Helicobacter pylori infection modifies gastric and plasma ghrelin dynamics in Mongolian gerbils

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Background and aim: Although ghrelin, a novel growth hormone releasing peptide localised mainly in the gastric fundus, is reported not only to accelerate food passage and gastrointestinal motility but also to affect appetite and weight control, regulation of gastric ghrelin secretion under the conditions of gastric Helicobacter pylori infection is unknown. The present study was designed to investigate plasma and gastric ghrelin levels in Mongolian gerbils with H pylori colonisation of the gastric mucosa.

Methods: Gerbils orally inoculated with H pylori were examined after inoculation. To examine preproghrelin mRNA expression in the gastric mucosa, cDNA encoding the gerbil preproghrelin and glyceraldehyde-3-phosphate dehydrogenase homologue was isolated and a quantitative reverse transcription-polymerase chain reaction system was established.

Results: In gerbils showing H pylori colonisation (H pylori group), expression of preproghrelin mRNA and total ghrelin levels were significantly decreased 17 and 23 weeks later (p<0.01). Although the number of ghrelin immunoreactive cells decreased as the stomach weight increased, the gastric contents of total and active ghrelin in this group were the same as those in controls. Gastric myeloperoxidase activity showed a positive correlation with plasma ghrelin levels. On the other hand, at 17 weeks, plasma ghrelin levels were significantly increased in the H pylori group (p<0.05), suggesting a compensatory increase in secretion of the peptide at this time point.

Conclusion: The present experimental study demonstrated that gastric and plasma ghrelin dynamics are altered in response to H pylori infection.

Methods: Cloning of gerbil ghrelin and GAPDH cDNA

Total RNA was obtained from the Mongolian gerbil (Meriones unguiculatus) stomach using the RNEasy Mini-kit (Qiagen, Hilden, Germany). In order to clone a gerbil ghrelin homologue, degenerative primers were designed from human and mouse ghrelin cDNA (forward primer M01: 5‘-CCGGAATTCCTCACTGATGCCTGGATGGAGCATGGCCATGGCAGG-3‘ and reverse primer M02: 5‘-CCGGAATTCTCAGCCTGCCGGCCTTGACCC-3‘). Approximately 2 μg of total RNA were used as the template and one step reverse transcription-polymerase chain reaction (RT-PCR) was performed with the M01 and M02 primers (50°C for 30 minutes, 95°C for 15 minutes, and 35 cycles of 55°C for 30 seconds, 72°C for one minute, and 94°C for 20 seconds). The size of the PCR product was close to the expected size (332 bp); the product was subcloned into the pBluescript SK vector (Stratagene, La Jolla, California, USA), and the DNA sequences were determined using the 310 Genetic Analyzer (PE Applied Biosystems, Norwalk, Connecticut, USA).

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription-polymerase chain reaction; RIA, radioimmunoassay; DAB, 3,3′-diaminobenzidine tetrahydrochloride; MPO, myeloperoxidase
To obtain the full length cDNA of ghrelin, we performed 5'-RACE and 3'-RACE with primers designed based on the sequences obtained. For 5'-RACE, two gene specific antisense primers, M06 (5'-TACGCTGGCCGCTTTGACTCCTT-3') and M07 (5'-GCTCCTGAGACCTTTGACCCAAGT-3'), were designed, and 5'-RACE was performed using the 5'-RACE system for rapid amplification of the cDNA ends (Invitrogen Corp., Carlsbad, California, USA). For 3'-RACE, a gene specific sense primer, M05 (5'-TGGGACTGTGCAGCTCCTCA-GGCTTT-3') was designed and 3'-RACE was performed using the SMART RACE cDNA Amplification Kit (BD Biosciences Clontech, Palo Alto, California, USA). Each amplified fragment was directly sequenced using the 310 Genetic Analyzer (PE Applied Biosystems).

For cloning of the gerbil homologue of GAPDH, the same method as that described above for ghrelin was used. In brief, to generate a fragment of GAPDH, forward primer M08 (5'-GGGGGATCCGTATTGGGCGCCTGGTCACCAGGGC-3') and reverse primer M11 (5'-CCGGAATCTTCTTTGAGGCCAAGGCCCCAT-3') were used. The sizes of the PCR products were close to the expected size (990 bp). For 5'-RACE, a gene specific antisense primer, M17 (5'-CTCAGTGTAGCCGCCAGG-ATGCCCTT-3'), was designed, and for 3'-RACE, a gene specific sense primer, M18 (5'-CTTCACCCACCAGG-GAACGGCGG-3'), was used. Each amplified fragment was directly sequenced using the 310 Genetic Analyzer (PE Applied Biosystems).

Quantitative RT-PCR

Total RNA was extracted from the stomach of the Mongolian gerbils using the RNeasy Mini Kit (Qiagen). A TaqMan quantitative real time RT-PCR was performed to detect preproghrelin mRNA and GAPDH mRNA with the ABI Applied Biosystems). For 3'-RACE, two gene specific antisense primers, M06 (5'-CCGGATCCGTATTGGGCGCCTGGTCACCAGGGC-3'), GAPDH-R (5'- GCCCTTCCGAGGGAGACAGTTCTTT-3'), and reverse primer M11 (5'-CCCGGAATCTTCTTTGAGGCCAAGGCCCCAT-3') were used. The sizes of the PCR products were close to the expected size (990 bp). For 5'-RACE, a gene specific antisense primer, M05 (5'-TGGGACTGTGCAGCTCCTCA-GGCTTT-3'), was designed, and 3'-RACE was performed using the SMART RACE cDNA Amplification Kit (BD Biosciences Clontech, Palo Alto, California, USA). Each amplified fragment was directly sequenced using the 310 Genetic Analyzer (PE Applied Biosystems).

Radioimmunoassay (RIA) for ghrelin

Stomach, duodenum, or jejunum sampling
Fresh whole anterior wall specimens of the glandular stomach, duodenum (5–15 mm from the pyloric ring), or jejunum (120–130 mm from the pyloric ring) were frozen immediately after collection and stored at –80°C. Each sample was boiled for five minutes in a 10-fold volume of water to inactivate the intrinsic proteases. The solution was adjusted to 1 M acetic acid after cooling, and the tissue was homogenised since acidification of sample solution prevented the loss of extraction recovery. The supernatant was lyophilised and then subjected to ghrelin RIA. The extraction efficiency of tissue ghrelin was more than 95%.

Plasma sampling
Whole blood samples (1 ml) were obtained from the right ventricle in tubes containing EDTA-2Na (1 mg/ml) and aprotinin (500 kIU/ml). Each plasma sample (500 μl) was treated with a C18 Sep-Pak cartridge (Waters, Milford, Massachusetts, USA) for peptide extraction. The cartridge was washed and eluted; the eluate (50 μl equivalent of plasma) was lyophilised and subjected to ghrelin RIA. The extraction efficiency of plasma ghrelin was approximately 90%.

RIA for ghrelin

The two RIA techniques were used for measuring ghrelin, as described previously. Briefly, ghrelin levels were measured using two polyclonal rabbit antibodies raised against the N terminal (1–11) (Gly1-Lys11) or C terminal (13–28) (Gln13-Arg28) fragment of rat ghrelin. Two tracer ligands were synthesised: [Tyr29]-rat ghrelin for antirat ghrelin (1–11) antiserum and [Tyr12]-rat ghrelin (13–28) for antirat ghrelin (13–28) antiserum. RIA incubation mixtures, containing 100 μl of either standard ghrelin or unknown sample with 200 μl of antiserum diluted in RIA buffer containing 0.5% normal rabbit serum, were initially incubated for 12 hours. Then, 100 μl of 125I labelled tracer (15 000 cpm) were added and the mixture was incubated for 36 hours. Antirabbit IgG goat serum (100 μl) was added prior to a further 24 hour incubation period. Free and bound tracers were then separated by centrifugation at 3000 rpm for 30 minutes. Following aspiration of the supernatant, radioactivity in the pellet was quantitated using a gamma counter (ARC-600; Aloka, Tokyo, Japan). All assays were performed at 4°C. The antirat ghrelin (1–11) antiserum specifically recognised the n-octanoylated form of rat ghrelin but not the des-acyl form. The antirat ghrelin (13–28) antiserum recognised both the acylated and des-acyl forms of rat ghrelin equally. Both antiserum were equally cross reactive with human and gerbil ghrelin and did not recognise the other enteric peptides. The respective intra- and interassay coefficients of variation for the N terminal RIA were 3% and 6%, and for the C terminal RIA, 6% and 9%.

Mongolian gerbil model of H pylori infection

All experiments and procedures carried out on animals were approved by the Keio University Animal Research Committee (No 023009). Thirty one specific pathogen free male Mongolian gerbils (MGS/Sea, five week old; Seac Yoshitomi, Fukuoka, Japan) were administered H pylori suspensions (ATCC43504: 106 colony forming units (CFU)/ml, 15 ml/kg) while 27 control gerbils were administered buffer solution alone after overnight deprivation of food. All animals were allowed free access to water and a standard pellet diet (CE-2; Clea Japan, Tokyo, Japan). Four, 17, and 23 weeks after inoculation, the gerbils were examined under ether anaesthesia after 16 hours of food deprivation, and sacrificed by an overdose of ether. H pylori infection at each time point was examined by determining the number of CFU in a microaerobic bacterial culture. Briefly, the diluted homogenates of the stomachs were plated onto Brucella agar plates containing 10% horse blood, 2.5 μg/ml amphotericin B, 9 μg/ml vancomycin, 0.32 μg/ml polymixin B, 5 μg/ml trimethoprim, and 50 μg/ml 2, 3, 5-triphenyl-tetrazolium chloride. The plates were then incubated at 37°C in a microaerobic atmosphere for seven days. The number of colonies was counted, and the amount of viable H pylori was expressed as the number of CFU/g of tissue.
pH 6.0). After cooling, endogenous peroxidase was quenched by 0.3% hydrogen peroxide. After washing, non-specific binding was blocked by a blocking reagent (BlockAce; Dainippon Pharm, Osaka, Japan). All sections were incubated overnight at 4°C with antighrelin (13–28) antiserum (Dainippon Pharm, Osaka, Japan). All sections were incubated with a mouse anti-H^+,-K^+ ATPase (Zymed, South San Francisco, USA). Total protein in the homogenates was measured using the modified Lowry method, as described by Smith and colleagues.23 Myeloperoxidase (MPO) activity, an index of polymorphonuclear cell accumulation, was determined using a previously described method, with some modifications.24 Samples of mucosal homogenates were centrifuged at 8000 g for 15 minutes at 4°C to pellet the insoluble cellular debris. The pellet was then resuspended in an equal volume of 0.05 M potassium phosphate buffer (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide. Samples were centrifuged at 8000 g for 15 minutes at 4°C and the supernatants were saved. MPO activity was assessed by measuring H_2O_2 dependent oxidation of 3,3',5,5'-tetramethylbenzidine. One unit of enzyme activity was defined as the amount of MPO that caused a change in absorbance of 1.0/minute at 655 nm at 25°C.

**RESULTS**

**cDNA and protein sequences of the Mongolian gerbil preproghrelin and characterisation of the peptide**

The isolated full length Mongolian gerbil preproghrelin cDNA was 460 bp long, consisting of 17 bp in the 5'-untranslated region, 354 bp of coding region, and 89 bp in the 3'-untranslated region. The predicted initiation methionine was located at nucleotide position 18–20. An AATAAA polyadenylation signal was identified in the 3' non-coding region (position 452–457) (fig 1A) (Suzuki et al, EMBL/GenBank: AF442491). The deduced amino acid sequence of the coding region indicated that gerbil preproghrelin was composed of 117 amino acid residues, the same length as that of the mouse, rat, and human preproghrelin (fig 1B) (AF442491). At the nucleotide level, the gerbil preproghrelin sequence exhibited an average homology score of 85% with human (AJ252278), 90% with mouse (AJ243503), and 92% with rat (AB029433) preproghrelin (fig 1B). The gerbil preproghrelin was highly conserved, the protein sharing 83% (97/117) identity with its human counterpart, 89% (104/117) identity with its mouse counterpart, and 88% (103/117) identity with its rat counterpart. The unidentified third residue of the purified peptide was determined to be serine, the same as that in the case of other mammalian ghrelin. As gerbil ghrelin was determined to be entirely homologous with rat ghrelin apart from one amino acid (Thr^{12}); two polyclonal antibodies against rat ghrelin were used to measure the gerbil ghrelin in the present study.

**GAPDH and characterisation of this peptide**

The isolated full length Mongolian gerbil GAPDH cDNA was 1265 bp long, consisting of 71 bp in the 5'-untranslated region, 1002 bp in the coding region, and 192 bp in the 3'-untranslated region (Suzuki et al, EMBL/GenBank: AY066607). The predicted initiation methionine was located at nucleotide position 72–74. The deduced amino acid sequence of the coding region indicated that the gerbil GAPDH may be composed of 333 amino acids residues, the same length as that of the mouse or rat GAPDH, and two amino acids shorter than the human GAPDH. The deduced amino acid sequence of gerbil GAPDH was 97.6% (325/333) identical to that of its mouse counterpart, 97.3% (324/333) identical to that of its rat counterpart, and 95.2% (319/335) identical compared with its human counterpart.

**Preproghrelin mRNA expression and ghrelin contents in the stomach**

The level of preproghrelin mRNA as a ratio of the level of GAPDH mRNA (preproghrelin/GAPDH mRNA) in the H pylori group was significantly decreased compared with that in controls, both at 17 and 23 weeks after H pylori inoculation (fig 2).

Although there were no significant differences in total gastric and active ghrelin levels (fmol/mg) between the control and H pylori groups at four weeks after inoculation, these levels were significantly decreased in the H pylori group compared with those in the control group at both 17 and 23 weeks after inoculation (fig 3A, B).

**Ghrelin immunoreactive cells**

As shown in fig 4A, ghrelin immunoreactive cells were located in the mucosal neck glands of the gastric corpus, especially in the vicinity of H^+,-K^+ ATPase positive parietal cells, in non-H pylori infected gerbils. Under higher magnification, ghrelin immunoreactive cells were found to be in contact with parietal cells in the same glands (fig 4B, C). In the gastric corpus mucosa of H pylori colonised gerbils, although the density of H^+,-K^+ ATPase positive cells was...
of human, rat, mouse, and Mongolian gerbil preproghrelin protein sequences. Matching residues are indicated as homologous (∗). Residues constituting the matured ghrelin peptides are printed in bold type. The EMBL/GenBank accession number for this sequence is AF442491. (B) Alignment of the human, rat, mouse, and Mongolian gerbil preproghrelin protein sequences. Numbered amino acids are printed in the signal peptide sequence are printed in italics. The amino acid residues in the signal peptide sequence are printed in italics.

Figure 1: cDNA and protein sequences of preproghrelin and their homology with those of other species. (A) Nucleotides and amino acids are numbered on the left and right, respectively. The amino acid residues in the signal peptide sequence are printed in italics. The amino acid residues constituting the matured ghrelin peptides are printed in bold type. The EMBL/GenBank accession number for this sequence is AF442491. (B) Alignment of human, rat, mouse, and Mongolian gerbil preproghrelin protein sequences. Matching residues are indicated as homologous (∗). Amino acids corresponding to the ghrelin peptide are shaded as shown in the area.

Figure 2: Preproghrelin mRNA expression levels normalised to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression levels in the stomach of Mongolian gerbils. **p<0.01 compared with control at 17 weeks; †††p<0.001 compared with controls at 23 weeks.

**Gastric mucosal inflammation**

During the observation period, persistent *H pylori* colonisation was confirmed in all *H pylori* inoculated gerbils. No significant difference in body weight was observed between...

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A
1 TC ACC ACC CCC AAG GCC ATG ATG TCT TCA GGG ACC ATC TGCA GTG GTA CTC CTT GGC
M M S S G T I C S L L L G
14
60 GTG CTC TGG ATG GAC GTG GCC ATG GCA GGC TCC AGC TTC AGC CCA GAG CAC CAG AAA
V L W M D V A M A G S S F L S P E H Q K
34
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T Q Q R K E S K K P P A K L Q P R A L E
54
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G W L H P D G R G Q A E G A E D E L E I
74
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R F N A P F D V G I K L S G A Q Y Q Q H
94
300 GGC CGG GCC CTG GGA AGG TTC CTC CAG GAC ATC CCT TGG GAA GAG GTC AAA GAG GGG GCA
G R A L G K F L Q D I L W E E V K E E A
114
360 ACT GAC AGA TGA CCA CTG ACA TGA CTG GTC CAC TGT GCT TCT CTG CCG TCT TAG CCT CCT
T D K
117
420 GTG TAA CTC CCA GAC TCT CCT ATT ACC TIA AGA ATA AAT GT

B

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<td>Rat</td>
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<td></td>
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<tr>
<td>Consensus</td>
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* H pylori + ATPase positive cells was noted and the number of ghrelin immunoreactive cells was also decreased (fig 4E). Representative photomicrographs of ghrelin immunohistochemistry in the corpus revealed a decrease in the number of brown stained ghrelin immunoreactive cells in *H pylori* colonised mucosa (fig 5B, D). Morphometric analysis also revealed that the number of ghrelin immunoreactive cells/mm² was significantly decreased in *H pylori* colonised mucosa of gastric corpus, both at 17 and 23 weeks after *H pylori* inoculation (table 1). In contrast, the total cell number was found to be higher in *H pylori* colonised mucosa (table 1), possibly due to inflammatory cell infiltration. Taking these parameters into consideration, it was found that the density of ghrelin immunoreactive cells (%) was decreased in *H pylori* infected gastric corpus mucosa (table 1), suggesting a possible relationship between the increase in the number of inflammatory cells and decrease in the number of ghrelin immunoreactive cells.

**Gastric mucosal inflammation**

During the observation period, persistent *H pylori* colonisation was confirmed in all *H pylori* inoculated gerbils. No significant difference in body weight was observed between...
the control and \( H \) pylori groups (table 2), suggesting that although \( H \) pylori altered the ghrelin dynamics, it probably did not influence the eating behaviour or body mass of the animals in such a short period of time after the onset of infection. In this situation, some compensatory mechanism may be evoked to regulate ghrelin independent growth hormone secretion and body mass. In contrast, stomach wet weight was significantly increased in the \( H \) pylori group (table 2), which could reflect the macroscopic findings of severe thickening and oedematous changes associated with severe inflammation observed in \( H \) pylori colonised gastric mucosa. Consistent with the increase in stomach weight, gastric mucosal MPO activity, which reflects polymorphonuclear infiltration, was increased significantly compared with that in controls 17 and 23 weeks after \( H \) pylori inoculation (table 2). At four weeks after inoculation, although the weight of the stomach was significantly increased in the \( H \) pylori group (table 2), no significant increase in MPO activity was observed, reflecting the merely oedematous changes of the gastric mucosa in the acute phase of infection.

Notwithstanding the above data on stomach weight, total and active ghrelin levels in each whole stomach specimen were determined to be the same in the control and \( H \) pylori groups (table 3).

**Plasma ghrelin levels**

Although the fasting plasma total ghrelin level was significantly increased in the \( H \) pylori group compared with that of controls at 17 weeks after \( H \) pylori inoculation, the difference was not significant at four or 23 weeks (fig 6A). In contrast, fasting plasma active ghrelin levels were significantly increased in the \( H \) pylori group at both 17 and 23 weeks after \( H \) pylori inoculation compared with those in each control group, although no significant increase in plasma active ghrelin level was shown at four weeks (fig 6B).

Gastric mucosal MPO activity was correlated with plasma levels of total and active ghrelin (fig 7).

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**Figure 3** Levels of ghrelin in the stomach of Mongolian gerbils. (A) Total ghrelin levels in the stomach of Mongolian gerbils with or without \( H \) pylori colonisation at 17 and 23 weeks after bacterial inoculation. \( **p < 0.01 \) compared with controls at each of the time points. (B) Active ghrelin levels in the stomach of Mongolian gerbils with or without \( H \) pylori colonisation at 17 and 23 weeks after bacterial inoculation. \( *p < 0.05 \) compared with controls at 17 weeks; \( \dagger p < 0.01 \) compared with controls at 23 weeks.

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**Figure 4** Colocalisation of ghrelin immunoreactive cells with parietal cells in the gastric fundic mucosa of Mongolian gerbils. The brown coloured cells represent ghrelin immunoreactive cells and the pink coloured cells represent \( H \)\(^+\),K\(^+\)-ATPase positive parietal cells. (A) Control fundic mucosa; magnification 20×. (B) Control fundic mucosa; magnification 100×, longitudinal view. (C) Control fundic mucosa; magnification 100×, cross sectional view. (D) \( H \) pylori colonised fundic mucosa showing inflammatory cell infiltration; magnification 20×. (E) \( H \) pylori colonised fundic mucosa showing severe gastric mucosal atrophy; magnification 20×.
Ghrelin levels in the duodenum and jejunum
Figure 8 demonstrates levels of total ghrelin in the stomach, duodenum, and jejunum in both the control and H pylori groups at four weeks after H pylori inoculation. Although no significant difference in the content of ghrelin in any part of the intestine was observed between the control and H pylori groups at this time point, levels were significantly reduced to 0.55% and 0.16% in the duodenum and jejunum, respectively, compared with stomach levels, suggesting predominant localisation of ghrelin in the stomach of gerbils.

DISCUSSION
Modified ghrelin dynamics in H pylori infection were demonstrated in the present study using an experimental animal model, and reduced preproghrelin mRNA expression and ghrelin levels were clearly shown in the stomach of gerbils with H pylori colonisation, in association with a significant increase in inflammatory cell infiltration.

The amino acid sequence corresponding to the ghrelin peptide in the gerbil was 93% identical to that of humans and 96% identical to that of its mouse or rat counterpart (fig 1B),

Table 1

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<tr>
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<th>Ghrelin immunoreactive cells</th>
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<td></td>
<td>17 weeks</td>
<td>23 weeks</td>
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<tr>
<td></td>
<td>Control (n = 9)</td>
<td>H pylori (n = 6)</td>
<td>Control (n = 12)</td>
</tr>
<tr>
<td>Ghrelin immunoreactive cells (No/0.1 mm²)</td>
<td>16.1 (1.0)</td>
<td>10.1 (1.0)*</td>
<td>17.3 (0.8)</td>
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<tr>
<td>Total cell number (No/0.1 mm²)</td>
<td>677.2 (21.4)</td>
<td>792.3 (15.0)</td>
<td>681.5 (12.8)</td>
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<td>Ghrelin immunoreactive cells (%)</td>
<td>2.4 (0.1)</td>
<td>1.3 (0.1)*</td>
<td>2.6 (0.1)</td>
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* p<0.05, **p<0.01 compared with controls at 17 weeks; ††p<0.01 compared with control at 23 weeks.

Table 2

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<th>4 weeks</th>
<th>17 weeks</th>
<th>23 weeks</th>
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<td>Control (n = 10)</td>
<td>H pylori (n = 10)</td>
<td>Control (n = 9)</td>
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<tr>
<td>Body weight (g)</td>
<td>58.3 (0.7)</td>
<td>55.5 (0.8)</td>
<td>76.9 (2.2)</td>
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<tr>
<td>Wet weight of stomach (mg)</td>
<td>632.1 (14.1)</td>
<td>765.2 (53.9)*</td>
<td>619.1 (16.2)</td>
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<tr>
<td>Gastric MPO activity (mU/mg protein)</td>
<td>0.9 (0.2)</td>
<td>0.6 (0.1)</td>
<td>6.9 (0.6)*</td>
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* p<0.05 compared with controls at four weeks, †††p<0.001 compared with control at 17 weeks; ††††p<0.001 compared with control at 23 weeks.
suggesting functional homogeneity of this peptide in various animals. Differences in the amino acid sequences among the four species examined were found only at position 34 or 35 (fig 1B), a rather small segment of the molecule, probably insignificant in relation to biological function. Indeed, studies on the structure-activity relationship of the peptide showed that the octanoyl group on Ser is the essential moiety for biological activity of this peptide, and that the N-terminal fragments showing conservation of the first five amino acids exhibited full functional activity.

Using the same experimental animal, we previously reported a significant increase in the extent of inflammatory cell infiltration and oxidative stress in Helicobacter pylori colonised gastric mucosa, which validated the usefulness of this model for the study of Helicobacter pylori associated gastritis. The present study also showed marked gastric inflammation induced following Helicobacter pylori inoculation (table 2), which was reflected by an increase in stomach weight (table 2).

As ghrelin levels in the whole stomach specimens with Helicobacter pylori infection were in the same range as those in controls, even after release of the peptide into the bloodstream in response to food deprivation (table 3), it would be difficult to propose that the increase in plasma ghrelin levels in gerbils with Helicobacter pylori infected gastric mucosa (fig 6) could be the result of increased gastric total ghrelin production. Increase in plasma ghrelin levels, particularly active ghrelin, may be due to enhancement of degradation of the ghrelin producing A-like cells induced by inflammatory stimuli, such as cytokines or free radicals. As plasma ghrelin elevation has been reported to occur in association with an increase in tumour necrosis factor-α, the increase in Helicobacter pylori associated tumour necrosis factor-α production may play a role in the enhanced ghrelin release noted following food deprivation. These contents are endorsed by the significant correlation between gastric MPO activity (table 1) and plasma ghrelin levels (fig 7). Even though the underlying mechanisms have still to be elucidated in detail, there may be a compensatory increase in levels of plasma ghrelin following food deprivation in Helicobacter pylori colonised gerbils, in association with a decrease in gastric ghrelin density. Such elevation of plasma ghrelin levels may attenuate the reduction in appetite and

![Figure 6](link)  
**Figure 6** Plasma levels of ghrelin in Mongolian gerbils. (A) Total plasma ghrelin levels in Mongolian gerbils with or without Helicobacter pylori colonisation at 17 and 23 weeks after Helicobacter pylori inoculation, *p* < 0.05 compared with controls at 17 weeks. (B) Plasma active ghrelin levels in Mongolian gerbils with or without Helicobacter pylori colonisation at 17 and 23 weeks after Helicobacter pylori inoculation. *p* < 0.05 compared with controls at the time points indicated.

![Figure 7](link)  
**Figure 7** Relationship between gastric myeloperoxidase (MPO) activity and plasma ghrelin level. (A) Gastric mucosal MPO activity was well correlated with total plasma levels of ghrelin. (B) Gastric mucosal MPO activity was well correlated with plasma levels of active ghrelin.

![Figure 8](link)  
**Figure 8** Ghrelin contents in the glandular stomach, duodenum, and jejunum, four weeks after Helicobacter pylori inoculation (fmol/mg).
body weight in \textit{H pylori} infected gerbils until 23 weeks (table 2). As longer term \textit{H pylori} infection (72 weeks) than in the cohorts examined in the present study (23 weeks) reportedly evoked significant weight loss in gerbils, further extension of gastric inflammation and atrophy may nullify the effects of the compensatory increase in plasma ghrelin levels and lead to reduction of both appetite and body weight.

As the average intestinal wet weight in uninfected gerbils at four weeks after inoculation was 1540 mg, the total ghrelin content in the small intestine was computed to be less than 0.029 nmol (fig 8). In contrast, the average wet weight of the stomach in uninfected gerbils at four weeks was 632.1 mg (table 2) and the average gastric total ghrelin content was computed to be 2.168 nmol. The gastric total ghrelin content was estimated to be at least 74-fold greater than that of the small intestine. Therefore, it would be difficult to consider an extragastric source of ghrelin as the origin of the increased plasma ghrelin in \textit{H pylori} colonised gerbils.

The present data in gerbils were inconsistent with the results of two recent clinical studies,\(^1\)\(^2\) the results of which were also discrepant. These discrepancies might be attributable to differences in the methods of measurement of ghrelin peptide or patient selection, or interspecies difference in the distribution of extragastric ghrelin.

In the present study, plasma total ghrelin levels were not significantly increased in \textit{H pylori} colonised cohorts at 23 weeks after \textit{H pylori} inoculation (fig 6). Among the \textit{H pylori} colonised cohorts at 23 weeks, four of 11 gerbils showed plasma total ghrelin levels below the average value observed in controls at the corresponding time point. Among these four gerbils with \textit{H pylori} infection, two showed severe gastric atrophy, with the number of ghrelin immunoreactive cells being less than 1.2\%, as well as lower than average values for gastric ghrelin and preproghrelin mRNA. Severe atrophy might account for the absence of a statistically significant difference between infected and uninfected gerbils at 23 weeks. These results suggest that the ghrelin content in the stomach decreases in response to \textit{H pylori} infection, and with further extension of gastric atrophy, plasma ghrelin levels also decrease. Although there does seem to be differential regulation of preproghrelin mRNA, mucosal ghrelin, and possibly secretion of active ghrelin, the molecular physiology underlying ghrelin dynamics is outside the purview of the current study. Further investigation of ghrelin dynamics based on long term \textit{H pylori} infection could help to clarify these issues.

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\textbf{REFERENCES}


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