Comparative Effects of Roux-en-Y Gastric Bypass and Sleeve Gastrectomy on Glucose Homeostasis and Incretin Hormones in Obese Type 2 Diabetic Patients: A One-Year Prospective Study

Abstract
The aim of the work was to compare the hormonal and the metabolic mechanisms involved in weight loss and remission of T2DM one year after Roux-en-Y gastric bypass (RYGB) and vertical sleeve gastrectomy (VSG) in morbidly obese type 2 diabetic (T2DM) patients. Insulin sensitivity, insulin secretion, insulin secretion, and insulin secretion improved similarly after either procedures with a similar rate in T2DM remission (86% in RYGB and 76% in VSG).

Introduction
The increasing epidemic of obesity and type 2 diabetes (T2DM) is long recognized as a major healthcare problem. Bariatric surgery has emerged as the most successful therapeutic option for morbid obesity, since it results in remarkable and sustained weight loss and a dramatic improvement of glucose control in patients with T2DM [1]. The improvement/resolution of T2DM is associated with the extent of weight loss and the type of surgery ranging from 55% after restrictive procedures to 95% after malabsorptive interventions [2]. The beneficial effects on glucose metabolism occur early after surgery, before any substantial weight loss, suggesting a role of weight loss-independent mechanisms, possibly related to changes in gastrointestinal (GI) hormones in response to ingested nutrients [3–5]. There is now ample evidence that both obesity and T2DM are associated with impaired secretion/action of GI hormones, namely glucagon-like peptide 1 (GLP-1) and glucose-depend-
remission similar to that of RYGB, but without the potential complications inherent to intestinal bypass procedures [7]. Previous studies have examined the effects of the 2 procedures with regard to diabetes remission or the changes in GI hormones, but limited information is available on the contribution of intervention-specific changes in GI hormonal pattern to the remission of diabetes. To this end, in this prospective study we compared the changes in insulin sensitivity, insulin secretion, and post-meal GI hormone levels in obese patients with T2DM 1-year after VSG or RYGB, to evaluate the hormonal and metabolic mechanisms involved in weight loss and remission of T2DM.

Materials and Methods

Selection and description of participants
The study group included 33 obese patients with T2DM (M/F: 14/19; mean age: 46 ± 9 years, BMI: 44 ± 8 kg/m²), who were on a waiting list for bariatric surgery. Inclusion criteria included: age 30–65 years, body mass index (BMI) > 40 or ≥ 35 kg/m² with uncontrolled T2DM under medical treatment, no contraindications to VSG or RYGB. The choice of surgical procedure was made by the patient together with the surgeon after a full explanation of the risks and benefits of each procedure. In total, 14 subjects underwent RYGB and 19 subjects underwent VSG. All participants were examined by a multidisciplinary and integrated medical team consisting of a diabetologist, a bariatric surgeon, a psychiatrist, and a dietician. The clinical and metabolic evaluation of participants were conducted at the Department of Clinical Medicine and Surgery of Federico II University while surgical procedures were performed at the Department of Surgery, San Giovanni Bosco Hospital, Naples. Antidiabetic treatment was oral hypoglycemic agents (OAD) in 24 patients, combined OAD plus bedtime insulin in 5 patients and diet alone in 4 patients. None was on multiple insulin injection regimen. Fourteen patients (74%) in VSG and 9 (64%) in RYGB were on antihypertensive drugs; 5 patients (36%) in the RYGB group and 3 patients (16%) in the VSG group were on hypolipidemic treatment. The protocol was approved by the local Ethics Committee; all patients were informed of the risks and benefits of each procedure and provided written, informed consent before the study.

Study design
Before and one year after the bariatric procedure, anthropometric, clinical and routine laboratory parameters were collected together with data on medication use. On both occasions, in the morning after a 12-h overnight fast, a standard glucose tolerance test (OGTT, 75 g of glucose) was performed to evaluate insulin secretion and insulin sensitivity. The day after, a mixed-meal test (MMT) was performed to evaluate GI hormonal response. The week before the tests all patients consumed a standardized hypocaloric diet (1200Kcal) containing 52% CHO, 18% protein, 30% fat. To avoid possible confounders, OAD were withheld 2 days before the MMT, while long-acting insulin was discontinued for 24h.

Mixed meal test (MMT)
The liquid mixed meal (Resource® ENERGY Nestle Nutrition, 304kcal in total), containing 41 g carbohydrate (glucose), 13 g protein, and 9 g fat, was consumed within a maximum of 15 min. Blood samples were drawn through an indwelling cannula at times 0, 30, 60, 90, 120, and 180 min for the measurement of glucose, insulin, active GLP-1 and total GIP concentrations at 0, 60, 120, and 180 min for the measurement of total ghrelin. Blood samples were collected in BD Vacutainer® EDTA Aprotinin Tube containing K3EDTA (1.6 mg per ml blood) and Aprotinin protease inhibitor (50KIU per ml blood) and immediately centrifuged at +4 °C and 3000 rpm. Plasma samples were stored at −80 °C and rigorously kept at +4 °C during assay. The collection of blood samples with EDTA/Aprotinin under cooled conditions was appropriate to maintain GLP-1 and ghrelin stability as described by Di Marino et al. [8] and Tvarijonaviciute et al. [9].

Operative techniques
All operations were performed laparoscopically by the same surgery team, as previously described [10]. There were no major intra-operative complications or conversions to laparotomy.

Analytical procedures
Plasma glucose concentration was measured by the glucose oxidase method. Plasma insulin and C-peptide were determined by ELISA. Plasma lipids were measured by Roche Cobas analyzer using a colorimetric assay. HbA1c was measured by high-performance liquid chromatography [Diamat HPLC, Bio-Rad Laboratories (Canada) Ltd., Mississauga, Canada] [11].

Active GLP-1 was assayed by a nonradioactive, highly specific sandwich ELISA method (Merck-Millipore, Darmstadt, Germany) with 100% cross-reactivity with 7–36 amide and 7–37 glycine-extended, but no reactivity with 9–36 amide and 9–37 glycine-extended GLP-1 isomers, GLP-2 or glucagon. Total GIP was assayed by a nonradioactive highly specific sandwich ELISA method with (Merck-Millipore, Darmstadt, Germany) 100% cross-reactivity to human GIP (1–42) and GIP (3–42). Human total ghrelin (both intact and des-octanoyl forms) was assayed by a nonradioactive, highly specific sandwich ELISA method (Merck-Millipore, Darmstadt, Germany) with 100% cross-reactivity with des-octanoyl human ghrelin, 80% human ghrelin (active), and 70% canine ghrelin (active). The intra- and intersay coefficient of variation for the GLP-1 assay was <5% and for GIP and ghrelin assays was <10%.

Measurements
Weight loss was expressed as % change in BMI and as percent excess weight loss (%EWL) calculated by the following formula: (preoperative weight – current weight)/(preoperative weight – ideal weight)×100 [12]. Insulin sensitivity and insulin secretion indexes were derived from glucose, insulin, and C-peptide values measured every 30 min for 3 h during the OGTT. Insulin sensitivity was assessed as the oral glucose insulin sensitivity (OGIS), which has been validated vs. the hyperinsulinemic euglycemic clamp demonstrating a good correlation between the 2 measurements of insulin sensitivity [13]. Insulin secretion as total amount of the hormone released by the beta cells (ISR) was calculated from C-peptide with the deconvolution method [14]. Beta-cell function, which reflects the release of the hormone normalized to the glycemnic stimulus, was assessed as “early” (IGIearly=ratio between incremental C-peptide concentration and incremental glucose concentration at 30 min) and “total” insulinogenic index (IGItotal=AUCCpeptide/AUCGlucose). The interplay between insulin sensitivity and secretion, that describes the beta-cell adaptive response to changes of insulin sensitivity, was determined by the product OGIS×AUCCpeptide (adaptation index, Al) [15].
Estimation of insulin secretion was based on plasma C-peptide concentrations (prehepatic) to circumvent possible changes in insulin clearance after surgery, which may influence peripheral insulin concentrations. The hormonal responses to the mixed meal were evaluated as the incremental area under the curves (IAUC) for 3 h, calculated with the trapezoidal rule. IAUC was also assessed as maximal increase (peak) during MMT. The remission of diabetes (partial plus total) was defined as a HbA1c < 42.1 mmol/l and fasting glucose < 125 mg/dl in the absence of antidiabetic medications. Complete remission was defined as a HbA1c < 42.1 mmol/l and fasting glucose < 125 mg/dl in the absence of antidiabetic medications.

Statistical analysis
All statistical analyses were performed using the statistical software package, SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as means ± standard deviation. Group comparisons were performed by the χ2 test for categorical variables, the Wilcoxon test or the Mann-Whitney test. Time course effect of glucose and hormones was analyzed by general linear model (GLM) for repeated-measures with the aim of testing whether the changes in hormone levels between pre- and post-surgery differed among types of surgery at each time point of MMT. GLM included weight loss (EWL %) as covariate. A p-value < 0.05 was considered statistically significant.

Results

Weight loss and metabolic control

Table 1 provides the main clinical and metabolic characteristics of participants before and one year after surgery. Age, BMI, duration of diabetes, glucose control, and lipid profile at baseline were similar between RYGB and VSG groups. At one-year, weight loss expressed as percent change in BMI was −32 ± 10 % after RYGB and −30 ± 7 % after VSG (p = 0.546). Likewise, excess weight loss (EWL %) was similar after the 2 interventions (78 ± 15 and 70 ± 23 %, p = 0.146). Antihypertensive medications were discontinued in all except 3 patients of the RYGB group and in 2 patients of the VSG group. Four patients of the RYGB group and one patient of the VSG group discontinued hypolipidemic treatment.

Insulin secretion and insulin sensitivity (OGTT)

Table 2 provides the main clinical and metabolic characteristics of participants before and one year after surgery. Age, BMI, duration of diabetes, glucose control, and lipid profile at baseline were similar between RYGB and VSG groups. At one-year, weight loss expressed as percent change in BMI was −32 ± 10 % after RYGB and −30 ± 7 % after VSG (p = 0.546). Likewise, excess weight loss (EWL %) was similar after the 2 interventions (78 ± 15 and 70 ± 23 %, p = 0.146). Antihypertensive medications were discontinued in all except 3 patients of the RYGB group and in 2 patients of the VSG group. Four patients of the RYGB group and one patient of the VSG group discontinued hypolipidemic treatment.

Glucose and hormone profile (MMT)

IAUC_{Glucose} decreased while IAUC_{Insulin} increased after surgery with no difference between interventions (Fig. 1).
Meal-stimulated GLP-1 concentrations were flat in all patients preoperatively. Following RYGB, both GLP-1 peak and IAUC increased markedly (p = 0.001), while after VSG, the release of GLP-1, although increased compared to presurgery, was much lower in patients operated of RYGB (p = 0.0001). Mead GIP response after surgery decreased by 50 % (p = 0.001 after RYGB and p = 0.05 after VSG) with no difference between interventions. Neither fasting nor nadir ghrelin during MMT changed after RYGB; in contrast, a marked suppression in both variables occurred after VSG with a significant difference between the 2 intervention (p = 0.013 for fasting ghrelin and p = 0.035 for nadir ghrelin concentrations) (\( \text{Fig. 1} \)) and \( \text{Table 2} \). The increase in insulin sensitivity and beta-cell function was correlated with weight loss (\( R = 0.425, p = 0.014 \) and \( R = 0.461, p = 0.035 \), respectively) (\( \text{Fig. 2} \)) while no association was found with GI hormone concentrations.

Discussion and Conclusion

In this study, we evaluated glucose homeostasis and the profile of GI hormones in severely obese patients with T2DM before and one year following either RYGB or VSG – 2 of the most frequently performed bariatric procedures – to gain insight into the physiological mechanisms behind weight loss and remission of T2DM. The 2 interventions resulted to be equally effective in terms of weight loss and improvement of glycemic control, with a similar rate of T2DM remission at 1 year (76 % after VSG and 86 % after RYGB). Actually, the 2 major determinants of glucose homeostasis, that is, beta-cell function and insulin sensitivity, improved to a similar extent after either procedures. Interestingly, total insulin secretion remained unchanged while beta-cell function increased significantly after surgery, indicating an amelioration of the pancreatic glucose sensitivity, since a similar secretion occurs with much lower blood glucose.

These results are in agreement with those of Keidar A et al. [16] and Nannipieri et al. [17] but differ from those of Kashyap et al. [18], Lee et al. [19] and Schauer et al. [20] who demonstrated that RYGB is more effective than VSG in terms of metabolic improvement. Differences in the degree of weight loss achieved with the 2 procedures, study population, length of follow-up and experimental methods to assess metabolic functions may contribute to these variable results. A distinct GI hormonal pattern followed the 2 procedures. After RYGB, we found a marked increase in meal-induced GLP-1 response, a significant reduction in post-meal GIP and ghrelin response. Increased GLP-1 levels are well documented after RYGB, due to the accelerated nutrient entry into the small intestine [3, 4]. Data regarding GIP are more inconsistent, with some studies reporting an increased postprandial GIP level early after RYGB [21] and others no change [22] or even an early decline in fasting GIP levels in diabetic but not in nondiabetic patients [23]. This discrepancy may be due to differences in analytical methods to measure GIP (total vs. active form), the characteristics of the population studied (diabetics vs. nondiabetic), the length of the limbs of the Roux anastomosis and the duration of the follow-up. Following VSG, a marked suppression of both fasting and post-meal ghrelin levels occurred as a consequence of gastric fundus removal; GLP-1 concentration increased although to a much lower extent than RYGB while GIP levels decreased by 50 %. The finding that RYGB and VSG are equally effective on weight loss and metabolic improvement in the face of a different pattern of GI hormone profile, lead us to hypothesize that the changes in gut hormones are not the main determinant of the metabolic improvement, at least several months after surgery. However, since our evaluations were performed one year after surgery we cannot rule out that the changes in GI hormonal profile may have contributed to diabetes remission early after surgery. This hypothesis is in line with a recently published commentary, which underlined that the mechanisms behind the remission of diabetes after VSG or RYGB may differ in relation to the time at which they are studied. Early after surgery, the improvement of glycemic control is due to increased hepatic insulin sensitivity and to the improved beta-cell function conse-

Table 2  Insulin sensitivity and insulin secretion, and glucose and hormonal response to MMT before and one year after surgery.

<table>
<thead>
<tr>
<th></th>
<th>RYGB (n = 14)</th>
<th>VSG (n = 19)</th>
<th>RYGB vs. VSG</th>
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<tbody>
<tr>
<td></td>
<td>Presurgery</td>
<td>One year Δ</td>
<td>Presurgery</td>
</tr>
<tr>
<td>OGTT</td>
<td></td>
<td></td>
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<tr>
<td>IGI 30 min (nmol/l/mg/dl)</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.2 **</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>IGI 180 min (nmol/l/mg/dl)</td>
<td>324 ± 111</td>
<td>1603 ± 1577 *</td>
<td>1279 ± 439</td>
</tr>
<tr>
<td>ISR (nmol/m²)</td>
<td>65 ± 21</td>
<td>58 ± 18</td>
<td>−7 ± 16</td>
</tr>
<tr>
<td>OG1S (ml min⁻¹ m⁻²)</td>
<td>287 ± 90</td>
<td>549 ± 82 **</td>
<td>262 ± 88</td>
</tr>
<tr>
<td>Adaptation index</td>
<td>0.5 ± 0.2</td>
<td>0.7 ± 0.3 *</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>MMT</td>
<td></td>
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<td></td>
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<tr>
<td>Fasting glucose (mg/dl)</td>
<td>173 ± 50</td>
<td>96 ± 13 **</td>
<td>−76 ± 50</td>
</tr>
<tr>
<td>IAUC glucose (mg/dl·180’)</td>
<td>6.2 ± 4.5</td>
<td>3.0 ± 0.347</td>
<td>−3.230 ± 5.710</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>30 ± 18</td>
<td>11 ± 4 *</td>
<td>−19 ± 17</td>
</tr>
<tr>
<td>IAUC insulin (µU/ml·180’)</td>
<td>4.3 ± 5.12</td>
<td>5.118 ± 2.076</td>
<td>−805 ± 2.808</td>
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<tr>
<td>Fasting GIP (pmol/l)</td>
<td>4.7 ± 4.5</td>
<td>4.3 ± 3.7</td>
<td>−0.2 ± 5.1</td>
</tr>
<tr>
<td>Glucagon (pmol/l·180’)</td>
<td>2.7 ± 130</td>
<td>2.119 ± 1.200 **</td>
<td>2.092 ± 1.266</td>
</tr>
<tr>
<td>GLP-1 30-min peak (pmol/l)</td>
<td>7 ± 6</td>
<td>47 ± 22 **</td>
<td>40 ± 21</td>
</tr>
<tr>
<td>Fasting GIP (pg/ml)</td>
<td>108 ± 130</td>
<td>42 ± 19 *</td>
<td>−66 ± 128</td>
</tr>
<tr>
<td>IAUC GIP (pg/ml·180’)</td>
<td>25.344 ± 15.169</td>
<td>13.234 ± 4.713 **</td>
<td>−12110 ± 15.733</td>
</tr>
<tr>
<td>Fasting ghrelin (pg/ml)</td>
<td>391 ± 264</td>
<td>443 ± 169</td>
<td>52 ± 290</td>
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<tr>
<td>Ghrelin nadir (pg/ml)</td>
<td>309 ± 72</td>
<td>278 ± 38</td>
<td>31 ± 75</td>
</tr>
</tbody>
</table>

Data are expressed as means (± SD); OGTT: Oral glucose tolerance test; MMT: Mixed meal test; RYGB: Gastric bypass; VSG: Vertical sleeve gastrectomy; OG1S: Oral glucose insulin sensitivity; ISR: Insulin secretion rate; IGI: Insulinogenic index; AI: Adaptation index

* p<0.05, † p≤0.001 vs. presurgery
quent to the exaggerated postprandial GLP-1 secretion. Later on, with progressive weight loss the improvement in peripheral insulin sensitivity becomes the prevalent mechanism [24]. On the other hand, a number of mechanisms have been highlighted which may contribute to the improvement of glucose tolerance after BS, including neural activation [25], modifications of intestinal microbiota [26], and changes in the expression of genes regulating glucose and fatty-acid metabolism induced by low nutrient availability [27].

A significant reduction in fasting triglycerides occurred in our patients after either procedure, as reported in previous studies [10,28,29], whereas total and LDL-cholesterol decreased after RYGB but not VSG. This finding is in line with recent studies demonstrating that bariatric procedures differentially affect cholesterol metabolism with malabsorptive procedures (biliointestinal bypass) providing a much greater reduction than restrictive surgery independent of weight loss and insulin resistance [30].

A weakness of this study might be the lack of patient randomization to the 2 types of operations. However, since the 2 procedures differ in terms of unwanted effects and frequency of monitoring during follow-up, the patient’s preference should not be ignored. Noteworthy is the fact that the 2 groups were comparable for anthropometric and biochemical measures, as
well as for medication use, thus minimizing the possibility of selection bias.

In conclusion, RYGB and VSG exert similar beneficial effects in terms of weight loss and remission of T2DM in the face of remarkable differences in GI hormone profile. These findings highlight the importance of weight loss and challenge a primary role of incretins in mediating the metabolic improvement achieved in obese patients with T2DM one year after VSG or RYGB.

Conflict of Interest

The authors declare no conflict of interest.

References
