_4 concentrations were measured with breath analyzer TGA-2000 (TERAMECS, Kyoto) and expressed in parts per million (ppm). Linear accuracy response range was 2 to 150 ppm. An increase of at least 10 ppm within a 2-h period is indicative of bacterial overgrowth or glucose malabsorption[19]. Venous blood samples were obtained before ingestion and at 30, 60, 90, and 120 min after ingestion and blood glucose and serum insulin concentrations were measured.

Calculations

The homeostasis model assessment (HOMA), fasting serum insulin, the sum of serum insulin during the test, and the Δinsulin/Δglucose ratio were used as indexes of insulin resistance. The HOMA value was calculated as follows: HOMA = fasting plasma glucose (mg/dL) × fasting insulin (pmol/L)/405. The Δinsulin/Δglucose ratio was calculated as follows: (insulin at 30 min - insulin at baseline)/(glucose at 30 min - glucose at baseline).

Statistical analysis

All values were expressed as means ± SD. Comparisons of groups were made using the Mann-Whitney U test. A P value less than 0.05 was considered statistically significant.

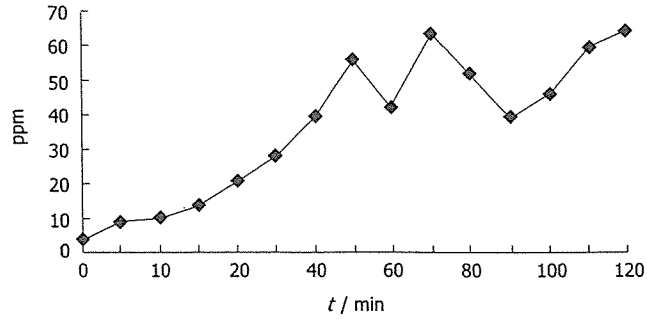

Figure 1 Changes in breath hydrogen concentration during OGTT in the patient with suspicious of small bowel bacterial overgrowth. An increase of H_2 greater than 10 ppm above the baseline was found at 15 min.

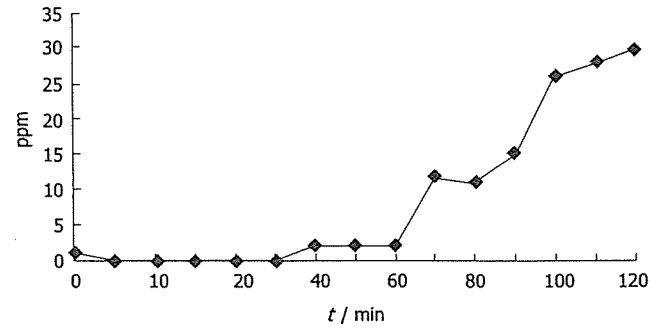

Figure 2 Changes in breath hydrogen concentration during OGTT in the patient with suspicious of carbohydrate malabsorption. Breath H_2 concentration increased gradually from the beginning and reached 10 ppm over the baseline at 70 min and later.

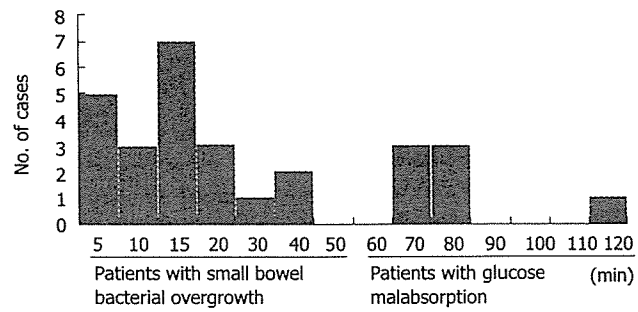

Figure 3 Distribution of time when breath hydrogen and/or methane levels increased more than 10 ppm over baseline.

RESULTS

Incidence of carbohydrate malabsorption

When carbohydrate malabsorption was defined as subjects with an increase of at least 10 ppm within a 2-h period, 34% (28/82) patients had carbohydrate malabsorption. As shown in Figures 1 and 2, small bowel bacterial overgrowth was defined as an increase of H_2 and/or CH_4 greater than 10 ppm above the baseline before the first 40 min, whereas carbohydrate malabsorption without small bowel bacterial overgrowth was defined as an increase of H_2 and/or CH_4 greater than 10 ppm above the baseline at 70 min and later. Of 28 patients with an increase of H_2 and/or CH_4, 21 (75%) patients were classified as small bowel bacterial overgrowth and 7 (25%) as carbohydrate malabsorption (Figure 3). All

Table 1 Characteristics of 82 patients with diabetes mellitus classified according to carbohydrate malabsorption status

	Carbohydrate malabsorption (+)	Carbohydrate malabsorption (-)	*P* value
n	28	54	
Age	63.3 ± 10.3	59.2 ± 9.7	0.34
HbA1c	7.1 ± 1.7	6.4 ± 1.5	0.17
HOMA	2.0 ± 2.1	1.9 ± 1.9	0.22
ΣIR	107.0 ± 95.3	117.0 ± 76.2	0.08
ΔIR/ΔBG	0.18 ± 0.27	0.14 ± 0.21	0.06

28 patients had no abdominal symptom during OGTT.

As shown in Table 1, the values of HbA1c were 7.11% ± 1.70% and 6.43% ± 1.46% in patients with and without carbohydrate malabsorption (including small bowel bacterial overgrowth), respectively. Patients with carbohydrate malabsorption were older and had poor glycemic control when compared with those without carbohydrate malabsorption.

Carbohydrate malabsorption and insulin resistance

The HOMA values in the patients with carbohydrate malabsorption (2.0 ± 2.1) were greater than those without carbohydrate malabsorption (1.94 ± 1.86), but did not reach statistical significance ($P = 0.22$). Similarly, the sum of serum insulin during the test and the Δinsulin/Δglucose ratio were also greater in patients with carbohydrate malabsorption than those without carbohydrate malabsorption (Table 1), but the difference was not significant.

DISCUSSION

Impaired intestinal and gastric motility are frequent findings in diabetic patients[19,20]. However, there is a wide range of symptoms in gastrointestinal motility disorders, and the degree of motility disorders correlate poorly with severity of symptoms. Intestinal peristalsis as well as gastric acid secretion are the most important factors protecting against the small bowel bacterial overgrowth[21]. Although delayed gastrointestinal transit potentially causes bacterial overgrowth, in contrast, a rapid transit may also cause diarrhea due to an increase in intraluminal contents that reach the cecum. It is, therefore, possible that both rapid and delayed gastrointestinal transit cause diarrhea because of bacterial overgrowth or carbohydrate malabsorption. However, patients with bacterial overgrowth may also be asymptomatic[22]. Although gastrointestinal symptoms are present in 50%-70% of diabetic patients, the association between symptoms and bacterial overgrowth in diabetic patients has been unknown.

In the present study, 34% (28/82) patients without abdominal symptoms, including diarrhea, had carbohydrate malabsorption, including 7 patients classified as small bowel bacterial overgrowth. It has been unclear how much of small bowel bacterial overgrowth is opposed to carbohydrate malabsorption. The results of the present study suggest that carbohydrate malabsorption occurs more often in diabetic patients than small bowel bacterial overgrowth.

There is increasing evidence that postprandial hyperglycemia has a major role in the pathogenesis of diabetic macrovascular complications[23,24]. It is widely recognized that postprandial glycemia is potentially dependent on a number of factors, including the rate of carbohydrate entry into the small intestine, small intestinal digestion and absorption, insulin secretion, peripheral insulin sensitivity, and hepatic and muscle glucose metabolism[25]. In addition, postprandial secretion of insulin is prompted as much by the incretin hormones as by entry of glucose into the blood, and the release of incretins is dependent on rates of nutrient entry into the small intestine[26,27]. Our observations confirmed that one third of patients with diet-controlled type 2 diabetes and without abdominal symptoms had carbohydrates malabsorption, which might contribute to postprandial hyperglycemia[25]. Some previous studies also described approximately 35% of the variance in initial postprandial blood glucose concentrations after a 75-g oral glucose load[28,29]. Since patients with carbohydrate malabsorption tended to be older and to have poor glycemic control as compared with those without carbohydrate malabsorption, it is possible that patients with long-standing or poor-controlled diabetes may more often have carbohydrate malabsorption. Conversely, the result and its interpretation of 75-g OGTT might be influenced by carbohydrate malabsorption.

A standard 75-g OGTT has also been used to assess insulin resistance and insulin release. For example, fasting plasma insulin concentrations have been used as an index of insulin resistance, and the 30-min ratio of changes in plasma insulin and glucose has been used as an index of β-cell function[30,31]. Insulin resistance, evaluated by the HOMA value, the sum of serum insulin during the test, and the Δinsulin/Δglucose ratio were greater in patients with carbohydrate malabsorption than those without carbohydrate malabsorption. However, these differences were not significant. This suggests that insulin resistance might be overestimated by using these markers if the patient has carbohydrate malabsorption, or that carbohydrate malabsorption might be present prior to the development of insulin resistance. In conclusion, it is more desirable that carbohydrate malabsorption should be taken into account for estimating insulin resistance and β-cell function.

REFERENCES

1 **Reaven GM,** Brand RJ, Chen YD, Mathur AK, Goldfine I. Insulin resistance and insulin secretion are determinants of oral glucose tolerance in normal individuals. *Diabetes* 1993; **42:** 1324-1332
2 **Stephen AM,** Haddad AC, Phillips SF. Passage of carbohydrate into the colon. Direct measurements in humans. *Gastroenterology* 1983; **85:** 589-595
3 **Mcdonald GW,** Fisher GF, Burnham C. Reproducibility of the oral glucose tolerance test. *Diabetes* 1965; **14:** 473-480
4 **Thompson DG,** Wingate DL, Thomas M, Harrison D. Gastric emptying as a determinant of the oral glucose tolerance test. *Gastroenterology* 1982; **82:** 51-55
5 **Hoekstra JH.** Fructose breath hydrogen tests in infants with chronic non-specific diarrhoea. *Eur J Pediatr* 1995; **154:** 362-364
6 **Levitt MD,** Donaldson RM. Use of respiratory hydrogen (H2)

excretion to detect carbohydrate malabsorption. *J Lab Clin Med* 1970; **75**: 937-945
7 **Bond JH Jr,** Levitt MD. Use of pulmonary hydrogen (H 2) measurements to quantitate carbohydrate absorption. Study of partially gastrectomized patients. *J Clin Invest* 1972; **51**: 1219-1225
8 **Maffei HV,** Metz GL, Jenkins DJ. Hydrogen breath test: Adaptation of a simple technique to infants and children. *Lancet* 1976; **1**: 1110-1111
9 **Bond JH Jr,** Levitt MD, Prentiss R. Investigation of small bowel transit time in man utilizing pulmonary hydrogen (H2) measurements. *J Lab Clin Med* 1975; **85**: 546-555
10 **La Brooy SJ,** Male PJ, Beavis AK, Misiewicz JJ. Assessment of the reproducibility of the lactulose H2 breath test as a measure of mouth to caecum transit time. *Gut* 1983; **24**: 893-896
11 **Gotze H,** Ptok A. Orocaecal transit time in patients with Crohn disease. *Eur J Pediatr* 1993; **152**: 193-196
12 **Rhodes JM,** Middleton P, Jewell DP. The lactulose hydrogen breath test as a diagnostic test for small-bowel bacterial overgrowth. *Scand J Gastroenterol* 1979; **14**: 333-336
13 **Le Marchand L,** Wilkens LR, Harwood P, Cooney RV. Use of breath hydrogen and methane as markers of colonic fermentation in epidemiologic studies: circadian patterns of excretion. *Environ Health Perspect* 1992; **98**: 199-202
14 **Perman JA,** Modler S, Barr RG, Rosenthal P. Fasting breath hydrogen concentration: normal values and clinical application. *Gastroenterology* 1984; **87**: 1358-1363
15 **McKay LF,** Eastwood MA, Brydon WG. Methane excretion in man--a study of breath, flatus, and faeces. *Gut* 1985; **26**: 69-74
16 **Bjorneklett A,** Jenssen E. Relationships between hydrogen (H2) and methane (CH4) production in man. *Scand J Gastroenterol* 1982; **17**: 985-992
17 **Stadtman TC.** Methane fermentation. *Annu Rev Microbiol* 1967; **21**: 121-142
18 **Bond JH Jr,** Engel RR, Levitt MD. Factors influencing pulmonary methane excretion in man. An indirect method of studying the in situ metabolism of the methane-producing colonic bacteria. *J Exp Med* 1971; **133**: 572-588
19 **Abrahamsson H.** Gastrointestinal motility disorders in patients with diabetes mellitus. *J Intern Med* 1995; **237**: 403-409
20 **Iber FL,** Parveen S, Vandrunen M, Sood KB, Reza F, Serlovsky R, Reddy S. Relation of symptoms to impaired stomach, small bowel, and colon motility in long-standing diabetes. *Dig Dis Sci* 1993; **38**: 45-50
21 **Kirsch G.** Bacterial overgrowth. *Am J Gastroenterol* 1990; **85**: 231-237
22 **Camilleri M.** Gastrointestinal problems in diabetes. *Endocrinol Metab Clin North Am* 1996; **25**: 361-378
23 **Del Prato S.** In search of normoglycaemia in diabetes: controlling postprandial glucose. *Int J Obes Relat Metab Disord* 2002; **26 Suppl 3**: S9-S17
24 **Saydah SH,** Miret M, Sung J, Varas C, Gause D, Brancati FL. Postchallenge hyperglycemia and mortality in a national sample of U.S. adults. *Diabetes Care* 2001; **24**: 1397-1402
25 **Rayner CK,** Samsom M, Jones KL, Horowitz M. Relationships of upper gastrointestinal motor and sensory function with glycemic control. *Diabetes Care* 2001; **24**: 371-381
26 **Herrmann C,** Goke R, Richter G, Fehmann HC, Arnold R, Goke B. Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* 1995; **56**: 117-126
27 **Schirra J,** Katschinski M, Weidmann C, Schafer T, Wank U, Arnold R, Goke B. Gastric emptying and release of incretin hormones after glucose ingestion in humans. *J Clin Invest* 1996; **97**: 92-103
28 **Horowitz M,** Edelbroek MA, Wishart JM, Straathof JW. Relationship between oral glucose tolerance and gastric emptying in normal healthy subjects. *Diabetologia* 1993; **36**: 857-862
29 **Jones KL,** Horowitz M, Carney BI, Wishart JM, Guha S, Green L. Gastric emptying in early non-insulin-dependent diabetes mellitus. *J Nucl Med* 1996; **37**: 1643-1648
30 **Haffner SM,** Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. *Diabetes Care* 1997; **20**: 1087-1092
31 **Haffner SM,** Kennedy E, Gonzalez C, Stern MP, Miettinen H. A prospective analysis of the HOMA model. The Mexico City Diabetes Study. *Diabetes Care* 1996; **19**: 1138-1141

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