Ghrelin gastric tissue expression and wall thickness in patients submitted to laparoscopic sleeve gastrectomy as the primary weight loss procedure

Ghada Morshed, Laila Rashed, Mohamed Hafez

Departments of General Surgery, Biochemistry and Histology, Faculties of Medicine, Fayoum University, Cairo University (Kasr El Aini), Egypt

Correspondence to Ghada Morshed, MD, MRCS, 9 Said Zo El Fokkar, Manial, 589 Cairo, Egypt
Tel: +20 122 587 0476, +20 223 645 694;
e-mail: ghadamorshed@yahoo.com

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Background
Ghrelin (Ghr) plays a role in the regulation of food intake. Laparoscopic sleeve gastrectomy is used for treatment of morbid obesity following which the expression of ghrelin can be modulated. The aim of the present study was to analyse the expression of ghrelin in three areas of resected stomach specimens from morbid obese patients and correlate these data with plasmatic ghrelin levels before and after surgery and measure the wall thickness of the fundus, body and prepyloric area of the resected stomach and its relation to the stapler thickness (green or gold cartridge) used.

Patients and methods
Thirty morbidly obese patients were subjected to laparoscopic sleeve gastrectomy, and tissue samples were obtained from the fundus, body and prepyloric area of the resected stomach for mRNA and protein expression analysis. Blood samples were collected before and 1 month after surgery to evaluate the plasmatic ghrelin levels and for histologic examination to detect its wall thickness.

Results
Ghrelin protein expression was higher in the fundus than in the other areas. Total ghrelin plasma levels decreased significantly from 70.2 ± 80.4 pg/ml before surgery to 12.2 ± 29.3 pg/ml after surgery. The wall thickness of the prepyloric area was higher than that of the body and fundus, which is the reason for the use of a green cartridge at the prepyloric area (higher thickness) and a gold cartridge at the body and fundus (less thickness).

Conclusion
Ghrelin protein expression was higher in the fundus than in the body and prepyloric areas. The wall thickness of the prepyloric area is higher than that of the body and fundus.

Keywords:
Ghrelin, laparoscopic sleeve gastrectomy, wall thickness

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Introduction

Ghrelin (Ghr) is a 28-amino acid acylated peptide. Ghrelin stimulates appetite by acting on the hypothalamic arcuate nucleus. Ghrelin is secreted from the stomach and circulates in the blood stream under fasting conditions [1].

Ghrelin is a natural leptin antagonist [2]. In general, plasma ghrelin levels are low in obese human subjects and after food intake, and it increases during starvation and in patients with mental anorexia. In addition, ghrelin plasma levels are negatively correlated with BMI, amount of body fat, adipocyte size, and leptin, insulin and glucose levels. The ghrelin hormone not only stimulates the brain, giving rise to an increase in appetite, but also favours the accumulation of lipids in visceral fatty tissue, located in the abdominal zone and considered to be the most harmful [3,4].

The release of GH from the pituitary gland might be regulated not only by the GH-releasing hormone but also by ghrelin produced by the stomach, intestine, placenta, pituitary gland and possibly in the hypothalamus [1,5,6]. Ghrelin and its receptor are widely distributed in the body; however, the greatest expression of ghrelin is in stomach endocrine cells.

Administration of exogenous ghrelin has been shown to stimulate pituitary growth hormone (GH) secretion, appetite, body growth and fat deposition. Thus, it is an anabolic hormone [7].

Aim of the study

Laparoscopic sleeve gastrectomy (LSG) is used for treatment of morbid obesity and the aim of this study

was to analyse the expression of ghrelin in three areas of resected stomach specimens from patients after LSG and determine the wall thickness of the fundus, body and pylorus in normal stomach.

Patients and methods

This was a prospective study comprising 30 morbid obese patients, seven men and 23 women, with a median age of 40 years (range 24–63 years), who had consecutively undergone LSG. Exclusion criteria were presence of noncompensated chronic liver or renal disease, BMI>60 kg/m^2, and age less than 18 and more than 65 years. Median presleeve BMI value was 42.5 kg/m^2 (range 35–55 kg/m^2) and patients (15%) presented type 2 diabetes mellitus.

Ethical committee approval was obtained before study initiation, and all participants signed an informed consent form.

Laparoscopic sleeve gastrectomy

LSG was performed under general anaesthesia, and started with division of the greater curvature blood supply (Figs 1 and 2). This was followed by resection of the fundus and greater curvature from 6 cm from the pylorus until the angle of His using an EndoGIA stapler green cartridge (4.8/60 mm) at the prepyloric area (higher thickness) and a gold cartridge (3.8/60 mm) at the body and fundus (less thickness), along with a bougie (36 Fr) (Fig. 3).

Prolene sutures were used to reinforce the staple line; methylene blue was injected intraoperatively to check for any leakage. Postoperative gastrografin study was carried out on all patients. The patients started eating on the seventh postoperative day.

Plasma ghrelin determination

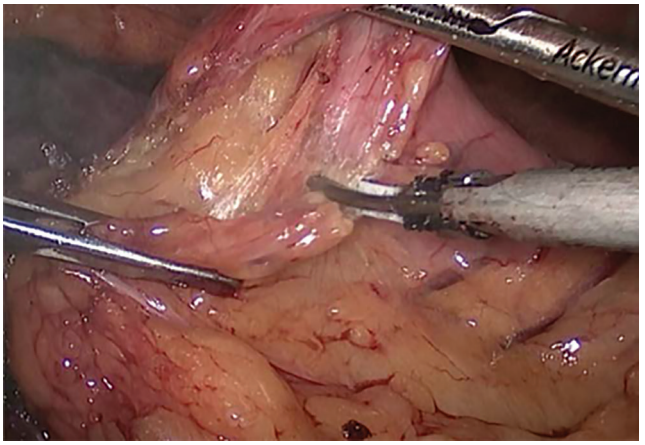
The plasmatic levels of ghrelin were measured with a commercial radioimmunoassay kit (LINCO Cat# GHRT-89HK, USA). From all overnight fasted patients a sample of peripheral blood was obtained on the day of surgery and 1 month after surgery.

Quantitative real-time PCR for ghrelin in gastric tissue

Total RNA was extracted from gastric homogenate by using the SV total RNA isolation system supplied by Promega (Madison, Wisconsin, USA) according to the manufacturer's protocol. Extracted RNA was quantified by means of a spectrophotometer at 260 nm.

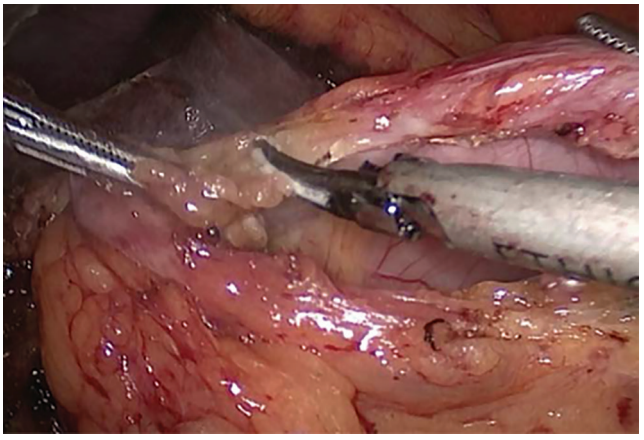
The total RNA (0.5~2 μg) was used for cDNA conversion using high capacity cDNA reverse transcription kit (#K1621, Fermentas, USA). cDNA was generated from 1 mg of total RNA extracted according to the

Figure 1



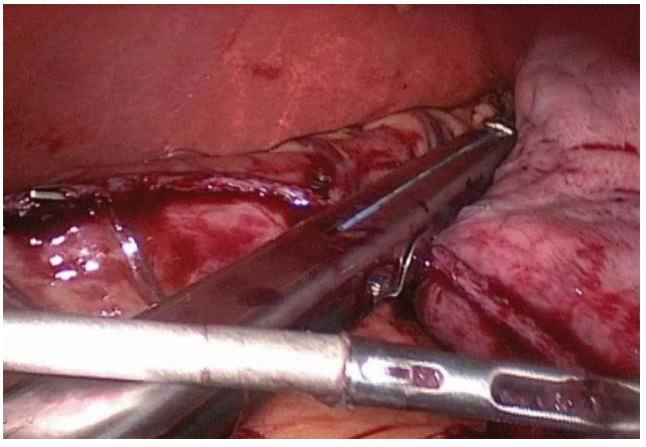
Division of the vascular supply of the greater curvature of the stomach.

Figure 2



Division of the vascular supply of the greater curvature of the stomach

Figure 3



Gastrectomy using a stapler 6 cm proximal to the pylorus laparoscopic sleeve gastrectomy (LSG).

manufacturer's instructions. The relative abundance of mRNA species was assessed using the SYBR Green method on an ABI prism 7500 sequence detector system (Applied Biosystems, Foster City, California, USA). PCR primers were designed with Gene Runner Software (Hasting Software Inc., Hasting, New York, USA) from RNA sequences from GenBank (Table 1). All primer sets had a calculated annealing temperature of 60°. Quantitative RT-PCR was performed in duplicate in a 25 ml reaction volume consisting of 2X SYBR Green PCR Master Mix (Applied Biosystems), 900 nmol/l of each primer and 2–3 ml of cDNA. Amplification conditions were 2 min at 50°C, 10 min at 95°C and 40 cycles of denaturation for 15 s and annealing/extension at 60° for 10 min. Data from real-time assays were calculated using the v1·7 Sequence Detection Software from PE Biosystems (Foster City, California, USA). Relative expression of pro-ANP, SDF-1, MMP-9, Bax and bcl2 mRNA was calculated using the comparative C_t method. All values were normalized to the β-actin genes and reported as fold change (Table 1).

Histologic examination

Stomach specimens resected during sleeve gastrectomy (SG) were fixed in 10% buffered formalin for 24 h. After fixation, three tissue samples were obtained from three areas in each stomach: the fundus, body and the prepyloric area. The specimens were then trimmed using a scalpel to enable them to fit into an appropriately labelled tissue cassette. The filled tissue cassettes were stored in formalin until they were processed into thin microscopic sections using a paraffin block. Tissue specimens were then cut into sections that could be placed on a slide. Histochemical stains (typically haematoxylin and eosin) were used for staining (Figs 4–6).

Morphometric study

Using a Leica Qwin 500 LTD computer-assisted image analysis system (Glory Science Co Ltd, Del Rio, TX, USA), the wall thickness (indicated by the distance parameter) was measured in H&E-stained sections using the interactive measuring menu. This was examined at magnification ×100.

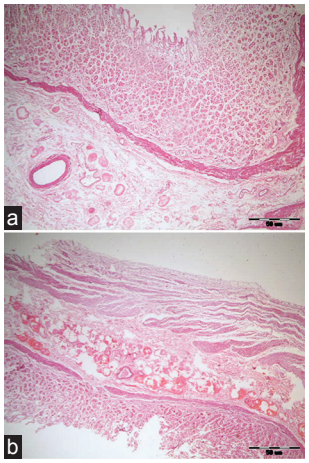
Results

Ghrelin protein expression was higher in the fundus than in the other areas (Fig. 7), and total ghrelin plasma levels decreased significantly from 70.2 ± 80.4 pg/ml before surgery to 12.2 ± 29.3 pg/ml after surgery as a result of proper total fundectomy. The wall thickness of the prepyloric area was higher than that of the body and fundus; the mean antral thickness was 4.2 mm (range 3.2–4.6 mm), the mean body thickness was 2.56 mm (range 1.5–3.56 mm) and the mean fundus thickness was 2.14 mm (range 1.7–2.7 mm) (Fig. 8). We also found that gastric smooth muscle, particularly the circular layer, is thicker and denser around the gastric antrum than around the rest of the stomach; this explains the use of the green cartridge (4.8/60 mm) at the prepyloric area (higher thickness) and the gold cartridge (3.8 mm/60 mm) at the body and fundus (less thickness). The correlations between PCR and wall thickness are shown in Fig. 9 and Table 2.

Table 1 Oligonucleotide primer sequence used for real-time PCR

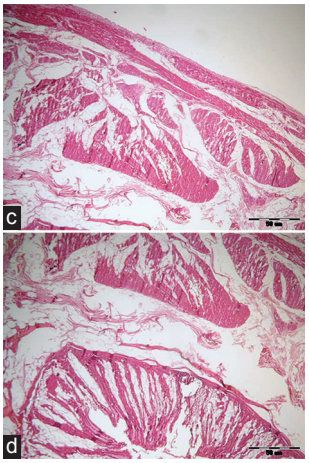
Gene	Primer sequence
Ghrelin	Forward primer 5′-TGCAGAAACCCTGGCTGA-3′Reverse primer 5′-CACGTGGTCTCGGAAGTG-3′
β-Actin	Forward primer 5′-TGCTGGTGCTGAGTATGTCG-3′Reverse primer 5′-TTGAGAGCAATGCCAGCC-3′

Figure 4



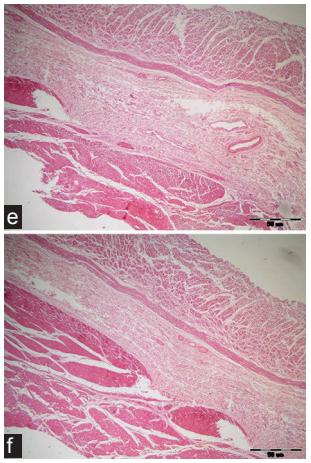
Wall thickness in the fundus (a, b).

Figure 5



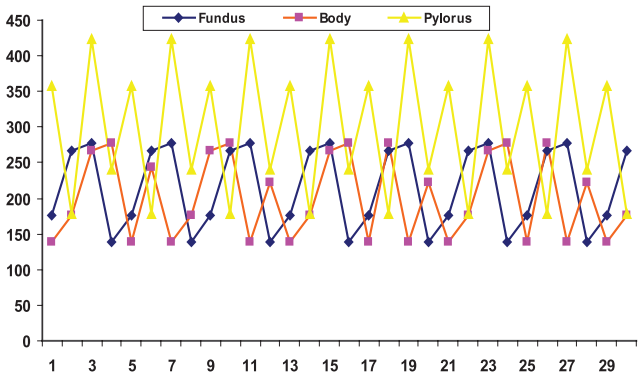
Wall thickness in the body (c, d).

Figure 6



Wall thickness in the pylorus (e, f).

Figure 8



Wall thickness in the fundus, body and pylorus

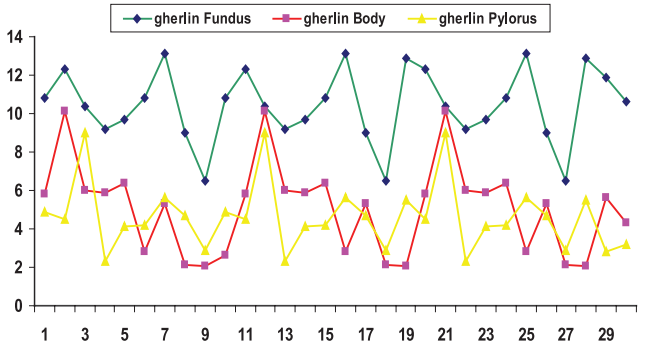
Table 2 Correlations between PCR and wall thickness

Items	Thickness	PCR
Thickness		
Pearson's correlation	1	177
Significance (two-tailed)	90	0.095
N		90
PCR		
Pearson's correlation	177	1
Significance (two-tailed)	0.095	90
N	90	

Statistical analysis

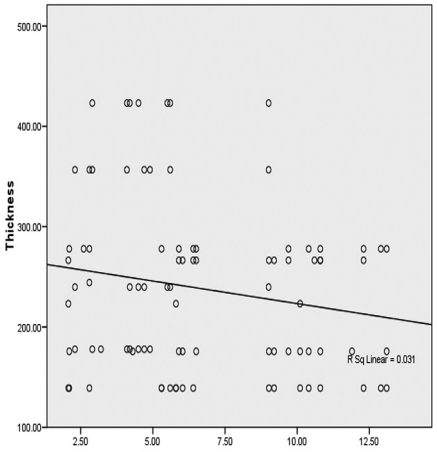
All data obtained from the various analyses were entered into a customizable database built with Microsoft Access (Microsoft Corporation). Relevant data were extracted from the database with appropriate queries and exported in Microsoft Excel (Microsoft Corporation, USA) and SPSS v.08 for further manipulations and statistical analyses (IBM SPSS Statistics, London).

Figure 7



PCR for Ghrelin expression in the fundus,body and pylorus

Figure 9



Correlations between PCR & Wall thickness

Discussion

The decrease of plasma ghrelin after LSG is advocated as one of the hormonal mediators of weight loss and glucose homeostasis in the early phase in the absence of significant weight loss [8–11].

In this prospective study, ghrelin expression and tissue distribution of cells producing this protein were evaluated to better understand the ghrelin distribution in different areas of the stomach and to correlate those findings with ghrelin plasmatic levels in patients who had undergone LSG. The total plasma ghrelin level in all patients before surgery was correlated to BMI, as found in other studies [12,13].

The ghrelin plasma level significantly decreased after surgery in all patients as a result of proper total fundectomy, as found in other studies [14,15].

In contrast, several studies have not found a modification of ghrelin plasma levels, and some authors have reported controversial results, including an increment of ghrelin

plasma level after surgery [16,17]. The contradictory results could be due to differences in study design, follow-up periods, measurement methods, surgical intervention and circadian rhythm [14,18].

Our immunohistochemical results showed that ghrelin protein expression was higher in the fundus than in the body and prepyloric area, although this difference was not statistically significant, in agreement with the findings of Goitein *et al.* [19].

Miyazaki *et al.* [18] hypothesized that greater the number of positive cells present in the stomach, better the surgical outcome, and if the number of positive cells in the stomach correlates with the mRNA ghrelin expression, this value could be considered a favourable predictor of LSG outcome. We found a distinct correlation between ghrelin mRNA expression and ghrelin protein expression in the fundus and a weak correlation between ghrelin mRNA expression and the mean value of ghrelin protein expression obtained from the three areas (fundus, body and prepyloric area). Nevertheless, as previously reported [19], levels of ghrelin mRNA did not correlate with plasmatic protein levels, which could be due to compensatory productions from extragastric organs.

Recently, there was a debate on the extension of the antral resection during SG: some authors recommend preservation of the antrum because this site is important as a pumping mechanism for gastric emptying, because the partial antrum resection does not significantly affect the long-term pouch volume [20,21] and because the removal of antral tissue allows a more extensive reduction of ghrelin-producing cells [19]. In contrast, other authors argue that the cell population present on antral tissue cannot be the real population of cells producing ghrelin because these cells are very different from those seen in other areas and also because their volume is poor [22,23]. According to this, we believe that it is not necessary to remove antral tissue as the majority of positive cells were present on fundus tissue, as previously described in other studies [22,23].

In our study patients without suspected gastric disease there was relative wall thickening of the distal gastric antrum compared with the proximal stomach as a normal finding; the mean antral thickness was 4.2 mm (range 3.2–4.6 mm), the mean body thickness was 2.56 mm (range 1.5–3.56 mm) and the mean fundus thickness was 2.14 mm (range 1.7–2.7 mm). We also found that gastric smooth muscle, particularly the circular layer, was thicker and denser around the gastric antrum than around the rest of the stomach, as found in other studies [24] and in studies using multidetector CT (MDCT) [25,26]. This explains the use of green cartridge (4.8/60 mm) at the prepyloric area (higher thickness) and gold cartridge (3.8/60 mm) at the body and fundus (less thickness).

Conclusion

The preliminary results of the ongoing prospective study confirm that ghrelin protein expression was higher in the fundus than in the body and prepyloric areas. Moreover, smooth and uniform wall thickening of the distal gastric antrum relative to the proximal stomach is a normal finding. Normal antral wall thickening is likely caused by an anatomic component (muscular thickening), which is the reason for the use of the green cartridge (4.8/60 mm) at the prepyloric area (higher thickness) and the gold cartridge (3.8/60 mm) at the body and fundus (less thickness).

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Conflicts of interest

There are no conflicts of interest.

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