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Population Study | Articles

Helicobacter pylori infection and serum leptin, obestatin, and ghrelin levels in Mexican schoolchildren

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BACKGROUND: There is little information about the possible role of *Helicobacter pylori* infection on appetite-regulating peptides in children. This study evaluated the association between *H. pylori* infection and serum levels of ghrelin, leptin, and obestatin in schoolchildren.

METHODS: One hundred seventy-eight schoolchildren, students at boarding schools in Mexico City, participated. *H. pylori* infection status was determined every 6 months for 1 year by a breath test using ^{13}C-urea; schoolchildren with consistently positive or negative results were selected to participate. Age, sex, and body mass index (BMI) were recorded. Serum concentrations of total ghrelin, leptin, and obestatin via specific enzyme-linked immunosorbent assays were determined.

RESULTS: Schoolchildren with *H. pylori* infection had lower concentration of leptin, −0.54 pg/ml (95% CI: −0.98 to −0.09), compared to the schoolchildren without infection, after adjustment by age, gender, and BMI. And the children with the infection had a median of obestatin lower in 0.99 ng/ml (95% CI: −1.93 to −0.06) compared with the uninfected children after adjustment by BMI.

CONCLUSION: Association was found between *H. pylori* infection and decreased serum concentrations of leptin and obestatin. These results suggest that in schoolchildren, *H. pylori* infection affects the levels of hormones implicated in regulating appetite and energy homeostasis.

Ghrelin, leptin, and obestatin are peptides involved in regulating appetite and satiety. The stomach is the main source of ghrelin and obestatin, which are synthesized in the gastric oxyntic mucosa (1). Ghrelin has an orexigenic effect in the hypothalamic regulation of metabolic control and energetic balance; its serum levels are inversely correlated with body mass index (BMI) and change depending on energy requirements (2,3). Obestatin is derived from pro-ghrelin and exerts an effect opposite to that of ghrelin. Obestatin is a growth hormone antagonist that reduces gastric motility, increases release of gastric juice, and reduces food intake; however, its anorexigenic property remains controversial (4). Leptin has an anorexigenic effect; although leptin is mainly released by adipose tissue, it is also produced by P cells of the gastric epithelium and it has a direct correlation with BMI (3–5).

The stomach is an organ with endocrine functions, and *Helicobacter pylori*, a bacterium associated with chronic gastritis and gastric cancer, can affect some of its functions because the gastric mucosa is the site of its colonization (6). According to the Centers for Disease Control in the United States, *H. pylori* infection is prevalent in 30–40% of the population in developed countries, and can reach 80% in developing countries (http://wwwnc.cdc.gov/travel/yellowbook/2016/infectious-diseases-related-to-travel/helicobacter-pylori). Infection is usually acquired in childhood (7,8).

Some studies of children infected with *H. pylori* have revealed an association between infection and iron deficiency, low height and weight, and decreased growth rate (9–15). It has been reported that *H. pylori* infection can influence the levels of ghrelin, leptin, and obestatin. Therefore, this infection could have a role in regulating appetite and satiety, affecting body weight and height (16,17). In the adult population, it has been found that the serum level of ghrelin is lower and the concentration of leptin is higher in individuals infected with *H. pylori* compared with those in uninfected individuals; no difference has been found in the serum concentration of obestatin (18–22). A relationship has also been observed between the degree of gastric inflammation and the level of ghrelin expression (23,24). Other studies have shown changes in the expression levels of ghrelin after bacterial eradication as well as changes in the serum levels of ghrelin and leptin, and in BMI (19,25–29).

There is little information about the association between *H. pylori* infection and the concentration of appetite-regulating peptides in children. Some reports have shown that the concentrations of ghrelin and leptin are lower in infected children vs. those in uninfected children (27,30), but a higher concentration of leptin was also found in infected

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children (17). However, there is no information about what happens to obestatin in children in relation to *H. pylori* infection. It is important to determine whether *H. pylori* infection in children may be associated with ghrelin, leptin, and obestatin levels because this could affect the food intake and nutritional status of children (31). Therefore, the aims of this study were to evaluate the association between *H. pylori* infection and the serum levels of ghrelin, leptin, and obestatin in schoolchildren.

METHODS

Study Population

For this cross-sectional study, 178 schoolchildren (103 girls and 75 boys) ranging in age from 5 to 13 years were selected. They had initially participated in a longitudinal study on the effect of *H. pylori* infection on the growth of schoolchildren. The children were enrolled in three public elementary boarding schools in Mexico City. The study population was described in detail elsewhere (13,32). In brief, the participant schoolchildren remain at the boarding school 5 days per week, and go home on weekends and vacations. This boarding school program is offered by the Secretary of Education, and it is oriented toward children of low socioeconomic level. One of those boarding schools is attended only by girls; the other two are coeducational.

The selection criterion of the 178 participants was that they had maintained a constant *H. pylori* infection status, either positive or negative, for at least 1 year. In addition, schoolchildren without infection were selected based on the consideration that this group had a similar frequency of overweight/obesity, and were similar in low height-to-age and sex as the schoolchildren with infection.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Research and Ethics Committee of Mexican Institute of Social Security. The use of serum samples and data aimed at this specific study was approved by the Research and Ethics Committee of the National Institute of Pediatrics. Written informed consent was signed by the parents; agreement to participate was obtained from the children.

Determination of *H. pylori* Infection Status and Serum Total Ghrelin, Leptin, and Obestatin

H. pylori infection was determined by a breath test with ^{13}C-urea every 6 months, as previously published (32). The schoolchildren included in this study had consistent consecutive results, either positive or negative for infection, for at least three ^{13}C-urea consecutive breath tests.

The measurements of total ghrelin, leptin, and obestatin were performed in the serum. Serum was obtained from the main study in which blood samples were taken every 6 months, on the same day in which the breath sample was collected for detecting *H. pylori* infection. The blood samples were taken under 8-h fasting conditions and stored in aliquots at −80 °C until used. To determine the concentrations of the three peptides, the first of the three sera obtained semiannually was selected, but if the amount of serum available was insufficient, one of the other two serum samples was used. In 74% (131/178) of the cases, the first serum sample was used. Serological concentrations of total ghrelin, leptin, and obestatin were determined in duplicate via enzyme-linked immunosorbent assays (ELISAs), using the Human Total Ghrelin and Leptin commercial kits from EMD Millipore (Billerica, MA); the minimum detectable limits are 50 pg/ml for total ghrelin and 0.2 ng/ml for leptin. For obestatin, an ELISA kit from ALPCO Diagnostics (Salem, NH) was used, with a detection range of 0.231–25 ng/ml.

Anthropometric Measurements

We used information on weight, height, and BMI from each child; these measurements were obtained each time a serum sample was taken, and the *H. pylori* infection status was determined. Students were classified according to their BMI as overweight, obese, or normal BMI, according to the cutoff proposed by WHO (33). A *Z*-score of height-for-age indicator was obtained with WHO-Anthro software 2007, and schoolchildren with height-to-age equal to or < -2 *Z*-score were considered as stunted (34). A similar proportion of children who were overweight, obese, and stunted in the group with *H. pylori* infection was selected in children without infection.

Statistical Analysis

The ghrelin, leptin, and obestatin levels did not have a normal distribution; therefore, we reported the median and its interquartile range (IQR). The concentrations of these peptides were compared, stratifying by gender, age tertile, and BMI categories in relation to the infection status. In all these comparisons, the Wilcoxon rank-sum test for independent samples or Kruskal–Wallis equality-of-populations rank test were used.

Multivariate analysis was performed to evaluate the association between *H. pylori* infection and the concentrations of ghrelin, leptin, and obestatin. At first, we tried using linear regression models, but because these models showed lack of adherence to the assumptions of linear regression, multivariate analysis was performed using quantile regression models for the 50th quantile or median (35), employing a model for each peptide, adjusting for age, sex, and BMI, when necessary, according to the results obtained using univariate and stratified analyses. All analyses were performed using Stata software (version 12.0 STATA Corporation, College Station, TX).

RESULTS

Study Population Characteristics

The study population was 178 schoolchildren. The average age of the schoolchildren was 9.6 ± 1.8 years; 57.9% ($n = 103$) were girls. A total of 39.9% of our study population were overweight or obese; 60.1% ($n = 107$) had a normal BMI. Five percent (9/178) of schoolchildren had stunting. There was no statistically significant difference between these characteristics by *H. pylori* status, as mentioned in the Methods section; in selecting participants, we attempted to avoid significant differences in this regard. With respect to persistent infection status with *H. pylori* as determined by three consecutive breath tests (^{13}C-Urea) performed every 6 months, 50.6% were positive for infection in all three tests (**Table 1**).

Serum Levels of Total Ghrelin by *H. pylori* Infection Status

Schoolchildren with *H. pylori* infection had lower levels of ghrelin than those without infection. At stratified analysis by age, schoolchildren with *H. pylori* infection had lower levels of ghrelin from the second tertile of age (**Table 2**). The boys with *H. pylori* infection had lower levels of ghrelin than boys without *H. pylori* infection had, they had median ghrelin levels of 158.89 pg/ml (interquartile range (IQR) 147.20–166.44) vs. 167.56 (IQR 156.83–221.11), $P = 0.004$. For BMI, there was no difference in the levels of ghrelin between schoolchildren with the infection and those without the infection (**Table 2**).

Table 1. Study population characteristics

Characteristics	*H. pylori* positive ($n=90$, 50.6%)		*H. pylori* negative ($n=88$, 49.4%)		All $n=178$	
	n	%	*n*	%	*n*	%
Age (years)[a]	9.6 ± 1.8		9.5 ± 1.9		9.6 ± 1.8	
Age tertiles						
5.7–8.49 years	25	27.8	34	38.6	59	33.2
8.5–10.5 years	35	38.9	23	26.1	58	32.6
10.6–13.7 years	30	33.3	31	35.2	61	34.3
Sex						
Female	55	61.1	48	54.6	103	57.9
Male	35	38.9	40	45.4	75	42.1
Body mass index (*Z*-score)[a]	0.78 ± 1.02		0.80 ± 1.08		0.79 ± 1.05	
Body mass index						
Normal[b]	55	61.1	52	59.1	107	60.1
Overweight[b]	19	21.1	24	27.3	43	24.2
Obesity[b]	16	17.8	12	13.6	28	15.7
Height-for-age (*Z*-score)[a]	− 0.48 ± 0.90		− 0.21 ± 0.97		− 0.34 ± 0.94	
Height-for-age						
Normal[c]	85	94.4	84	95.4	169	94.9
Low height-for-age[c]	5	5.6	4	4.6	9	5.1

[a](Mean ± SD).
[b]WHO 2007 categories. Normal: $-2 < Z\text{-score} < 1$, overweight: $1 \leqslant Z\text{-score} < 2$, obese: $Z\text{-score} \geqslant 2$.
[c]WHO 2007 categories. Normal: $Z\text{-score} \geqslant -2$; low height-for-age: $Z\text{-score} < -2$.

Serum Levels of Leptin by *H. pylori* Infection Status

In univariate analysis, the levels of leptin were lower in schoolchildren with *H. pylori* infection. In the stratified analysis by age, in the group of children in the third tertile of age, 10.6–13.7 years, those with the infection had lower levels of leptin than those without the infection—1.75 ng/ml (IQR 1.18–2.62) vs. 3.59 (IQR 2.05–6.52), $P=0.005$.

By gender, girls with the infection had lower levels of leptin than those without the infection—1.84 ng/ml (IQR 1.16–3.28) vs. 2.47 (IQR 1.58–4.74), $P=0.023$.

On the basis of BMI categories, in children with normal BMI, those with *H. pylori* infection had lower levels of leptin than those without the infection—1.29 ng/ml (IQR 1.07–1.84) vs. 1.61 (IQR 1.33–2.10), $P=0.015$ (**Table 3**).

Serum Levels of Obestatin by *H. pylori* Status

Levels of obestatin were lower in schoolchildren with the infection. Children in the third tertile of age with the infection had lower levels than those without the infection—2.12 ng/ml (IQR 1.48–3.64) vs. 4.84 ng/ml (IQR 2.90–6.38) $P<0.001$.

By gender, girls with the infection had lower levels of obestatin than those without the infection—2.08 ng/ml (IQR 1.38–4.40) vs. 4.32 (IQR 2.38–6.02), $P=0.001$ (**Table 4**).

On the basis of BMI categories, in participants with normal BMI, those with *H. pylori* infection had significantly lower levels of obestatin than those without infection—2.10 ng/ml (IQR 1.49–4.02) vs. 2.99 ng/ml (IQR 2.34–5.04), $P=0.008$ (**Table 4**).

Differences in the Serum Median Levels of Ghrelin, Leptin, and Obestatin by *H. pylori* Status in the Multivariate Analysis

The results from the multivariate analysis showed that *H. pylori* infection in this population was associated with lower levels of leptin and of obestatin, but not with lower levels of ghrelin (**Table 5**). After adjusting for gender, age, and BMI, we found that children with *H. pylori* infection had lower levels of leptin, − 0.54 ng/ml (95% CI − 0.98 to − 0.09), $P=0.019$, than children without the infection. Also, gender was associated with leptin levels: girls had a median of leptin higher at 0.74 ng/ml (95% CI 0.23–1.26) than boys had. And schoolchildren who were overweight had a median of leptin higher at 1.40 ng/ml (95% CI 0.72–2.08) than schoolchildren with normal BMI had; schoolchildren who were obese had a median of leptin higher at 3.64 ng/ml (95% CI 1.49–5.79) than schoolchildren with normal BMI had.

There was also an association between infection and levels of obestatin. Schoolchildren with *H. pylori* infection have a median of obestatin concentration lower than that children without the infection have, − 0.99 ng/ml (95% CI − 1.93 to − 0.06), $P=0.037$ after adjusting by BMI categories.

DISCUSSION

In the present study, we evaluated the association between *H. pylori* infection and the serum levels of ghrelin, leptin, and obestatin in schoolchildren after adjusting for confounding variables such as age, gender, and BMI. We found an association between *H. pylori* infection and levels of leptin and obestatin. This report is the first to describe results relating the association between *H. pylori* infection and levels of serum obestatin in schoolchildren.

H. pylori Infection and Serum Total Ghrelin Levels

In this study, we did not find an association between *H. pylori* infection and median ghrelin levels after adjusting for possible confound factors. Our results are consistent with those described by Pacifico and Erdemir in children (27,36) and by Roper in adults (20); they also did not find differences in levels of ghrelin between subjects with and without *H. pylori* infection. But our results are contrary to those reported by Konturek and Plonka, who reported that children with *H. pylori* infection had lower ghrelin concentrations than healthy controls had (17,30). However, these authors did not present results of multivariate analysis; univariate analysis in our study also showed that ghrelin levels were lower in schoolchildren with *H. pylori* infection, but this was not maintained after adjustment by age and gender.

Table 2. Serum levels of ghrelin (pg/ml, median, and IQR), by *H. pylori* status

Variable	With *H. pylori* infection ($n = 90$)	Without *H. pylori* infection ($n = 88$)	*P*-value[a]
Ghrelin levels[a]	159.34 (145.56–167.56)	161.92 (152.25–190.00)	0.019
Age tertile			
5.7–8.4 years	165.17 (160.89–175.17)	161.83 (152.25–168.67)	0.113
8.5–10.5 years	157.56 (148.50–165.33)	165.16 (155.33–218.02)	0.027
10.6–13.7 years	146.20 (127.78–163.11)	160.89 (148.03–183.50)	0.003
P-value[b]	0.001	0.439	
Gender			
Males	158.89 (147.2–166.44)	167.56 (156.83–221.11)	0.004
Females	159.78 (138.50–168.50)	157.66 (147.57–167.83)	0.545
P-value[a]	0.616	0.002	
Body mass index			
Normal BMI	158.50 (146.83–167.56)	161.83 (148.30–218.02)	0.056
Overweight	164.12 (136.67–179.78)	160.89 (155.53–179.54)	0.448
Obesity	162.00 (138.62–166.44)	164.22 (160.89–168.67)	0.288
P-value[b]	0.814	0.973	

IQR, interquartile range.
[a]Two-sample Wilcoxon rank-sum test.
[b]Kruskal–Wallis equality-of-populations rank test.

In some clinical studies that include patients with gastric symptoms, the association between *H. pylori* infection and ghrelin levels, stratifying by the gastric inflammation grade, has been evaluated. Chuang *et al.* found lower levels of ghrelin only in men with *H. pylori* infection; in women, the levels of ghrelin were similar for *H. pylori* infection, regardless of the inflammation grade. Also, they found only lower ghrelin levels in male patients with marked acute inflammation and in those with chronic inflammation compared with patients with little inflammation. The authors concluded that there are gender differences in circulating ghrelin levels in response to chronic *H. pylori* infection (37).

In addition, in Pacifico's study, the participants were children 3–8 years old and were undergoing upper gastrointestinal endoscopy for evaluation of symptoms related to the upper gastrointestinal tract. An inverse correlation between ghrelin levels and the grade of gastritis was found in both groups with and without *H. pylori*, but the antral and corpus gastritis score were significantly higher in *H. pylori*-positive children than in *H. pylori*-negative children. The authors concluded that in children, serum ghrelin concentrations are inversely related to the severity of *H. pylori*-associated gastritis (27). Similar results were reported by other authors in adult populations (21,23,38). These results suggest that the association between *H. pylori* infection and ghrelin levels can differ in relation to the grade of activity and chronic inflammation. It is possible that in our study, because of population characteristics, which includes asymptomatic, apparently healthy schoolchildren, the ghrelin levels are not different between those with and without *H. pylori* infection. In addition, several factors could explain the discrepancy in the results of some studies about the effect of *H. pylori* infection on ghrelin levels. These factors could include differences in gender, age, gastric-related diseases, and the extent and severity of *H. pylori*-related gastritis in the study populations (24,27,31).

H. pylori Infection and Serum Leptin Levels

Leptin is synthesized and secreted by adipocytes; serum concentrations reflect the amount of energy stored in adipose tissue. Leptin binds to the receptor in the hypothalamus and influences the expression of several neuropeptides that regulate energy intake and energy expenditure (3). In our study, leptin concentrations in schoolchildren with *H. pylori* infection were lower than in those without the infection, after adjustment by important confounding factors. Our results are consistent with those of Pacifico in children 3–8 years old with gastrointestinal symptoms (27), and those of Plonka, in children and adolescents 6–17 years old (30), who also found lower concentration of leptin in children with *H. pylori* infection.

In adults, Roper also found lower concentration of leptin in those with *H. pylori* infection (20). This can be explained in part because, in addition to the regulation of food intake and energy homeostasis, it has been proposed that leptin participates in acute phase responses to inflammation, and

Table 3. Serum levels of leptin (ng/ml, median, and IQR), by *H. pylori* status

Variable	With *H. pylori* infection ($n=90$)	Without *H. pylori* infection ($n=88$)	*P*-value[a]
Leptin levels	1.75 (1.16–2.72)	2.13 (1.52–3.72)	0.011
Age tertiles			
5.7–8.4 years	1.50 (1.28–2.39)	1.96 (1.50–2.95)	0.139
8.5–10.5 years	1.84 (1.14–3.4)	1.80 (1.13–2.72)	0.911
10.6–13.7 years	1.75 (1.18–2.62)	3.59 (2.05–6.52)	0.005
P-value[b]	0.975	0.006	
Gender			
Males	1.64 (1.14–2.23)	2.01 (1.40–2.84)	0.114
Females	1.84 (1.16–3.28)	2.47 (1.58–4.74)	0.023
P-value[a]	0.378	0.065	
Body mass index			
Normal BMI	1.29 (1.07–1.84)	1.61 (1.33–2.10)	0.015
Overweight	2.72 (2.04–4.20)	3.08 (2.27–5.06)	0.282
Obesity	4.07 (2.06–5.87)	6.39 (3.34–10.44)	0.068
P-value[b]	<0.001	<0.001	

IQR, interquartile range.
[a]Two-sample Wilcoxon rank-sum test.
[b]Kruskal–Wallis equality-of-populations rank test.

Table 4. Serum levels of obestatin (ng/ml, median, and IQR), by *H. pylori* status

Variable	With *H. pylori* infection ($n=90$)	Without *H. pylori* infection ($n=88$)	*P*-value[a]
Obestatin levels	2.47 (1.52–4.69)	3.66 (2.41–5.56)	<0.001
Age tertile			
5.7–8.4 years	3.02 (1.52–4.31)	3.25 (1.79–4.96)	0.591
8.5–10.5 years	2.44 (1.69–5.00)	2.99 (2.27–5.25)	0.283
10.6–13.7 years	2.12 (1.48–3.64)	4.84 (2.90–6.38)	<0.001
P-value[b]	0.398	0.035	
Gender			
Males	3.83 (1.76–5.28)	2.97 (2.48–5.11)	0.698
Females	2.08 (1.38–4.40)	4.32 (2.38–6.02)	<0.001
P-value[a]	0.034	0.297	
Body mass index			
Normal BMI	2.10 (1.49–4.02)	2.99 (2.34–5.04)	0.008
Overweight	4.46 (1.93–5.00)	4.55 (2.85–6.41)	0.204
Obesity	2.64 (1.48–4.77)	3.78 (1.96–6.76)	0.287
P-value[b]	0.220	0.204	

IQR: interquartile range.
[a]Two-sample Wilcoxon rank-sum test.
[b]Kruskal–Wallis equality-of-populations rank test.

the leptin produced and released exerts a potent gastroprotective action. In addition, the presence of leptin, as well as the expression of its receptors, has been detected in the human stomach (39). The decreased serum leptin in schoolchildren may be related to the inflammatory response during *H. pylori* infection, and it may be mediated through interaction with pro-inflammatory cytokines (30). Further studies are needed to evaluate the possible consequences of a lower serum leptin concentration in schoolchildren with *H. pylori* infection.

H. pylori Infection and Serum Obestatin Levels

Our study is the first report of the serum concentration of this peptide in children infected with *H. pylori*. Obestatin concentration was lower in schoolchildren with the infection than in those without *H. pylori* infection. In adults, Ulasoglu *et al.* did not find differences between patients with the infection and those without the infection at baseline. However, 8 weeks after eradication therapy, there was an increase in concentration of obestatin in those who eradicated the bacterium compared with the control and compared with those who failed to eradicate *H. pylori* (38). It has been proposed that ghrelin and obestatin are peptides that have opposing roles in the regulation of appetite and satiety. Both are derivatives of preproghrelin and are secreted mainly from gastric oxyntic mucosa. Although ghrelin is a peptide with a role as an orexigenic signal and is a growth hormone release stimulator, obestatin has a role in the satiety pathways (38). Currently, there is a controversy about the effects of obestatin on food intake; some studies suggest that it can inhibit food intake and others suggest no effect. Studies in humans have shown that plasma obestatin levels do not vary significantly with a fixed energy meal but they are significantly lower in obese subjects compared with the lean controls, indicating a role for obestatin in long-term body weight regulation (40). As higher obestatin levels have been found in obese adults and children, it has been proposed that elevated obestatin levels may indicate a feedback response to obesity in an attempt to curtail appetite and food intake (41).

As mentioned, in our study the association between *H. pylori* infection and the levels of the three peptides after considering age, gender, and BMI showed that the presence of *H. pylori* in schoolchildren was associated with decreased serum concentrations of leptin and obestatin; no such association was observed for ghrelin. These results show that with *H. pylori* infection, changes occur in serum levels of peptides related to appetite, energy homeostasis, growth, immune, and reproductive functions (39,42). The magnitude of these changes could depend on the duration of the infection, age, and gender, among other factors. In this study, we included *H. pylori* infected children who had maintained this infection for at least 1 year, but we do not know the total time

Table 5. Multivariate models on the association between *H. pylori* infection and serum levels of ghrelin, leptin, and obestatin

Variables	Difference in the median[a]	95% CI	*P*-value
Model to ghrelin			
H. pylori (positive vs. negative)	−0.99	−8.43 to 6.45	0.794
Age tertile			
5.7–8.4 years	1.00		
8.5–10.5 years	−3.28	−11.28 to 4.72	0.419
10.6–13.7 years	−6.46	−15.85 to 2.92	0.176
Gender: females vs. males	−4.33	−13.64 to 4.98	0.360
Model to leptin			
H. pylori (positive vs. negative)	−0.54	−0.98 to −0.09	0.019
Age tertile			
5.7–8.4 years	1.00		
8.5–10.5 years	0.29	−0.26 to 0.83	0.304
10.6–13.7 years	0.64	−0.01 to 1.27	0.051
Gender: females vs. males	0.74	0.23 to 1.26	0.005
Body mass index			
Normal weight	1.00		
Overweight	1.40	0.72 to 2.08	<0.001
Obesity	3.64	1.49 to 5.79	0.001
Model to obestatin			
H. pylori (positive vs. negative)	−0.99	−1.93 to −0.06	0.037
Body mass index			
Normal weight	1.00		
Overweight	1.74	0.32 to 3.16	0.017
Obesity	0.52	−0.83 to 1.87	0.449

[a]Multivariate analysis, coefficient adjusted using quantile regression models for the conditional median in each peptide: model to median levels of ghrelin, adjusted for gender and tertile of age. Model to median levels of leptin, adjusted for gender, tertile of age, and categories of BMI. Model to median levels of obestatin, adjusted for categories of BMI.

of the infection. In children with *H. pylori* infection, lower levels of obestatin and leptin compared with children without the infection may represent a regulation to prevent deterioration in the nutritional status. We believe that further studies should be conducted about these peptides and their relationships with *H. pylori* infection in children. Our study design does not permit examining the mechanisms for these findings.

Our study has two strengths. (1) The statistical analysis includes multivariate analysis in which confounding variables such as age, sex, and BMI were taken into account; this is important because of metabolic and endocrine changes that occur at specific ages (41,42). (2) Participants maintained their status of *H. pylori* infection for at least 1 year; in addition, most cases of infection were prevalent cases. This is important because, in developing countries, most children get infected by *H. pylori* by age 5, and nearly all children get infected by age 10 (8,31). However, also in this population, we described the occurrence of incidence and spontaneous elimination of the *H. pylori* bacterium (32).

Our study had some limitations. (1) We measured only total ghrelin levels; however, as mentioned by Roper and Wali *et al.*, most studies on ghrelin have assessed total, not-acyl, ghrelin (20,41). And total ghrelin levels reflect the complex functions of the various ghrelin isoforms, including acyl and des-acyl ghrelin; in addition, the major percentage of circulating ghrelin, 90%, is as not-acyl ghrelin (38). (2) We did not evaluate the puberty stage of development in the schoolchildren, and leptin, obestatin, and ghrelin levels can be influenced by puberty changes. However, the adjustment by age and sex can be important in this aspect. (3) The serum samples were kept frozen for 8 years on average before being used to make ghrelin, leptin, and obestatin determinations for this study; however, these were at −80 °C in appropriate airtight tubes to prevent freeze-drying and had no freezing and thawing cycles as samples were separated in various aliquots before freezing (43). The results of this study have internal validity as procedures were identical for schoolchildren with and without *H. pylori* infection. (4) The temporal ambiguity inherent in cross-sectorial studies; however, according to studies on the association between *H. pylori* infection and nutritional status in children, it would be expected that *H. pylori* infection affects levels of peptides that have a role in appetite-satiety regulation. The possible mechanisms should be probed in basic and clinical studies.

CONCLUSION

H. pylori infection in schoolchildren was associated with low serum levels of leptin and obestatin. These results suggest that *H. pylori* infection may have an effect on the serum levels of hormones implicated in the regulation of appetite and growth. Further studies are needed to determine the role and interaction of these peptides in children to understand the health implications of changes in their serum levels in relation to *H. pylori* infection.

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