CLINICAL STUDY

Long-term effects of Helicobacter pylori eradication on circulating ghrelin and leptin concentrations and body composition in prepubertal children

Lucia Pacifico1,2, Caterina Anania1, John F Osborn3, Eva Ferrara1, Elisa Schiavo1, Margherita Bonamico1 and Claudio Chiesa1,2

1Department of Pediatrics, La Sapienza University of Rome, Viale Regina Elena 324, 00161 Rome, Italy, 2Institute of Neurobiology and Molecular Medicine, National Research Council, Via del Fosso del Cavaliere 100, 00133, Rome, Italy and 3Department of Public Health Sciences, La Sapienza University of Rome, 00185 Rome, Italy

(Correspondence should be addressed to L Pacifico; Email: lucia.pacifico@uniroma1.it)

Abstract

Background: Helicobacter pylori, and the chronic gastric inflammation that it causes, may compromise the function and survival of ghrelin-producing cells, resulting in a decrease of circulating ghrelin levels. This finding raises the possibility that the infection might affect growth in children by reducing the ghrelin production.

Aims: To determine baseline circulating levels of ghrelin and leptin in prepubertal children with and without H. pylori infection and to evaluate the long-term effects of H. pylori eradication on circulating levels of ghrelin and leptin as well as on body composition.

Patients: Thirty children with H. pylori-associated gastritis, 35 children with H. pylori-negative gastric mucosa, and 20 healthy controls were studied.

Results: At baseline, while leptin levels were significantly lower in H. pylori-positive patients, ghrelin concentrations did not differ among the three study groups. However, a significant inverse correlation between ghrelin concentrations and histological severity of gastritis was found. Eradication of the organism was associated with a progressive decrease in ghrelin concentrations over baseline at both 6- and 12-month follow-ups. SDS-body mass index (BMI), lean and fat mass, as well as leptin concentrations, significantly increased over baseline at both follow-ups.

Conclusions: In prepubertal children, serum ghrelin concentrations are inversely related to the severity of H. pylori-associated gastritis. In these youngsters, long-term eradication of H. pylori infection is associated with a significant increase in BMI, lean and fat mass along with a significant decrease in circulating ghrelin levels and an increase in leptin levels.

European Journal of Endocrinology 158 323–332

Introduction

Helicobacter pylori infection is the major etiologic agent for chronic active gastritis and also plays a crucial role in gastric and duodenal ulcer, as well as in gastric cancer. Once acquired, the infection lasts several decades, with a very low spontaneous eradication rate (1, 2). H. pylori is generally acquired early in life and unfavorable socio-economic conditions are regarded as the most important risk factors for acquisition of the infection (3). Recently, some reports have identified potentially important associations with H. pylori infection in children, including protein losing enteropathy, iron deficiency anemia, and diarrhea and malnutrition (4–6). It has also been suggested that, like other chronic diseases, H. pylori infection might impair growth (7–11).

Ghrelin, a 28-amino acid peptide possesses strong growth hormone (GH)-releasing activity and plays both central and peripheral roles in food intake, gastric motility, and acid secretion (12–17). Plasma ghrelin concentrations rise before meals and fall after meals (18). This peptide also contributes to the regulation of both somatic growth and adipose tissue mass and is therefore a short-term meal-related orexigenic as well as a long-term regulator of body weight. In children and adults, plasma ghrelin concentrations are lower in obese subjects, compared to those with normal body weight and lean subjects (19). The decrease of plasma ghrelin concentrations appears to compensate for the positive energy balance in obese individuals. The majority of circulating ghrelin is produced in the mammalian gastric mucosa by enteroendocrine cells/oxyntic glands, the X/A-like cells (14). Thus, there exists the possibility that chronic persistent damage of the gastric mucosa, such as chronic gastritis, might affect ghrelin production, leading to changes in food intake and body weight.
Leptin is a protein product of the obese gene and is expressed primarily by adipocytes (20). Leptin expression has also been detected in the lower half of the gastric fundic glands (21, 22). This protein is thought to provide feedback information to the central nervous system (CN) regarding the size of energy stores and thus is involved in controlling food intake, energy expenditure, and body weight homeostasis (23). Leptin signals satiety to the hypothalamus, resulting in reduced food intake, increased energy expenditure, reduced gastrin and acid secretion, and increased gastric mucosal cell proliferation (22).

Leptin and ghrelin actions are mediated by the hypothalamic neurons in the arcuate nucleus containing neuropeptide Y (NPY) and agouti-related peptide, which induce feeding, and neurons containing proopiomelanocortin and cocaine and amphetamine-regulated transcript, which inhibit feeding. Leptin inhibits NPY neurons and stimulates proopiomelanocortin neurons to suppress feeding; whereas ghrelin activates NPY neurons and inhibits proopiomelanocortin neurons to promote food intake (24).

There are data in adults to suggest that H. pylori may modify plasma concentrations and/or gastric mucosal expression of ghrelin (25–31) as well as gastric mucosal leptin levels (32). However, to our knowledge, only one cross-sectional study has examined, by means of 13C-urea breath test, the influence of H. pylori infection on serum ghrelin and leptin concentrations in children (33), but there are no longitudinal data on the effect of H. pylori eradication on serum ghrelin and leptin in children.

Thus, the two major objectives of our study were 1) To compare the baseline levels of circulating ghrelin and leptin in three groups of prepubertal children. The first two groups consisted of patients with symptoms related to the upper gastrointestinal tract, one with H. pylori-associated gastritis and the other with H. pylori-negative gastric mucosa. The third group included healthy children. 2) To evaluate in patients the long-term effects of H. pylori eradication on circulating ghrelin and leptin levels as well as on body composition.

Materials and methods

Subjects

Over a 2-year period, children undergoing upper gastrointestinal endoscopy for evaluation of symptoms related to the upper gastrointestinal tract, such as epigastric pain, nocturnal or burning abdominal pain, chronic vomiting, or hematemesis were enrolled at the Pediatric Department of La Sapienza University of Rome. A detailed clinical history which included patient socioeconomic status (on the basis of the scores of educational and occupational levels of both parents) (9) was obtained. Exclusion criteria were H. pylori eradication therapy at any time or antibiotics, antisecretory or bismuth treatment, during the last 3 months preceding the study, recent corticosteroid or non-steroidal anti-inflammatory drug use, immunocompromised state including known infectious or systemic disorders, gastrointestinal surgery, structural abnormalities of the gastrointestinal tract, and major neurological impairment. We also excluded patients with serological evidence of celiac disease. To avoid confounding factors associated with the pubertal growth spurt, only prepubertal (Tanner growth stage 1) subjects were included.

The standing height (cm) and the weight (kg) of each subject were recorded at enrollment. Body mass index (BMI) and SDS-BMI using Cole’s least mean square method were calculated (34). Body composition, including validated measurements of fat and lean body mass, was determined by a total body scanner (Hologic QDR-4500W, Waltham, MA, USA, which uses fan-beam scanning) in array mode. This equipment uses a switched pulse stable dual energy X-ray operating at 100 and 140 kV. The data were analyzed using the software version 11.2. The precision error of dual energy X-ray absorptiometry (DXA) has been reported to be 425 g for whole-body fat and fat-free mass (35), with a correlation of 0.99 with a four-compartment model body composition method for measuring fat-free mass and 0.93–0.97 with multislice computed tomography for measuring regional fat-free mass (36). Whole-body DXA is recommended for determining body composition in children (37, 38), and the coefficients of variation (CV) for lean body and fat mass by DXA in the pediatric weight range have been reported as 1.0 and 4.1% respectively (39, 40).

Over the same study period, healthy children with BMI appropriate for sex and age who were attending the Department of Pediatrics for checkup visits were recruited to the study if they had no history of gastrointestinal symptoms, recent therapy with antibiotics or non-steroidal anti-inflammatory drugs, no family history of peptic ulcer disease, and negative 13C-urea breath test at enrollment.

The research protocol was approved by the ethical committee of our institution. An informed written consent was obtained from parents or guardians.

At baseline (as well as at follow-ups), blood samples were collected from each subject after an overnight fast, and centrifuged within 30 min of collection. Serum was aliquoted and frozen at −70 ºC for later duplicate measurements of ghrelin, leptin, insulin, and insulin-like growth factor-I (IGF-I).

Endoscopy

All patients, fasting overnight, underwent endoscopy after narcosis. Pediatric size gastросcopes (Olympus XP10 and PQ20, Olympus Italia S.r.l., Segrate, Milano, Italy) were used. Biopsy specimens were taken in antrum and corpus, at least three from each site, for histological examination (hematoxylin–eosin and Giemsa stains),

www.eje-online.org
culture, and urease testing. According to the updated Sydney system (41), each histological parameter of activity (polymorphonuclear cell infiltration), chronic inflammation (mononuclear cell infiltration), glandular atrophy, and intestinal metaplasia was graded on a four-point scale: 0, absent; 1, mild; 2, moderate; and 3, severe. Patients were considered infected with *H. pylori* when either culture or both histological examination and a rapid urease test were positive. The negative *H. pylori* status was considered when all the three analyses resulted negative. Patients with only a positive urease rapid test or a positive histology were considered to be in an indeterminate *H. pylori* status, and were excluded from study analysis. Patients positive for *H. pylori* received therapy with omeprazole (20 mg once daily), amoxicillin (50 mg/kg per day), and clarithromycin (15 mg/kg per day) for 2 weeks (42). Cure of *H. pylori* infection was evaluated using the 13C-urea breath test 2 months after the end of therapy. If patients were still positive, a second therapeutic course with bismuth citrate, clarithromycin, and metronidazole for 2 weeks was given, and treatment success was again evaluated by the 13C-urea breath test 2 months after the end of therapy.

**Follow-up**

Patients were followed up at 6 and 12 months after enrollment. At both follow-up visits, we evaluated their *H. pylori* status by the 13C-urea breath, monitored their serum ghrelin, leptin, insulin, and IGF-I concentrations, and assessed changes in their BMI and total body composition (fat and lean tissue mass).

**Serum assays**

Immunoreactive ghrelin levels were assessed with an RIA that recognizes both acylated and des-acyl forms (Phoenix Pharmaceuticals, Belmont, CA, USA; sensitivity, 10 pg/ml; inter- and intra-assay CVs, 9.0–13.6 and 4.5–5.3%, respectively) (43). The RIA was also used to measure human (total) leptin (DRG Diagnostica, Marburg, Germany; detection limit, 0.5 ng/ml; inter- and intra-assay CVs, 3.0–6.2 and 3.4–8.3%, respectively), and insulin (CIS Bio International, Schering S.A., Gif-Sur-Yvette Cedex, France; detection limit, 2.0 μU/ml; inter- and intra-assay CVs, 6.4–8.8 and 4.2–8.2%, respectively). An IRMA (ImmunoTech, Beckman Coulter Inc., Marseilles, France) was used to measure IGF-I (detection limit, 2.0 ng/ml; inter- and intra-assay CVs, ≤ 6.8 and ≤ 6.3% respectively).

**Statistical analysis**

Statistical analyses were performed using the SPSS package, SPSS Inc., Chicago, IL, USA. The data are expressed as either frequencies or means with 95% confidence intervals (CIs). The measured leptin, ghrelin, insulin, and IGF-I were distributed with a long tail to the right (positive skew), but their logarithms were approximately normally distributed. Thus, mean values with 95% CI are reported as geometric means for leptin, ghrelin, insulin, and IGF-I values. The differences in quantitative variables among the groups were evaluated using ANOVA or Kruskal–Wallis, as appropriate. Proportions were compared by the χ²-test. Pearson’s correlation and linear regression coefficients were used to examine relationships between variables. Pairwise comparisons were performed using paired t-test or Wilcoxon’s rank sum test, as appropriate. Multiple regression analysis was used to identify variables associated with ghrelin changes over the 12-month follow-up.

**Results**

**Subjects demographic data**

We studied 65 patients (mean age: 6.0 years; range: 3–8 years: 31 females and 34 males) of whom 30 were found to be positive and 35 negative for *H. pylori* infection. Diagnoses in these 35 *H. pylori*-negative children included gastroesophageal reflux disease (n = 10) or food allergy (n = 25). At baseline, a control group of 20 healthy children was also studied. Table 1 summarizes the age, gender, ethnic background, socioeconomic class, and anthropometric characteristics of the three groups of children. Age and gender did not differ significantly among the three study groups. The prevalence of *H. pylori* infection was higher in non-Caucasian (Asian descent) children than in Caucasian children. Likewise, the prevalence of *H. pylori* infection was higher in children of lower socioeconomic class. BMI and SDS-BMI were significantly lower for patients with *H. pylori* infection compared with uninfected patients and healthy children. After correction for both ethnicity and socioeconomic class, BMI and SDS-BMI were no longer significant.

**Serum concentrations of peptides and *H. pylori* status**

At baseline, there were no significant differences in serum ghrelin, insulin, and IGF-I concentrations among the three groups of children (Table 2). Also after adjustment for SDS-BMI, serum ghrelin, insulin, and IGF-I did not differ among the three study groups. In contrast, leptin concentrations were significantly lower in *H. pylori*-positive than in *H. pylori*-negative patients as well as in controls, even after adjustment for SDS-BMI (Table 2).

Within the entire study population, baseline characteristics, including age (P < 0.01), weight (P < 0.05), and height (P < 0.01) were negatively associated with serum ghrelin concentrations. Ghrelin levels were also associated with gender, where males have higher levels than...
females (geometric mean, 459 (CI, 369–579) pg/ml versus 323 (CI, 267–387) pg/ml, P < 0.05). If these relationships were re-examined in the H. pylori-negative group as well as in the control group, there was still a negative association between ghrelin levels and age (P < 0.05 and P < 0.05 respectively), gender (P < 0.05 and P < 0.05 respectively), weight (P < 0.05 and P < 0.05 respectively), and height (P < 0.05 and P < 0.05 respectively). In comparison, within the H. pylori-positive group, ghrelin levels did not correlate with age, gender, weight, and height. Among the evaluated peptides, IGF-I was the only one found to be negatively associated with ghrelin (P < 0.05) within the entire study population as well as in the H. pylori-positive and -negative children.

Within the entire study population, at baseline serum leptin concentrations were positively associated with SDS-BMI (P < 0.01). There was no association of leptin concentrations with age or gender. Within the H. pylori-negative group as well as in the control group, there was still a positive association between leptin levels and SDS-BMI (P < 0.05 and P < 0.05 respectively). In comparison, within the H. pylori-positive group, leptin levels did not correlate with SDS-BMI. Among the evaluated peptides, insulin was the only one found to be positively associated with leptin (P < 0.05) within the entire study population as well as in the H. pylori-positive and -negative children.

There were significant differences in ghrelin, but not leptin, levels based on the grade of each histological parameter: activity in the antrum and corpus, and chronic inflammation in the antrum and corpus (Table 3). Histological examination showed no case of atrophy or intestinal metaplasia. Completely normal histology of the gastric mucosa was found in one of the 30 (3.3%) patients with H. pylori infection, and in 26 of the 35 (74.3%) children without evidence of H. pylori infection. The antral and corpus gastritis scores (mean (s.d.)) were significantly higher in H. pylori-positive children than in H. pylori-negative children (3.3 (1.7) vs 0.8 (1.3), P < 0.0001 and 2.1 (1.2) vs 0.3 (0.4), P < 0.0001 respectively). In particular, inflammation and activity scores were both higher in H. pylori-positive children either in the antrum (P < 0.0001 and P < 0.001 respectively) or in the corpus (P < 0.0001 and P < 0.05 respectively). The mean baseline ghrelin level differed significantly according to the severity of gastritis. As such,

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>H. pylori-positive (n=30)</th>
<th>H. pylori-negative (n=35)</th>
<th>Healthy children (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>6.1 (5.3–6.9)</td>
<td>5.9 (5.1–6.8)</td>
<td>6.3 (5.7–7.5)</td>
</tr>
<tr>
<td>Sex – n (%)</td>
<td>Female 17 (56.6)</td>
<td>14 (40.0)</td>
<td>9 (45.0)</td>
</tr>
<tr>
<td></td>
<td>Male 13 (43.3)</td>
<td>21 (60.0)</td>
<td>11 (55.0)</td>
</tr>
<tr>
<td>Ethnic group – n (%)</td>
<td>White Caucasian 17 (56.6)* †</td>
<td>30 (85.7)</td>
<td>20 (100)</td>
</tr>
<tr>
<td></td>
<td>Asian 13 (43.3)</td>
<td>5 (14.3)</td>
<td>0</td>
</tr>
<tr>
<td>Socioeconomic class</td>
<td>I+II 14 (46.7)* †</td>
<td>9 (25.7)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>III 16 (53.3)</td>
<td>26 (74.3)</td>
<td>7 (35.0)</td>
</tr>
<tr>
<td></td>
<td>IV 0</td>
<td>0</td>
<td>13 (65.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Mean (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.9 (13.9–15.8)* †</td>
<td>15.3 (14.9–15.8)</td>
<td>16.1 (15.2–17.1)</td>
</tr>
<tr>
<td>SDS-BMI</td>
<td>Mean (95% CI)</td>
<td>–1.0 (–1.57 to –0.50)* †</td>
<td>–0.26 (–0.65 to 0.12)</td>
</tr>
</tbody>
</table>

*P < 0.01 versus control subjects, †P < 0.05, ‡P < 0.01 versus uninfected patients. CIs, confidence intervals; BMI, body mass index; SDS-BMI, standard deviation score - body mass index.
antral ($P<0.01$ and $P<0.05$ respectively) as well as corpus inflammation ($P<0.01$ and $P<0.05$ respectively) scores were negatively associated with ghrelin concentration within both groups of $H. pylori$-positive and -negative patients. Likewise, antral ($P<0.01$ and $P<0.05$ respectively) as well as corpus activity ($P<0.01$ and $P<0.05$ respectively) scores were negatively associated with ghrelin concentration within both patient groups. However, repeat analysis of the association between antral inflammation and ghrelin levels after correction for corpus inflammation showed that there was no independent association.

Effects of eradication therapy on serum levels of hormones

Of the 30 $H. pylori$-positive children, 22 (73%) were cured of the organism after the first therapeutic course, while 8 were after the second therapeutic course. At baseline, there were no significant differences in BMI, BMI-SDS, lean and fat mass, as well as in leptin, insulin, and IGF-I concentrations, between children with and without early eradication of $H. pylori$. However, baseline ghrelin concentrations were higher in those with early eradication of $H. pylori$ (455 (324–632) pg/ml versus 245 (CI, 176–337) pg/ml, $P<0.05$). We used the multiple regression analysis to identify the variables having a relation with treatment failure. Low socioeconomic status ($P<0.05$) and ethnicity ($P<0.05$) were the significant risk factors for poor treatment results. Early $H. pylori$ eradication was associated with a progressive decrease in serum geometric mean ghrelin concentrations over baseline, achieving statistical significance at both 6-month (−147 (CI, 118–173) pg/ml; $P<0.01$) and 12-month (−213 (CI, 176–249) pg/ml; $P<0.01$) follow-ups (Fig. 1). Conversely, serum geometric mean leptin concentrations were significantly increased from baseline at both 6-month (+1.7 (CI, 0.9–2.6) ng/ml; $P<0.01$) and 12-month (+3.3 (CI, 1.6–9) ng/ml; $P<0.01$) follow-ups. In the 22 children with early $H. pylori$ eradication, BMI (Fig. 1) and BMI-SDS, as well as lean and fat mass also increased over baseline at both follow-up visits (Table 4). In contrast, the eight children with late $H. pylori$ eradication did not display significant changes in ghrelin or leptin concentrations at both 6- and 12-month follow-ups; BMI, SDS-BMI, lean and fat mass also remained unchanged (Table 4).

Of the 35 $H. pylori$-negative children, 20 completed both the 6- and 12-month follow-ups, and all 20 remained uninfected. No significant changes in ghrelin or leptin concentrations as well as BMI, lean and fat mass

<table>
<thead>
<tr>
<th>Parameter</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>$P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric mean (CI) ghrelin levels in pg/ml (No. of specimens)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>459 (354–595) (27)</td>
<td>512 (331–788) (12)</td>
<td>330 (245–403) (16)</td>
<td>230 (148–357) (10)</td>
<td>0.01</td>
</tr>
<tr>
<td>Corpus</td>
<td>493 (387–626) (30)</td>
<td>365 (278–544) (12)</td>
<td>299 (237–365) (15)</td>
<td>181 (125–299) (8)</td>
<td>0.01</td>
</tr>
<tr>
<td>Activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>445 (354–493) (34)</td>
<td>330 (242–445) (14)</td>
<td>245 (164–299) (10)</td>
<td>200 (143–268) (7)</td>
<td>0.01</td>
</tr>
<tr>
<td>Corpus</td>
<td>454 (347–488) (34)</td>
<td>299 (235–365) (16)</td>
<td>245 (164–330) (10)</td>
<td>181 (97.5–337) (5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Geometric mean (CI) leptin levels in ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>4.7 (3.3–6.6) (27)</td>
<td>4.0 (2.7–5.6) (12)</td>
<td>3.2 (2.6–4.0) (16)</td>
<td>4.3 (2.6–7.0) (10)</td>
<td>0.35</td>
</tr>
<tr>
<td>Corpus</td>
<td>4.8 (4.3–6.8) (30)</td>
<td>4.3 (3.3–5.5) (12)</td>
<td>3.4 (2.7–4.1) (15)</td>
<td>6.0 (3.2–9.9) (8)</td>
<td>0.09</td>
</tr>
<tr>
<td>Activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>4.8 (4.3–6.8) (34)</td>
<td>4.0 (3.4–4.9) (14)</td>
<td>3.4 (2.1–5.5) (10)</td>
<td>4.5 (2.4–7.0) (7)</td>
<td>0.49</td>
</tr>
<tr>
<td>Corpus</td>
<td>4.7 (3.3–6.6) (34)</td>
<td>3.9 (3.3–4.8) (16)</td>
<td>3.1 (1.4–9.0) (10)</td>
<td>5.9 (3.0–9.9) (5)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

$^a$ANOVA.
were observed at each follow-up, though a trend for BMI to rise was observed after 1 year (mean + 0.43 kg/m² (95% CI, 0.13–0.87) from baseline; \( P = 0.06 \)), along with a decrease in ghrelin concentrations (mean–80 pg/ml (95% CI, –41 to –155) from baseline; \( P = 0.08 \); Table 4 and Fig. 1). To identify in \( H. pylori \)-positive patients with early \( H. pylori \) eradication parameters that were associated with the decrease in ghrelin levels, we performed a multiple regression analysis with ghrelin changes at 6 and 12 months as the dependent variable, and age, gender, eradication status, and changes in measures of body weight, body composition, and leptin at 6 and 12 months as the independent variables. The changes in ghrelin at 6 months correlated significantly with changes in BMI, total fat mass, and eradication status, while at 1 year correlated with changes in BMI, fat, and lean mass, but no longer with eradication status.

### Discussion

The present study failed to show that baseline serum ghrelin concentrations in children with \( H. pylori \) infection are different from those found in \( H. pylori \)-negative children and in healthy controls. However, we found a significant inverse correlation between ghrelin levels and histological severity of gastritis induced by \( H. pylori \). This may imply that ghrelin production may be reduced in children in the presence of severe \( H. pylori \)-associated gastritis, as has been found in studies of adult patients. For example, Tatsuguchi et al. found that ghrelin-positive cells in the gastric mucosa were significantly lower for \( H. pylori \)-infected adult patients than for healthy controls, while in patients infected with \( H. pylori \), an inverse correlation was seen between ghrelin immunoreactivity and inflammation/activity grade (26). Similarly, Isomoto et al. demonstrated that an increasingly severe degree of \( H. pylori \) gastritis correlated with progressively lower plasma ghrelin levels in adult patients (28). In children, a very recent study showed that both serum ghrelin and leptin concentrations were significantly reduced in \( H. pylori \)-infected children when compared with those in \( H. pylori \)-negative children (33). However, no information was given on the histological findings as well as on the association between \( H. pylori \) status and anthropometric measures.

Despite the small size of \( H. pylori \)-negative children with gastric inflammation, our study also highlights the possibility that ghrelin levels may be reduced in children even in the presence of other causes of gastric inflammation. Consistent with our findings, recent observations demonstrated a low plasma ghrelin in a young woman with an evolving autoimmune gastric process (18). Overall, this is of interest because most studies on the effects of \( H. pylori \) infection on gastric or circulating ghrelin dynamics in adults have included in
the H. pylori-negative group subjects with no gastric inflammation or an unspecified histological status of the gastric mucosa (26, 27, 30, 44, 45). Therefore, further investigation is clearly required to understand the role of gastric and circulating ghrelin in children as well as in adults with gastritis due to any cause, including H. pylori.

As expected, in our study population including prepubertal young children, there was no evidence of gastric atrophy with/without intestinal metaplasia. Generally, these H. pylori-related lesions occur decades after the acquisition of infection (46). Potential mechanisms accounting for reduction in ghrelin production in the absence of the above lesions may include functional impairment of ghrelin-producing cells due to inflammation, perhaps mediated by cytokines (28, 47).

There are contradictory reports on the influence of H. pylori eradication on ghrelin concentrations (25, 26, 29, 48, 49). In most studies, the follow-up was too short to see any change in BMI. Nwokolo et al. first reported that following eradication of H. pylori from ten asymptomatic adult patients, plasma ghrelin increases profoundly (25). Since that report, it has been suggested that plasma ghrelin levels increase after H. pylori cure for an increase in gastric ghrelin production, leading to body weight gain. For example, Tatsuguchi et al. reported that the number of gastric ghrelin-positive cells increased in adult patients after H. pylori eradication, but they neither monitored circulating ghrelin levels nor assessed changes in BMI after H. pylori eradication (26). Another study showed that circulating ghrelin levels tended to be increased in adult patients, who eradicated the organism, but the differences and changes of BMI were insignificant at 4 weeks after cessation of treatment (29). In contrast, our findings show that H. pylori eradication is not accompanied by an increase in circulating ghrelin. Indeed, our present results show that after H. pylori eradication serum ghrelin concentrations in children who are prepubertal inversely correlate with measures of body composition, and are altered in a compensatory manner by changes in body weight. This is in line with the finding of a decrease in circulating ghrelin levels with weight gain caused by forced overfeeding, high-fat diets, excessive glucocorticoids, or successful treatment of celiac disease and anorexia nervosa (50). Together, these observations suggest that ghrelin levels respond in a compensatory fashion to bidirectional alterations in body weight, consistent with the hypothesis that ghrelin contributes to the known adaptive metabolic responses to such alterations (51). Yet, in agreement with our data, Osawa et al. recently showed that mean plasma ghrelin concentrations significantly decreased after successful H. pylori treatment, though with wide variation (48). In that report, changes in plasma ghrelin concentrations before and 12 weeks after H. pylori cure were inversely correlated with body weight change but not with gastric ghrelin production.

Like Osawa et al. (48), we measured total ghrelin concentrations but not the active form of ghrelin. This raises the possibility that changes in total ghrelin concentrations might have not reflected changes in active ghrelin concentrations. Thompson et al. (52) have shown that both forms of ghrelin may promote bone marrow adipogenesis, whereas only acylated ghrelin probably acts centrally to suppress GH lipolytic activity during prolonged starvation. Thus, the proportion of total ghrelin to des-acylated ghrelin could be relevant for regulating the balance between adipogenesis and lipolysis in response to nutritional status (53). Though recent studies (54, 55) have reported similar changes in total and active ghrelin concentrations in control and anorexic adults, suggesting that total ghrelin is a good marker for active ghrelin (56), we recognize that additional studies are warranted to investigate whether the ratio of the circulating active and inactive forms of ghrelin before and after H. pylori eradication in the pediatric as well as adult population could be relevant for modulating energy balance by two different mechanisms, appetite and adipogenesis (57).

It is well documented that the immune and inflammatory response against H. pylori affects various cell types in gastric mucosa including somatostatin (SST)-producing cells (58, 59). H. pylori gastritis causes a reduction of mucosal SST levels (58, 59). Also, recent evidence has revealed that systemic infusion of SST suppresses plasma ghrelin concentrations (60). Thus, it is possible that an increase in SST following H. pylori eradication might be a negative regulator of ghrelin secretion. But this speculation needs to be elucidated by further investigations.

The physiologic relevance of a functional link between ghrelin and somatotrope secretion is still unclear. Although ghrelin is a potent GH secretagogue, there have been conflicting data on the relationship between ghrelin levels and IGF-I or GH levels (44, 61–66). Our data showed a negative association between ghrelin and IGF-I, but this correlation did not persist after adjustment for age, gender, and BMI. In the paper by Whatmore et al. in healthy children and adolescents total ghrelin concentrations (measured in the same assay as used in our study) remained negatively correlated with IGF-I, but adjustments were not performed for gender (63). In line with this, it may be of interest that Chan et al. (67) found in healthy subjects a negative association between ghrelin (measured by a commercial assay using a MAB specific for the active form of this hormone) and IGF-I that did not persist after adjusting for gender, age, and adiposity. The interaction between ghrelin, GH, and IGF-I probably involves a complex system that is regulated by stimulatory and inhibitory factors in the hypothalamus and influenced by effects of gender, age, and BMI.

In this study, no relationship was found between ghrelin and insulin, but the influence of insulin on ghrelin remains controversial (68, 69). Though there is evidence that ghrelin and leptin exert opposite actions in nutrient...
intake and metabolic balance (17, 70), in our clinical setting including children with H. pylori-associated gastritis (without atrophic changes or long-term history of gastritis), ghrelin and leptin responses appeared to be independent of one another.

In conclusion, our findings show that in prepubertal children serum ghrelin concentrations are inversely related to the severity of H. pylori-associated gastritis. In these youngsters, long-term eradication of H. pylori infection is associated with a significant increase in BMI, lean and fat mass along with a significant decrease in circulating ghrelin levels and an increase in leptin levels. Our observations may provide novel insights for understanding ghrelin and its functions in relation to various diseases.

References


7 Klein PD, Graham DK, Gaillour A, Opekun AR & Smith EO. Water intake and metabolic balance (17, 70), in our clinical setting including children with H. pylori-associated gastritis (without atrophic changes or long-term history of gastritis), ghrelin and leptin responses appeared to be independent of one another.


www.eje-online.org
Ghrelin and H. pylori infection in children

331


www.eje-online.org
67 Chan JL, Bullen J, Lee JH, Yiannakouris N & Mantzoros CS. Ghrelin levels are not regulated by recombinant leptin administration and/or three days of fasting in healthy subjects. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 335–343.


Received 3 September 2007
Accepted 5 December 2007