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Ghrelin-producing cells distribution in the stomach and the relation with *Helicobacter pylori* in obese patients

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Abstract

Objective: Ghrelin is believed to influence weight evolution after bariatric surgery. *Helicobacter pylori* (*H. pylori*) infection may influence ghrelin plasma levels by affecting the ghrelin-producing cells (GPC) in the stomach. The purpose of the study was to characterize the GPC distribution in the stomach in overweight patients and the influence of *H. pylori* infection on them. *Patients, Materials and Methods*: The study group included 21 obese patients undergoing bariatric surgery with ghrelin levels and anti-*H. pylori* antibodies previously measured, and upper gastrointestinal endoscopy with histological evaluation of *H. pylori* infection performed. Immunohistochemical expression of ghrelin was quantified in gastric resection specimens. *Results*: The results showed a higher number of GPC in the obese women than in men ($p>0.05$). The highest number of GPC was detected in the gastric body, followed by the fundus and antral region ($p<0.001$). GPC number correlated inversely with anthropometric parameters: weight ($p=0.011$), body mass index (BMI) ($p=0.017$), waist circumference (WC) ($p=0.066$) was lower in patients with *H. pylori* infection ($p>0.05$) or gastritis ($p>0.05$), the number decreasing with the increase in depth of gastritis lesion ($p>0.05$). *Conclusions*: The present study fulfills the characterization of GPC in obese patients, showing a higher number in women than in men, their predominant location in the gastric body, and their relationship with the anthropometric parameters (weight, BMI, WC), *H. pylori* infection and gastritis lesions. These results open broad perspectives for a deeper understanding of the ghrelin involvement in the obesity pathogenic mechanism, associated or not with other gastric conditions.

Keywords: ghrelin-producing cells, gastritis, *Helicobacter pylori*, immunohistochemistry, obesity.

Introduction

Obesity is a chronic condition that according to *World Health Organization* (*WHO*) statistics has become an epidemic [1], and the importance of this pathology derives from the life-threatening cardiometabolic consequences of excessive fat accumulation [2].

Currently, the metabolic surgery is the only therapeutic method effective in achieving weight control, determining significant and sustained weight loss [3]. The beneficial metabolic effects occur well before weight loss is observable, which prompted the introduction of this method in the international guidelines for the treatment of diabetes mellitus [4, 5].

The mechanisms by which metabolic surgery improves the metabolic abnormalities are not fully understood. Numerous hypotheses have been suggested: from the restrictive mechanism determined by the reduced capacity of the digestive tract and absorption surface, changes in digestive secretions, to changes in gut microbiota and stomach-gut-brain axis influencing food intake and energy expenditure [6–8]. Worldwide, the most commonly used surgical method seems to be laparoscopic sleeve gastrectomy [9], either as a sole surgical procedure or as an intermediate step in extreme obesity. Sleeve gastrectomy is a procedure by which a large portion of the stomach is removed, including the resection of a portion of the antrum, most of the corpus and all of the fundus [10], so that the resected stomach specimen is suitable for the research of peptides secreted at this level, peptides involved in the regulation of energy balance.

Ghrelin is a peptide firstly isolated by Kojima *et al.* [11], its expression is mainly located in the gastric cells distinct from other peptide-secreting cell types [12]. Ghrelin-immunopositive cells are found throughout the length of the gastrointestinal tract and their number gradually decreases from the stomach to the duodenum, ileum, cecum and colon [13], ghrelin being the peripherally-secreted hormone that acts centrally to increase appetite [11]. To become active, ghrelin requires acylation under the action of ghrelin O-acyltransferase (GOAT) enzyme [14], the substrate for acylation being the dietary medium-chain fatty acids [15]. Regarding the relationship between serum ghrelin level, number of ghrelin-producing cells (GPC) and weight status, a study conducted on a group of patients with Prader–Willi syndrome showed that plasma ghrelin level and density of GPC are 2–3 times higher in these patients compared to normal weight individuals, even at young ages [16].

The majority of circulating ghrelin is produced in the gastric mucosa [17] and, therefore, persistent, chronic gastric mucosal lesions (such as chronic gastritis) could influence ghrelin production, with possible consequences on food intake and body weight change. *Helicobacter pylori* (*H. pylori*) is a Gram-negative bacterium which is involved in the pathogenesis of gastritis, gastric and duodenal ulcer, gastric carcinoma and lymphoma of mucosa-associated lymphoid tissue (MALT) type [18]. Ghrelin production, its acylation and/or secretion may be compromised by the presence of chronic gastritis and atrophy [19], the consequences being changes in appetite and weight. *H. pylori* infection can cause endocrine cell destruction by inflammation and atrophy. In addition, *H. pylori* infection is accompanied by malabsorption (secondary to hypochlorhydria, vomiting, dyspepsia, increased susceptibility to other enteric pathogens), which gave rise to speculation that altered food dietary intake or even dysregulated absorption of medium-chain fatty acid substrate for GOAT enzyme could influence the acylated/ total ghrelin ratio during *H. pylori* infection [20].

Controversial data exist in the literature regarding the relationship between plasma ghrelin level and number of GPC and *H. pylori* infection, both in normal-weight and obese individuals. *H. pylori* infection may influence weight status and metabolic profile of patients, and treatment of *H. pylori* infection would lead to a subsequent change in the level of appetite-regulating peptides, such as ghrelin.

The aim of this study was to analyze the number and distribution of GPC in the stomach and the plasma acyl ghrelin level, and the relationship with the *H. pylori* infection in a group of obese patients who underwent laparoscopic sleeve gastrectomy.

Patients, Materials and Methods

Patients

The study group included 21 patients with obesity (16 women) who underwent metabolic surgery (September 2014–November 2015) at the Center for Obesity and Bariatric Surgery, "St. Spiridon" Clinical Emergency Hospital, Iaşi, Romania. The Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, granted approval for the research and patients gave their consent to participate. Patients included in the study met the current metabolic surgery eligibility criteria [21] and were followed up using the same algorithm for the complex preoperative multidisciplinary assessment. Patients were submitted to an upper digestive endoscopy (UDE), performed by experienced gastroenterologists who evaluated the gastric mucosa appearance and harvested biopsies for the morphological assessment. Patients with histological evidence of *H. pylori* infection received the same eradication treatment regime (the triple therapy) and surgery was performed only after completion of the treatment. All patients underwent laparoscopic sleeve gastrectomy including the greater curvature of fundus and stomach body.

Patient's characteristics (gender, age, weight, and the anthropometric parameters – body mass index (BMI) and waist circumference (WC) assessed according to *WHO* recommendations [22]) are shown in Table 1.

Table 1 – ***General characteristics of the study group***

Characteristics	Unit	Total	Women (16; 76.2%)	Men (5; 23.8%)
Age [years]	mean± SD	43.9± 12.522	43.63± 12.612	44.8± 13.646
	min.; max.	18; 61	18; 58	25; 61
Weight [kg]	mean± SD	120.5± 21.74	112.9± 16	144.7± 21.11
	min.; max.	90; 175	90; 145	117; 175
BMI [kg/m^2]	mean± SD	43.24± 5.51	42.77± 6.03	44.74± 3.46
	min.; max.	35.05; 53.911	35.05; 53.91	40.48; 48.47
WC [cm]	mean± SD	126.35± 13.55	123± 13.15	136.4± 9.91
	min.; max.	104; 147	104; 144	122; 147

BMI: Body mass index; WC: Waist circumference; SD: Standard deviation.

Histopathological methods

All endobiopsies were routinely processed by fixation in 10% neutral buffered formalin, pH 6, for six hours and paraffin embedding. All fresh surgical specimens were measured and the gross appearance was described. Fixation was done for 18–24 hours in 10% neutral buffered formalin, pH 6. From each gastric sample, three fragments were selected from the proximal, middle and distal sites. They were routinely processed by paraffin embedding, using a Leica ASP200 tissue processor. Five μm thin sections were cut using a Leica RM2135 manual rotary microtome and stained with Hematoxylin–Eosin (HE) and Giemsa.

Microscopic examination and image acquisition were done using a Leica DM 3000 motorized light microscope with Leica camera and Leica Application Suite (LAS) image acquisition software. For the classification and grading of gastritis, we used the updated Sydney System [23].

Immunohistochemical methods

Representative tissue sections placed on special slides coated with poly-L-lysine were dewaxed, hydrated, and then immersed in Target Retrieval Solution pH 6 (code S1700, DAKO, Denmark), at 96^0C, for 25 minutes, to unmask the antigen [heat-induced epitope retrieval technique (HIER)]. Tissue sections were incubated overnight with the primary anti-ghrelin monoclonal antibody (mouse anti-human, 1:150, IgG2a, ab57222, Abcam, Cambridge, MA, USA), at 4^0C. The detection of the immunoreaction was performed using NovoLink™ Polymer Detection System (Leica Biosystems, Germany). Sections were developed using 3,3'-Diaminobenzidine tetrahydrochloride chromogen (DAB, code K5001, DAKO, Denmark) and counterstained with Lille's Hematoxylin.

Histopathological and immunohistochemical assessments

The length and width of the resected gastric specimen were measured and based on them its volume was calculated, and then each specimen was subdivided into three regions: proximal (fundus), medium (corpus) and distal (antral region).

The histological examination of the gastric samples

included the classification and grading of the inflammation (resulting in three categories: normal, superficial chronic gastritis and profound chronic gastritis), and assessment of *H. pylori* infection presence [24].

For each region of the resected specimen, five high power fields (HPFs), randomly chosen, were evaluated by counting the number of GPC with cytoplasmic ghrelin-positive immunoexpression.

Immunological methods

Serological diagnosis of H. pylori infection

The serological diagnosis of *H. pylori* infection was based on the detection of anti-*H. pylori* immunoglobulin G (IgG) antibodies, in blood samples of each patient. Titers ≥1.1 U/mL were considered to be "positive", titers <0.9 U/mL were considered as "negative" and titers values ≥0.9 U/mL and <1.1 U/mL were considered to be "indeterminate" and were subject to retesting. Negative results do not preclude recent primary infection.

The dosage of plasma ghrelin

Acyl ghrelin level was preoperatively determined in all patients. Plasmatic acylated ghrelin was quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits (BioVendor Laboratory, USA) based on a double-antibody sandwich technique.

Statistical analysis

Data were analyzed using Microsoft Office Excel and Statistical Package for the Social Sciences (SPSS) version 17.0. Numerical data were expressed as means and standard deviation (SD), minimum and maximum. Significant differences between numerical data were found using Student's *t*-test. For the description of categorical variables, we used frequencies and percentages and the significant differences were assessed using the *chi*-square (χ^2) test (or Fisher test for small samples) with a significance value of $p<0.05$.

Results

The macroscopic aspects observed during the UDE were divided into four categories: normal, congestion, gastritis, and other lesions (granular aspect, hypertrophic folds, and biliary reflux).

Histopathological examination of the gastric resection specimens showed the presence of gastritis lesions in 14 (66.7%) patients, in 10 patients the lesions being superficial, and normal gastric mucosa in seven (33.3%) patients. In the entire study group, the *H. pylori* infection was identified only in two (9.5%) patients. Statistical analysis between the anthropometric parameters and size of the resected gastric specimen revealed a positive correlation between patient height and specimen length (p=0.023) and specimen volume (p=0.051), and between specimen width and patient weight (p=0.042) and WC (p=0.023).

GPC were mainly identified in the basal half of the gastric mucosa. They were present in all cases, both in those with normal aspect (Figures 1 and 2) as in those with chronic gastritis, superficial (Figure 3) or profound (Figure 4).

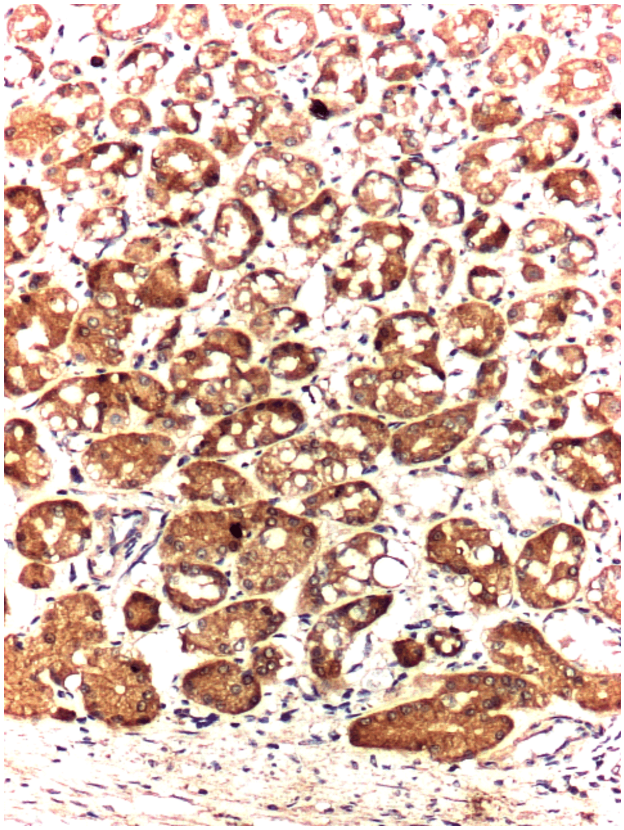

Figure 1 – ***Normal gastric mucosa, fundic region, with low number (2) of GPC (Anti-ghrelin antibody immunomarking, ×100). GPC: Ghrelin-producing cells.***

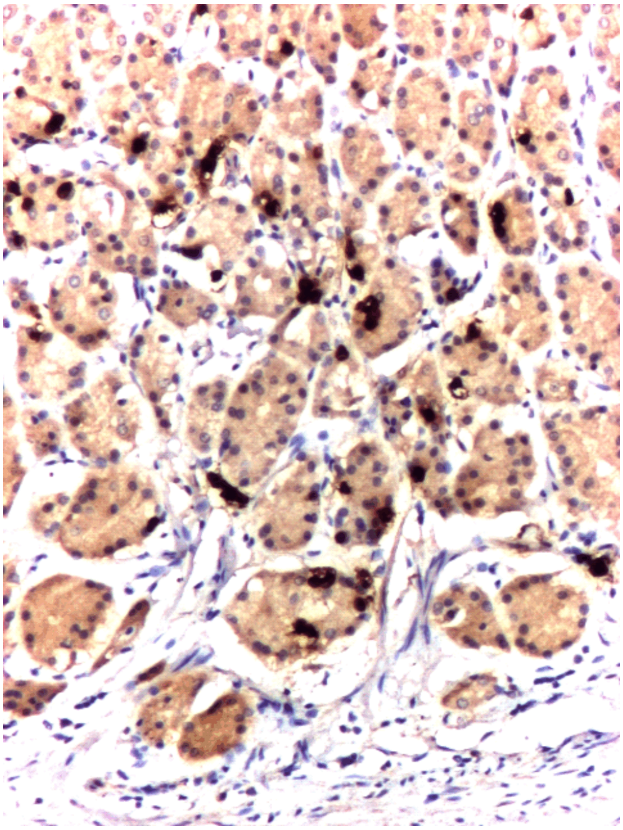

Figure 2 – ***Normal gastric mucosa, fundic region, with high number (37) of GPC (Anti-ghrelin antibody immunomarking, ×100). GPC: Ghrelin-producing cells.***

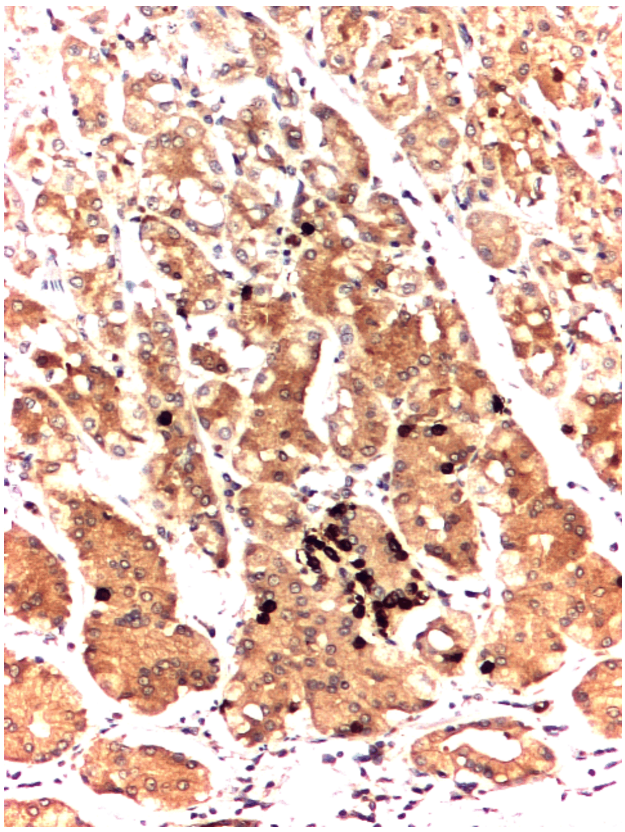

Figure 3 – ***Superficial chronic gastritis, fundic region, with high number (28) of GPC (Anti-ghrelin antibody immunomarking, ×100). GPC: Ghrelin-producing cells.***

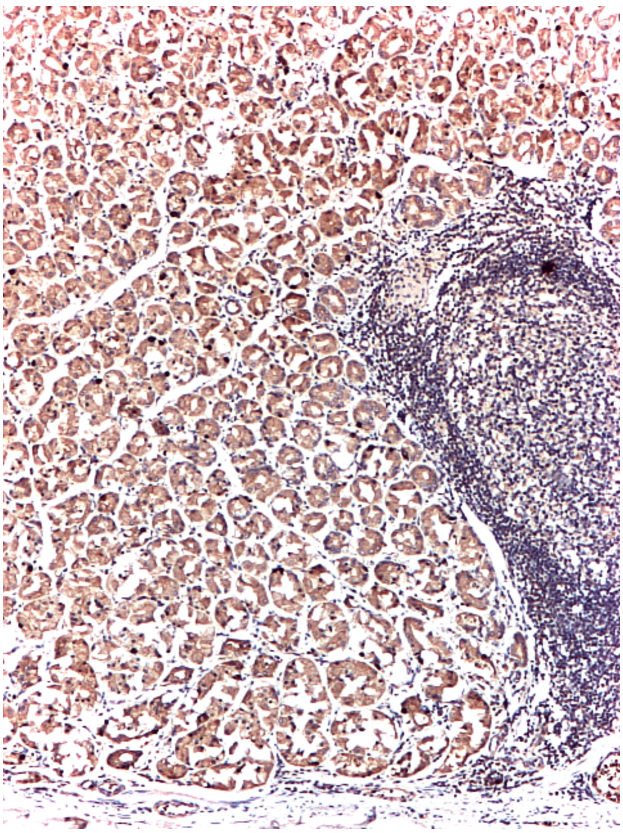

Figure 4 – ***Profound chronic follicular gastritis, body region, with moderate number (12) of GPC (Anti-ghrelin antibody immunomarking, ×40). GPC: Ghrelin-producing cells.***

The total mean number of GPC in the gastric resection specimen of the study group was 15.06±5.97, the highest number of cells being found in the gastric body (16.6± 7.2), followed by fundus (14.85±7.02) and antral region (13.75±8.12) – the differences being statistically significant (p<0.001) (Table 2). The total number of GPC was higher in women compared to men, but the differences both in total number, and by each region (fundus, body and antral region) were not statistically significant (Table 2).

Table 2 – ***The number of GPC according to patients' gender and gastric region***

Characteristics	Unit	Total	Women (16; 76.2%)	Men (5; 23.8%)	p*
GPC average no. in fundus	mean±	14.85±	16.05±	11.04±	<0.001
	SD	7.02	7.49	3.47	
	min.;	4.4;	4.4;	5.6;	
	max.	31.2	31.2	15.2	
GPC average no. in body	mean±	16.6±	17.92±	12.36±	<0.001
	SD	7.2	7.2	5.9	
	min.;	3.6;	6;	3.6;	
	max.	28.2	28.2	18.4	
GPC average no. in antrum region	mean±	13.75±	13.83±	13.48±	<0.001
	SD	8.12	7.17	11.7	
	min.;	3.8;	3.8;	4.6;	
	max.	33.6	28.8	33.6	
Total average GPC	mean±	15.06±	15.93±	12.29±	<0.001
	SD	5.97	5.77	6.4	
	min.;	4.6;	6;	4.6;	
	max.	23.26	23.26	21.93	

GPC: Ghrelin-producing cells; SD: Standard deviation; *Comparison between regions.

The number of GPC in the gastric body correlated inversely with weight (p=0.011) (Figure 5), BMI (p=0.017) (Figure 6) and WC (p=0.066) (Figure 7).

The anti-*H. pylori* antibody was detected in 14 (66.7%) patients and these patients had a smaller number of GPC compared to patients with *H. pylori* absent (14.78±6.27 *vs.* 15.64±5.76, p>0.05).

The number of GPC was lower in the patients with *H. pylori* infection and in those with gastritis lesions (14.928±6.38 *vs.* 15.35±5.53), decreasing with the increase in depth of gastritis lesion (p>0.05).

In the study group the mean plasma acyl ghrelin level was 14.95 pg/mL [95% confidence interval (CI) 4.88–25.02], a slightly lower level being found in women compared to men – 14.37 pg/mL (95% CI 2.77–25.96) and 16.82 pg/mL (95% CI 15.24–48.88), respectively, without significant correlation between gender (p>0.05). No significant correlations were registered between the acyl ghrelin level and anthropometric parameters, or GPC, except for patients' age (r^2=-0.437, p=0.048).

Discussions

Numerous research teams showed interest in GPC immediately after its isolation and description by Kojima *et al.*, in 1999 [11], and a year later it has already been demonstrated that the expression of ghrelin is located mostly within certain gastric cells distinct from other peptide-secreting cell types [12]. In the following years, several studies have attempted to characterize these cells by various methods, both in laboratory animals [13, 25, 26] and human subjects [27].

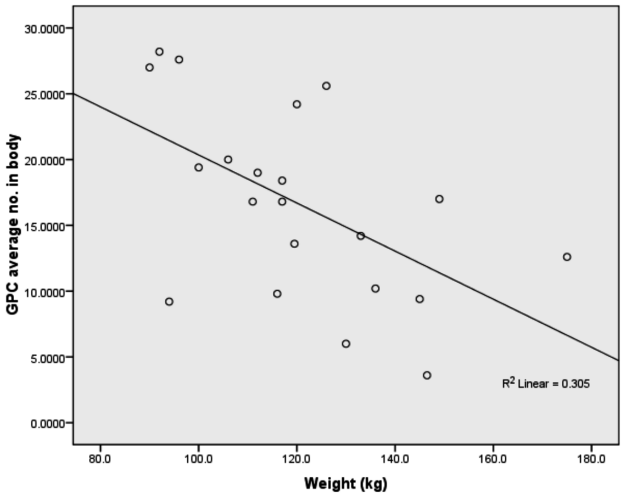

Figure 5 – ***Correlation of ghrelin-producing cells (GPC) number with weight (p=0.011).***

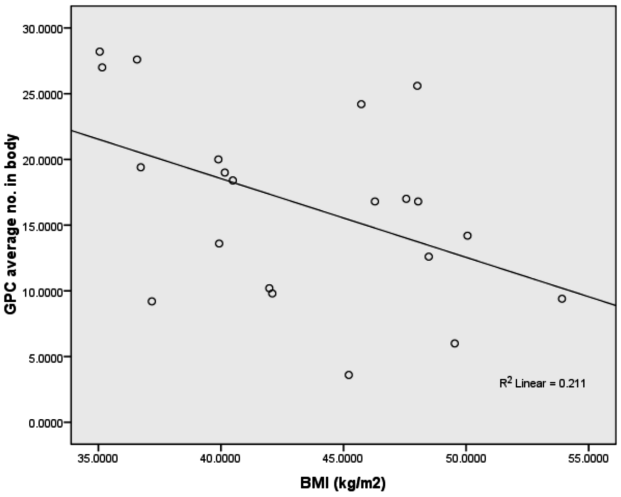

Figure 6 – ***Correlation of ghrelin-producing cells (GPC) number with body mass index (BMI) (p=0.017).***

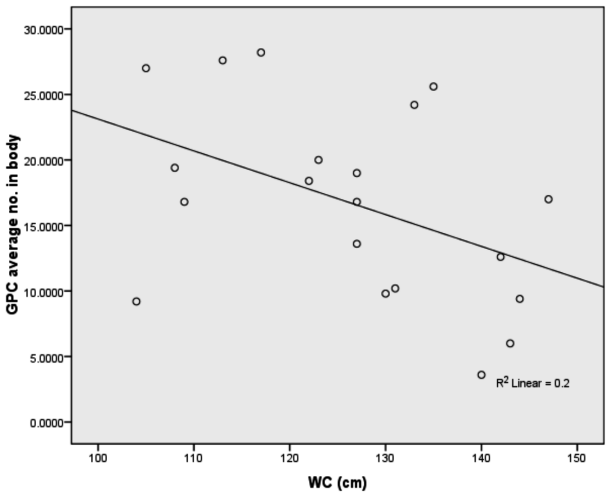

Figure 7 – ***Correlation of ghrelin-producing cells (GPC) number with waist circumference (WC) (p=0.066).***

The distribution of GPC is still debatable. The highest number of GPC was reported in the upper part of the stomach [28, 29], fundus [30], or gastric body [31]. In our study on obese patients, the main location of GPC was in the gastric body, with statistically significant differences between the number of the cells present in this site and fundus and antral region, respectively. Relatively few data are available in the literature on the number of GPC in the stomach of obese patients. A study published in 2009 including 18 morbidly obese patients who underwent metabolic surgery showed hypoactivity of gastric X/A-like cells in the obese group compared to controls [32], the interpretation of these results suggesting the existence of an adaptive mechanism in obese people, which decreases ghrelin levels and thus food intake in order to restore the body's energy balance. In 2012, Goitein *et al.* published the results of a study on plasma ghrelin level, distribution of ghrelin messenger ribonucleic acid (mRNA) expression by reverse transcription polymerase chain reaction (RT-PCR) and distribution of GPC by immunohistochemistry in resected stomach specimens from 20 morbidly obese patients who underwent sleeve gastrectomy [33]. The results showed that the expression of ghrelin mRNA decrease gradually from the fundus towards the preantral region. The prevalence of GPC shared the same pattern, with the highest percentage of positive ghrelin cells in the fundus, intermediate in the body, and the lowest in the preantral region (p=0.08) [33]. Similar results were also obtained by Abdemur *et al.*, who reported a higher number of GPC in the gastric fundus compared to the body and antral region in obese patients who underwent metabolic surgery [34]. Our results complete the ongoing profile of GPC in obesity, while we demonstrated an inverse correlation between their number and the anthropometric parameters (namely weight, BMI and WC).

Our results also show gender differences in the number of GPC, data in agreement with those obtained by a group of Turkish authors who showed that the number of ghrelin-immunopositive cells was higher in women (p=0.007) [35]. The authors showed an increased number in the fundus-proximal body *vs.* distal body (p=0.0001) [35], consistent with our findings.

Surprisingly, the results of the plasma acyl ghrelin analysis were not concordant with those obtained through the immunohistochemical assessment of GPC; we cannot demonstrate correlations between the plasma acyl ghrelin level, the patients' gender or the specific parameters for obesity.

Data on the relationship between *H. pylori* infection and plasma ghrelin level are controversial. Thus, some authors reported that *H. pylori* infection is associated with a significant decline in plasma ghrelin level, and plasma ghrelin level increases after *H. pylori* eradication [18, 36–38], while other studies show that plasma ghrelin level is higher in patients with *H. pylori* infection [39] or ulcers [40] and the eradication of *H. pylori* infection would be associated with reduced plasma ghrelin levels [41, 42], or even that there is no relationship between *H. pylori* infection and ghrelin level [43, 44]. These contradicting results may be explained by the differences in study population cohorts, inclusion criteria, methods of *H. pylori* infection diagnosis, etc. as well as by the fact that most studies measured total ghrelin levels rather than active acyl ghrelin. The study by Campana *et al.*, published in 2007, showed that plasma acyl ghrelin level is higher in patients with chronic atrophic gastritis [45]. This opposite dynamic of acyl ghrelin compared to plasma total ghrelin level could be a compensatory increase in plasma active ghrelin level in response to gastric atrophy [37]. Moreover, a recent

study showing that plasma acyl ghrelin levels have increased one year after *H. pylori* eradication and the expression of gastric pre-proghrelin mRNA was upregulated [46].

Moreover, the literature focusing on the relationship between *H. pylori* infection and the number of GPC in the stomach is rather scarce. The published studies suggest that *H. pylori* infection causes a decrease in the number of ghrelin-immunoreactive cells in the oxyntic mucosa both in normal weight [18, 47] and obese patients [48–50], and the number of GPC and plasma ghrelin levels increase significantly after *H. pylori* infection eradication [51, 52], thus confirming the hypothesis according to which the reduced number of GPC in the gastric mucosa due to *H. pylori* infection causes a decrease in plasma ghrelin level [37].

Our results are consistent with the findings reported in the literature, and show that the number of GPC is lower in *H. pylori*-infected individuals and in those with gastritis lesions, the number of cells being lower the greater the depth of gastritis. Also, our results are similar with those reported by Maksud *et al.*, in that the density of ghrelin-immunoreactive cells was higher in the oxyntic mucosa of *H. pylori*-negative compared with *H. pylori*-positive patients, those with morbid obesity included [53]. The same relationship between *H. pylori* infection and the number of GPC has been reported by Isomoto *et al.* [38], in this case the decrease in the number of GPC being more significant in patients with severe glandular atrophy and intestinal metaplasia of the gastric body mucosa.

Conclusions

The present study fulfills the characterization of GPC in obese patients, showing a higher number in women than in men, their predominant location in the gastric body, and their relationship with the anthropometric parameters (weight, BMI, WC), *H. pylori* infection and gastritis lesions. These results open broad perspectives for a deeper understanding of the ghrelin involvement in the obesity pathogenic mechanism, associated or not with other gastric conditions.

Conflict of interests

The authors declare no conflict of interests.

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