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ORIGINAL CONTRIBUTIONS

Analysis of the Immunohistochemical Expression of Ghrelin in the Gastric Mucosa and Correlation with Weight Loss After Sleeve Gastrectomy

Reynaldo Martins e Quinino[1,2] · André Luis Costa Barbosa[3] · Maíra de Araújo Barros Xavier[4] · Romero de Lima França[4] · Mirella Patrícia Cruz de Freitas[4] · Alberto Goldenberg[5]

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Abstract

Introduction Sleeve gastrectomy is one of the main techniques used to treat severe obesity. The study of the immunohistochemical expression of ghrelin in the gastric mucosa has already been related to weight loss and can be a promising method to predict the surgical outcome.

Purpose To analyze the immunohistochemical expression of ghrelin in the gastric mucosa and its correlation with weight loss, comorbidities, and inflammatory changes after sleeve gastrectomy.

Methods Thirty-five patients submitted to sleeve gastrectomy were evaluated, 29 of whom were female (82.9%), with a mean age of 35.2 years and an average body mass index of 38.1 kg/m^2. Endoscopic samples of the mucosa were collected, whose ghrelin expression was evaluated in a semi-quantitative way through the stained antibody area. These data were correlated with weight loss at 3, 6, and 12 months and with the control of comorbidities, and inflammatory alterations.

Results The average total weight loss (TWL%) was 17.7, 26.4, and 32.1%, respectively, at 3, 6, and 12 months. A negative correlation was found between the immunohistochemical expression of ghrelin in the endoscopic biopsy of the fundus and weight loss at 3 ($s = -0.536$; $p = 0.001$) and 6 months ($s = -0.339$; $p = 0.047$).

Conclusion The immunohistochemical expression of ghrelin in the mucosa of the gastric fundus was negatively correlated with early weight loss after sleeve gastrectomy.

Keyword Bariatric Surgery · Ghrelin · Immunochemistry

Introduction

According to the World Health Organization, in 2015, there were 2.3 billion overweight adults in the world, and 700 million considered adults with obesity.

The sleeve gastrectomy (SG) was initially conceived and first described by Hess and Marceau in 1988, and, in 1999, it was performed as a single first stage procedure in high-risk

Key Points
- The expression of ghrelin may help to predict the outcome of sleeve gastrectomy.
- The result of antral resection may be influenced by the production of ghrelin.
- The gastric fundus is the ideal place to study the expression of ghrelin.

✉ Reynaldo Martins e Quinino
reynaldoquinino@yahoo.com.br

[1] Department of Integrated Medicine at the Universidade Federal Do Rio Grande Do Norte, Rua Raimundo Chaves 1652, Casa i 13 Natal, Candelária, RN 59064-390, Brazil

[2] Department of Digestive System Surgery at Hospital, Universitário Onofre Lopes, Av. Nilo Peçanha, 620 Natal, Petrópolis, RN 59012-300, Brazil

[3] Surgery Service for Obesity and Associated Diseases at Hospital, Universitário Onofre Lopes, Av. Nilo Peçanha, 620 Natal, Petrópolis, RN 59012-300, Brazil

[4] Hospital Universitário Onofre Lopes, Av. Nilo Peçanha, 620 Natal, Petrópolis, RN 59012-300, Brazil

[5] Department of Surgery, Escola Paulista de Medicina, Universidade Federal de São Paulo, R. Napoleão de Barros, 715 Vila Clementino, São Paulo, SP 04024-002, Brazil

patients [1, 2]. Despite the good results and its increasing use as a single procedure, there is no consensus as to which patients may have an unsatisfactory weight loss follow-up with sleeve gastrectomy [3].

Currently, it is known that sleeve gastrectomy leads to weight loss due to a combination of several factors, such as volume restriction, creation of a high-pressure system that favors gastric emptying, and induction of favorable hormonal changes [4, 5].

Among the main hormonal changes involved in the result of the SG is the suppression of ghrelin levels. The study of its immunohistochemical expression, as opposed to serum levels, seems to reflect the potential production capacity in response to weight loss, as already demonstrated in the literature, but with divergence [6, 7].

In addition, other factors have not been adequately studied, such as the correlation with antral resection [8] and the influence in inflammatory changes and infection by *Helicobacter pylori* (HP) [9, 10].

This work aims to correlate the immunohistochemical expression of ghrelin in the gastric mucosa with weight loss, comorbidities, and inflammatory changes after the SG.

Materials and Methods

The study included 40 patients with severe obesity who were candidates for surgical treatment through SG at Hospital Universitário Onofre Lopes, Universidade Federal do Rio Grande do Norte and Hospital Unimed from Natal, of both sexes, who agreed to participate in the study after signing the Free and Informed Consent Form (FICF). The study was approved by the ethics committee of the Universidade Federal de São Paulo (UNIFESP) under number: 1,844,885.

The inclusion criteria were BMI between 35 and 49.9 kg/m^2; age between 16 and 65 years; and availability for outpatient follow-up. We used as exclusion criteria: presence of Barrett's esophagus, insulin-dependent diabetes mellitus (DM), chronic use of immunosuppressants or corticosteroids having previously undergone gastric surgery or another bariatric procedure; occurrence of surgical complications such as fistulas and strictures; and Prader-Willi syndrome. During the initial consultation, the weight and presence of comorbidities were recorded. Four biopsy fragments of the gastric mucosa were randomly collected in preoperative endoscopy, two from the antrum and two from the fundus of the stomach.

The SG was performed according to the standardized technique in the service: the gastric resection starting 2–3 cm from the pylorus using a disposable endoscopic stapler (Victor Medical Instruments Co., Ltd.), and a 32F calibration Fouchet probe, the surgical specimen was removed without trauma by enlarging the umbilical incision.

Patients were evaluated on an outpatient basis when they completed 1, 3, 6, and 12 months of surgery. If the patient did not show up after three telephone contacts, data were collected through a telephone interview.

The surgical specimens were opened and two fragments of the mucosa were removed, the first 1 cm adjacent to the most proximal part of the stapling line (funds) and another 1 cm adjacent to the most distal part of the staple line (antrum). The specimens were analyzed by a single pathologist using conventional histological analysis in hematoxylin-eosin, staining by Giemsa to assess the presence of HP, and immunohistochemistry for ghrelin.

Immunohistochemical staining was performed using the monoclonal anti-ghrelin antibody produced in mice (monoclonal anti-GHRL antibody produced in mouse, clone 2F4, Sigma Aldrich, Germany). After being included in paraffin, the specimens were sectioned 3.5 µm thick, and then deparaffinized and dehydrated. The slides were then incubated with the described antibody. The sections were finally washed and contrasted with hematoxylin. Negative controls were treated identically but without the primary antibody (Fig. 1).

To evaluate ghrelin expression in the mucosa, two micrographs were performed randomly under a light microscope (Carl Zeiss AB, Primo Star, Sweden) coupled to a digital capture system (Motic, Moticam 205A, China) in 400× magnification. The analysis was performed according to the technique described by Ruifrok and Johnston [11] using the Color Deconvolution 2 [12] plugin installed in the public domain software ImageJ (National Institutes of Health, USA). The semi-quantification was calculated as described below: The total area of the tissue captured in the image was isolated and calculated to obtain the measurement by

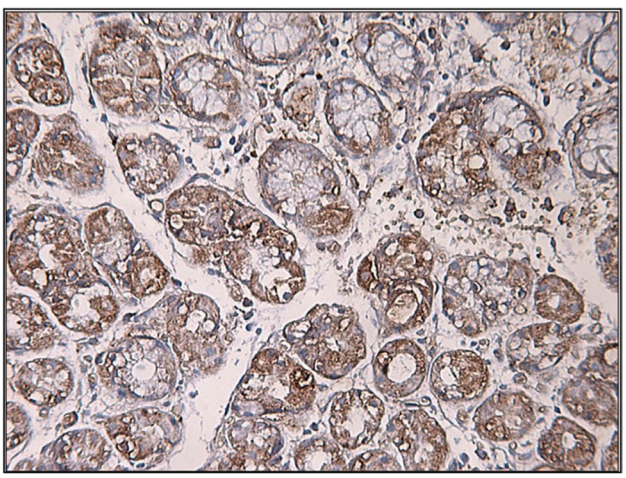


Fig. 1 Immunohistochemistry staining

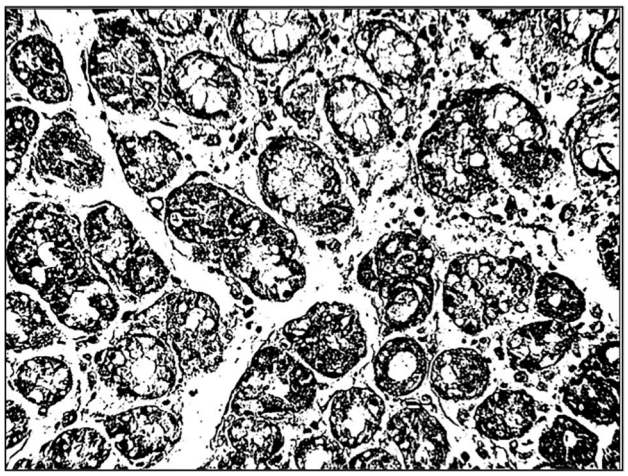


Fig. 2 Image with subtraction of the slide background

subtracting the slide background; subsequently, the area of tissue stained by the antibody was isolated from the application of the aforementioned plugin, the resulting brown positive sign had its area calculated. Finally, the result was expressed in the total stained area (μm^2) and relative percentage (%) of the area stained by the antibody by the total area of tissue captured in the image (Fig. 2).

The statistical analysis inferential was performed employed were *t*-Student for independent samples, Mann–Whitney, analysis of variance with a fixed factor, Kruskal–Wallis, Spearman's correlation coefficients, and intraclass. In all conclusions, an alpha significance level of 5% was applied. The analyses were performed using the IBM-SPSS Statistics version 24 statistical program.

Results

Of the 40 patients initially selected, two patients were excluded, one due to surgical complication (fistula) and another who received a diagnosis of systemic lupus erythematosus and starting a therapy with immunosuppressants and corticosteroids. Of the remaining patients, three did not complete the 1-year follow-up. The 35 remaining patients were followed for 12 months had a mean age of 35.2 years, ranging from 23 to 65 years (Table 1). Most of the 35 patients were female (82.9%) and without any associated surgery (94.3%). The average operative time was 48 min, ranging from 34 to 82 min, and the average hospital stay was 1.8 days, ranging from 0.9 to 3.4.

At the beginning of this follow-up, six patients had diabetes (17.1%); however, at 6 and 12 months, none of these patients remained with diabetes. Regarding systemic arterial hypertension, 11 (31.4%) patients were hypertensive; however, at 6 and 12 months, none of these patients remained hypertensive. Of the nine patients (25.7%) who had dyslipidemia at the beginning of the follow-up, only one (2.9%) remained diagnosed at 6 and 12 months. The average initial weight was 101.7 kg, whereas the average initial BMI was 38.1 kg/m^2. The mean BMI at 3, 6, and 12 months was 31.4 kg/m^2, 28.1 kg/m^2, and 25.9 kg/m^2, respectively, and the total weight loss (TWL%) was 17.7% in the third month, 26.4% at the sixth, and 32.1% at 12 months (Graph. 1).

Table 1 General characteristics of patients

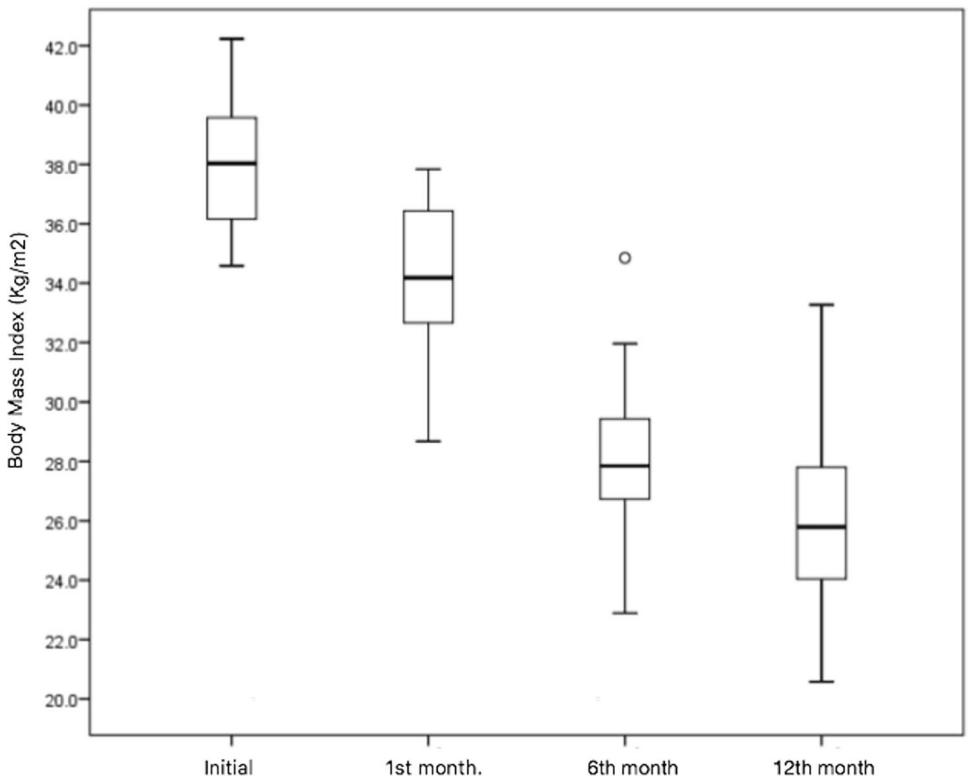
Age (years) ($n=35$)	Mean	35.2	
Gender ($n=35$)	Female	29	82.9%
	Male	6	17.1%
Associated surgeries ($n=35$)	Cholecystectomy	1	2.9%
	Hiatorraphy	1	2.9%
	None	33	94.3%
Operative time (min)	Mean	48	
Hospital stay (days)	Mean	1.8	

n = number of patients

The immunohistochemical expression of ghrelin in the gastric mucosa measured is summarized in Table 2. The mean area stained by ghrelin and percentage in the endoscopic biopsy was, respectively, 4.8 μm^2 and 47.9% in the fundus and 4.1 μm^2 and 41% in the antrum, whereas, in the surgical specimen, the result found was 5.44 μm^2 and 54.5% in the fundus and 5.57 μm^2 and 55.7% in the antrum.

The relationship between ghrelin immunohistochemistry and weight loss in the 3rd, 6th, and 12th months was performed using Spearman's correlation coefficient (s). In Table 3, we observed that the greater the weight loss, the lower the immunohistochemical expression in the endoscopic biopsy of the fundus in the 3rd month ($s=-0.536$; $p=0.001$) and the 6th month ($s=-0.339$; $p=0.047$) (Graph. 2 and 3).

The presence of mild gastritis was found in 12 patients (34.3%) and moderate in seven (20%). In 16 patients (45.7%), there was no inflammation in the gastric mucosa. Follicular hyperplasia was found in seven patients with gastritis (20%), six with moderate gastritis, and one with mild gastritis. The presence of HP was not detected in the surgical specimen by Giemsa staining in any patient. The presence of these histopathological findings and the presence of DM, systemic arterial hypertension (SAH), and dyslipidemia (DLP) were also compared with the immunohistochemical expression of ghrelin; however, no significant association was found between these variables. Specimen and endoscopic biopsy showed relative agreement for the fundus region, as was observed in the Bland–Altman diagrams of Graph. 4, as can be seen in the numerical difference is concentrated close to the line zero; otherwise, in Graph. 5, which represents the samples from the antrum, most points are above the dashed line, that is, they are away from the line zero.



Graph. 1 Body mass index boxplot (kg/m^2) of patients in the follow-up period

Table 2 Summary measures of the results of ghrelin immunohistochemical expression (biopsy and specimen) from patients

		Mean	Median	Minimum	Maximum	Standard deviation
Endoscopic biopsy	UDE fundus area ($n=35$)	4.80	5.20	0.30	7.90	1.67
	UDE fundus (%) ($n=35$)	47.9%	52.0%	2.5%	78.5%	16.7%
	UDE antrum area ($n=35$)	4.10	4.40	0.30	6.30	1.58
	UDE antrum (%) ($n=35$)	41.0%	44.0%	3.1%	63.1%	15.8%
Surgical specimen	Fundus area ($n=35$)	5.44	5.30	3.10	8.30	1.25
	Fundus (%) ($n=35$)	54.5%	53.3%	31.5%	82.9%	12.6%
	Antrum area ($n=35$)	5.57	5.40	3.10	7.60	1.12
	Antrum % ($n=35$)	55.7%	53.8%	31.2%	76.1%	11.2%

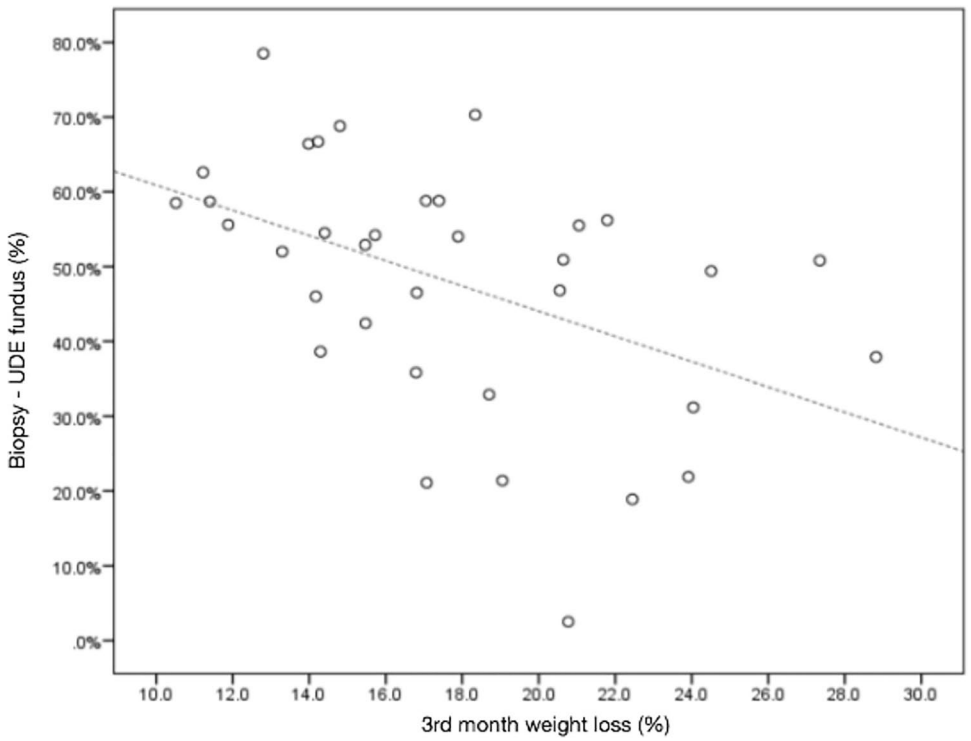
n, number of patients; *UDE*, upper digestive endoscopy

Table 3 Estimates of Spearman's correlation coefficients (*s*) between immunohistochemistry results (endoscopic and specimen biopsy) and weight lost in the 3rd, 6th, and 12th months

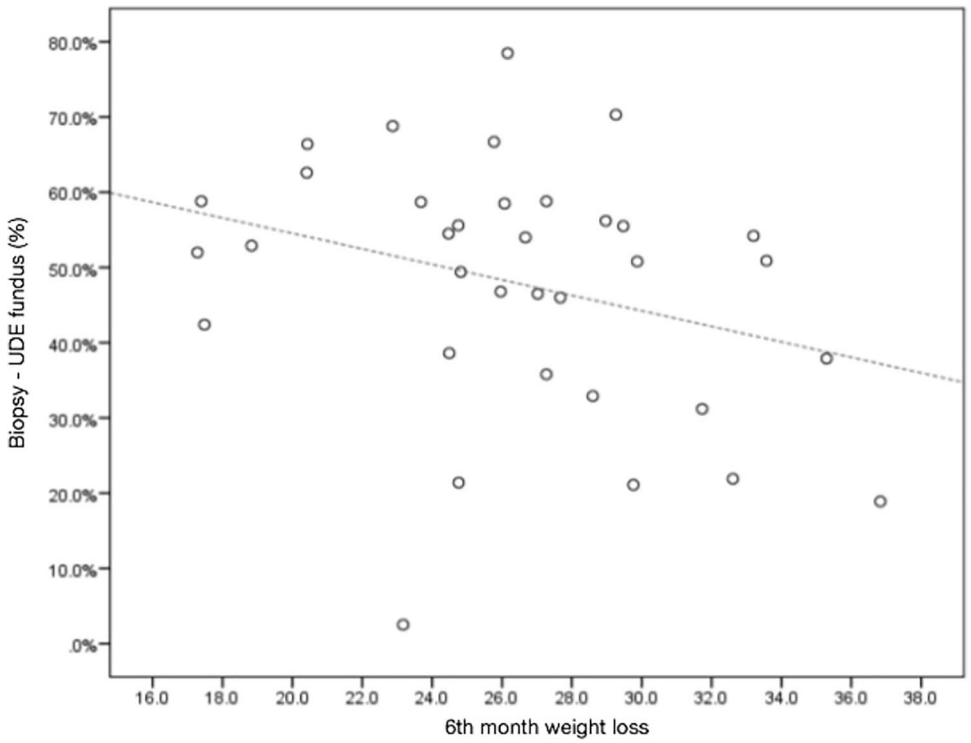
		3rd month Weight loss (%)	6th month Weight loss (%)	12th month Weight loss (%)
Biopsy				
UDE fundus area	*s*	−0.536	−0.339	−0.198
	p	**0.001***	**0.047***	0.253
UDE antrum area	*s*	−0.167	−0.265	−0.186
	p	0.336	0.124	0.285
Surgical specimen				
Fundus area	*s*	−0.221	−0.325	−0.313
	p	0.202	0.057	0.067
Antrum area	*s*	−0.171	0.092	0.047
	p	0.327	0.599	0.790

UDE, upper digestive endoscopy

*Statistically significant



Graph. 2 Two-dimensional dispersion diagram between weight lost in the 3rd month (%) and endoscopic biopsy of the fundus (%)

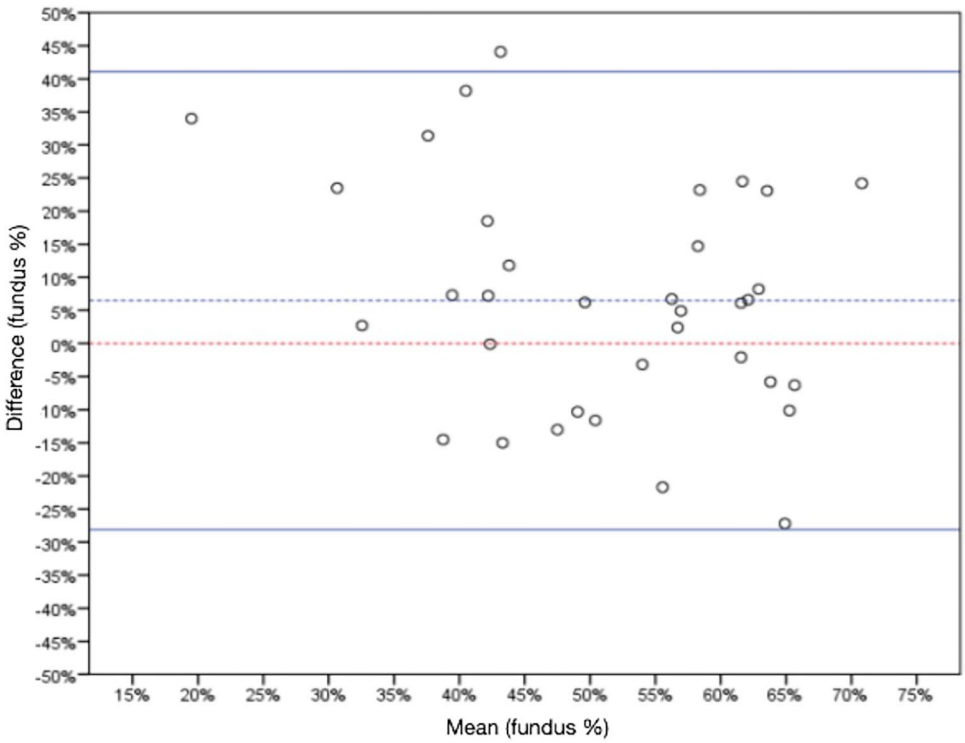


Graph. 3 Two-dimensional dispersion diagram between weight lost in the 6th month (%) and endoscopic biopsy of the fundus (%)

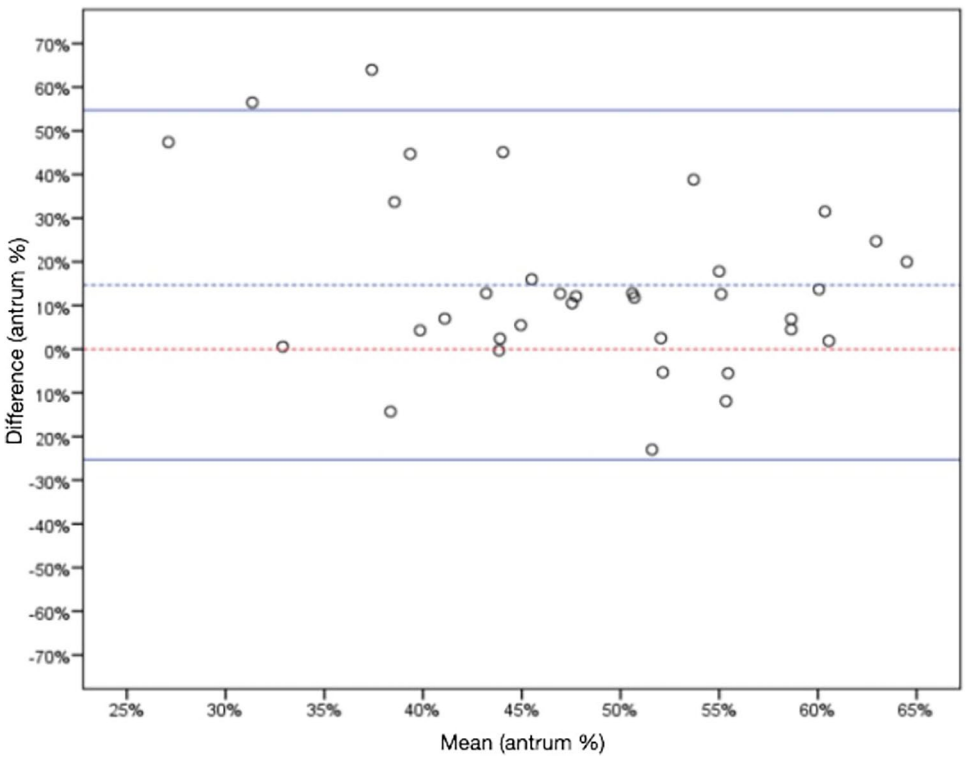
Discussion

Our study aimed to assess whether the immunohistochemical expression of ghrelin could predict the surgical outcome. This would make it possible, in the preoperative period, to identify patients who may have an unsatisfactory weight loss or weight regain with sleeve gastrectomy and, with this, help in the choice of the technique to be used.

The sample showed the proportion of women (82.9%) and the average age (35.2 years) that does not differ



Graph. 4 Bland–Altman diagram of the fundus measurements (%) in the surgical specimen and endoscopic biopsy



Graph. 5 Bland–Altman diagram of antrum measurements (%) in the surgical specimen and endoscopic biopsy

significantly from other published studies [13, 14]. Our average operative and hospital stay was also similar to that found in the literature [15, 16]. We use anti-ghrelin monoclonal antibody produced in mice because its use was already validated [17]. The quantification of the total area of stained cells was carried out through a semi-quantitative analysis due to the type of staining [18, 19].

Concerning to comorbidities, the remission of SAH and DM occurred early in all patients followed, while only one patient remained with DLP (88.9% remission). The

incidence of remission of comorbidities was above the average found in other analyses, probably due to the low BMI of the sample [20–24].

The initial mean weight of the patients was 101.7 kg and the average BMI = 38.1 kg/m^2. This characteristic was reflected in the excellent result in terms of weight loss, with an average BMI of 31.4 kg/m^2, 28.1 kg/m^2, and 25.9 kg/m^2 at 3, 6, and 12 months and an average TWL% of 17.7%, 26.4%, and 32.1% to the same period. Although most of the studies in the literature demonstrate different percentages [23, 25], when analyzing some publications with a similar profile to this study, similar results can be observed [20, 26].

When we correlated ghrelin expression in the gastric mucosa, we found a negative correlation between expression of ghrelin endoscopic biopsy of the fundus and the total weight loss, inferring that the less ghrelin is expressed in this region, the better the surgical result. Itlaybah et al., in a study with 39 patients undergoing sleeve gastrectomy, found a similar result [7].

Currently, ghrelin secretion and plasma ghrelin levels are known to be reduced in individuals with obesity compared to normal body weight controls, as an adaptation to a positive energy balance [27]; however, when ghrelin expression is evaluated in the gastric mucosa, the results are divergent: Goitein et al. demonstrated a decrease in ghrelin-producing cells count in the fundus in relation to the body and antrum [17]; Maksud et al. described a high percentage of ghrelin-producing cells in individuals with obesity, with several foci of ghrelin production in the antrum [8]; similarly, in our sample, ghrelin expression was identified in the antrum mucosa, both in the endoscopic biopsy and in the one obtained from the surgical specimen; Miyazaki et al. similarly described, in patients submitted to SG with or without duodenojejunal bypass (DJB), a greater expression of ghrelin-producing cells in individuals with extreme obesity, in relation to controls considered without obesity [6]; more recently, Itlaybah et al., evaluating 39 patients submitted only to SG, found a result similar to ours, with a weak negative correlation ($r = -0.1837$) between ghrelin expression measured through the number of producing cells [7].

The presence of comorbidities (SAH, DM, and DLP) was also correlated with the expression of ghrelin in the mucosa and no significant association was found.

The presence of inflammatory changes in the gastric mucosa can alter the expression of ghrelin [28, 29]. The result found in our sample, without any case of atrophic gastritis, intestinal metaplasia, or dysplasia, is compatible with other publications [30–32] and probably did not influence the expression of ghrelin in the evaluated mucosa. Follicular hyperplasia was found in eight patients with gastritis (22.9%), similar to Clapp et al. who, in a study evaluating 145 patients, found follicular hyperplasia in 11 [33]. The presence of follicular hyperplasia was also correlated with the ghrelin immunohistochemical expression; however, no association was found. Regarding the presence of HP, nine patients (25.7%) had a positive urease test preoperatively. Although the need for treatment in patients undergoing SG is controversial, both because of the prevention of changes in the mucosa and because it may even modify the outcome of the surgery in terms of the metabolic response [34–36] or alter the incidence of complications [37], all patients were treated. After surgery, we confirm the high efficacy of the eradication treatment [38].

Finally, we only found agreement in biopsies collected in the gastric fundus. In reality, this was an expected result, given that the occurrence of ghrelin expression in the gastric antrum, now known [17], is more irregular, and more susceptible to variation between the collection site (endoscopy or in the specimen). Among the limitations of our study are the small sample size, the low incidence of comorbidities, the low mean BMI, and the small BMI variation, which does not allow us to carry out some analyses. Additional studies with larger samples and with greater variability are necessary to be able to extrapolate the results to daily clinical practice; however, we believe that the evaluation of ghrelin expression in the gastric mucosa can be promising to identify groups of patients with unsatisfactory results.

Conclusions

The immunohistochemical expression of ghrelin in the gastric fundus mucosa was negatively correlated with early weight loss after sleeve gastrectomy. The gastric fundus was considered the ideal site to analyze the immunohistochemical expression of ghrelin. There was no significant correlation between the immunohistochemical expression of ghrelin in the gastric mucosa with the presence of SAH, DM, DLP, and histopathological findings, probably due to the low incidence of these variables in the sample.

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Author Contribution All authors have contributed equally to this work.

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Declarations

Ethics Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the insti-

tutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Conflict of Interest The authors declare no competing interests.

References

1. Regan JP, Inabnet WB, Gagner M, Pomp A. Early experience with two-stage laparoscopic Roux-en-Y gastric bypass as an alternative in the super-super obese patient. Obes Surg. 2003;13(6):861–4.
2. Milone L, Strong V, Gagner M. Laparoscopic sleeve gastrectomy is superior to endoscopic intragastric balloon as a first stage procedure for super-obese patients (BMI > or =50). Obes Surg. 2005;15(5):612–7.
3. Cheung D, Switzer NJ, Gill RS, Shi X, Karmali S. Revisional bariatric surgery following failed primary laparoscopic sleeve gastrectomy: a systematic review. Obes Surg. 2014;24(10):1757–63.
4. Akkary E, Duffy A, Bell R. Deciphering the sleeve: technique, indications, efficacy, and safety of sleeve gastrectomy. Obes Surg. 2008;18(10):1323–9.
5. Herron DM, Roohipour R. Bariatric surgical anatomy and mechanisms of action. Gastrointest Endosc Clin N Am. 2011;21(2):213–28.
6. Miyazaki Y, Takiguchi S, Seki Y, Kasama K, Takahashi T, Kurokawa Y, et al. Clinical significance of ghrelin expression in the gastric mucosa of morbidly obese patients. World J Surg. 2013;37(12):2883–90.
7. Itlaybah A, Elbanna H, Emile S, Thabet W, Elkashef W, Abdelsalam N, et al. Correlation between the number of ghrelin-secreting cells in the gastric fundus and excess weight loss after sleeve gastrectomy. Obes Surg. 2019;29(1):76–83.
8. Maksud FA, Alves JS, Diniz MT, Barbosa AJ. Density of ghrelin-producing cells is higher in the gastric mucosa of morbidly obese patients. Eur J Endocrinol. 2011;165(1):57–62.
9. Kasai C, Sugimoto K, Moritani I, Tanaka J, Oya Y, Inoue H, et al. 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):119.
10. Liew PL, Lee WJ, Lee YC, Chen WY. Gastric ghrelin expression associated with Helicobacter pylori infection and chronic gastritis in obese patients. Obes Surg. 2006;16(5):612–9.
11. Ruifrok AC, Johnston DA. Quantification of histochemical staining by color deconvolution. Anal Quant Cytol Histol. 2001;23(4):291–9.
12. Landini G, Martinelli G, Piccinini F. Colour deconvolution: stain unmixing in histological imaging. Bioinformatics. 2021;37(10):1485–7.
13. Juodeikis Ž, Brimas G. Long-term results after sleeve gastrectomy: a systematic review. Surg Obes Relat Dis. 2017;13(4):693–9.
14. Kikkas E, Sillakivi T, Suumann J, Kirsimägi Ü, Tikk T, Värk P. Five-year outcome of laparoscopic sleeve gastrectomy, resolution of comorbidities, and risk for cumulative nutritional deficiencies. Scand J Surg. 2019;108(1):10–6.
15. Carandina S, Montana L, Danan M, Zulian V, Nedelcu M, Barrat C. Laparoscopic sleeve gastrectomy learning curve: clinical and economical impact. Obes Surg. 2019;29(1):143–8.
16. Małczak P, Pisarska M, Piotr M, Wysocki M, Budzyński A, Pędziwiatr M. Enhanced recovery after bariatric surgery: systematic review and meta-analysis. Obes Surg. 2017;27(1):226–35.
17. Goitein D, Lederfein D, Tzioni R, Berkenstadt H, Venturero M, Rubin M. Mapping of ghrelin gene expression and cell distribution in the stomach of morbidly obese patients—a possible guide for efficient sleeve gastrectomy construction. Obes Surg. 2012;22(4):617–22.
18. Taylor C, Levenson RM. Quantification of immunohistochemistry—issues concerning methods, utility and semiquantitative assessment II. Histopathol. 2006;49(4):411–24.
19. Rizzardi AE, Johnson AT, Vogel RI, Pambuccian SE, Henriksen J, Skubitz AP, et al. Quantitative comparison of immunohistochemical staining measured by digital image analysis versus pathologist visual scoring. Diagn Pathol. 2012;7(1):1–10.
20. Berry MA, Urrutia L, Lamoza P, Molina A, Luna E, Parra F, et al. Sleeve gastrectomy outcomes in patients with BMI between 30 and 35–3 years of follow-up. Obes Surg. 2018;28(3):649–55.
21. Fuchs T, Loureiro M, Both GH, Skraba HH, Costa-Casagrande TA. The role of the sleeve gastrectomy and the management of type 2 diabetes. ABCD Arquivos Brasileiros de Cirurgia Digestiva (São Paulo). 2017;30(4):283–6.
22. Guraya SY, Strate T. Surgical outcome of laparoscopic sleeve gastrectomy and Roux-en-Y gastric bypass for resolution of type 2 diabetes mellitus: a systematic review and meta-analysis. World J Gastroenterol. 2020;26(8):865.
23. Nudotor RD, Canner JK, Haut ER, Prokopowicz GP, Steele KE. Comparing remission and recurrence of hypertension after bariatric surgery: vertical sleeve gastrectomy versus Roux-en-Y gastric bypass. Surg Obes Relat Dis. 2021;17(2):308–18.
24. Strain GW, Saif T, Ebel F, Dakin GF, Gagner M, Costa R, et al. Lipid profile changes in the severely obese after laparoscopic sleeve gastrectomy (LSG), 1, 3, and 5 years after surgery. Obes Surg. 2015;25(2):285–9.
25. Peterli R, Wölnerhanssen BK, Peters T, Vetter D, Kröll D, Borbély Y, et al. Effect of laparoscopic sleeve gastrectomy vs laparoscopic Roux-en-Y gastric bypass on weight loss in patients with morbid obesity: the SM-BOSS randomized clinical trial. JAMA. 2018;319(3):255–65.
26. Csendes A, Burgos AM, Martinez G, Figueroa M, Castillo J, Díaz JC. Loss and regain of weight after laparoscopic sleeve gastrectomy according to preoperative BMI. Obes Surg. 2018;28(11):3424–30.
27. Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. Diabetes. 2001;50(4):707–9.
28. Panarese A, Romiti A, Iacovazzi PA, Leone CM, Pesole PL, Correale M, et al. Relationship between atrophic gastritis, serum ghrelin and body mass index. Eur J Gastroenterol Hepatol. 2020;32(10):1335–40.
29. Takiguchi S, Adachi S, Yamamoto K, Morii E, Miyata H, Nakajima K, et al. Mapping analysis of ghrelin producing cells in the human stomach associated with chronic gastritis and early cancers. Dig Dis Sci. 2012;57(5):1238–46.
30. Safaan T, Bashah M, El Ansari W, Karam M. Histopathological changes in laparoscopic sleeve gastrectomy specimens: prevalence, risk factors, and value of routine histopathologic examination. Obes Surg. 2017;27(7):1741–9.
31. Demirbas BT, Erdim A, Celikel C, Akbas G, Cingi A. Is it necessary to send the sleeve gastrectomy specimens to pathology? Surg Laparosc Endosc Percutaneous Tech. 2019;29(2):117–9.
32. Ge L, Moon RC, Nguyen H, de Quadros LG, Teixeira AF, Jawad MA. Pathologic findings of the removed stomach during sleeve gastrectomy. Surg Endosc. 2019;33(12):4003–7.

33. Clapp B. Histopathologic findings in the resected specimen of a sleeve gastrectomy. JSLS. 2015;19(1):e2013.00259.
34. Di Palma A, Alhabdan S, Maeda A, Chetty R, Serra S, Quereshy F, et al. Preoperative Helicobacter pylori screening and treatment in patients undergoing laparoscopic sleeve gastrectomy. Obes Surg. 2020;30(7):2816–20.
35. Goday A, Castañer O, Benaiges D, Pou AB, Ramón JM, del Mar IM, et al. Can Helicobacter pylori eradication treatment modify the metabolic response to bariatric surgery? Obes Surg. 2018;28(8):2386–95.
36. Emile SH. Should We Test for and Eradicate Helicobacter pylori Before Bariatric Surgery? Obes Surg. 2021;31(4):1841–2.
37. Shanti H, Almajali N, Al-Shamaileh T, Samarah W, Mismar A, Obeidat F. Helicobacter pylori does not affect postoperative outcomes after sleeve gastrectomy. Obes Surg. 2017;27(5):1298–301.
38. Hernández MC, Peña CP, Martín PM, García LC, Pérez-Ferre N, Sánchez-Pernaute A, et al. Helicobacter pylori (HP) infection in obese patients undergoing Roux-en-Y gastric bypass; efficacy of two different treatment regimens in HP eradication. Nutr Hosp. 2015;32(2):600–5.