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DECREASED SERUM GHRELIN FOLLOWING *HELICOBACTER PYLORI* ERADICATION

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Abstract – *Objective:* Ghrelin is an appetite-modulating peptide mainly produced in the stomach, with levels reported to be lower in *Helicobacter pylori*-positive subjects. This pre-post study aimed to evaluate the effect of *H. pylori* eradication on circulating total ghrelin, gastric histopathology, appetite, and nutritional status of dyspeptic adults.

Patients and Methods: Weight, height, appetite and nutrient intake were determined using validated tools. Gastric biopsies were obtained for histopathology and *H. pylori* diagnosis. Fasting serum ghrelin was measured by ELISA. *H. pylori*-positive subjects received eradication therapy and returned ≥12 weeks later for re-evaluation.

Results: Of 117 screened individuals, 47 who were *H. pylori*-positive were included, and the organism was eradicated in 28 (59.6%). Pathologic findings decreased significantly after treatment in subjects with eradication ($p<0.0001$) but did not in those without. Appetite and nutrient intake did not differ significantly after therapy in either group; however, body weight increased in both ($p=0.02$ and $p=0.03$). Fasting serum ghrelin significantly decreased in subjects with eradication [345.0 pg/mL (IQR 373.0-517.8) before, 298.5 pg/mL (IQR 251.0-383.5) after; $p=0.0007$] but remained unchanged in those without.

Conclusions: The decrease in fasting ghrelin after *H. pylori* eradication indicates its involvement in the regulation of this hormone, which may be mediated by the inflammatory responses in tissues to the organism.

Keywords: *Helicobacter pylori*, Ghrelin, Eradication, Gastric pathology, Nutritional status.

INTRODUCTION

Ghrelin, a 28-amino acid peptide hormone mainly synthesized in the gastric oxyntic glands[1,2], is an endogenous ligand for the growth hormone secretagogue receptor and is related to physiological processes, including energy metabolism, cell proliferation, bone formation, inflammation, sleep regulation, thermogenesis and blood circulation[3]. Two main forms of ghrelin have been described in humans: acyl ghrelin, with an n-octanoyl-modification at the serine-3 that confers its activity, and des-acyl ghrelin[4]. Ghrelin increases appetite sensation and food intake, stimulates gastric acid secretion and gut motility, promotes fatty acid storage and reduces lipid oxidation[4,5]. Given these actions on food intake and fat deposition, the overall effect of ghrelin is to increase body weight[6].

Helicobacter pylori are bacteria that colonize the gastric mucosa of about 50% of the world's population, with higher prevalence in developing countries[7]. Although colonization with *H. pylori* causes inflammatory cell infiltration into the gastric mucosa (i.e., gastritis) in all colonized patients[8], most of the affected population is asymptomatic. A combination of bacterial, host and environmental factors can develop gastrointestinal pathologies such as peptic ulcer disease, atrophic gastritis, MALT lymphoma, and/or gastric cancer[7,9].

It has been hypothesized that *H. pylori* infection may impair ghrelin production, resulting in decreased circulating concentration[10]. However, studies of the influence of *H. pylori* on gastric ghrelin production and body weight have yielded conflicting results; *H. pylori*-positive subjects have had lower, higher or similar ghrelin levels compared to *H. pylori*-negative individuals[11-13]. We previously conducted a cross-sectional study which revealed that *H. pylori* colonization was associated with lower serum ghrelin values[14], consistent with the conclusions from a systematic review and meta-analysis[15].

Since ghrelin gastric synthesis has been found to be increased after *H. pylori* eradication[11,16], this could lead to a rise in circulating ghrelin, increasing appetite and body weight[17,18]. However, inconsistencies between results from different reports on *H. pylori* eradication have been found, indicating the need for more research in this field[15,19]. Nwokolo et al[17] described an increase in plasma ghrelin but unchanged body mass index (BMI) six weeks after *H. pylori* eradication in asymptomatic subjects and Choi et al[20] also observed a rise in acyl ghrelin without significant BMI modification one year after eradication. Francois et al[21] reported a significant increase in BMI and post-prandial (but not pre-meal) acyl ghrelin levels a median of seven months after *H. pylori* eradication, and a randomized controlled trial also found BMI to be higher following *H. pylori* cure[22]. However, other authors reported a decrease in acyl ghrelin[23,24] or in total ghrelin[25,26] after eradication. Therefore, the current study aimed to evaluate the effect of *H. pylori* eradication on serum ghrelin levels, appetite sensation, nutritional status, and gastric histopathology in dyspeptic adults in Argentina.

MATERIALS AND METHODS

Participants and Protocol Design

This longitudinal analytical study was conducted over a 2-year period at the Hospital de Gastroenterología "Dr. Carlos Bonorino Udaondo", a tertiary referral hospital located in Buenos Aires, Argentina. The protocol was approved by the Institutional Review Board (Comité de Ética en Investigaciones, CEI, Hospital de Gastroenterología "Dr. Carlos Bonorino Udaondo") in April 2015 and was performed in accordance with the principles of the Declaration of Helsinki and the Guidelines of Good Clinical Practice. Overnight fasting dyspeptic adults (18-70 years) referred for an upper-digestive-endoscopy were prospectively enrolled. Patients who were pregnant or had active gastrointestinal (GI) bleeding, history of GI surgery, neoplastic disease, diabetes, thyroid, renal or hepatic pathologies, celiac disease, coagulopathy, drug abuse, or treatment with antimicrobials or acid suppressants within a month before enrollment were excluded. Subjects provided written informed consent, which detailed the objectives, procedures and outcomes of the study; confidentiality of the data was assured.

All individuals completed an epidemiological questionnaire and underwent upper GI endoscopy with biopsy. In this pre-post therapy study, baseline determinations included energy and macronutrient intake, appetite sensation, weight, height, waist circumference, serum ghrelin concentration after an overnight fast, type of gastric pathology and presence of *H. pylori* and its genotype. Those

subjects who were *H. pylori*-positive received eradication therapy and returned for reevaluation at least twelve weeks later[15]. Initial *H. pylori* status was based on microscopic assessment of stained gastric tissue by histologic examination. At the follow-up, in addition to repeated gastric biopsy and histological examination, the ^{13}C-Urea Breath Test (^{13}C-UBT) was performed to ascertain *H. pylori* status.

Eradication Therapy

Given the high level of *H. pylori* resistance to clarithromycin and metronidazole in Argentina[27] and the unavailability of bismuth, patients who tested positive for *H. pylori* received eradication treatment consisting of pantoprazole (40 mg twice daily for 30 d), amoxicillin (1 g twice daily for 10 d), levofloxacin (500 mg once daily for 10 d), as suggested by the Maastricht Consensus Report[8]. After a minimum of twelve weeks, all treated patients returned for re-evaluation. Treatment compliance and protocol adherence were evaluated by an interview as well as count of remaining tablets in medication containers at the programmed control visit.

Food Intake and Appetite Sensation

Each volunteer completed a 24 h dietary recall to assess energy and macronutrient intake. Subjects were provided with picture charts for estimation of portion size[28]. Food composition was analyzed using a 2007 database compiled by the Argentine Ministry of Health[29].
The Simplified Nutritional Appetite Questionnaire (SNAQ), a short, validated assessment tool, was used for evaluating appetite sensation and predicting weight loss risk. A calculated score ≤14 indicates a significant risk of at least 5% weight loss within six months[30].

Anthropometry

Body weight was measured using a portable mechanical scale (CAM, Buenos Aires, Argentina) to the nearest 100 g, and height was determined with a stadiometer (Stanley, Morangis, France) to the nearest 0.1 cm. Anthropometric techniques were previously standardized according to the CDC anthropometry procedure manual[31]. Body Mass Index (BMI) was calculated as weight in kilograms divided by the square of height in meters and underweight, stunting, overweight and obesity were classified according to World Health Organization standards[32]. Abdominal adiposity, a cardiovascular disease risk predictor, was evaluated by measuring waist circumference using a stretch-resistant tape[33].

Ghrelin Determination

Venous blood samples were obtained from the fasted patients before endoscopy. The serum was separated by centrifugation and stored at -80ºC. Total ghrelin serum concentration was determined by Enzyme-linked-Immunosorbent-Assay (ELISA) using a commercial kit (EMD Millipore Corporation, MO, USA). Absorbance values were obtained using a FlexStation 3 Multi-Mode Microplate Reader (Molecular Devices, LLC., CA, United States) and were processed with the Cembal 2.2® program (Cembal Applications 2000-2001, Argentina) to calculate ghrelin concentrations.

Endoscopy

All subjects underwent esophagogastroduodenoscopy (EGD), during which four gastric biopsies were taken: two each from the antrum and corpus. Gastric mucosal histology was evaluated using one biopsy from each site, and the two remaining samples were used for molecular biology evaluation.

Histological Analysis

Gastric biopsies were processed by formol immersion for 2h, dehydration in 96% ethanol for 6h, 100% ethanol for 4h and xylene for 3h, with immersion in paraffin at 56-58ºC for 3h and at 62ºC for 3h. Consecutive 4 μm sections were obtained using a spin tissue processor (MicromSTP120, ThermoScientific Corp., Walldorf, Germany) for hematoxylin-eosin and Giemsa histologic staining. Microscopic assessment allowed *H. pylori* diagnosis and gastritis grading according to the updated Sydney System Classification[34].

^{13}C-Urea Breath Test

We used a commercial ^{13}C-Urea Breath Test (^{13}C-UBT) kit (TAU-KIT, Isomed Pharma, Madrid, Spain) as described in our prior study[14]. Fasted patients drank 100 mL of a citric acid-enriched beverage. Ten minutes later, two basal exhaled air samples were collected in hermetically sealed containers. Then each patient ingested 100 mg ^{13}C-urea dissolved in 50 mL water. After 30 minutes, two post-dose breath samples were collected. Samples were measured in an isotope ratio mass spectrometer coupled to a gas chromatograph (Finnigan MAT GmbH, Thermo Fisher Scientific, Bremen, Germany). A change of 3.5‰ or more in the Delta Over Baseline value was considered positive for the presence of *H. pylori*[35].

H. pylori Genotype

H. pylori vacA and *cagA* genotypes were evaluated. Prior to PCR amplification, bacterial DNA was isolated from antrum and corpus biopsies using the QIAamp Mini Kit (QIAGEN, INC., CA, United States). Amplification was performed using the following primer sequences: va1F (5'-ATGGAAATACAACAAACACAC-3') and va1XR (5'-CCTGAGACCGTTCCTACAGC-3') for *vacA*, which amplified the *vacA*S1 (176 bp) and *vacA*S2 (203 bp) alleles[36]; *cagA*22 (5'-GATCCTGCTAGTTTGTCAGCGA-3') and *cagA*23 (5'-CTTATCATTCACGAGTTTGAGC-3') for the *cagA* gene (127 bp product)[37]. Reagent concentrations and conditions were as described[14].

Statistical Analysis

Sample size was estimated using Statcalc (Epi Info 3.2, Georgia, USA), considering $\alpha = 0.05$, $\beta = 0.20$, and a 30% variation in ghrelin level after successful therapy[21]. Enrolment of 22 eradicated patients was calculated to be necessary to find a statistically significant change in ghrelin concentration after *H. pylori* eradication. Considering an *H. pylori* eradication rate of 70% and a 20% loss to follow-up, we calculated 40 *H. pylori*-positive patients would be needed for this study. Mann-Whitney test was used to compare differences in weight between subjects with successful eradication or not. The Wilcoxon-Signed-Rank test was used to analyze paired measures of energy and macronutrient intake, appetite sensation and ghrelin concentration before and after *H. pylori* treatment. Relationships between variables were evaluated by Spearman correlation. Friedman test was used to analyze differences concerning gastric pathology. All statistical analyses were performed using SPSS software version 19.0 (IBM SPSS, Chicago, IL, USA), setting significance levels at $p < 0.05$.

RESULTS

Patients

The screening included 117 subjects with a median age of 44.0 years (IQR 33.5-53.0 y), of which 66.7% (CI95% 57.7-74.5%) were female. Anthropometric evaluation showed that 25.9% of the screened patients were under or normal weight, 48.2% were overweight, and 25.9% were obese. *H. pylori* prevalence in this population was 68.4% [CI95% 59.5-76.1%]. Seventy-two *H. pylori*-positive subjects received eradication therapy and 47 returned at least twelve weeks later for re-evaluation, exceeding the calculated sample size for statistical significance. Eradication was successful

in 28 (59.6%) subjects in which the variables under analysis were compared before and after treatment. In addition, variables were also analyzed in the 19 subjects in which eradication failed. The *H. pylori* eradicated group had a median age of 51.5 y (IQR 36.5-60.3y) and a 16/12 female/male ratio, while in the *H. pylori* non-eradicated group, the median age was 47.0 y (IQR 35.0-52.0y) and female/male ratio was 13/6. There were no statistically significant differences between groups in terms of age (p=0.20) or sex (p=0.44).

Dietary Assessment and Appetite Evaluation

Energy, macronutrient intake, and SNAQ scores in the *H. pylori* eradicated and non-eradicated groups are presented in Table 1. There was a tendency towards increased post-treatment nutrient intake in both groups; however, differences with pre-treatment values were not statistically significant. Appetite sensation did not change significantly after treatment in either of the two groups.

Anthropometric Evaluation

H. pylori eradicated patients showed a median initial weight of 70.0 kg (IQR 60.3-79.0) very similar to the non-eradicated patients, with 69.4 kg (IQR 60.0-84.7) (p=0.90). A significant increase in body weight following therapy was observed in both the eradicated (p=0.02) and non-eradicated subjects (p=0.03). We further analyzed whether weight gain was different according to treatment outcome finding no significant differences between the two groups: 0.7 kg median weight gain (IQR 0.0-2.9) in eradicated and 1.4 kg (IQR -0.1-3.8) in non-eradicated subjects (p=0.71).

Ghrelin Levels

There was no significant difference in the baseline ghrelin levels in the eradicated and non-eradicated groups. However, median ghrelin concentration decreased from 345.0 pg/mL (IQR 273.0–517.7) to 298.5 pg/mL (IQR 251.0–383.5) after *H. pylori* eradication (p=0.0007), whereas it did not change significantly in the non-eradicated patients (Table 1). Ghrelin changes inversely correlated with baseline levels in the *H. pylori* eradicated group (r=-0.81; p<0.0001), consistent with a prior report[38], while no significant correlation was found in the non-eradicated group (p=0.053) (Figure 1A). We found a significant correlation between changes in ghrelin and body weight as a percent in the eradicated group (r=-0.41; p=0.035) but not in non-eradicated subjects (Figure 1B).

TABLE 1. NUTRIENT INTAKE, APPETITE SENSATION AND GHRELIN LEVELS BEFORE AND ≥12 WEEKS AFTER *H. PYLORI* TREATMENT.

Variable	*H. pylori* eradicated (n = 28)			*H. pylori* non-eradicated (n = 19)		
	Baseline	Post-treatment	p	Baseline	Post-treatment	p
Energy (kcal/d)[a]	1469.0 [1311.0-1777.5]	1693.0 [1279.0-2040.0]	0.75	1400.0 [1107.5-1992.0]	1896.5 [1344.0-2381.3]	0.32
Carbohydrate (g/d)[a]	200.0 [167.3-241.8]	215.0 [138.0-350.0]	0.72	170.0 [130.5-247.7]	260.5 [153.5-286.5]	0.42
Protein (g/d)[a]	57.5 [43.8-82.0]	74.0 [51.0-86.0]	0.20	55.5 [37.5-79.7]	59.0 [49.5-88.7]	0.55
Fat (g/d)[a]	50.5 [42.3-66.0]	55.0 [45.0-64.0]	0.75	44.0 [28.0-72.2]	75.0 [60.0-100.0]	0.06
SNAQ score[b,c]	14.9 ± 2.2	15.4 ± 1.9	0.19	14.8 ± 2.3	14.8 ± 2.1	0.99
Fasting ghrelin (pg/mL)[a]	345.0 [273.0 – 517.7]	298.5 [251.0 – 383.5]	0.0007*	485.0 [333.0 – 629.0]	413.0 [291.0 – 594.0]	0.11

[a]Median [IQR]; [b]Mean ± SD; [c]Simplified Nutritional Appetite Questionnaire score; *p < 0.05, statistically significant (Wilcoxon-Signed-Rank).

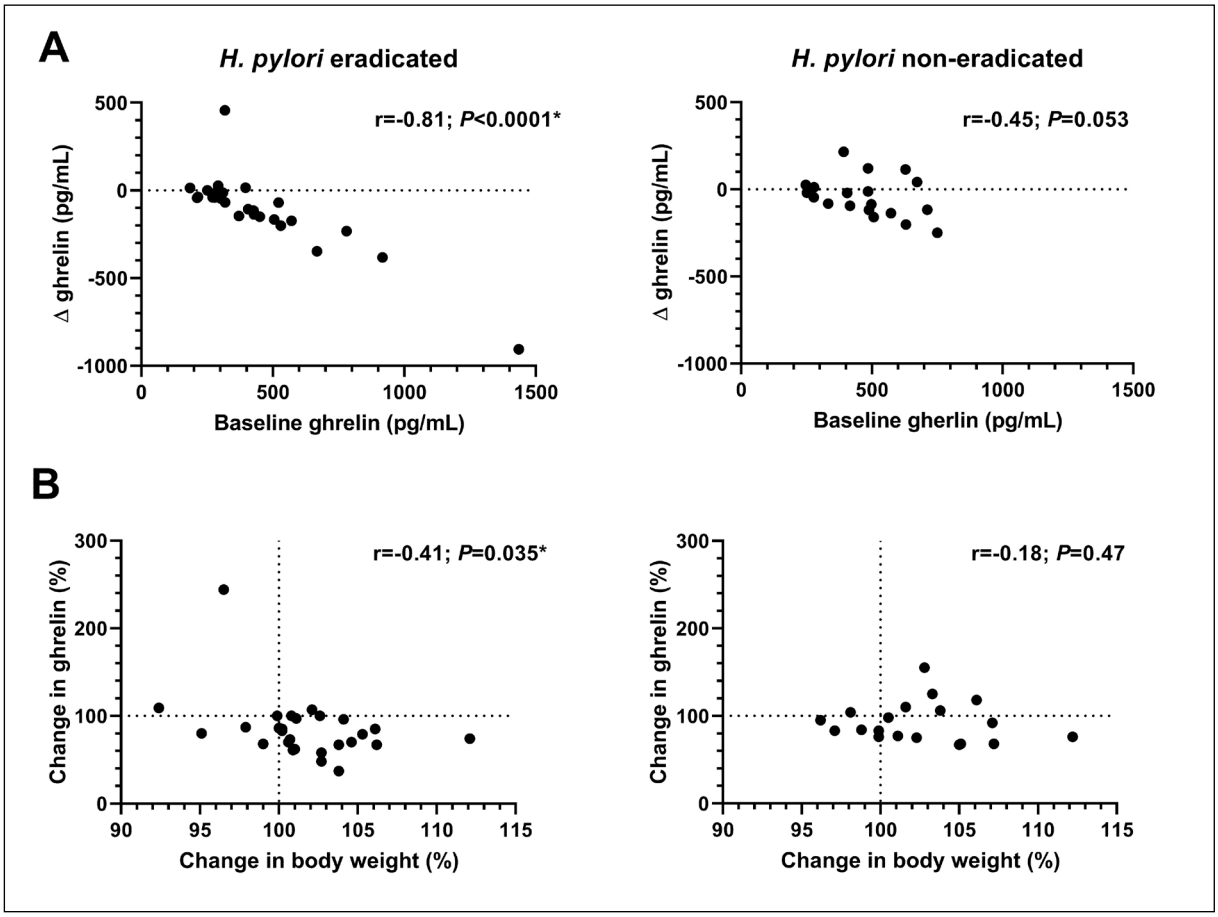

Figure 1. Relationship between baseline ghrelin levels and ghrelin variation (Δ) after *H. pylori* treatment in eradicated and non-eradicated patients (**A**). Relationship between changes in body weight and ghrelin concentration as a percent (**B**). R-values represent Spearman Rank coefficients, and *p*-values were calculated. *Statistically significant (Spearman correlation).

Gastric Histopathology

As expected, there was higher prevalence of active chronic gastritis before treatment in the *H. pylori*-positive subjects than in those without (Table 2). After antibiotic therapy, the prevalence of chronic inactive gastritis and normal mucosa both increased with a decrease in chronic active gastritis, both in antrum ($p<0.0001$) and corpus ($p<0.0001$), in those patients who eradicated the infection. In the non-eradicated group, there was no significant change.

H. pylori Strains

Distribution of *H. pylori vacA* and *cagA* genotypes among the 47 treated patients was as follows: 58.7% *vacA*S1 *cagA*-positive, 32.6% *vacA*S2 *cagA*-negative, 6.5% *vacA*S1 *cagA*-negative, and 2.2% *vacA*S2 *cagA*-positive. Ghrelin concentration decreased significantly after eradication in patients carrying *cagA*-positive strains ($n=15$) [371.0 pg/mL (IQR 271.0-571.0) before *vs.* 299.0 pg/mL (IQR 232.0-399.0) after, $p=0.0032$]. There were no statistically significant differences in subjects with *cagA* negative strains ($n=13$) [319.0 pg/mL (IQR 276.5-513.5) before *vs.* 298.0 pg/mL (IQR 251.0-395.0) after, $p=0.08$]. In the non-eradicated group, ghrelin variation was not influenced by the *H. pylori cagA* genotype.

TABLE 2. GASTRIC PATHOLOGY BEFORE AND ≥12 WEEKS AFTER *H. PYLORI* ERADICATION TREATMENT.

Variable	Eradicated (*n* = 28)			Non-eradicated (*n* = 19)		
	Baseline	Post-treatment	*p*	Baseline	Post-treatment	*p*
Antrum	*n* = 26	*n* = 28		*n* = 18	*n* = 18	
No inflammatory cell infiltration[a]	0 (0.0)	8 (28.6)		0 (0.0)	0 (0.0)	
Chronic inactive gastritis[a]	2 (7.7)	16 (57.1)	[<0.0001*]	2 (11.1)	6 (33.3)	[0.06]
Chronic active gastritis[a]	22 (84.6)	3 (10.7)		15 (83.3)	12 (66.7)	
Atrophy or intestinal metaplasia[a]	2 (7.7)	1 (3.6)		1 (5.6)	0 (0.0)	
Corpus	*n* = 26	*n* = 28		*n* = 18	*n* = 17	
No inflammatory cell infiltration[a]	0 (0.0)	11 (39.3)		0 (0.0)	0 (0.0)	
Chronic inactive gastritis[a]	1 (3.8)	16 (57.1)	[<0.0001*]	3 (16.7)	5 (29.4)	[0.41]
Chronic active gastritis[a]	25 (96.2)	1 (3.6)		15 (83.3)	12 (70.6)	
Atrophy or intestinal metaplasia[a]	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	

[a]*n* (%); *$p < 0.05$, statistically significant (Friedman Test).

DISCUSSION

The goal of this study was to analyze the effect of *H. pylori* eradication on serum ghrelin concentration, gastric mucosa status, anthropometry, appetite and food intake. Our results showed a statistically significant decrease in circulating total ghrelin levels after successful eradication of *H. pylori* infection, which was not observed in subjects with maintenance of *H. pylori* (non-eradicated), and the inverse correlation between total ghrelin variation and baseline ghrelin concentration was only observed in the *H. pylori* eradicated group.

Previous studies[15] concerning ghrelin after *H. pylori* eradication showed conflicting results: a meta-analysis found no significant differences in circulating ghrelin levels after *H. pylori* eradication. Subsequent reports continued showing discrepant results[20,21,23-26], probably due to the heterogeneity of subject sex, age, ethnicity, diseases, *H. pylori* strain, measured ghrelin entity, sample type, follow-up duration, study design, eradication therapy, all of which hinder comparisons.

When we analyzed ghrelin variation after eradication in relation to the *H. pylori* strain, we observed ghrelin decreased significantly only in eradicated patients who carried *cagA*-positive strains; however, sample size was not calculated for this sub-analysis, which may have prevented observing changes in ghrelin among subjects with *cagA*-negative strains.

A significant increase in body weight was observed after *H. pylori* eradication. Although weight gain following *H. pylori* eradication has been widely described[11,39], we also observed a weight increase in the non-eradicated group. A possible explanation for this outcome may be the resolution of dyspepsia due to eradication therapy itself, independent of its effectiveness. Despite our attempt to evaluate dyspepsia symptoms through the administration of a validated questionnaire, enrollment of subjects with low education levels precluded properly obtaining data. If dyspeptic symptoms improved, appetite sensation and food intake would be expected to increase; however, this was not observed.

An alternative explanation for the weight gain would be the alteration of intestinal microbiota composition by the eradication therapy[24,40], regardless of the effects on *H. pylori*. Since microbiota composition affects the amount of energy extracted from diet and fat deposition[41,42], eradication

therapy may affect weight increase even when food intake remains unchanged. Reduction in plasma ghrelin level after *H. pylori* eradication also could be due to an antimicrobial-induced alteration of the gut microbiota[24]. Although we did not evaluate the microbiota composition, we only found ghrelin decrease in successfully eradicated patients. Since the majority of human ghrelin production is in the stomach[1,2], a gastric-specific process, such as the eradication of *H. pylori*, would likely have had a more profound effect. Circulating ghrelin concentration is known to be strongly correlated with body weight change[11]; therefore, decreased ghrelin concentration could be a consequence of the body weight increase. We observed a significant inverse correlation between ghrelin and body weight changes only in eradicated subjects. However, the non-eradicated group in our study did not have significant decreases in ghrelin concentration despite having a significant body weight increase. These considerations suggest that the mechanisms tying the antimicrobial effects, *H. pylori* eradication, ghrelin levels, and weight changes are not linearly related across a heterogeneous human population.

Since decreased inflammatory cell infiltration of the gastric mucosa was observed only in *H. pylori* eradicated patients, as expected[8,43], the ghrelin decrease is related to the change in the gastric tissue responses; consistent with this view, the strongest effects were seen with eradication of *cagA* positive *H. pylori* strains, which are known to be the most interactive with host tissues. Ghrelin itself has gastroprotective and anti-inflammatory effects[3,19,44]; therefore, any changes in fasting ghrelin after *H. pylori* eradication may be mediated by the inflammatory responses. In this way, the lack of assessment of pro- or anti-inflammatory cytokines could be a limitation of this study. This change in circulating ghrelin concentration, however, may be different in the short-term or the long-term after *H. pylori* eradication, as reported by other authors[20,21].

CONCLUSIONS

In conclusion, our results provide further evidence that gastric colonization with *H. pylori*, especially *cagA* positive strains, affects ghrelin physiology. The effects of these changes may not be limited to the stomach, but affect systemic phenomena, like circulating ghrelin levels and body weight homeostasis, furthering our view of the integrated role of *H. pylori* with human physiology.

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Author’s Contributions

Paula Mantero, investigation, formal analysis, writing-original draft preparation; Liliana Marchesi Olid, investigation; Candela Giacomantone, investigation; Ana M. Cabanne, investigation; Marcela B. Zubillaga, formal analysis; Martin J. Blaser, writing-review and editing; Mariana A. Janjetic, formal analysis, writing-original draft preparation conceptualization; Cinthia G. Goldman, investigation, formal analysis, writing-original draft preparation, conceptualization, project administration, funding acquisition. All authors have read and approved the final manuscript.

Ethics Statements

The protocol was approved by the Institutional Review Board (Comité de Ética en Investigaciones, CEI, Hospital de Gastroenterología “Dr. Carlos Bonorino Udaondo”) in April 2015 and was performed in accordance with the principles of the Declaration of Helsinki and the Guidelines of Good Clinical Practice.

Informed Consent

Subjects provided written informed consent, which detailed the objectives, procedures and outcomes of the study; confidentiality of the data was assured.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the corresponding author on reasonable request.

REFERENCES

1. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999; 402: 656-660.
2. Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology 2000; 141: 4255-4261.
3. Müller TD, Nogueiras R, Andermann ML, Andrews ZB, Anker SD, Argente J, Batterham RL, Benoit SC, Bowers CY, Broglio F, Casanueva FF, D'Alessio D, Depoortere I, Geliebter A, Ghigo E, Cole PA, Cowley M, Cummings DE, Dagher A, Diano S, Dickson SL, Diéguez C, Granata R, Grill HJ, Grove K, Habegger KM, Heppner K, Heiman ML, Holsen L, Holst B, Inui A, Jansson JO, Kirchner H, Korbonits M, Laferrère B, LeRoux CW, Lopez M, Morin S, Nakazato M, Nass R, Perez-Tilve D, Pfluger PT, Schwartz TW, Seeley RJ, Sleeman M, Sun Y, Sussel L, Tong J, Thorner MO, van der Lely AJ, van der Ploeg LH, Zigman JM, Kojima M, Kangawa K, Smith RG, Horvath T, Tschöp MH. Ghrelin. Mol Metab 2015; 4: 437-460.
4. Kojima M, Kangawa K. Ghrelin: Structure and function. Physiol Rev 2005; 85: 495-522.
5. Theander-Carrillo C, Wiedmer P, Cettour-Rose P, Nogueiras R, Perez-Tilve D, Pfluger P, Castaneda TR, Muzzin P, Schürmann A, Szanto I, Tschöp MH, Rohner-Jeanrenaud F. Ghrelin action in the brain controls adipocyte metabolism. J Clin Invest 2006; 116: 1983-1993.
6. Cummings DE. Ghrelin and the short- and long-term regulation of appetite and body weight. Physiol Behav 2006; 89: 71-84.
7. Czinn SJ. *Helicobacter pylori* infection: Detection, investigation, and management. J Pediatr 2005; 146: 21-26.
8. Malfertheiner P, Megraud F, O'Morain C, Gisbert JP, Kuipers EJ, Axon A, Bazzoli F, Gasbarrini A, Atherton J, Graham DY, Hunt R, Moayyedi P, Rokkas T, Rugge M, Selgrad M, Suerbaum S, Sugano K, El-Omar E. Management of *Helicobacter pylori* infection - the Maastricht V/Florence Consensus Report. Gut 2017; 66: 6-30.
9. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Schistosomes, Liver Flukes and *Helicobacter pylori*. IARC Monogr Eval Carcinog Risks Hum 1994; 61: 1-241.
10. Osawa H, Nakazato M, Date Y, Kita H, Ohnishi H, Ueno H, Shiiya T, Satoh K, Ishino Y, Sugano K. Impaired production of gastric ghrelin in chronic gastritis associated with *Helicobacter pylori*. J Clin Endocrinol Metab 2005; 90: 10-16.
11. Osawa H. Ghrelin and *Helicobacter pylori* infection. World J Gastroenterol 2008; 14: 6327-6333.
12. Roper J, Francois F, Shue PL, Mourad MS, Pei Z, Olivares De Perez AZ, Perez-Perez GI, Tseng CH, Blaser MJ. Leptin and ghrelin in relation to *Helicobacter pylori* status in adult males. J Clin Endocrinol Metab 2008; 93: 2350-2357.
13. Stec-Michalska K, Malicki S, Michalski B, Peczek L, Wisniewska-Jarosinska M, Nawrot B. Gastric ghrelin in relation to gender, stomach topography and *Helicobacter pylori* in dyspeptic patients. World J Gastroenterol 2009; 15: 5409-5417.
14. Mantero P, Matus GS, Corti RE, Cabanne AM, Zerbetto De Palma GG, Marchesi Olid L, Piskorz MM, Zubillaga MB, Janjetic MA, Goldman CG. *Helicobacter pylori* and corpus gastric pathology are associated with lower serum ghrelin. World J Gastroenterol 2018; 24: 397-407.
15. Nweneka CV, Prentice AM. *Helicobacter pylori* infection and circulating ghrelin levels - A systematic review. BMC Gastroenterol 2011; 11: 7.

16. Lee ES, Yoon YS, Park CY, Kim HS, Um TH, Baik HW, Jang EJ, Lee S, Park HS, Oh SW. Eradication of *Helicobacter pylori* increases ghrelin mRNA expression in the gastric mucosa. J Korean Med Sci 2010; 25: 265-271.
17. Nwokolo CU, Freshwater DA, O'Hare P, Randeva HS. Plasma ghrelin following cure of *Helicobacter pylori*. Gut 2003; 52: 637-640.
18. Tatsuguchi A, Miyake K, Gudis K, Futagami S, Tsukui T, Wada K, Kishida T, Fukuda Y, Sugisaki Y, Sakamoto C. Effect of *Helicobacter pylori* infection on ghrelin expression in human gastric mucosa. Am J Gastroenterol 2004; 99: 2121-2127.
19. Peeters TL. Ghrelin: A new player in the control of gastrointestinal functions. Gut 2005; 54: 1638-1649.
20. Choi YJ, Kim N, Yoon H, Shin CM, Park YS, Park JH, Nam RH, Lee DH, Jung HC. Increase in plasma acyl ghrelin levels is associated with abatement of dyspepsia following *Helicobacter pylori* eradication. J Gastroenterol 2016; 51: 548-559.
21. Francois F, Roper J, Joseph N, Pei Z, Chhada A, Shak JR, de Perez AZO, Perez-Perez GI, Blaser MJ. The effect of *H. pylori* eradication on meal-associated changes in plasma ghrelin and leptin. BMC Gastroenterol 2011; 11: 1-9.
22. Lane JA, Murray LJ, Harvey IM, Donovan JL, Nair P, Harvey RF. Randomised clinical trial: *Helicobacter pylori* eradication is associated with a significantly increased body mass index in a placebo-controlled study. Aliment Pharmacol Ther 2011; 33: 922-929.
23. Ulasoglu C, Isbilen B, Doganay L, Ozen F, Kiziltas S, Tuncer I. Effect of *Helicobacter pylori* eradication on serum ghrelin and obestatin levels. World J Gastroenterol 2013; 19: 2388-2394.
24. Yanagi H, Tsuda A, Matsushima M, Takahashi S, Ozawa G, Koga Y, Takagi A. Changes in the gut microbiota composition and the plasma ghrelin level in patients with *Helicobacter pylori*-infected patients with eradication therapy. BMJ Open Gastroenterol 2017; 4: 1-8.
25. Ando T, Mizuno S, Ishida T, Kondo Y, Miki I, Yoshida M, Azuma T, Ishikawa T, Takagi T, Yagi N, Kokura S, Naito Y, Yoshikawa T, Asakawa A, Inui A. Plasma ghrelin isoforms and gastric ghrelin O-acyltransferase expression are influenced by *Helicobacter pylori* status. Nutrition 2012; 28: 967-972.
26. Kasai C, Sugimoto K, Moritani I, Tanaka J, Oya Y, Inoue H, Tameda M, Shiraki K, Ito M, Takei Y, Takase K. Changes in plasma ghrelin and leptin levels in patients with peptic ulcer and gastritis following eradication of *Helicobacter pylori* infection. BMC Gastroenterol 2016; 16: 1-8.
27. Zerbetto De Palma G, Mendiondo N, Wonaga A, Viola L, Ibarra D, Campitelli E, Salim N, Corti R, Goldman C, Catalano M. Occurrence of mutations in the antimicrobial target genes related to levofloxacin, clarithromycin, and amoxicillin resistance in *Helicobacter pylori* isolates from Buenos Aires city. Microb Drug Resist 2017; 23: 351-358.
28. Avila T, Chiappe C. Atlas Fotográfico de Preparaciones de Alimentos. 1st ed. Buenos Aires: Editorial Akadia; 2010: 1-11.
29. Ministerio de Salud, Presidencia de la Nación Argentina. SARA: Sistema de Análisis y Registro de Alimentos. Available from: https://datos.dinami.gov.ar/sara/.
30. Wilson M mary G, Thomas DR, Rubenstein LZ, Chibnall JT, Anderson S, Baxi A, Diebold MR, Morley JE. Appetite assessment: simple appetite questionnaire predicts weight loss in community-dwelling adults and nursing home residents 1 – 3. Published online 2005: 1074-1081.
31. CDC. National Health and Nutrition Examination Survey. Anthropometry Procedures Manual. Atlanta: CDC, 2007: 1.1-3.26.
32. World Health Organization. Report of a WHO Expert Committee. Physical Status: The Use and Interpretation of Anthropometry. Geneva: WHO Technical Report Series 854, 1995: 4-33.
33. World Health Organization. Report of a WHO Expert Consultation. Waist Circumference and Waist-Hip Ratio Geneva: World Health Organization, 2008: 1-34.
34. Stolte M, Meining A. The updated Sydney system: classification and grading of gastritis as the basis of diagnosis and treatment. Can J Gastroenterol 2001; 15: 591-598.
35. Gisbert JP, Pajares JM. Review article: ^{13}C-urea breath test in the diagnosis of *Helicobacter pylori* infection - A critical review. Aliment Pharmacol Ther 2004; 20: 1001-1017.
36. van Doorn LJ, Figueiredo C, Sanna R, Pena S, Midolo P, Ng EKW, Atherton JC, Blaser MJ, Quint WIMG V. Expanding Allelic Diversity of *Helicobacter pylori vacA*. J Clin Microbiol 1998; 36: 2597-2603.
37. Akopyants NS, Clifton SW, Kersulyte D, Crabtree JE, Youree BE, Reece CA, Bukanov NO, Drazek ES, Roe BA, Berg DE. Analyses of the *cag* pathogenicity island of *Helicobacter pylori*. Mol Microbiol 1998; 28: 37-53.
38. Osawa H, Kita H, Ohnishi H, Nakazato M, Date Y, Bowlus CL, Ishino Y, Watanabe E, Shiiya T, Ueno H, Hoshino H, Satoh K, Sugano K. Changes in plasma ghrelin levels, gastric ghrelin production, and body weight after *Helicobacter pylori* cure. J Gastroenterol 2006; 41: 954-961.
39. Boltin D, Niv Y. Ghrelin, *Helicobacter pylori* and Body Mass: Is There an Association? IMAJ Isr Med Assoc J 2012; 14: 130-132.
40. Mikkelsen KH, Allin KH, Knop FK. Effect of antibiotics on gut microbiota, glucose metabolism and body weight regulation: A review of the literature. Diabetes, Obes Metab 2016; 18: 444-453.
41. Krajmalnik-Brown R, Ilhan ZE, Kang DW, DiBaise JK. Effects of gut microbes on nutrient absorption and energy regulation. Nutr Clin Pract 2012; 27: 201-214.
42. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature 2012; 489: 242-249.
43. Jonaitis L, Pellicano R, Kupcinskas L. *Helicobacter pylori* and nonmalignant upper gastrointestinal diseases. Helicobacter 2018; 23: 1-5.
44. Slomiany BL, Slomiany A. Role of LPS-elicited signaling in triggering gastric mucosal inflammatory responses to *H. pylori*: modulatory effect of ghrelin. Inflammopharmacology 2017; 25: 415-429.