Ghrelin, glucagon-like peptide-1, and peptide YY secretion in patients with and without weight regain during long-term follow-up after bariatric surgery: a cross-sectional study

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Abstract

Introduction: Weight loss after bariatric surgery is attributed, at least in part, to the altered gastrointestinal (GI) hormone secretion, which is thought to be responsible for a number of beneficial metabolic effects.

Material and methods: We conducted a cross-sectional study. Twelve patients who underwent laparoscopic sleeve gastrectomy (SG) and 20 patients who underwent a variant of biliopancreatic diversion with Roux-en-Y gastric bypass and long limbs (BPD/RYGB-LL) were evaluated ≥ 7 years postoperatively. Ghrelin, glucagon-like peptide-1 (GLP-1), and peptide YY (PYY) secretion were compared between patients with successful weight loss maintenance (WM group) and patients with weight regain (WR group).

Results: In both types of surgery, standard liquid mixed meal (SLMM) ingestion did not result in significant changes in fasting GI hormone levels. Fasting ghrelin levels did not differ between the WM group and the WR group in both types of surgery. In SG patients, SLMM ingestion elicited greater suppression of ghrelin levels in the WM group ($p = 0.032$). No difference in GLP-1 secretion was observed between the 2 groups of patients in both types of surgery. When patients were examined, regardless of the type of bariatric surgery they had undergone, postprandial PYY levels were lower in the WM group ($p < 0.05$), while fasting and postprandial PYY levels were correlated positively with an increase in body mass index (BMI) in the evaluation (Spearman's rho ≥ 0.395, $p < 0.03$).

Conclusions: Our data do not support the hypothesis that long-term weight regain after bariatric surgery is associated with an unfavourable GI hormone secretion pattern.

Key words: GLP-1, ghrelin, gastrointestinal hormones, weight regain.

Introduction

Weight loss failure following bariatric surgery may be due to either insufficient weight loss or weight regain. Insufficient weight loss is characterized by a maximum achieved percentage loss of excess body weight (%EWL) of less than 50%. On the other hand, weight regain is characterized by successful initial weight loss (maximum achieved %EWL greater than or equal to 50%) followed by progressive weight gain [1]. Weight regain is the most important long-term complication following bariatric surgery. It is associated with relapse of obesity-related comorbidities and is one of the most common indica-

tions for revisional surgery [2]. The prevalence of weight regain following bariatric surgery is difficult to determine because there is currently no consensus on what constitutes clinically significant weight regain and no commonly accepted definition of weight regain [3]. Nevertheless, weight regain is a common phenomenon after all types of bariatric surgery, and it depends on the type of bariatric surgery and the duration of follow-up [4]. The aetiology of weight regain following bariatric surgery has not been elucidated, and the underlying pathophysiological mechanisms are poorly understood. Factors implicated in weight regain include anatomic and surgical factors, hormonal and metabolic alterations, nutritional

non-compliance, physical inactivity, and mental health and psychological factors [5].

In the past, bariatric surgical procedures were considered to reduce body weight and treat obesity-related comorbidities simply by restricting meal size and/or by reducing the absorption of macronutrients. Nowadays, however, it is recognized that bariatric surgery results in several beneficial metabolic effects such as reduced hunger, increased satiety, increased energy expenditure, and increased insulin sensitivity, which help weight loss and contribute to the resolution of comorbidities [6]. It has been proposed that these metabolic effects are mediated, at least in part, by altered gastrointestinal (GI) or gut hormone secretion that occurs after bariatric surgery [7]. Ghrelin, peptide YY (PYY), and glucagon-like peptide-1 (GLP-1) are GI hormones involved in energy balance regulation and glucose homeostasis, and their changes after bariatric surgery have been extensively studied over the past 2 decades.

Ghrelin is an orexigenic hormone that stimulates food intake. Conversely, both PYY and GLP-1 exert anorexic effects and reduce food intake [8]. Ghrelin levels increase preprandially and may participate in initiating a meal, whereas PYY and GLP-1 increase during meal consumption and may help to terminate the meal in healthy normal-weight subjects. Bariatric surgery has been shown to alter GI hormone secretion. Fasting ghrelin levels decrease during the first years after sleeve gastrectomy (SG) and during the first months after Roux-en-Y gastric bypass (RYGB). Postprandial PYY and GLP-1 levels increase at many times after RYGB and to a lesser extent after SG [9–11]. Enhanced postprandial PYY and GLP-1 release have been associated with favourable weight loss outcomes during the first years after RYGB [12, 13]. However, it is largely unknown whether these changes are critical to the long-term success of bariatric surgery.

The aim of the present study was to investigate whether long-term weight regain after bariatric surgery is associated with increased fasting ghrelin levels and/or diminished postprandial GLP-1 and PYY release. To test the above hypothesis, we compared ghrelin, GLP-1, and PYY secretion between patients with successful weight loss maintenance and patients with significant weight regain during long-term follow-up after SG or a variant of biliopancreatic diversion with Roux-en-Y gastric bypass and long limbs (BPD/RYGB-LL). All patients had successful initial weight loss.

Material and methods

Subjects and study design

Two types of bariatric surgeries performed on a large scale in our institution in the last 20 years are SG and BPD/RYGB-LL. Sleeve gastrectomy was performed in morbidly obese patients with a body mass index (BMI) of ≤ 50 kg/m^2 who were not "sweet-eaters" ("sweet-eaters" were defined as subjects who consumed of more than 300 kcal of sweet foods or beverages at least 3 times a week) and had no symptoms of gastroesophageal reflux disease. BPD/RYGB-LL was performed in super obese patients (BMI ≥ 50 kg/m^2), in morbidly obese patients who were "sweet-eaters", and in those with severe metabolic complications. The vast majority of patients undergoing SG or BPD/RYGB-LL exhibited excellent weight loss outcomes during the first postoperative years [14, 15]. However, during long-term follow-up, we observed that a proportion of them regained a significant amount of body weight. To determine whether these patients were characterized by an unfavourable GI hormone secretion pattern, we designed a cross-sectional study.

Successful initial weight loss was defined as %EWL greater than or equal to 50% during the first 2 postoperative years. Significant weight regain was defined as a BMI increase greater than or equal to 4 kg/m^2 once maximum weight loss was achieved. Minimum BMI and maximum %EWL were achieved at the same time that the weight loss was at its maximum. We chose to define weight regain based on BMI because BMI is strongly associated with all-cause mortality [16]. The eligibility and exclusion criteria for patient selection were defined as follows: Patients should be at least 18 years old at the time of surgery, should have undergone either SG or BPD/RYGB-LL at least 7 years before the evaluation, should have successful initial weight loss, and should have maintained stable body weight (±5 kg of total body weight) during the 3 months leading up to the evaluation. Also, patients should not have undergone another bariatric surgery before SG or BPD/RYGB-LL, should not have undergone any other surgery (e.g. enterectomy), should not have complications (e.g. gastrogastric fistula) that alters the anatomy of the digestive tract after SG or BPD/RYGB-LL, should not suffer from uncontrolled endocrine disorder, active malignancy, or other disease known to affect appetite and intestinal motility, and should not be pregnant. Patients with controlled diabetes mellitus were eligible to participate unless they were receiving GLP-1 analogues.

From the registry of our unit, we found patients who met the above criteria and presented significant weight regain. Eligible patients were invited to participate, and finally 6 patients who had undergone SG and 10 patients who had undergone BPD/RYGB-LL formed the weight-regain group (WR group). Patients who also met the above criteria and presented successful weight loss maintenance were selected to match the WR group for type of surgery, preoperative characteristics (age, gender, height, weight, BMI, ideal weight, excess weight, and status of diabetes mellitus), maximum %EWL, minimum BMI, and length of postoperative follow-up. In the same way as above, 6 patients who had under-

gone SG and 10 patients who had undergone BPD/RYGB-LL formed the "successful weight loss maintenance" (WM) group. Ideal body weight was determined according to the Metropolitan Life Insurance Company 1983 height/weight tables.

Patients attended the outpatient clinic of our hospital on a single occasion after an overnight fast between July and October 2019. All patients underwent a complete evaluation that included anthropometric, clinical, and behavioural parameters and medications. Venous blood samples were obtained in a fasting state. Patients were then asked to ingest a standard liquid mixed meal (SLMM; Fortimel Extra, Nutricia, Netherlands; containing 300 kcal, with 45% calories as carbohydrates, 24% as protein, and 31% as fat) over 10 minutes, and additional venous blood samples were obtained at 30 and 90 minutes after meal ingestion. The main outcome variables were as follows: a) fasting and postprandial total ghrelin, total GLP-1, and total PYY levels; b) total ghrelin, total GLP-1, and total PYY response to the SLMM. Gastrointestinal hormone levels were determined using commercially available kits, while the incremental area under the curve (iAUC) was calculated to assess the response of GI hormones to the SLMM.

Surgical technique

ll SG patients underwent laparoscopic surgery by the same surgeon. During SG, about 90% of the stomach was removed by dividing the greater curvature of the stomach from the distal antrum to the left crus of the diaphragm. The dissection of the stomach began with a 60 mm linear stapler about 3 cm from the pylorus close to a 32F bougie up to the angle of His. Continuously applied linear staplers (45 mm and 60 mm) were used for the complete transection of the stomach so that a gastric sleeve tube with an estimated volume of 40–60 ml was created (Fig. 1).

ll BPD/RYGB-LL patients underwent open surgery by the same surgeon. BPD/RYGB-LL is a combination of distal RYGB and BPD (Scopinaro operation) and involves a gastric pouch of 40 ±10 ml with complete separation from the bypassed stomach, an alimentary limb of 400 cm, a common limb of 100 cm, and biliopancreatic limb the remainder of the small intestine. The gastric pouch was constructed by initially placing a 45 mm linear stapler horizontally along the lesser curvature of the stomach, about 7 cm from the gastroesophageal junction. The pouch capacity was determined by filling a 33F orogastric tube with water, and a second 60 mm linear stapler was then placed vertically, aiming towards the angle of His. Measurements of intestinal limb lengths were always performed by the surgeon with the small bowel fully stretched (Fig. 1).

Blood samples and biochemical analysis

Blood samples were collected in prechilled heparin coated tubes containing 1.8 trypsin inhibitor units of aprotinin (Trasylol®). All samples were immediately put on ice and were centrifuged at 4°C for 20 min and 1600 relative centrifugal force. The resulting supernatant (plasma) was immediately frozen at –70°C until assayed. Gastrointestinal hormones (ghrelin, GLP-1, and PYY) were measured with commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Korain Biotech Co., Ltd., Shanghai, China) and analysed in an automatic analyser using the instructions provided by the manufacturer. The limit of detection of the assay was 0.05 ng/ml for total ghrelin, 2 pmol/l
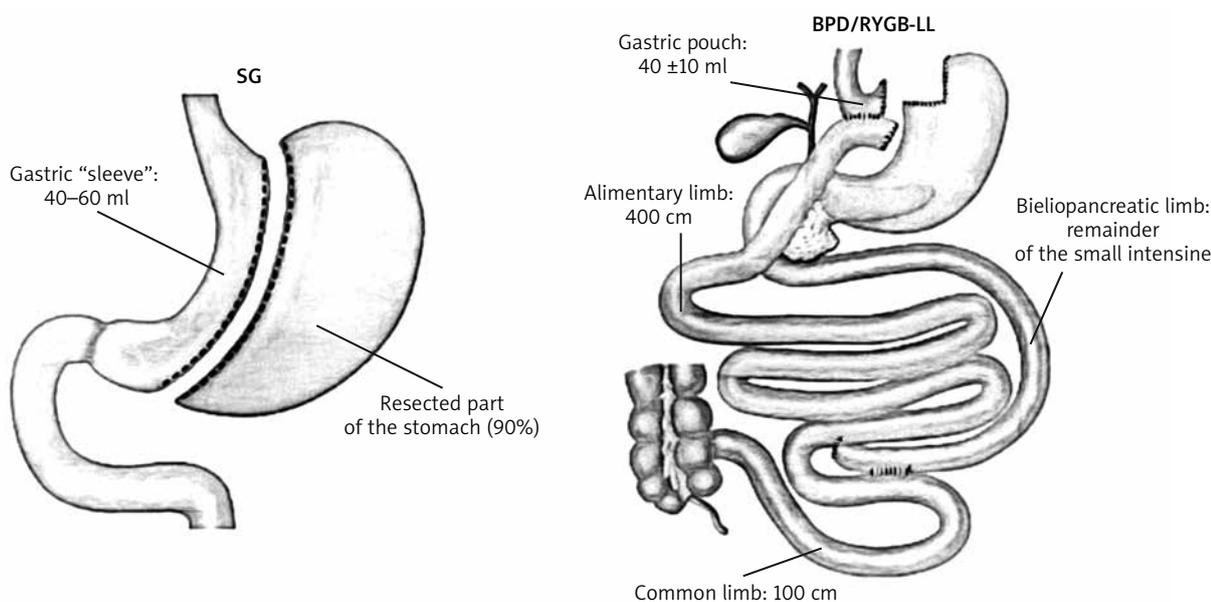

Fig. 1. Schematic representation of the studied procedures

for total GLP-1, and 3 pg/ml for total PYY. The intra- and inter-assay coefficients of variation were < 8% and < 10%, respectively. We chose to determine the total ghrelin, total GLP-1, and total PYY concentrations because acylated ghrelin and PYY (3–36) are technically more difficult to quantitate, while GLP-1 secretion has been shown to be best estimated by determining total GLP-1 in plasma [17]. To validate the effect of the SLMM on GI hormone secretion, we also measured all 3 GI hormones with different commercial ELISA kits (Thermo Fisher Scientific, Inc., Bartlesville, USA) in the serum of a representative subgroup of patients.

Statistical analysis

Statistical analysis was performed using SPSS version 27.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were expressed as n (%). Continuous parametric variables were expressed as mean ± standard deviation (SD) and continuous non-parametric variables as median with interquartile range (IQR). The area under the curve (AUC) was calculated using the trapezoidal method, while the iAUC for the hormonal response was calculated using the formula: iAUC = AUC – (90 × hormonal plasma concentration at baseline). Data were investigated for normality using the Shapiro-Wilk test.

Initial data analysis was performed depending on the type of surgery. Comparisons between the WR group and the WM group were made using the χ^2 test or Fisher's exact test for categorical variables, the Student's t-test for continuous parametric variables, and the Mann-Whitney U test (Wilcoxon rank-sum test) for continuous non-parametric variables. Comparisons between fasting and postprandial GI hormone levels were performed using a paired (dependent) sample t-test for parametric variables or Wilcoxon signed-rank test for non-parametric variables. For all analyses, p-values < 0.05 were considered significant. Comparisons with p-values between 0.10 and 0.05 were considered to show a trend worthy of interest.

Results

Sleeve gastrectomy patients

Per study design, patients in both groups did not differ in preoperative characteristics (gender, age, weight, height, BMI, ideal weight, excess weight, and status of diabetes mellitus), minimum BMI and maximum %EWL achieved postoperatively, and duration of follow-up. %EWL (P 0.002) in the evaluation was lower, whereas BMI (P 0.003), BMI increase (p < 0.001), and weight regain (p < 0.001) in the evaluation were higher

Table 1. Clinical characteristics of patients

	SG patients			BPD/RYGB-LL patients		
	WR group (n = 6)	WM group (n = 6)	p-value	WR group (n = 10)	WM group (n = 10)	p-value
Gender: female/male [n (%)]	5 (83.3)/1 (16.7)	5 (83.3)/1 (16.7)	1.000	6 (60)/4 (40)	6 (60)/4 (40)	1.000
Age [year] at surgery	36.7 ±8.6	32.5 ±9.0	0.428	35.7 ±10.9	34.3 ±6.0	0.740
Status of diabetes mellitus: diabetic/non-diabetic [n (%)]	2 (33.3)/4 (66.6)	2 (33.3)/4 (66.6)	1.000	1 (10)/9 (90)	1 (10)/9 (90)	1.000
Preoperative BMI [kg/m²]	43.3 ±2.9	42.4 ±1.7	0.537	50.4 ±6.4	54.8 ±7.2	0.165
Preoperative weight [kg]	121.3 ±8.0	119.0 ±9.3	0.652	146.0 ±24.3	165.7 ±30.6	0.129
Preoperative height [cm]	167.5 ±4.2	167.5 ±6.5	1.000	170.1 ±8.3	173.6 ±10.5	0.419
Ideal weight [kg]	61.8 ±3.4	61.8 ±5.1	1.000	60.5 (12.8)	67.3 ±9.5	0.529
Excess weight [kg]	59.5 ±6.7	54.9 (6.8)	0.394	81.3 ±20.7	98.4 ±24.4	0.108
Nadir weight [kg]	71.1 ±8.2	68.5 6.2	0.551	75.4 ±6.3	70.6 ±13.9	0.805
Time [year] to achieve nadir weight	1.6 (1.1)	4.6 (10.6)	0.699	1.5 (0.3)	1.8 (6.3)	0.190
Minimum BMI [kg/m²]	25.3 ±3.0	24.5 ±3.1	0.654	26.1 ±2.4	25.3 ±2.6	0.449
Maximum %EWL	85.3 ±13.0	88.0 ±14.8	0.743	86.6 ±8.6	90.3 ±8.4	0.339
Time [year] after surgery in the evaluation	11.8 ±1.6	11.0 ±1.3	0.390	8.9 ±1.1	8.9 ±0.9	0.988
Weight [kg] in the evaluation	94.8 ±11.4	72.8 ±3.6	0.004	98.3 (10)	82.2 ±16.4	0.063
%EWL in the evaluation	44.7 ±18.3	80.5 ±10.5	0.002	60.2 ±16.2	84.2 ±12.6	0.002
BMI [kg/m²] in the evaluation	33.8 ±4.1	26.0 ±2.4	0.003	33.0 ±3.7	27.1 ±3.5	0.002
Increase in BMI in the evaluation (BMI in the evaluation minus minimum BMI) [kg/m²]	8.5 ±3.3	1.5 ±1.3	< 0.001	6.9 ±2.1	1.8 ±1.1	< 0.001
Weight [kg] regain in the evaluation	23.7 ±9.3	4.3 ±3.6	< 0.001	19.8 ±5.6	5.6 ±3.5	< 0.001

BMI – body mass index

in the WR group compared to the WM group (Table 1). Regardless the degree of weight regain, postprandial GI hormone levels after SLMM ingestion did not differ significantly compared to fasting levels. These results were validated after measurements with different commercial ELISA kits in the serum of a representative subgroup of patients. No differences in fasting and postprandial ghrelin and GLP-1levels were observed between the 2 groups of patients. However, SLMM ingestion elicited greater suppression of the orexigenic hormone ghrelin in the WM group compared to the WR group ($p = 0.032$). Glucagon-like peptide-1 response to SLMM did not differ between the 2 groups of patients. Contrary to what might be expected, PYY response to SLMM tended to be greater in the WR group ($p = 0.093$) (Table 2).

BPD/RYGB-LL patients

In the same way as above, preoperative characteristics, minimum BMI and maximum %EWL achieved postoperatively, and duration of follow-up were comparable between the 2 groups of patients. %EWL ($p = 0.002$) in the evaluation was lower, whereas BMI ($p = 0.002$), BMI increase ($p < 0.001$), and weight regain ($p < 0.001$) in the evaluation were higher in the WR group compared to the WM group (Table 1). As in the case of SG patients and regardless the degree of weight regain, postprandial GI hormone levels after SLMM ingestion did not differ significantly compared to fasting levels. These results were also validated after measurements with different commercial ELISA kits in the serum of a representative subgroup of patients. No differences in fasting ghrelin, GLP-1 and PYY levels, and postprandial ghrelin and GLP-1 levels were observed

between the 2 groups of patients. However, absolute PYY levels at 30' (P 0.089) and 90' (P 0.052) after SLMM ingestion tended to be higher in the WR group. No differences in ghrelin, GLP-1, and PYY responses to SLMM were found between the studied groups (Table 2).

Peptide YY levels and response to standard liquid mixed meal

As already mentioned, SLMM tended to elicit a greater response of PYY in the WR group compared to the WM group in patients who had undergone SG. Also, absolute postprandial PYY levels tended to be higher in the WR group compared to the WM group in patients who had undergone BPD/RYGB-LL. To further evaluate the importance of these observations, we compared PYY levels and response to SLMM between the WR and WM groups, regardless of the type of bariatric surgery. Preoperative characteristics, type of bariatric surgery, minimum BMI – maximum %EWL achieved postoperatively, and duration of follow-up did not differ between the 2 new groups formed. %EWL in the evaluation was lower, whereas BMI, BMI increase, and weight regain in the evaluation were higher in the WR group compared to the WM group (all $p < 0.001$). Absolute postprandial PYY levels differed significantly between the 2 groups of patients. In particular, PYY levels at 30' (P 0.043) and 90' (P 0.035) after SLMM ingestion were higher in the WR group compared to the WM group (Table 3). Also, the increase in BMI in the evaluation was correlated positively with fasting PYY levels (Spearman's rho = 0.416, P 0.02) and PYY levels at 30' (Spearman's rho = 0.460, $p < 0.01$) and 90' (Spearman's rho = 0.395, $p = 0.03$) after SLMM ingestion (Fig. 2).

Table 2. Gastrointestinal hormone levels and response to the standard liquid mixed meal

	SG patients			BPD/RYGB-LL patients		
	WR group (n = 6)	WM group (n = 6)	*p*-value	WR group (n = 10)	WM group (n = 10)	*p*-value
Ghrelin-0' [ng/ml]	1.60 (0.83)	1.80 (2.33)	0.394	1.63 (0.19)	1.62 ±0.11	0.436
Ghrelin-30' [ng/ml]	1.61 (0.91)	1.61 (2.36)	0.937	1.64 (0.21)	1.62 ±0.09	0.436
Ghrelin-90' [ng/ml]	1.63 (0.80)	1.67 (2.00)	0.818	1.60 (0.18)	1.63 ±0.11	0.684
iAUC Ghrelin [ng × ml^{-1} × min]	2.3 ±2.6	−10.6 ±10.8	0.032	0.65 (3.63)	0.03 ±5.08	0.971
GLP-1-0' [pmol/l]	34 ±10	31 (39)	0.310	31 (4)	30 ±2	0.631
GLP-1-30' [pmol/l]	34 ±10	30 (46)	0.818	31 (5)	30 ±1	0.436
GLP-1-90' [pmol/l]	33 ±11	30 (44)	0.485	31 (5)	30 ±2	0.481
iAUC GLP-1 [pmol × l^{-1} × min]	29 ±54	−70 (544)	0.180	13 ±45	19 ±50	0.783
PYY-0' [pg/ml]	124 (101)	119 (157)	0.310	123 (39)	120 ±20	0.165
PYY-30' [pg/ml]	127 (116)	116 (166)	0.310	125 (37)	119 ±19	0.089
PYY-90' [pg/ml]	134 (125)	114 (167)	0.394	128 (37)	120 ±19	0.052
iAUC PYY [pg × ml^{-1} × min]	276 (1686)	−132 ±109	0.093	273 ±630	−42 ±649	0.286

GLP-1 – glucagon-like peptide-1, iAUC – incremental area under the curve, PYY – peptide YY

Table 3. Clinical characteristics, peptide YY levels, and response to the standard liquid mixed meal of patients, regardless of the type of bariatric surgery

	WR group (*n* = 16)	WM group (*n* = 16)	*p-value*
Gender: female/male [*n* (%)]	11 (68.8)/5 (31.2)	11 (68.8)/5 (31.2)	1.000
Type of surgery: SG/BPD/RYGB-LL [*n* (%)]	6 (37.5)/10 (62.5)	6 (37.5)/10 (62.5)	1.000
Age [year] at surgery	36.1 ±9.8	33.7 ±7.0	0.432
Status of diabetes mellitus: diabetic/non-diabetic [*n* (%)]	3 (18.8)/13 (81.2)	3 (18.8)/13 (81.2)	1.000
Preoperative BMI [kg/m²]	47.7 ±6.3	50.1 ±8.4	0.362
Preoperative weight [kg]	136.8 ±23.0	148.2 ±33.7	0.271
Preoperative height [cm]	167.5 (12.0)	171.3 ±9.4	0.590
Ideal weight [kg]	61.1 (10.6)	63.2 (10.1)	0.696
Excess weight [kg]	73.2 ±19.8	83.0 ±28.1	0.262
Nadir weight [kg]	73.7 ±7.1	71.8 (15.9)	0.423
Time [year] to achieve nadir weight	1.5 (1.0)	1.8 (7.2)	0.210
Minimum BMI [kg/m²]	25.8 ±2.6	25.0 ±2.7	0.374
Maximum %EWL	86.1 ±10.0	89.5 ±10.8	0.370
Time [year] after surgery in the evaluation	10.0 ±1.9	9.7 ±1.5	0.645
Weight [kg] in the evaluation	95.0 ±9.7	78.6 ±13.7	0.001
%EWL in the evaluation	54.4 18.1	82.8 ±11.7	< 0.001
BMI [kg/m²] in the evaluation	33.3 ±3.7	26.7 ±3.1	< 0.001
Increase in BMI in the evaluation (BMI in the evaluation minus minimum BMI) [kg/m²]	7.5 ±2.6	1.7 ±1.2	< 0.001
Weight [kg] regain in the evaluation	21.2 ±7.2	5.1 ±3.5	< 0.001
PYY-0' [pg/ml]	123 (61)	118 (20)	0.094
PYY-30' [pg/ml]	126 (61)	114 (22)	0.043
PYY-90' [pg/ml]	128 (65)	116 (18)	0.035
iAUC PYY [pg × ml⁻¹ × min]	184 (887)	−76 ±688	0.051

BMI – body mass index, , iAUC – incremental area under the curve, PYY – peptide YY

Discussion

In the present study, we compared GI hormone secretion in patients with and without weight regain during long-term (7+ years) follow-up after 2 very different types of bariatric surgery. Sleeve gastrectomy restricts food intake and has been shown to accelerate the delivery of nutrients to the distal gut. BPD/RYGB-LL also restricts food intake, but in addition a large part of the small intestine is bypassed and nutrient absorption occurs mainly within 100 cm of the terminal ileum. Both types of surgery have been shown to reduce fasting ghrelin levels and increase postprandial GLP-1 and PYY release. This pattern of GI hormone secretion is thought to contribute to initial weight loss after bariatric surgery [18]. However, our data did not show increased fasting ghrelin levels or diminished postprandial GLP-1 and PYY release in patients with long-term weight regain compared to patients without weight regain after bariatric surgery.

Much to our surprise, and regardless of the type of surgery and the degree of weight regain, postprandial GI hormone levels after SLMM ingestion did not differ significantly compared to fasting levels. As mentioned above, these results were validated after measurements with different commercial ELISA kits. Both SG and RYGB are characterized by exaggerated postprandial GLP-1 and PYY release from the first postoperative days [9, 11]. Although enhanced postprandial release of these anorexic hormones is thought to persist indefinitely, there is some evidence that it may be less pronounced after the first postoperative year [19, 20]. Furthermore, Dar *et al.* showed similar GLP-1 levels at 30' after a mixed meal challenge between patients with type 2 diabetes who had undergone RYGB more than 10 years ago and lean, obese, and type 2 diabetic controls [21]. Taking into account that the measurement of GI hormones in our study was performed several years after the initial bariatric surgery (mean follow-up: 9.8 ±1.7 years) and that the first time point for measurements was 30' after SLMM ingestion, the above observations may interpret our results.

No difference in fasting ghrelin levels was observed between the WR group and the WM group after either SG or BPD/RYGB-LL during long-term follow-up. Although ghrelin is considered an orexigenic hormone, previous studies have shown similar fasting ghrelin levels between patients with good and poor
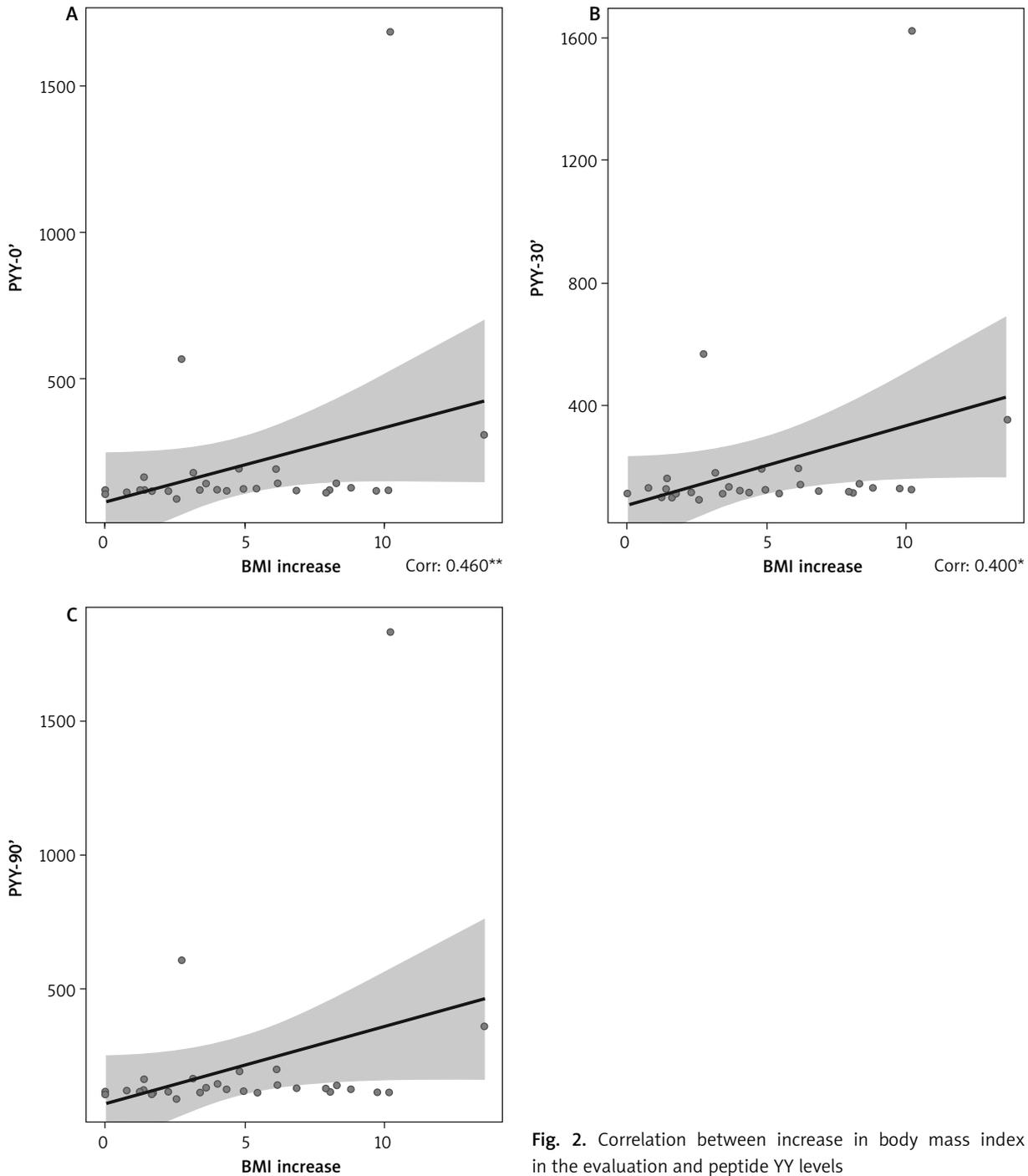
A

B

C

Fig. 2. Correlation between increase in body mass index in the evaluation and peptide YY levels

weight loss outcomes or higher levels in patients with good weight loss outcomes 2–12 years after RYGB [13, 22–26]. Only Bohdjalian *et al.* showed slightly higher, but statistically non-significant, fasting ghrelin levels in a small group of patients with weight regain 5 years after SG [27]. Despite the fact that no difference in fasting and postprandial ghrelin levels was found between the 2 groups, our data showed greater postprandial ghrelin suppression in patients with successful long-term weight loss maintenance after SG. This finding is obviously due to the slightly (not

statistically significant) higher fasting ghrelin levels in the WM group and should be interpreted cautiously. Although there is no clear data, postprandial ghrelin suppression does not appear to be greater during the first weeks after SG or RYGB compared to preoperatively [28–32]. On the other hand, postprandial ghrelin suppression has been shown to be greater at 12 months after RYGB compared to preoperatively [31, 32]. This happens when the loss of a significant proportion of excess body weight has been achieved. In other words, greater postprandial ghrelin suppression should be con-

sidered a consequence rather than a cause of weight loss after bariatric surgery. These observations do not support the hypothesis that ghrelin plays a major role as a determinant of good weight loss outcomes after bariatric surgery.

Enhanced postprandial GLP-1 release has been consistently associated with successful weight loss and successful weight loss maintenance 2–6 years after RYGB [12, 13, 22–24]. In addition, GLP-1 receptor agonists used to treat diabetes type 2 have been shown to produce weight loss compared to controls [33]. The above observations suggest that altered GLP-1 secretion may play a crucial role in weight loss after bariatric surgery. However, our data showed no differences in GLP-1 levels and GLP-1 response to SLMM between the WR group and the WM group after either SG or BPD/RYGB-LL during long-term follow-up. Recently, Sima et al. studied secretion of GI hormones in weight responders and non-responders at a mean of 11.7 years after RYGB and found similar active GLP-1 responses to oral glucose tolerance tests in both groups [25]. These results are similar to ours and indicate that long-term weight loss outcomes after bariatric surgery may not be determined by GLP-1 secretion.

Enhanced postprandial PYY release has also been associated with successful weight loss and successful weight loss maintenance 2–6 years after RYGB in some studies, but not in all [12, 13, 22, 23]. As in the case of GLP-1, Sima et al. found no differences in total PYY responses to oral glucose tolerance test in responders and non-responders at a mean of 11.7 years after RYGB [25]. Interestingly, our data showed higher postprandial PYY levels in the WR group compared to the WM group when patients were examined, regardless of the type of bariatric surgery they had undergone. Also, PYY levels were correlated positively with the increase in BMI in the evaluation. Contrary to what has been previously stated [34], favourable long-term weight loss outcomes following bariatric surgery may not be associated with increased postprandial PYY levels.

Patients undergoing bariatric surgery initially experience progressive and continuous weight loss. Minimum weight is usually achieved 18 to 24 months postoperatively followed by varying degrees of weight regain. The onset of weight regain from the second postoperative year resembles the weight regain seen in patients with short bowel syndrome and may be due to intestinal adaptation. Indeed, intestinal adaptation in adults with short bowel syndrome usually occurs 2 years after small bowel resection and is characterized by improved intestinal absorption, increased hormonal secretion, development of hyperphagia, and gut microbiota dysbiosis [35]. Such adaptive phenomena may be responsible, at least in part, for weight regain after bariatric surgery. One hypothesis to be considered further is that initial weight loss and long-term weight regain are independent phenomena regulated by different pathophysiological pathways.

Limitations

We acknowledge that our study has some limitations, possibly confounding interpretation of the results. The cross-sectional design of our study and the lack of longitudinal data limited our ability to draw definite conclusions about the relationship between evaluated GI hormones and weight regain. The study design does not allow the assessment of the effect of weight loss on GI hormone secretion, nor the assessment of the effect of GI hormone secretion on maximum weight loss postoperatively. The measurement of GI hormones at only 2 time points after SLMM ingestion may be another limiting factor for our study. One of the 2 types of bariatric surgery we studied was BPD/RYGB-LL. BPD/RYGB-LL is considered a less "restrictive" and more "malabsorptive" bariatric surgery compared to standard RYGB, which is widely performed nowadays. In addition, although both groups of patients who had undergone BPD/RYGB-LL differed in the degree of weight regain, they did not differ significantly in weight in the evaluation.

Conclusions

In conclusion, our data do not show that patients with long-term weight regain after bariatric surgery are characterized by increased fasting ghrelin levels or decreased postprandial GLP-1 and PYY levels compared to patients with successful weight loss maintenance. This indicates that ghrelin, GLP-1, and PYY may not play a major role as determinants of long-term weight regain, and other mechanisms than those contributing to the initial rapid weight loss may be involved in the process of weight regain after bariatric surgery. Further research is needed to investigate the importance of differences in GI hormone secretion after SG and BPD/RYGB-LL in order to accurately determine the role of them in the long-term weight loss maintenance and weight regain after bariatric surgery.

Disclosure

The authors report no conflict of interest.

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