CLINICAL RESEARCH

Effects of Sleeve Gastrectomy and Medical Treatment for Obesity on Glucagon-like Peptide 1 Levels and Glucose Homeostasis in Non-diabetic Subjects

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Abstract

Background The effects of medical and surgical treatments for obesity on glucose metabolism and glucagon-like peptide 1 (GLP-1) levels independent of weight loss remain unclear. This study aims to assess plasma glucose levels, insulin sensitivity and secretion, and GLP-1 levels before and after sleeve gastrectomy (SG) or medical treatment (MED) for obesity.

Methods This study is a prospective, controlled, non-randomised study. Two groups of non-diabetic obese patients with similar BMIs, including a SG group (BMI, 35.5 ± 0.9 kg/m^2; $n=6$) and a MED group (BMI, 37.7 ± 1.9 kg/m^2; $n=6$) and a group of lean subjects (BMI, 21.7 ± 0.7 kg/m^2; $n=8$).

Results Plasma glucose, insulin, and total GLP-1 levels at fasting and after the intake of a standard liquid meal at baseline and at 2 months post-intervention. At baseline, total GLP-1 levels were similar, but obese patients had lower insulin sensitivity and higher insulin secretion than lean subjects. At 2 months post-intervention, SG and MED patients achieved similar weight loss ($14.4\pm0.8\%$, $15.3\pm 0.9\%$, respectively). Insulin sensitivity increased in SG and

MED patients; however, postprandial insulin secretion decreased after MED, but not after SG. The incremental area under the curve of GLP-1 increased after SG ($P=0.04$), but not after MED.

Conclusions Weight loss by medical or surgical treatment improved insulin sensitivity. However, only MED corrected the hyperinsulinemic postprandial state associated to obesity. Postprandial GLP-1 levels increased significantly after SG without duodenal exclusion, which may explain why insulin secretion did not decrease following this surgery.

Keywords Glucagon-like peptide 1 · Sleeve gastrectomy · Diet · Insulin-resistance · Obesity · Weight loss · Insulin secretion · Surgery for obesity

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Introduction

The increase in obesity prevalence during recent decades has led to a rise in its associated conditions, including type 2 diabetes mellitus (T2D), hypertension, cardiovascular disease, fatty liver disease, and metabolic syndrome [1, 2]. Decreased insulin sensitivity and compensatory hyperinsulinemia are the main precursors to the development of these conditions in obese subjects. In particular, insulin hypersecretion can promote atherosclerosis via multiple mechanisms [3]. Significant weight loss has shown clear benefits in the improvement of all of these conditions [4]; however, medical interventions based on diet, exercise and behavioural therapy are associated with poor long-term results [5]. On the other hand, bariatric surgery results in significant, sustained long-term weight loss. Roux-en-Y gastric bypass (RYGB) and bilio-pancreatic diversion

(BPD) procedures provide the best results, especially regard to T2D remission [6], but there are concerns about the associated nutritional side effects [7]. Sleeve gastrectomy (SG) is a novel restrictive procedure that has shown positive results without the complaints associated with the derivative surgeries [8]. A recent study suggests that SG, like RYGB and BPD, can modify the secretion of entero-peptides, such as glucagon-like peptide 1 (GLP-1) [9].

GLP-1 is produced from the proglucagon gene in the pancreas, gut, and brain. In each tissue, the peptide undergoes different post-translational processing. This process, plus a rapid enzymatic plasmatic degradation by dipeptidyl peptidase 4, generates many circulating forms of GLP-1, mainly including the following peptides: (1–37), (1–36), (7–36), (7–37), (6–36), (6–37), (9–36), and (9–37). The active hormones, (7–36) and (7–37), represent less than 15% of all GLP-1 peptide forms [10]. The existence of these multiple circulating peptide forms of GLP-1 explains the measurement difficulties and the wide range of plasma concentrations obtained from the different assays employed [11]. However, the postprandial rise of GLP-1 reflects the L-cell response to the meal and accounts for the main effects of this hormone: it stimulates insulin secretion, inhibits glucagon secretion and bowel motility, and reduces appetite and food intake. Additionally, GLP-1 has trophic effects on the beta cell [12]. Thus, increased GLP-1 secretion following bariatric surgery can improve the glycemic control in diabetic patients [13]. However, the benefit of this effect in non-diabetic obese subjects remains controversial.

The aim of this study was to evaluate the metabolic effects of SG in non-diabetic obese subjects relative to a group of non-diabetic obese subjects who achieved a similar weight loss by medical treatment (MED) based on diet and exercise.

Subjects and Methods

Subjects

We designed a non-randomised prospective study with two groups of obese patients from the Obesity Treatment Program of Pontificia Universidad Catolica de Chile School of Medicine. Inclusion criteria were between the age of 18–60 years and a BMI of 30–50 kg/m^2. Exclusion criteria included severe medical conditions or psychiatric illness, substance abuse, previous gastrointestinal surgery, and use of medications known to affect intestinal function. The subjects were studied before and after 2 months of MED or SG. In addition, we evaluated eight healthy lean volunteers (lean group) who were 35.7± 3.0 years old (23–48) with a BMI of 21.3±0.7 kg/m^2

(18.2–24.5). The Ethics Committee of the Pontificia Universidad Catolica de Chile approved this study, and all patients provided written informed consent.

Surgical and Medical Treatment Protocols

Surgical and medical treatment methods were published previously [14]. In brief, SG was performed over a 60 French bougie, removing the complete gastric fundus and up to the angle of His together with the major part of the corpus and antrum. In this surgery, patients were not vagotomised. The post-surgical diet included a daily intake of 600–800 kcal during the first month and 1,000 kcal during the second month. MED included a low-calorie diet (20–25 kcal/kg of ideal body weight), a behavioural approach and a routine of 180 min/week of aerobic and resistance exercise. All subjects were examined by a physician and a registered dietician every 15 days.

Study Protocol

Patients arrived to the study facility at 8 AM, after a 12-h (overnight) fast. Weight and height were recorded. Excess weight was calculated as the difference between current body weight and the weight corresponding to a BMI of 25 kg/m^2. While the subjects were in a seated position, a topical anaesthetic (EMLA cream®; AstraZeneca, Wilmington, DE, USA) was applied to the forearm 15 min before inserting an intravenous catheter. Blood samples were drawn before and at 30, 60, 90, 120, and 180 min after the intake of 237 ml of a standard test meal (STM; Ensure Plus®: 355 kcal, 13 g protein, 50 g carbohydrate, and 11 g fat; Abbott Laboratories, Columbus, OH, USA).

Assays

Glucose was measured by the glucose oxidase method (Human, Wiesbaden, Germany). Insulin was measured by radioimmunoassay (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Plasma GLP-1 concentration was measured by an in-house sandwich-type enzyme-linked immunosorbent assay (ELISA) [15]. Briefly, the plate was coated overnight with anti-GLP-1 antibody, specific for the amidated C-terminus of GLP-1 (7–36) amide and GLP-1 (1–36) amide (HYB 147-06; AntibodyShop®, Denmark). Direct plasma (100 μL/well) was applied and incubated overnight at 4°C. The plate was washed and incubated for 2 h at room temperature with a detection antibody that reacts with all forms of GLP-1 (HYB 147-12B; AntibodyShop®, Denmark). The optical density was read at 450 nm in a multiscan reader (Labsystems Multiscan MS; Labsystems, Finland). The ability to bind GLP-1 in plasma was assessed using prepared standard solutions in the range 0.8–115 ng/dl

with GLP-1 (7–36) amide (Sigma G8147). The assay had an intra-assay variation coefficient of <10% and an inter-assay variation coefficient <10%; it detects all forms of GLP-1 truncated at the N-terminus (36).

Calculations

Indices to assess insulin sensitivity, insulin secretion and beta-cell function were obtained from the standard meal test. The homeostatic model assessment of insulin resistance (HOMA-IR) (Fasting glucose × fasting insulin/405) [16] and ISI(comp) or Matsuda index $(10,000/\sqrt{([(G_0 x I_0) \times (G_{mean} x I_{mean})])})$ [17] were calculated to estimate insulin resistance and sensitivity, respectively. First-phase insulin release $(1,283 + [1.829 \times Ins_{30}] - [138.7 \times Gluc_{30}] + [3.772 \times Ins_0])$ [18] and the area under the curve (AUC) for insulin were calculated to estimate insulin secretion. All of these indices calculated from oral glucose tolerance tests and meal tolerance tests exhibit good correlations with the estimates obtained from euglycemic insulin clamps and intravenous glucose tolerance tests [18–20] and were used in a previous bariatric study [21].

Statistical Analysis

Data are expressed as the mean±standard error of the mean. The Kolmogorov–Smirnov test was used to assess the normality of the residuals. Values for AUC for glucose, insulin, and total GLP-1 after a standard mixed liquid meal were calculated using the trapezoidal method and were compared by ANOVA or paired-samples t test. The incremental AUC for GLP-1 was calculated by using the first observation as the baseline value. Two-way ANOVA followed by a Bonferroni post hoc test was used to analyse the effect of the treatments and time points. Statistical analyses were performed using SPSS 15.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA). A P value <0.05 was considered significant.

Results

Six obese patients were recruited in the MED and SG groups. None of the subjects had T2D or impaired glucose tolerance during the initial evaluation. During the STM, one subject in the MED group and one subject in the SG group had impaired fasting glucose (100.6 and 106.0 mg/dl, respectively). None of the patients had plasma glucose levels >140 mg/dl at 120 min after STM. There were no significant differences between the MED and SG groups in age, weight, and BMI (Table 1).

Table 1 Clinical characteristics of obese groups before and at 2 months after interventions

N	SG 6	MED 6	P value
Baseline			
Age (years)	40.5±2.7	34.1±4.1	0.4
Weight (kg)	91.0±4.4	109.0±11.7	0.3
BMI (kg/m^2)	35.5±0.9	37.7±1.9	0.4
After 2 months			
BMI (kg/m^2)	30.4±0.6	32.0±1.6	0.4
% Weight loss	14.4±0.9	15.3±0.9	0.5
% EWL	48.9±3.2	47.3±4.4	0.8

Values are mean±SEM

BMI body mass index, EWL excess weight loss, MED medical treatment, SG sleeve gastrectomy

Effects on Weight Two months after the initial evaluation, each intervention was followed by a marked reduction in weight and BMI, without significant differences between groups in percentage of weight loss, although all the patients remained obese (Table 1).

Effects on Glycaemia Plasma glucose levels during STM are shown in Fig. 1a. Before the interventions, there was no difference in fasting plasma glucose levels between the MED, SG, and lean groups. Two months after the intervention, we observed a reduction in the fasting glucose levels of SG patients (Table 2 and Fig. 1a).

Effects on Insulin Sensitivity At baseline, the two obese groups had higher HOMA-IR and lower and ISI(comp) indices than the lean group, and there were no significant differences between the SG and MED groups (Table 2). After the interventions, both obese groups underwent a clear and similar diminution in HOMA-IR and a significant increase in ISI(comp) index, resulting in improved insulin sensitivity (Table 2, Fig. 2a–b).

Effects on Insulin Secretion Serum insulin levels during STM are shown in Fig. 1b. At baseline, the AUC for insulin was higher in the SG than in the MED group, and was higher in both obese groups compared to the lean group. The SG group had a first-phase insulin release that was higher than the lean group, but was not significantly different from that of the MED group (Table 2). The interventions were followed by a significant decrease in the AUC for insulin in MED subjects, but did not change in SG group (Table 2 and Fig. 2c). Furthermore, 2 months after the interventions, the SG group had a significantly higher first phase of insulin secretion than the MED group;

Fig. 1 Plasma glucose levels (**a**) and insulin serum levels (**b**) in response to STM in SG and MED groups before and at 2 months after interventions
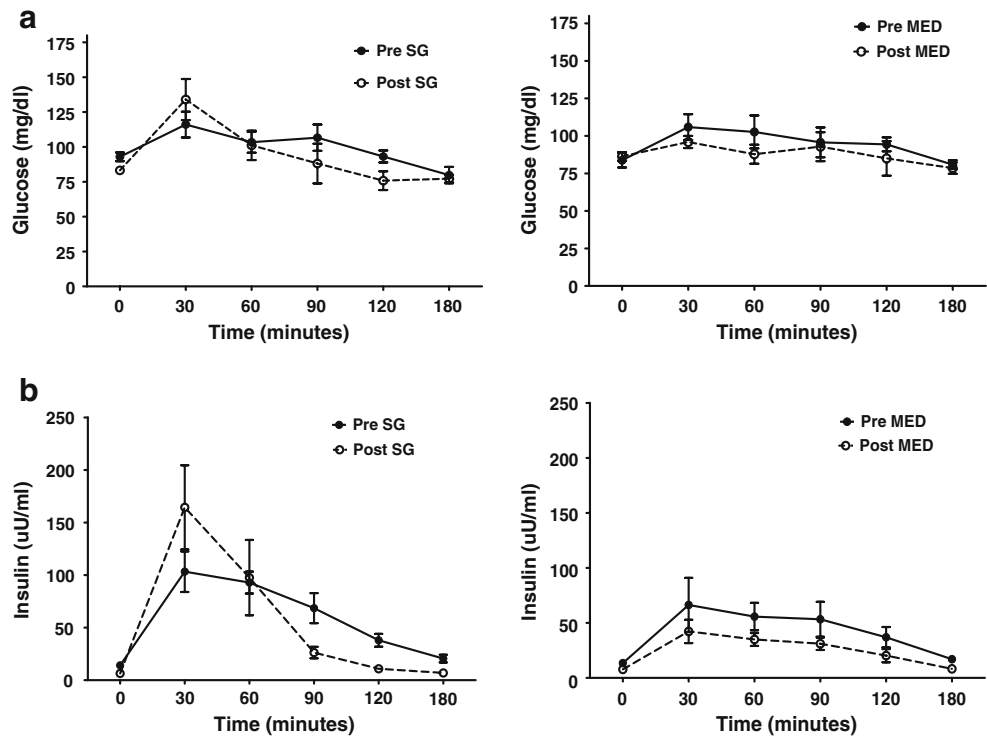
this difference was produced by a slight increase in the first phase of insulin secretion after SG and a slight decrease in the first phase of insulin secretion following MED (Table 2).

Effects on GLP-1 There were no differences between the three groups with regard to fasting total GLP-1 levels at baseline or after the interventions (Table 3). Following SG, there were increases in GLP-1 levels at 30 min after STM

Table 2 Insulin sensitivity and insulin secretion indices obtained from STM in lean, MED and SG groups before and at 2 months after interventions

	Lean $N=8$	SG $N=6$	MED $N=6$	P value[a]	P value[b]
Fasting glucose (mg/dl)					
Baseline	82.5±1.5	92.9±3.1	83.8±4.8	0.07	0.1
After 2 months		83.2±2.0	86.3±2.9		0.4
P value[c]		0.05	0.5		
HOMA-IR					
Baseline	1.35±0.09	3.27±0.26	2.82±0.33	0.0001	0.3
After 2 months		1.33±0.15	1.67±0.23		0.3
P value[c]		0.002	0.03		
ISI(comp)					
Baseline	11.50±1.0	3.87±0.44	5.42±0.86	0.0001	0.1
After 2 months		7.12±0.89	9.08±1.0		0.2
P value[c]		0.008	0.02		
Insulin AUC (uU•ml^{-1}•min^{-1})					
Baseline	3,869±432	10,476±983	7,636±1153		<0.001
After 2 months		9,439±2164	4,523±600		0.07
P value[c]		0.6	0.02		
First-phase insulin release					
Baseline	1,236±105	2,127±207	1,702±309	0.002	0.3
After 2 months		2,581±414	1,307±149		0.02
P value[c]		0.4	0.3		

Values are mean±SEM

AUC area under the curve

[a] *P* value for the difference between obese groups and lean group

[b] *P* value for the difference between SG and MED groups

[c] *P* value for the effect of weight loss within each group

Fig. 2 Indices for insulin resistance (**a** HOMA-IR), insulin sensitivity (**b** ISI(comp)), insulin secretion (**c** insulin AUC and **d** first-phase insulin release) obtained from STM in lean, SG and MED groups before and at 2 months after interventions
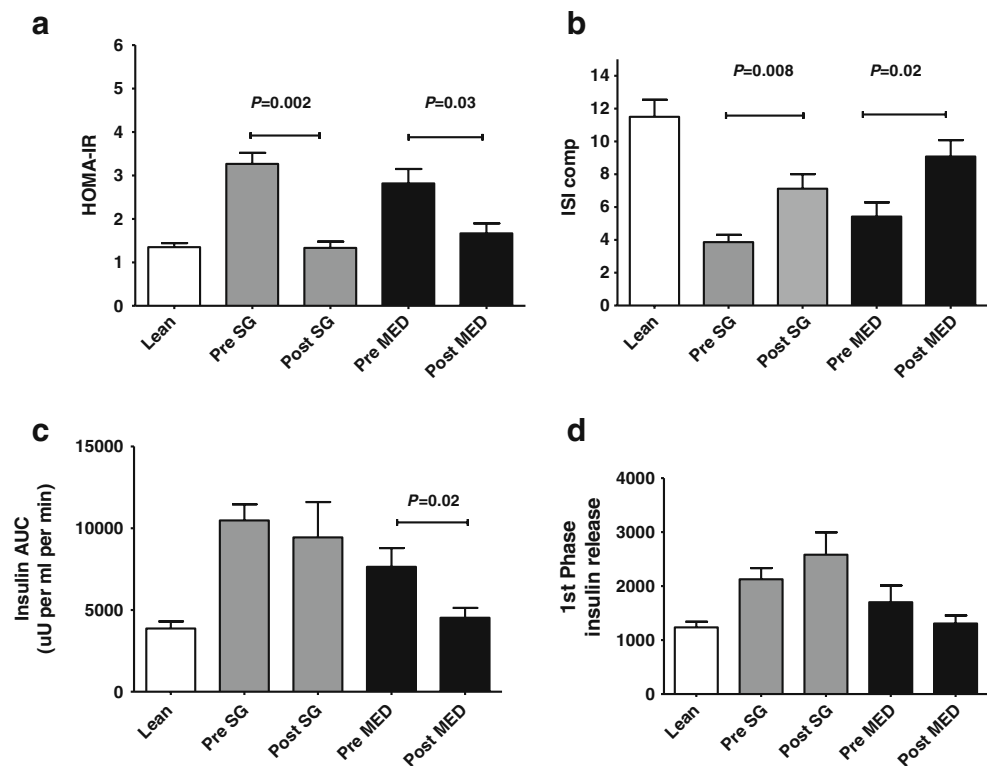
(Fig. 3a) and in the incremental AUC for GLP-1 (Fig. 3c) compared to baseline values. In the MED group, we did not observe changes in GLP-1 levels (Fig. 3b and c). We observed a no significant correlations between the first phase of insulin secretion and the GLP-1 AUC (r=0.5, P= 0.07) and between the insulin AUC and the GLP-1 AUC (r= 0.57, P=0.05), and there was a significant correlation between the change in the first phase of insulin secretion and the change in the GLP-1 AUC (r=0.58, P=0.04).

Discussion

In this study, we compared the effects of the weight loss on glucose homeostasis and GLP-1 levels in two groups of non-diabetic obese subjects who underwent SG, a restrictive surgery, or a MED. Both groups of obese patients had a decrease in insulin sensitivity compared to lean subjects, which improved significantly after each intervention. Specifically, subjects who underwent SG reached values

Table 3 Total GLP-1 values in lean, MED and SG groups before and at 2 months after interventions

|	Lean N=8	SG N=6	MED N=6	P value[a]	P value[b]
Fasting GLP-1 Total (ng/dl)					
Baseline	39.9±9.4	22.3±3.9	22.5±5.6	0.2	0.9
After 2 months		22.1±6.4	20.6±5.4		0.9
P value[c]		0.9	0.3		
GLP-1 Incremental AUC (ng•dl^{-1}•min^{-1})					
Baseline	625±638	2,002±497	1,492±463	0.2	0.5
After 2 months		7,221±2,065	949±171		0.01
P value[c]		0.04	0.3		

Values are mean±SEM

AUC area under the curve

[a] P value for the difference between obese groups and lean group

[b] P value for the difference between SG and MED groups

[c] P value for the effect of weight loss within each group

Fig. 3 Total GLP-1 levels in response to STM in SG (a) and MED (b) groups and incremental AUC of GLP-1 (c) in lean, SG and MED groups before and at 2 months after interventions. *P value<0.05 for comparisons at different time-points before and after intervention (ANOVA for repeated measures followed by Bonferroni post hoc analysis)
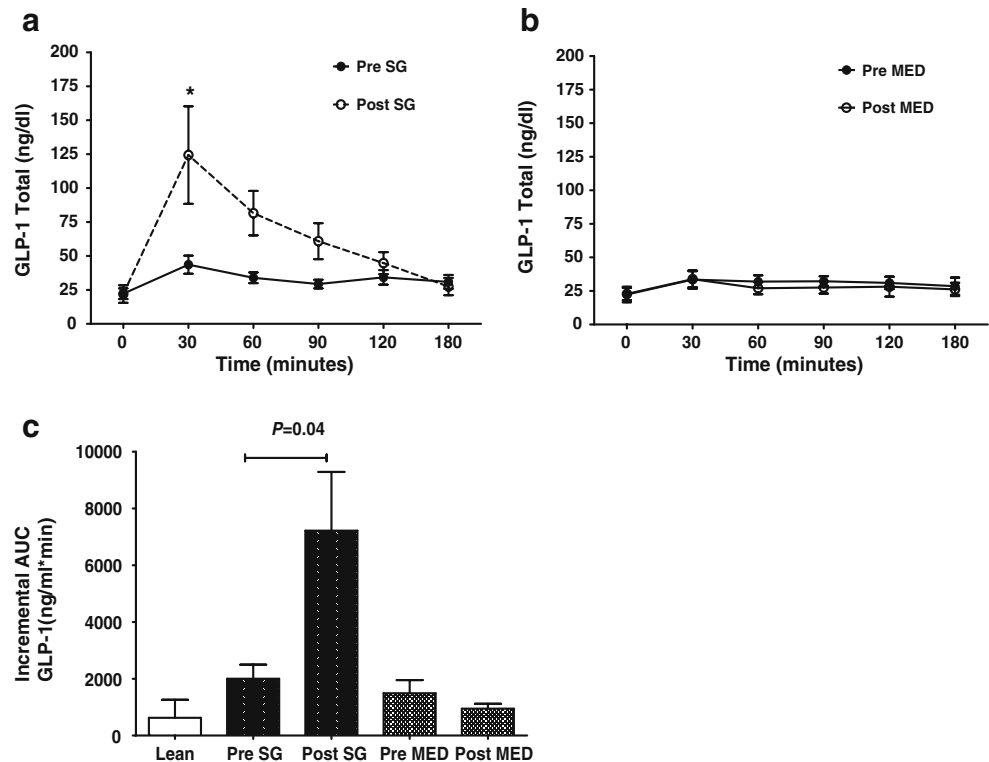
of HOMA-IR values similar to those of the lean group despite achieving a weight loss similar to that of the MED group, although they remained obese. A greater diminution of fasting glycemia in the SG group can explain this finding. A different effect on insulin secretion was founded between the SG and MED groups. Two months after these interventions, only subjects who reduced their weight by diet and exercise corrected the hyperinsulinemic postprandial state. Moreover, obese subjects who underwent SG exhibited a significantly higher insulin secretion than MED patients. Recent findings show that when obese normoglycemic subjects exhibit insulin hypersecretion, this decrease after weight loss due to the hyperbolic relationship between the insulin sensitivity and insulin secretion. This change has been observed after diet and some bariatric procedures [22]. Pereira et al. [23] described this phenomenon in nine patients at 6 and 14 months after undergoing vertical-banded gastroplasty-gastric bypass procedure. Jimenez et al. [24] observed a normalisation of the insulin response at 3and 12 months following vertical gastroplasty, and Letiexhe et al. [25] reported a similar finding in 12 obese non-diabetic subjects at 8 months after vertical-banded gastroplasty. Therefore, our finding that SG had a different effect on insulin secretion compared to MED is remarkable, since both groups of obese patients exhibited a similar weight loss. The different changes in GLP-1 secretion, a known insulinotropic hormone, may explain this observation.

In the current study, a clear increase in postprandial GLP-1 levels was observed at 2 months SG. This GLP-1 response reached supraphysiological levels in SG patients compared to lean subjects. This finding has been recently described by Peterli et al. [9]. Weight loss by MED failed to induce any change in fasting and postprandial GLP-1 levels. On the other hand, elevated postprandial GLP-1 levels have been described after RYGB [13]; however, the effect on insulin secretion differed if the subjects were normoglycemic or diabetic. Indeed, in normoglycemic obese patients with insulin hypersecretion, RYGB and BPD reduced the insulin response as it was observed after MED [26, 27]. In these studies, insulin secretion was determined using an intravenous glucose tolerance test (IVGTT), with a load that does not elicit the GLP-1 secretion and the incretin effect. Unfortunately, Morinigo et al. [26] did not report insulin secretion indices obtained from oral glucose tolerance test (OGTT), but Laferrere et al. [28] observed increased insulin levels at 15, 30, 45, and 60 min during OGTT 1 month after RYGB, without any changes in IVGTT. Therefore, if we would have performed an IVGTT in our patients after SG, we might have observed a different effect on insulin secretion compared to that observed in the STM.

Other mechanisms could affect glucose homeostasis after bariatric surgery. Faster gastric emptying has been described after RYGB and SG procedures, especially for liquid meals [29, 30]. This is very important because gastric emptying delay is one of the mechanisms employed by GLP-1 to reduce postprandial glucose levels [31]. The absence of change in the plasma glucose peak after SG in

the current study suggests that this effect was missing. On the other hand, faster gastric emptying might contribute the maintenance of a higher first-phase insulin release due to the insulinotropic effect of nutrients. However, it is difficult to distinguish between the nutrient insulinotropic effect and the GLP-1 effect on insulin secretion, which is beyond the scope of the current study.

Other entero-peptides may have been responsible for the finding in this study. Ghrelin, which exhibits severely decreased levels after SG [32], can reduce glucose-stimulated insulin secretion and glucose disappearance in healthy humans [33, 34]. Glucose insulinotropic peptide (GIP) postprandial levels increase after restrictive procedures, but do not change after derivative surgeries [35]. The insulinotropic and hypoglycaemic effects of GIP are well known [12]. Glucagon-like peptide 2 (GLP-2) can stimulate glucagon secretion and therefore regulates glycemic status [36, 37]. However, the effect of SG on GIP and GLP-2 levels has not yet been reported.

The increase in postprandial GLP-1 levels after SG could be explained by the prompt arrival of undigested nutrients secondary to accelerated gastric emptying, suggesting that food transit through the duodenum does not have an anti-incretin effect. Moreover, the SG procedure may modify some signals capable of altering postprandial GLP-1 secretion. Ghrelin, GIP and GLP-2 could be candidate modifiers; however, the effect of these hormones on GLP-1 secretion has not been clearly established. Some studies suggest that GIP may regulate GLP-1 secretion via a paracrine mechanism [38]. Further studies are needed to explain the increase in postprandial GLP-1 levels after SG.

This study has some weaknesses. Although there are no significant differences between the SG and MED groups at baseline in age, glucose and insulin levels, the SG subjects were older, had higher glucose levels and were more insulin resistant. The current study was designed with a short-term follow-up period in order to obtain a MED group that exhibited a similar degree of weight loss as a surgical treatment group. This was a non-randomised study because the risks and results associated with the alternative treatments were considered too different. The small sample size and the absence of other methods for evaluating beta cell function, limited the strength of the conclusions. Furthermore, the home-made sandwich-type ELISA employed to measure GLP-1 levels detects several forms of this peptide at concentrations similar to the early determinations reported [39] but higher than the values described for the recent specific radio-immunoassay methods [11].

We conclude that MED, which is based on diet and exercise, and SG treatments for obesity have similar effects on insulin sensitivity, but induce different changes in postprandial insulin secretion and GLP-1 levels. SG increases postprandial GLP-1 levels in the same manner as other bariatric procedures while maintaining nutrient transit by the duodenum. More studies focus on the entero-hormonal changes that may occur after SG surgeries are needed.

Disclosure Statement   The authors have nothing to disclose.

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