CLINICAL STUDY

Density of ghrelin-producing cells is higher in the gastric mucosa of morbidly obese patients

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Abstract

Background: Ghrelin is a peptide mainly secreted by gastric mucosa and has been implicated in the regulation of eating behavior and weight balance. Obesity and *Helicobacter pylori* infection are associated with changes in plasma ghrelin levels.

Objective: This study was designed to evaluate the density of ghrelin-producing cells in the gastric mucosa of morbidly obese and dyspeptic non-obese patients, with and without *H. pylori* infection.

Methods: Gastric biopsies of the antral and oxyntic mucosa were obtained from 50 morbidly obese patients (BMI > 40, 21 with metabolic syndrome (MS)), 17 dyspeptic overweight subjects (25 < BMI < 30), and 33 lean individuals (BMI < 25) and processed for histology and immunohistochemistry.

Results: Ghrelin-immunoreactive cell densities in the oxyntic mucosa were similar in morbidly obese patients with MS and in overweight and lean patients, whereas morbidly obese patients without MS presented higher ghrelin-immunoreactive cell density. The number of ghrelin cells in the oxyntic mucosa was significantly lower in obese and non-obese *H. pylori*-infected subjects. Ghrelin-immunoreactive cells, although sparse in the antral mucosa, were found more frequently in obese patients and their numbers did not seem to be affected by *H. pylori* infection.

Conclusions: The higher expression of ghrelin-immunoreactive cells in the oxyntic mucosa of morbidly obese patients compared with non-obese subjects or with morbidly obese subjects with MS and the finding of a higher number of small foci of ghrelin cells in the antral mucosa of obese patients may indicate an adaptive mechanism or an individual factor to be considered in the pathogenesis of obesity.

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Introduction

Obesity, a condition that presents an increasing prevalence worldwide, has become an alarming public health problem and is frequently associated with several pathological conditions such as diabetes mellitus, hypertension, coronary heart disease, and cancer, among others. The factors that affect the evolution of obesity are not well known, although it is believed that its pathogenesis may be multifactorial.

A number of gastrointestinal peptides have been reported as possible elements that could interfere with the disruption of the homeostasis between food intake and energy expenditure. Among the gastrointestinal hormones, the 28 amino acid peptide ghrelin appears to play a role in long-term appetite regulation and in the control of energy homeostasis. This peptide was discovered as the endogenous ligand of the GH secretagogue receptor, and soon afterward, it was observed that a gradient of ghrelin production occurs

along the gastrointestinal tract, with highest expression in the oxyntic gastric mucosa (1–3).

As gastric mucosa is a frequent site of chronic and persistent lesions, the gastric production of ghrelin may be affected along the evolution of these conditions, such as infection with *Helicobacter pylori* (4). The number of gastrin- and somatostatin-producing cells of the gastric mucosa has been reported to be affected in *H. pylori*-positive duodenal ulcer patients (5, 6). In addition, it has been reported that *H. pylori* infection seems to be associated with a significant decrease in plasma ghrelin levels that return to normal levels after eradication of this microorganism (7–10). In contrast, no difference in plasma ghrelin levels between *H. pylori*-positive and *H. pylori*-negative subjects matched for body mass index (BMI) has been shown by others (11, 12), leading to controversy about this subject. Furthermore, *H. pylori* infection may have a negative effect on the density of gastric ghrelin-immunoreactive cells. Impaired production of gastric ghrelin has been shown in parallel

to a decreased number of ghrelin-immunoreactive cells in the oxyntic mucosa of lean patients with chronic *H. pylori*-associated gastritis (7). These findings were also observed in obese patients infected with these microorganisms (13).

Recent reports suggest that hyperinsulinemia may interfere with serum ghrelin levels and also that ghrelin may modulate insulin secretion and contribute to obesity-associated insulin resistance in metabolic syndrome (MS) (14–18). On the other hand, obesity itself may interfere in some way with ghrelin production and release (19–22). Obese patients with Prader–Willi syndrome present increased plasma ghrelin concentrations and also an increased density of ghrelin-immunoreactive cells in the gastric mucosa (23–26). It should be mentioned that no comparative studies with a significant number of subjects are available regarding the density of ghrelin-immunoreactive cells in individuals with different BMI. This study was designed to evaluate the number of ghrelin-immunoreactive cells in the oxyntic and antral gastric mucosa of morbidly obese, overweight, and lean patients and its relationship to infection with *H. pylori*.

Materials and methods

Subjects

In this study, three groups of patients were studied: i) 50 obese patients (38 women, 12 men, mean age 35.4 years, BMI > 40) candidates for bariatric surgery. Among these obese patients, 21 (42%) had MS according to the International Diabetes Federation (IDF) and National Cholesterol Education Program (NCEP) criteria, i.e. all these patients had dyslipidemia (triglycerides ≥ 150 mg/dl or reduced high-density lipoprotein cholesterol), hypertension (systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg), and type 2 diabetes mellitus or hyperglycemia (fasting plasma glucose ≥ 100 mg/dl) in addition to a high waist circumference (27, 28); ii) 17 overweight dyspeptic patients (14 women, three men, mean age 47.8 years, 25 < BMI < 30); and iii) 33 lean dyspeptic patients (18 women, 15 men, mean age 41.7 years, BMI < 25) subjected to upper digestive endoscopy due to dyspeptic complaints and taking no medications. All patients were retrospectively and consecutively selected at the same general hospital Gastroenterology Unit. Patients with peptic ulcer or neoplastic lesions and patients presenting atrophic body gastritis at histology that could be suspected to be of autoimmune etiology were excluded. The clinical data of all patients were obtained from the hospital medical records. The Institutional ethics committee of the Federal University of Minas Gerais approved the study protocol, and written informed consent was obtained from all participants.

Tissue specimens, histology, and immunohistochemistry

A total of six tissue specimens of endoscopic gastric biopsies were obtained by the same endoscopist (J S A): four from the oxyntic mucosa (greater curvature, proximal smaller curvature, posterior and anterior walls) and two from the antral mucosa. The biopsy specimens were fixed in 4% formaldehyde and embedded in paraffin, and 4 µm thick sections from each specimen were stained with hematoxylin and eosin for histology and with Giemsa for histological evaluation of *H. pylori* infection and immunohistochemistry. The biopsy specimens were examined in a blind manner by a pathologist and scored to indicate absent, mild, moderate, and severe inflammatory infiltrates, atrophy, and intestinal metaplasia according to the updated Sydney system (29). Ghrelin immunoreactivity was determined on sections after microwave pretreatment (10 min in 0.01 M citric acid solution) and overnight incubation at 4 °C with human polyclonal antibody (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) diluted 1:1000. Diaminobenzidine–hydrogen peroxide was used as chromogen, and the sections were counterstained with diluted hematoxylin.

Evaluation of ghrelin cell density

Ghrelin-immunoreactive cell density was evaluated on acquired microscopic digital images (Axiocam camera and KS 300 digital system, Zeiss, Germany) in six consecutive 0.21 mm wide columns containing the full mucosa thickness at ×400 magnification. The results are expressed as the average number of cells/mm².

Statistical analysis

Statistical analysis was carried out using Epi-info version 6.04 and GraphPad Prism version 5.01 software (GraphPad Software Inc., La Jolla, CA, USA).

Table 1 Clinical and histological features of patients studied. Values are presented as n (%).

	Obese (BMI > 40)	Overweight (BMI 25–30)	Lean (BMI < 25)
Normal mucosa	19 (38)	4 (23.5)	5 (15.2)
Gastritis*			
Without atrophy	27 (54)	12 (70.6)	20 (60.6)
Atrophy	4 (8)	1 (5.8)	7 (21.2)
<i>H. pylori</i> -positive	25 (50)	8 (47)	22 (66.6)
Metabolic syndrome ^a	21 (41)	0 (0)	0 (0)
Total	50	17	13
Mean age (years)	35.4	47.8	41.7

*P = 0.36.
^aTriglycerides ≥ 150 mg/dl; high-density lipoprotein < 40 or 50; systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg; FPG ≥ 100 mg/dl (5.6 mmol/l) or type 2 diabetes mellitus.

Figure 1 Ghrelin cell densities in oxyntic mucosa (A) and in antral mucosa (B) of morbidly obese patients (BMI > 40) with and without metabolic syndrome (MS).

The χ^2 and Fisher tests were used for categorical comparisons and the Student's *t*-test was used to compare means among groups. The Kruskal–Wallis or Mann–Whitney *U* test was used to compare continuous versus categorical variables. A *P* value <0.05 was considered to be statistically significant.

Results

The frequency of chronic gastritis and the prevalence of *H. pylori* infection were similar in morbidly obese patients and in non-obese dyspeptic patients. In addition, mild atrophy of the gastric mucosa was observed in only four (8%) obese patients, in one (5.8%) overweight patient, and in seven (21.2%) lean patients (Table 1).

Ghrelin-immunoreactive cell density was lower in the oxyntic mucosa of morbidly obese patients with MS than in morbidly obese patients without this comorbidity ($P=0.04$). In the antral mucosa, the density of ghrelin-immunoreactive cells was similar in both morbidly obese patient groups ($P=0.84$; Fig. 1).

Ghrelin-immunoreactive cell density in the oxyntic mucosa was similar in morbidly obese patients with diabetes mellitus type 2 or MS and in overweight and lean patients. However, the density of ghrelin cells was higher in the oxyntic mucosa of morbidly obese patients without diabetes mellitus type 2 or MS compared with the two groups of non-obese patients. In the antral mucosa, the density of ghrelin-immunoreactive cells was higher in morbidly obese patients with or without diabetes type 2 or MS compared with the groups of overweight and lean subjects (Fig. 2).

The density of ghrelin-immunoreactive cells was higher in the oxyntic mucosa of *H. pylori*-negative patients than in *H. pylori*-positive patients of both the morbidly obese and the non-obese groups (Table 2). In the antral mucosa of lean patients, the density of ghrelin-immunoreactive cells was higher in *H. pylori*-negative subjects, whereas in the obese and overweight groups, there were no differences between *H. pylori*-negative and *H. pylori*-positive patients (Table 3).

As expected, almost all ghrelin-immunoreactive cells were seen in the oxyntic mucosa, whereas they were very few and scattered in the antral mucosa. Few small

foci of immunoreactive cells were observed in the antral mucosa of ten (20%) morbidly obese patients and in just one (5.8%) overweight patient, and no such small groups of cells were observed in lean patients.

Discussion

The 28 amino acid peptide ghrelin was identified by Kojima *et al.* (1) and soon found to be present in highest concentrations in tissues in the oxyntic gastric mucosa (2, 3, 30). In this region, the ghrelin-producing cells are located throughout the entire thickness of the mucosa and correlate topographically with the parietal cells (31).

The association between ghrelin and obesity started to emerge from experimental studies that showed an increase in appetite and adiposity after the administration of this peptide (32–34). In healthy subjects, the serum levels of ghrelin were demonstrated to be increased during periods of fasting and decreased after feeding, whereas in obese patients, the serum ghrelin levels usually did not present this behavior (21). Obese patients with Prader–Willi syndrome present increased plasma ghrelin concentrations and also have an increased density of ghrelin-producing cells in the gastric mucosa (23–26). Plasma ghrelin levels in children with Prader–Willi syndrome are elevated at any age, including during the first years of life and seem to precede the development of obesity (35). Thus, results of studies on the endocrine cell distribution in the gastric mucosa of obese patients may contribute to a better understanding of the role of this hormone regarding the mechanisms involved in the physiopathology of obesity.

Figure 2 Ghrelin cell densities in oxyntic (A and B) and antral mucosa (C and D) of morbidly obese patients (BMI > 40) with (A and C) and without (B and D) metabolic syndrome (MS), overweight (25 < BMI < 30), and lean patients (BMI < 25).

Table 2 Mean density of ghrelin-producing cells (cells/mm²) in oxyntic mucosa and *H. pylori* infection in morbidly obese (BMI > 40), overweight (25 < BMI < 30), and lean patients (BMI < 25).

<i>H. pylori</i> infection	Obese		Overweight		Lean	
	<i>n</i>	Density	<i>n</i>	Density	<i>n</i>	Density
Positive	25	87.1 ± 30.1*	8	71.8 ± 27.8†	22	70.74 ± 37.3‡
Negative	25	108.5 ± 34.2*	9	141.8 ± 50.8†	11	96.70 ± 27.2‡
Total	50		17		33	

*P = 0.026, †*P* = 0.0005, ‡*P* = 0.0001.

McLaughlin *et al.* (17) reported that mean plasma ghrelin concentration was lower in obese insulin-resistant than in obese insulin-sensitive patients. Recently, Tong *et al.* (18) found that ghrelin infusion did not alter fasting insulin or glucose concentration but significantly decreased the acute insulin response to i.v. glucose, and a similar suppression of C-peptide was observed. This study has shown that the distribution and density of ghrelin-producing cells in the oxyntic mucosa appears to be higher in morbidly obese patients without MS than in morbidly obese patients with this insulin resistance-associated condition, and also higher compared with two groups of dyspeptic non-obese patients, i.e. overweight and lean patients. The difference between density of ghrelin-producing cells between morbidly obese patients with and without MS may indicate that some factor in obese patients with MS would be related to activity of the ghrelin-producing cells, also reducing its expression. This finding certainly deserves further investigation.

It has been shown that plasma ghrelin levels are lower in obese than in non-obese patients (19, 20). As expected, this apparently low secretion of ghrelin could be due to a decreased number of gastric endocrine cells, a fact that did not seem to occur in the morbidly obese subjects studied here. The results obtained in the various studies are apparently conflicting. However, the fact that a larger number of ghrelin-immunoreactive cells were detected in obese patients does not necessarily mean that the number of these cells was increasing. Among other possibilities, ghrelin-producing endocrine cells may accumulate more peptide due to some interference with the mechanisms of secretion, becoming hypertrophic in obese patients and thus being more easily detected by immunohistochemical analysis. We have previously demonstrated

that hypertrophic neurons of the intramural plexuses of the intestine, when examined in histological sections, may simulate an increase in their numbers (36). Therefore, other factors could be responsible for the lower levels of serum ghrelin observed in obese patients, such as the presence of *H. pylori* infection, the most frequent worldwide pathogen affecting the gastric mucosa (7–10). Several gastric hormones have been reported to be affected by this microorganism, such as gastrin and somatostatin (4–6). Would ghrelin-producing cells also be affected by this infection? Interestingly, it has been reported that an increase in body weight was noted after eradication of this microorganism and that *H. pylori*-positive children had a higher incidence of growth retardation (37, 38). Furthermore, morbid obesity and *H. pylori* infection seem to have an inverse relationship, leading to the hypothesis that the absence of *H. pylori* infection during childhood may enhance the risk of developing morbid obesity (39). Nwokolo *et al.* (9) reported increased plasma concentrations of ghrelin after eradication of *H. pylori* in lean, asymptomatic individuals. It has been reported that the number of ghrelin-producing cells is significantly lower in patients with *H. pylori* infection than in subjects without *H. pylori* infection, and the same difference was observed in the plasma concentrations of this peptide (7, 8, 10, 11, 13). The results of this study are consistent with these findings. Therefore, *H. pylori* infection may affect the number of ghrelin-producing cells in the oxyntic mucosa. It is not known whether this may be the consequence of a direct influence of the microorganism or of factors related to the inflammatory reaction triggered by it. Gastric mucosa atrophy could explain a smaller number of a specific cell type in the gastric mucosa of *H. pylori*-infected patients. However, gastric mucosa atrophy was rarely observed in the present patient study. In addition, patients with atrophic body gastritis suggestive of autoimmune etiology were excluded from this study. Despite the rarity of ghrelin cells in antral mucosa, the presence of scattered foci of these cells in this region seems to be responsible for the wide dispersion of these cell counts, as shown in Figs 1B, 2C and D. Could these small foci of ghrelin cells play some role in obesity? It may be hypothesized that ghrelin could activate the hypothalamic hunger center via the afferent vagus nerve, independent of plasma ghrelin levels. The detection of ghrelin receptors on

Table 3 Mean density of ghrelin-producing cells (cells/mm²) in antral mucosa and *H. pylori* infection in morbidly obese (BMI > 40), overweight (25 < BMI < 30), and lean patients (BMI < 25).

<i>H. pylori</i> infection	Obese			Overweight			Lean		
	<i>n</i>	Mean	Median	<i>n</i>	Mean	Median	<i>n</i>	Mean	Median
Positive	25	50.9 ± 93.5	9.8*	8	4.5 ± 9.21	0.00†	22	7.5 ± 20.9	0.0‡
Negative	25	39.4 ± 108.9	7.8*	9	43.3 ± 93.0	6.66†	11	11.9 ± 18.1	4.4‡
Total	50			17			33		

*P = 0.37, †*P* = 0.10, ‡*P* = 0.04.

vagal afferent neurons in the rat nodose ganglion suggests that ghrelin signals from the stomach are transmitted to the brain via the vagus nerve and not only via the plasma levels that cross the blood–brain barrier (40). Some studies have shown that vagotomy inhibits the ability of ghrelin to stimulate food intake and GH release (41, 42).

In conclusion, the finding of a higher density of ghrelin-immunoreactive cells in the oxyntic mucosa of morbidly obese patients without diabetes mellitus type 2 or MS compared to lean subjects and also the finding of a higher number of small foci of ghrelin cells in the antral mucosa of obese patients may indicate an adaptive mechanism or an individual factor to be considered in the pathogenesis of obesity. We think that the present data could provide novel insights for understanding the role of gastric ghrelin and its relationship to obesity and insulin resistance.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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