Enhanced production of leptin in gastric fundic mucosa with *Helicobacter pylori* infection

Yoshito Nishi, Hajime Isomoto, Shigeo Uotani, Chun Yang Wen, Saburo Shikuwa, Ken Ohnita, Yohei Mizuta, Akio Kawaguchi, Kenichiro Inoue, Shigeru Kohno

**AIM:** To determine the concentrations of leptin in plasma and gastric fundic mucosa in humans, with reference to *Helicobacter pylori* (*H pylori*) infection, and their association with gastric mucosal levels of interleukin (IL)-1β, IL-6 and IL-8.

**METHODS:** Plasma leptin concentrations were determined in 135 outpatients with non-ulcer dyspepsia, consisting of 95 *H pylori*-infected and 40 uninfected subjects, and 13 patients before and after cure of the infection with anti-*H pylori* regimen. Using biopsy samples that were endoscopically obtained from the middle corpus along the greater curvature, gastric leptin contents were measured by radiomunoassay and the mucosal concentrations of IL-1β, IL-6 and IL-8 were measured by enzyme linked immunosorbent assay. We also analysed the expression of leptin in the fundic mucosa by reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry.

**RESULTS:** The mucosal levels of leptin in the fundic mucosa of *H pylori*-infected patients were significantly higher than those of uninfected patients. The amount of gastric leptin correlated positively with the mucosal levels of IL-1β and IL-6, but not IL-8. Circulating leptin correlated with body mass index, but not with *H pylori* status, and there was no change in plasma leptin levels following cure of the infection. Leptin immunoreactive cells were noted in the lower half of the fundic glands, and its expression of messenger ribonucleic acid in the oxyntic gland was detected by RT-PCR.

**CONCLUSION:** Leptin production is enhanced in *H pylori*-infected gastric mucosa. Gastric leptin may be involved in immune and inflammatory response during *H pylori* infection, through interaction with proinflammatory cytokines.
upper gastrointestinal endoscopy and diagnosed as having non-ulcer dyspepsia, between April 2000 and March 2003. The study was approved by Nagasaki University Human Ethics Committee. All samples were obtained with written informed consent of the patients prior to their inclusion, in accordance with the Helsinki Declaration. The exclusion criteria were: age <18 or >80 years, pregnancy, body mass index (BMI) >30 kg/m², diabetes mellitus, systemic infection, thyroid and liver diseases, renal impairment, use of medications effective against _H pylori_ during the preceding 3 mo, alcohol abuse, drug addiction, and long-term corticosteroid or nonsteroidal anti-inflammatory drug use. None had undergone gastrointestinal surgery.

During endoscopy, one biopsy specimen was obtained from the middle portion of the corpus along the greater curvature for the measurement of gastric leptin contents and cytokines, snap-frozen in an ethanol-dry ice mixture and then stored at -80 °C until use. Two additional biopsies were endoscopically taken from the antrum within 2 cm of the pyloric ring and the corpus along the greater curvature; one was for the rapid urease test (Helicocheck, Otsuka Pharmaceutical Co., Tokushima, Japan) and another for histopathological examination. In some cases, additional biopsy samples were obtained from the fundic gland mucosa for RT-PCR and immunohistochemical analysis. We treated 13 _H pylori_-positive patients with 7-d triple therapy consisting of rabeprazole, amoxicillin and clarithromycin. The sections were stained with haematoxylin and eosin. The grades of histological gastritis including activity (neutrophils) and chronic inflammation (mononuclear cells), was scored into 4 grades of histological gastritis including activity to 0, 1, 2 or 3 corresponding to none, mild, moderate or severe in accordance with the Sydney system.

**Immunohistochemistry**

Immunohistochemical staining was performed with the streptavidin-biotin-peroxidase-complex method (Histofine SAB-PO kit, Nichirei Co., Tokyo, Japan) as described previously. The following steps were performed at room temperature unless otherwise specified. Paraffin-embedded biopsy specimens were sectioned at 4-μm thickness, deparaffinized and rehydrated. After inhibition of endogenous peroxidase activity for 30 min with methanol containing 0.3% H₂O₂, the sections were reacted for 20 min with 10% normal goat serum to prevent non-specific binding. They were then incubated overnight with the rabbit polyclonal anti-leptin antibody (diluted 1:100, Santa Cruz Biotechnologies Inc., Santa Cruz, CA, USA) at 4 °C. On the next day, the sections were washed in 0.01 M PBS and incubated for 20 min with 10 μg/mL biotinylated goat anti-rabbit immunoglobulins (Nichirei Co.). After washed in PBS, the sections were re-incubated for 20 min with 100 μg/mL horseradish peroxidase (HRP)-conjugated streptavidin (Nichirei Co.) and stained with 0.02% 3,3'-diaminobenzidine tetrahydrochloride (Dojindo Co., Kumamoto, Japan) in 0.05 mol/L Tris-HCl buffer containing 0.03% H₂O₂. The sections were finally washed in PBS and counterstained with hematoxylin. Control studies were performed with normal rabbit serum or anti-leptin antisera (Santa Cruz Biotechnologies Inc.).

We calculated the leptin-labelling index, which was the numbers of immunoreactive cells for leptin per total numbers of cells within the fundic gland area (percentage). The calculation was performed by two investigators without knowledge of the experimental results.

**RT-PCR**

Total RNA from each biopsy sample was extracted using a commercial kit according to the instructions provided by the supplier (ISOGEN, Nippon Gene Co., Toyama, Japan). Equivalent amounts of RNA were monitored by absorption at 260 nm and by monitoring the density of 28S and 18S RNA detected after electrophoresis. One μg of total RNA was reverse transcribed into complementary deoxyribonucleic acid (cDNA) in a volume of 25 μL with M-ULV reverse transcriptase and random hexamers (both from PE Applied Biosystems, Warrington, UK). According to a previous report with a slight modification, the target sequence for leptin mRNA was amplified in 40 cycles, each consisting of 1 min at 94 °C for denaturation, 1 min at 62 °C for annealing and 1 min at 72 °C for extension, followed by a final extension for 10 min at 72 °C using a RT-PCR kit (Takara Bio, Kusatsu, Japan). Secondary primers, 5'-GTTTGTGCTTTTGAAAGTCG-3' (forward) and 5'-TGCAGTGCGGAGCAGTCC-3' (reverse), were used for amplification of a 224 bp product. A 10 μL aliquot of each PCR product was analysed by electrophoresis on 2% agarose gel containing ethidium bromide, and the bands were examined under ultraviolet light for the presence of amplified DNA. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene transcript was routinely amplified as described previously and used as an internal control of the processed RNA for each preparation.

**Statistical analysis**

Statistical analyses were performed using Fisher’s exact, χ², Student’s t, Mann-Whitney U, Kruskal-Wallis, Spearman rank...
and Wilcoxon signed ranks tests, as appropriate. A P value less than 0.05 was accepted as statistically significant. Data were expressed as mean±SD.

RESULTS

**Patient demographics**
The study population consisted of 69 men and 66 women, with a mean age of 54 years (range, 19-80). They consisted of 95 H pylori-infected and 40 uninfected subjects. There were no significant differences in age, sex, alcohol intake, smoking habit, BMI in terms of H pylori status.

**Plasma concentrations of leptin and H pylori status**
There was no significant difference in plasma leptin concentrations between H pylori-positive and -negative subjects (5.0±3.5 and 4.8±2.8 ng/mL, respectively). Successful eradication of the organism was confirmed in all of the 13 patients treated with anti-H pylori regimen. There was no significant difference in the leptin levels before and after cure of the infection (4.8±3.3 and 4.7±2.5 ng/mL, respectively). Successful eradication of the organism was confirmed in all of the 13 patients treated with anti-H pylori regimen. There was no significant difference in the leptin levels before and after cure of the infection (4.8±3.3 and 4.7±2.5 ng/mL, respectively).

**Gastric mucosal levels of leptin, IL-1β, IL-6 and IL–8 in relation to H pylori status**
Gastric leptin contents in patients with H pylori infection were significantly higher than those in uninfected subjects (P=0.05, Table 1). In addition, there were significant differences in the mucosal levels of IL-6 and IL-8 between H pylori-positive and -negative groups (P=0.0001 and P=0.0005, respectively, Table 1). The mucosal IL-1β-positive group tended to be higher than those in uninfected subjects, though the difference was insignificant (Table 1). There were no relationships between these cytokines and circulating leptin concentrations.

**Table 1** Gastric mucosal levels of leptin, interleukin 1β, interleukin 6 and interleukin 8 in terms to H pylori status (mean±SD)

|                      | H pylori-infected (n=95) | Uninfected (n=40) | P       |
|----------------------|--------------------------|-------------------|---------|
| Leptin (ng/mg protein) | 0.18±0.13                | 0.14±0.15         | <0.05   |
| Interleukin 1β (pg/mg protein) | 43.38±33.03            | 33.27±24.61       | NS      |
| Interleukin 6 (pg/mg protein) | 1.26±0.57              | 0.75±0.57         | <0.0001 |
| Interleukin 8 (pg/mg protein) | 70.42±66.12            | 1.5±0.13          | <0.0005 |

**Correlation between gastric mucosal levels of leptin and cytokines**
Gastric leptin contents correlated positively with the mucosal levels of IL-1β (correlation coefficient, r=0.600, P<0.0001) and IL-6 (r=0.475, P<0.0005), but not the mucosal levels of IL-8 (r=0.168).

**Correlation between gastric mucosal levels of leptin and activity and chronic inflammation**
Gastric leptin contents correlated positively with grading scores of chronic inflammation of gastritis (correlation coefficient, r=0.258, P<0.05), but not the scores of activity (r=0.111).

**Correlation between plasma and gastric leptin levels and baseline parameters**
Concentrations of leptin in plasma, but not in gastric mucosa, correlated positively with BMI (r=0.548, P<0.0001). Other baseline characteristics including age, sex, alcohol intake and smoking habit did not correlate with circulating and gastric leptin concentrations.

**Expression of leptin mRNA and protein in gastric mucosa**
Using RT-PCR, leptin mRNA was identified in the fundic gland mucosa, albeit the band intensities of the gastric mucosa from patients with and without H pylori infection were much weaker compared to that of omental fatty tissue (Figure 1).

In gastric biopsy specimens, leptin immunoreactive cells were detected in the lower half of the fundic glands (Figure 1). The leptin-labelling indices of H pylori-infected patients tended to be higher than those of uninfected subjects (22.4±14.3 and 18.1±13.9, respectively, P<0.10).

**DISCUSSION**
Bado and co-workers[29] were the first group to report the presence of leptin mRNA and protein in the fundic glands of rat stomachs, and that the chief cells were mainly immunoreactive for leptin[29].

![Figure 1](https://example.com/figure1.png)

**Figure 1** Leptin immunoreactive cells localized in the lower half of oxyntic glands (arrow head, magonification ×650). A: Patients with H pylori infection; B: Uninfected subjects; C: Patients who had successful eradication.

![Figure 2](https://example.com/figure2.png)

**Figure 2** Reverse transcriptase-polymerase chain reaction. M: size marker. Lane 1: H pylori-uninfected gastric biopsy sample; lane 2: omental adipose tissue sample; lane 3: H pylori-infected gastric biopsy sample; lane 4: non-template negative control.
Earlier studies failed to identify leptin mRNA in human gastric mucosa whereas the fundic epithelium exhibited the presence of immunoreactive leptin. Thus, it is suggested that leptin itself detected in gastric mucosa originates from the uptake one rather than representing local biosynthesis. However, we demonstrated here the expression of both leptin mRNA and its protein, providing further support for the recent results that leptin was a stomach-derived hormone in humans\(^{[9,10]}\).

In our study, gastric leptin contents in \textit{H pylori}-infected patients were significantly higher than those in uninfected patients. Using quantitative RT-PCR, Azuma et al\(^{[9]}\) demonstrated that \textit{H pylori} infection significantly increased the expression of leptin mRNA expression, and that cure of the infection significantly reduced it. Considered together, \textit{H pylori} infection seems to enhance local biosynthesis of leptin as well as its release into gastric juice in response to cholecystokinin or meal\(^{[7]}\). The finding that leptin was localized in the oxyntic gland area\(^{[10]}\), which is rarely colonized by the organism\(^{[11]}\), suggests that \textit{H pylori} itself may not affect gastric leptin levels.

Our results demonstrated significantly positive correlations between gastric leptin contents and the mucosal concentrations of IL-1\(\beta\) and IL-6. In fact, there is evidence for the expression of functional leptin receptor (Ob-R) in mononuclear cells\(^{[3,4,10,20,30]}\). Ob-R is homologous to members of class I cytokine receptor (gp130) superfamily including IL-6\(^{[3,4,31]}\). Several lines of evidence demonstrate that leptin could stimulate monocytes to produce IL-1, IL-6 and tumour necrosis factor \(\alpha\) (TNF-\(\alpha\))\(^{[5,6]}\). In turn, these cytokines could increase systemic leptin levels \textit{in vivo}\(^{[2,1]}\). It has been reported that IL-1\(\beta\) and IL-6 are elevated in gastric mucosa infected with \textit{H pylori}\(^{[6-8,20]}\), in line with this study. Thus, leptin released locally may be implicated in the immune and inflammatory responses to \textit{H pylori} infection, through interaction with proinflammatory cytokines. Moreover, it not only modulates the activation and proliferation of T lymphocytes but also skews cytokine responses towards a Th type by enhancing production of IL-2 and interferon\(\gamma\)\(^{[23,24]}\). It has been well accepted that mucosal cytokine profiles during \textit{H pylori} infection can imply Th1 predominance in human adults\(^{[13]}\). Considered together, these findings highlight the possible role of leptin as an immunomodulator in \textit{H pylori}-associated gastritis.

In our study, circulating leptin concentrations were not associated with \textit{H pylori} status and there was no significant alteration in their levels following cure of the infection, consistent with previous reports\(^{[19,33]}\). Gastric leptin may have a local rather than a systemic action, exerting paracrine effects within the gastric mucosa. On the other hand, plasma leptin concentrations significantly correlated with BMI, as the primary contributor of circulating leptin is exclusively the adipose tissue\(^{[1,2]}\).

In conclusion, we showed a significantly enhanced production of leptin in \textit{H pylori}-infected than uninfected gastric mucosa. The amount of gastric leptin correlated positively with the mucosal concentrations of IL-1\(\beta\) and IL-6, suggesting that local overproduction of leptin is likely to be involved in immune and inflammatory response during \textit{H pylori} infection.

**REFERENCES**

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse \textit{obese} gene and its human homologue. \textit{Nature} 1994; 372: 425-432
2. Coleman DL. Obese and diabetes: two mutant genes causing obesity-diabetes syndromes in mice. \textit{Diabetologia} 1978; 14: 141-148
3. Faggioni R, Feingold KR, Grunfeld C. Leptin regulation of the immune response and the immunodeficiency of malnutrition. \textit{FASEB J} 2001; 15: 2565-2571
4. Sanchez-Margalet V, Martin-Romero C, Santos-Alvarez J, Goberna R, Najib S, Gonzalez-Yanes C. Role of leptin as an immunomodulator of blood mononuclear cells: mechanisms of action. \textit{Clin Exp Immunol} 2003; 133: 11-19
5. Santos-Alvarez J, Goberna R, Sanchez-Margalet V. Human leptin stimulates proliferation and maturation of human circulating monocytes. \textit{Cell Immunol} 1999; 194: 6-11
6. Zarkesh-Esfahani H, Pockley G, Metcalfe RA, Bidlingmaier M, Wu Z, Ajami A, Weetman AP, Strasburger CJ, Ross RJ. High-dose leptin activates human leucocytes via receptor expression on monocytes. \textit{J Immunol} 2001; 167: 4593-4599
7. Konturek JW, Kowalczyk SJ, Kwiecien N, Bielskini W, Pawlik T, Rembiasz K, Domshcke W. Leptin in the control of gastric secretion and gut hormones in humans infected with \textit{Helicobacter pylori}. \textit{Scand J Gastroenterol} 2001; 36: 1148-1154
8. Bado A, Levassueur S, Attoub S, Kermorgant S, Lainegeau JP, Borotoulez MN, Moizo L, Lehy T, Guerre-Millo M, Le Marchand-Brustel Y, Lewin MJ. The stomach is a source of leptin. \textit{Nature} 1998; 394: 790-793
9. Azuma T, Suto H, Ito Y, Ohtani M, Dojo M, Kuriyama M, Kato T. Gastric leptin and \textit{Helicobacter pylori} infection. \textit{Gut} 2001; 49: 324-329
10. Sobhani I, Bado A, Vissuzaine C, Buyse M, Kermorgant S, Lainegeau JP, Attoub S, Lehy T, Henin D, Mignon M, Lewin MJ. Leptin secretion and leptin receptor in the human stomach. \textit{Gut} 2000; 47: 178-183
11. Blaser MJ. \textit{Helicobacter pylori} and the pathogenesis of gastroduodenal inflammation. \textit{J Infect Dis} 1990; 161: 626-633
12. Ernst PB, Gold BS. \textit{Helicobacter pylori} infection: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. \textit{Anna Rev Microbiol} 2000; 54: 615-640
13. Blaser MJ, Atherton JC. \textit{Helicobacter pylori} persistence: biology and disease. \textit{Clin Invest} 2004; 113: 321-333
14. Beales IL, Calam J. Interleukin 1 beta and tumour necrosis factor alpha inhibit acid secretion in cultured rabbit parietal cells by multiple pathways. \textit{Gut} 1998; 42: 227-234
15. Mossa SF, Legon S, Bishop JE, Pokak JM, Calam J. Effect of \textit{Helicobacter pylori} on gastric somatostatin in duodenal ulcer disease. \textit{Lancet} 1992; 340: 930-932
16. Isomoto H, Mizuta Y, Miyazaki M, Takekawa F, Omagari K, Murase K, Nishiyama T, Inoue K, Murata I, Kohn S. Implication of NF-kappaB in \textit{Helicobacter pylori}-associated gastritis. \textit{Am J Gastroenterol} 2000; 95: 2768-2776
17. Isomoto H, Miyazaki M, Mizuta Y, Takekawa F, Murase K, Inoue K, Yamazaki K, Murata I, Koji T, Kohn S. Expression of nuclear factor-kappaB in \textit{Helicobacter pylori}-infected gastric mucosa detected with southwestern histochemistry. \textit{Scand J Gastroenterol} 2000; 35: 247-254
18. Crabtree JE, Shallcross TM, Heatley RV, Wyatt JL. Mucosal tumour necrosis factor alpha and interleukin-6 in patients with \textit{Helicobacter pylori} associated gastritis. \textit{Gut} 1991; 32: 1473-1477
19. Noach LA, Bosma NB, Jansen J, Hoek FJ, van Deventer SJ, Tytgat GN. Mucosal tumour necrosis factor-alpha, interleukin-1 beta, and interleukin-8 production in patients with \textit{Helicobacter pylori} infection. \textit{Scand J Gastroenterol} 1994; 29: 425-429
20. Yamaoka Y, Kita M, Kodama T, Sawai N, Imanishi J. \textit{Helicobacter pylori} cagA gene and expression of cytokine messenger RNA in gastric mucosa. \textit{Gastroenterology} 1996; 110: 1744-1752
21. Murray CD, Kamm MA, Bloom SR, Emmanuel AV. Ghrelin for the gastroenterologist: history and potential. \textit{Gastroenterology} 2005; 125: 1492-1502
22. Gokcel A, Gumurdulu Y, Kayaselcuk F, Serin E, Ozer B, Ozsahin AK, Guvener N. \textit{Helicobacter pylori} infection modifies plasma ghrelin levels. \textit{Eur J Endocrinol} 2003; 148: 423-436
23. Nwokolo CU, Freshwater DA, O’Hare P, Randeva HS. Plasma ghrelin following cure of \textit{Helicobacter pylori} gastritis. \textit{Gut} 2003; 52: 637-640
24. Suzuki H, Masaoka T, Hosoda H, Ota M, Minegishi Y, Nomura S, Kanzawa K, Ishii H. \textit{Helicobacter pylori} infection modifies gastric and plasma ghrelin dynamics in Mongolian gerbils. \textit{Gut} 2004; 53: 187-194
25. Isomoto H, Furuhashi H, Morikawa T, Mizuta Y, Nishiyama T, Omagari K, Murase K, Inoue K, Murata I, Kohn S. 5-day vs. 7-day triple therapy with rabeprazole, clarithromycin and
amoxicillin for Helicobacter pylori eradication. *Aliment Pharmacol Ther* 2000; 14: 1619-1623

26 **Isomoto H**, Wang A, Mizuta Y, Akazawa Y, Ohba K, Omagari K, Miyazaki M, Murase K, Hayashi T, Inoue K, Murata I, Kohno S. Elevated levels of chemokines in esophageal mucosa of patients with reflux esophagitis. *Am J Gastroenterol* 2003; 98: 551-556

27 **Isomoto H**, Inoue K, Furusu H, Nishiyama H, Shikuwa S, Omagari K, Mizuta Y, Murase K, Murata I, Kohno S. Lafutidine, a novel histamine H2-receptor antagonist, vs lansoprazole in combination with amoxicillin and clarithromycin for eradication of *Helicobacter pylori*. *Helicobacter* 2003; 8: 111-119

28 **Ohara H**, Isomoto H, Wen CY, Ejima C, Murata M, Miyazaki M, Takeshima F, Mizuta Y, Murata I, Koji T, Nagura H, Kohno S. Expression of mucosal addressin cell adhesion molecule 1 on vascular endothelium of gastric mucosa in patients with nodular gastritis. *World J Gastroenterol* 2003; 9: 2701-2705

29 **Breidert M**, Miehlke S, Glasow A, Orban Z, Stolle M, Ehninger G, Bayerdorffer E, Nettesheim O, Halim U, Haidan A, Bornstein SR. Leptin and its receptor in normal human gastric mucosa and in *Helicobacter pylori*-associated gastritis. *Scand J Gastroenterol* 1999; 34: 954-961

30 **Madej T**, Boguski MS, Bryant SH. Threading analysis suggests that the obese gene product may be a helical cytokine. *FEBS Lett* 1995; 373: 13-18

31 **Tartaglia LA**, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995; 83: 1263-1271

32 **Sarraf P**, Frederich RC, Turner EM, Ma G, Jaskowiak NT, Rivet DJ, Flier JS, Lowell BB, Fraker DL, Alexander HR. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. *J Exp Med* 1997; 185: 171-175

33 **Lord GM**, Matarese G, Howard JK, Bloom SR, Lechler RI. Leptin inhibits the anti-CD3-driven proliferation of peripheral blood T cells but enhances the production of proinflammatory cytokines. *J Leukoc Biol* 2002; 72: 330-338

34 **Picker LJ**, Singh MK, Zdraveski Z, Treer JR, Waldrop SL, Bergstresser PR, Maino VC. Direct demonstration of cytokine synthesis heterogeneity among human memory/effector T cells by flow cytometry. *Blood* 1995; 86: 1408-1419

35 **Shimzu T**, Satoh Y, Yamashiro Y. Serum leptin and body mass index in children with *H pylori* infection. *Gut* 2002; 51: 142

*Edited by Wang XL*