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ARTICLE

Bariatric surgery

Gut hormone release after gastric bypass depends on the length of the biliopancreatic limb

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Abstract

Background/Objectives Changes in gut hormone secretion are important for the anti-diabetic effects of bariatric surgery. Roux-en-Y gastric bypass (RYGB) with extended biliopancreatic limb (BPL) length may improve the metabolic outcomes when compared to the classical procedure. The purpose of this study was to compare the gut hormone responses to a liquid mixed meal after RYGB with one of two different BPL lengths.

Subjects/Methods Non-diabetic weight-stable individuals previously submitted to classical RYGB ($n = 9$; BPL length: 87.8 ± 20.5 cm) or long BPL RYGB ($n = 11$; BPL length: 200 cm) underwent a liquid mixed-meal tolerance test (MMTT). Blood was sampled at baseline and 15, 30, 45, 60, 90 and 120 min later for measurement of plasma glucose, enteropancreatic hormones and total bile acids (TBA).

Results Plasma glucose excursion curves were similar in the two groups. The long BPL RYGB group displayed significantly higher fasting and post-prandial GLP-1 ($t = 0$ min, $p = 0.01$ and $t = 45$ min, $p < 0.05$; tAUC: 11,205 ± 3399 vs 7889 ± 1686 pmol/L × min, $p = 0.02$) and neurotensin ($t = 0$ min, $p = 0.02$; $t = 45$ min, $p < 0.05$ and $t = 60$ min, $p < 0.01$; tAUC: 18,392 ± 7066 vs 11,437 ± 3658 pmol/L × min, $p = 0.02$) levels, while responses of GIP ($t = 15$ min, $p < 0.01$), insulin and C-peptide ($t = 30$ min, $p < 0.001$) were lower as compared to classical RYGB. There were no differences in glucagon, PP, PYY and TBA between the groups.

Conclusions RYGB with a longer BPL results in a distinctive post-prandial hormone profile with augmented GLP-1 and neurotensin responses that could be beneficial for the metabolic outcomes of the surgery.

Introduction

Bariatric surgery, originally developed for severe obesity treatment, appears to be the most effective therapy not only in inducing considerable weight loss but also in improving obesity comorbidities and particularly type 2 diabetes (T2D) [1, 2].

Roux-en-Y gastric bypass (RYGB) surgery is a bariatric procedure extensively performed worldwide. RYGB results in a substantial and sustained weight loss, which was originally thought to be a consequence of the restrictive component derived from the creation of a small gastric pouch and the moderate malabsorption secondary to the partial exclusion of the digestive tract [3], although later the role of endocrine and metabolic changes induced by the operation also became evident [4]. RYGB surgery provides significant weight loss and T2D improvement in patients with a body mass index (BMI) <50 kg/m^2, with a side-effect profile that is more favourable when compared to more effective bariatric procedures, such as biliopancreatic diversion (BPD) or the duodenal switch (DS) [5].

In patients with T2D, weight loss is known to have profound effects on glucose metabolism [6]. Still, after

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procedures such as RYGB and BPD–DS, improvement in T2D is frequently observed before significant weight loss has occurred and the rate of T2D remission is higher than predicted for the extent of the weight reduction [7]. To explain the glycaemic effects of the surgeries two non-mutually exclusive hypotheses were raised, stating that these could be either triggered by excluding the proximal small gut from the intestinal transit or alternatively by the rapid delivery of nutrients to the distal intestine [8–10]. In both cases, the metabolic effects of bariatric surgery would be mediated by distinctive changes induced in the gastrointestinal (GI) hormone secretion profile [11]. In particular, RYGB would attenuate the release of GI hormones from the proximal gut, such as glucose-dependent insulinotropic peptide (GIP), while stimulating the secretion and release of distal GI hormones, such as glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and neurotensin (NT) [12–14]. In fact, favourable changes in GI hormone secretion after RYGB were extensively documented, suggesting that the surgery induces an anorexigenic and anti-diabetic hormonal profile [15]. Thus, by changing the length of the biliopancreatic limb (BPL) in RYGB, the gut hormone profile could theoretically be tailored.

Therefore, several surgical modifications involving variations of the different limb lengths were proposed to improve the outcomes of RYGB surgery in terms of weight loss and resolution of metabolic comorbidities. Among these, RYGB with longer BPL was implemented with encouraging weight loss and anti-diabetic outcomes [3, 16, 17]. However, the effect of these interventions on the postprandial hormone responses had not been characterized. Therefore, the objective of this study was to compare the GI hormone profiles in response to a mixed meal in subjects submitted to RYGB with two different BPL lengths.

Materials and methods

Participants

Subjects included in the study were selected among the post-bariatric surgery cohort under routine follow-up in a single bariatric centre at Hospital de São Sebastião, Centro Hospitalar de Entre o Douro e Vouga (CHEDV), Portugal. The study protocol and the patient information leaflet were approved by the CHEDV Institutional Ethical Review Board. Written informed consent was obtained from all participants before enrolment and the study was conducted according to the National Data Protection regulations.

Study design

Eligible subjects were selected from our prospective database of post-bariatric patients ($n = 2780$) and invited to participate in the study. Patients who accepted and met the established criteria were intentionally allocated to the two study groups in order to be matched according to the clinical and anthropometric postoperative features (Table 1). Subjects previously submitted either to a classical RYGB procedure ($n = 9$) or to a RYGB variant procedure with a longer BPL ($n = 11$), respectively, 3.8 ± 2.1 or 4.4 ± 1.7 years earlier, were enrolled. Exclusion criteria included past medical history of T2D or glucose intolerance, pregnancy and fasting plasma glucose >7.0 mmol/L. All participants had been weight-stable for at least 6 months before the study, had no past medical history suggestive of early or late dumping syndrome and met the established criteria for bariatric surgery before the operation [18].

For the mixed-meal tolerance test (MMTT), a standardized commercially available liquid meal (Fresubin Energy Drink, 200 mL, 300 kcal [50E% carbohydrate, 15E % protein, and 35E% fat]; Fresenius Kabi Deutschland, Bad Homburg, Germany) was ingested over a maximum period of 15 min after a 12-h overnight fast. Venous blood was sampled before and at regular intervals after the meal for a total of 2 h (0, 15, 30, 45, 60, 90 and 120 min). During the MMTT, participants were kept in a seated reclined position.

Surgical procedures

All surgeries were performed at the Department of General Surgery of CHEDV, using a standard laparoscopic RYGB technique only differing in the BPL length: 87.8 ± 20.5 cm (minimum 60 cm and maximum 90 cm) for the classical procedure and 200 cm for the long BPL RYGB variant, both with a constant 120-cm alimentary limb, as previously described [3]. Patients had been previously submitted to either surgical technique in a non-random way decided at the time of the procedure based on subjects' anatomical and clinical features according to the surgeon's preference (MN, MG).

The RYGB procedure involved the creation of a 15-mL gastric pouch by transecting the lesser curvature of the stomach distally to the cardia between the second and third vascular branch of the small gastric curvature, with a 45-mm endoscopic articulating linear cutter (Endopath ETS 3.5 mm; Johnson and Johnson, NJ, USA). Then, the stomach was intubated with a 36-Fr bougie and transected again vertically in the direction of the cardia. Afterwards, a calibrated (8–12 mm) gastro-enteric anastomosis, gastro-jejunal for classical or gastro-ileal for the long BPL, was made with a 45-mm linear stapler (Endopath ETS 2.5 mm; Johnson and Johnson, NJ, USA) and completed with a manual suture (Ethibond 2–0; Johnson and Johnson, NJ, USA). An ileal–ileal anastomosis was then performed with a 45-mm linear stapler (Endopath ETS 2.5 mm; Johnson

Table 1 Anthropometric and metabolic characteristics

	Classical BPL	Long BPL	*p*-Value
N (% of total)	9 (45%)	11 (55%)	–
Gender (men/women)	1/8 (11%/89%)	1/10 (9%/91%)	0.88
Age (years)	42 ± 9	47 ± 7	0.14
Preoperative body weight (kg)	111 ± 5	105 ± 4	0.34
Preoperative BMI (kg/m^2)	41.8 ± 1.1	40.6 ± 0.9	0.40
Postoperative body weight (kg)	74.8 ± 8.5	67.5 ± 8.3	0.07
Postoperative BMI (kg/m^2)	28.1 ± 2.3	26.2 ± 2.8	0.12
EBL (%)	81.7 ± 14.3	93.1 ± 18.0	0.14
TWL (%)	32.5 ± 2.0	35.3 ± 2.0	0.38
Postoperative HOMA-IR	1.4 ± 0.6	1.2 ± 0.4	0.41
Postoperative HOMA-β (%)	110.5 ± 25.6	115.5 ± 73.2	0.85
OGIS (mL × min^{-1} × m^2)	408 ± 9	449 ± 12	**0.02***
Postoperative time (years)	3.8 ± 2.1	4.4 ± 1.7	0.51
BPL length (cm)	88 ± 20	200	**<0.0001****

Anthropometric and metabolic characteristics of subjects previously submitted to classical RYGB ($n = 9$) or long BPL RYGB ($n = 11$) both at the time of the surgery (preoperative) and at the time of the mixed meal (postoperative). Data are presented as means ± SD or number (%) as appropriate, *$p < 0.05$, ****$p < 0.0001$ vs classical BPL RYGB (unpaired *t*-test)

The p-values in bold are the ones considered as significantly different between the groups.

BMI body mass index, *EBL* excess BMI loss, *TWL* total weight loss, *HOMA-IR* Homeostasis Model Assessment for Insulin Resistance, *HOMA-β* Homeostasis Model Assessment for β-cell function, *OGIS* oral glucose insulin sensitivity index, *BPL* biliopancreatic limb

and Johnson, NJ, USA) and completed with a manual suture (Ethibond 2–0; Johnson and Johnson, NJ, USA), thus creating a 60 or 200-cm BPL depending on the procedure, with a 120-cm alimentary limb (Fig. 1). This omega gastric bypass was then transformed into a RYGB by transecting the small intestine between the gastro-enteric and the entero-enteric anastomosis.

Biochemical measurements

Venous blood was collected from a catheter placed in a cubital vein into chilled EDTA tubes (S-Monovette® 7.5 mL, K2 EDTA Gel, 1.6 mg/mL, Sarstedt), centrifuged (Rotina 380R, Hettich, 2500 × *g*) and plasma stored frozen in Cryotubes (−20°C) pending analyses.

Plasma glucose was measured by the glucose oxidase method using a glucometer (YSI model 2300 STAT Plus; Yellow Springs Instruments, Yellow Springs, OH). Plasma samples for GLP-1, GIP, glucagon, PYY, pancreatic polypeptide (PP), and NT were extracted with ethanol (final concentration of 70%) prior to analysis.

Total GLP-1 was measured as described previously [19] using a C-terminal specific radioimmunoassay (RIA, antiserum 89390) which reacts with both intact GLP-1 and its primary N-terminally truncated metabolite. Total GIP was measured using the antiserum 867 directed at the C-terminal [20]. The glucagon assay was directed against the C-terminus of the glucagon molecule (antiserum 4305), therefore measuring glucagon of pancreatic origin [21]. RIA for total Peptide YY (PYY) in plasma was performed using a monoclonal antibody MAB8500 (Abnova, clone RPY-B12), which reacts equally well with $PY_{1–36}$ and $PYY_{3–36}$ [22]. Total NT was measured (antibody code 3D97) [23] as previously described [24]. PP was assayed using a monoclonal antibody HYB 347-07 (Statens Serum Institut, Copenhagen, Denmark), synthetic human PP (7TM Pharma A/S, Denmark) as standard and ^{125}I-PP (Perkin Elmer, Massachusetts, USA) as tracer. All plasma samples were handled identically and assayed in duplicate in one batch. For all assays, the free and bound moieties were separated with plasma-coated charcoal (E. Merck, Darmstadt, Germany). Intra-assay coefficients of variation were below 6% at 20–30 pmol/L.

Insulin and C-peptide levels were determined by electrochemiluminescence sandwich immunoassays on a Cobas 8000 e602 module (Roche Diagnostics, Mannheim, GmbH) according to the manufacturer's instructions. The coefficients of variation were below 5% and 8% for insulin and C-peptide, respectively, using liquid human serum-based controls (Liquichek™ Immunoassay Plus Control; Bio-Rad).

The total bile acid (TBA) content was measured using a commercial assay kit (Total Bile Acid Assay Kit, STA-631; Cell BioLabs, Inc., San Diego, CA, USA).

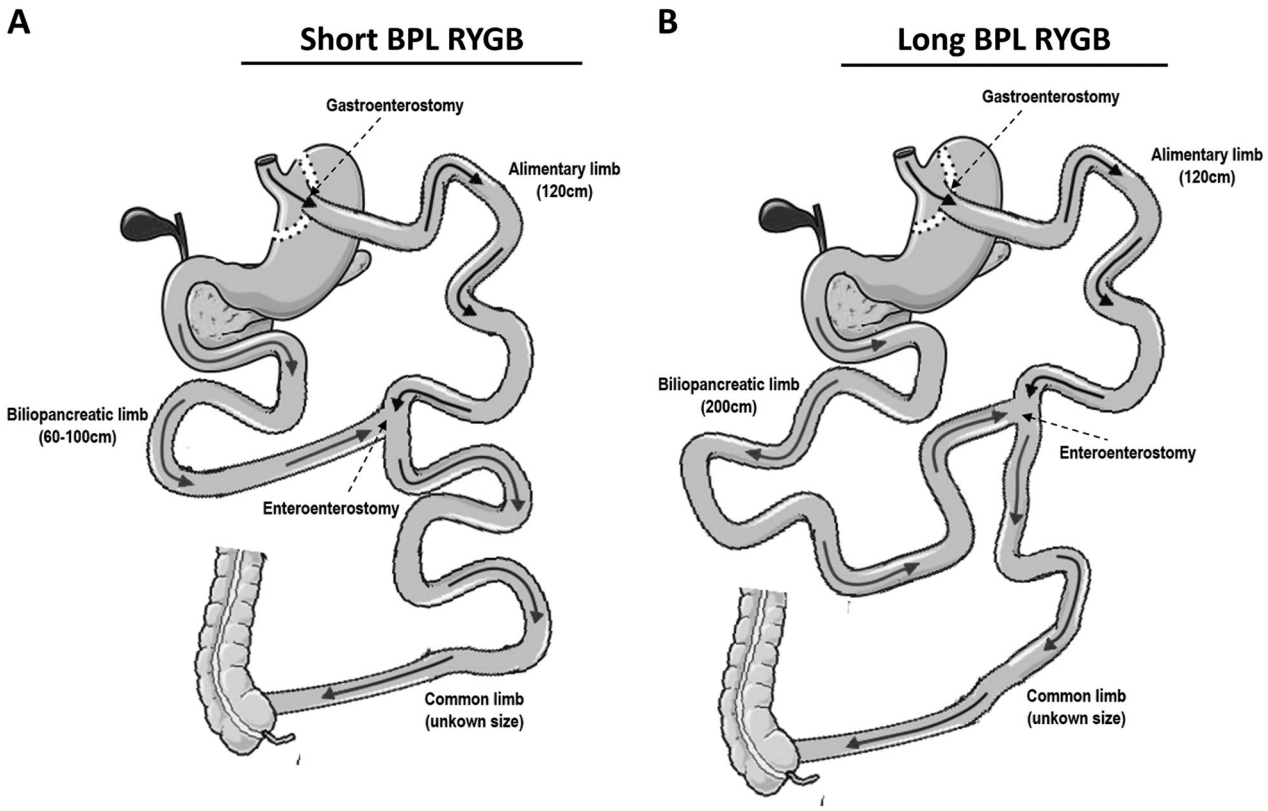

Fig. 1 **a** Short biliopancreatic limb (S-BPL) RYGB (classical RYGB) and **b** long biliopancreatic limb (L-BPL) RYGB

Calculations and statistical analysis

Data are presented as mean ± standard error of the mean (SEM) for figures and mean ± standard deviation (SD) for tables unless stated otherwise.

Total area under the curve (tAUC) was calculated using the trapezoidal rule. Excess BMI loss (EBL) was calculated using the formula (preoperative BMI − postoperative BMI)/(preoperative BMI − 25) × 100%. Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) and calculated according to the formula [fasting glucose (mmol/L) × fasting insulin (mIU/L)/22.5]. Pancreatic β-cell function was measured by the HOMA of β-cell function (HOMA-β) and was calculated according to the formula [20 × fasting insulin (mIU/L)/fasting glucose (mmol/L) − 3.5] [25]. To assess post-prandial insulin sensitivity based on the carbohydrate content of the mixed meal, oral glucose insulin sensitivity index (OGIS) was used as previously described [26].

The D'Agostino-Pearson test was used to verify the normality of the data. Clinical and anthropometric features, as well as fasting concentrations and areas under the curve (AUC) for the two groups, were compared with an unpaired two-tailed *t*-test. Comparisons between timepoints during the MMTT were performed using a two-way analysis of variance (ANOVA) with Sidak's post hoc multiple comparisons test. p-Values <0.05 were considered significant. All statistical analyses were performed using GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla, California, USA).

Results

Demographic data

Twenty subjects previously submitted to RYGB with a classical BPL ($n = 9$, 1 man and 8 women) or long BPL ($n = 11$, 1 man and 10 women) were included in the study. At the time of the MMTT, there were no significant differences regarding age, body weight, BMI, total weight loss (TWL), EBL, HOMA-IR, HOMA-β and time elapsed since surgery, except for the lengths of the BPL that were significantly different between the groups ($p < 0.0001$); there were also no differences in preoperative body weight or BMI (Table 1).

Fasting state

Fasting glucose, pancreatic and gut hormones were not significantly different between the groups, except for GLP-1

Table 2 Fasting glucose and enteropancreatic hormone concentrations

	Classical BPL	Long BPL	*p*-Value
Glucose (mmol/L)	5.1 ± 0.3	4.7 ± 0.6	0.18
GLP-1 (pmol/L)	12.9 ± 7.2	24.8 ± 10.8	**0.01***
GIP (pmol/L)	7.3 ± 2.6	9.5 ± 4.4	0.20
Glucagon (pmol/L)	6.4 ± 2.9	8.4 ± 3.9	0.22
Insulin (pmol/L)	46.0 ± 18.4	41.0 ± 15.7	0.52
C-peptide (pmol/L)	557.8 ± 114.8	484.4 ± 96.9	0.14
PP (pmol/L)	5.6 ± 3.1	7.6 ± 5.8	0.35
PYY (pmol/L)	12.0 ± 7.8	7.4 ± 6.3	0.16
NT (pmol/L)	8.7 ± 5.2	16.6 ± 8.0	**0.02***
TBA (μmol/L)	2.0 ± 0.5	3.0 ± 1.3	0.06

Fasting glucose and hormone concentrations in subjects previously submitted to classical RYGB ($n = 9$) or long BPL RYGB ($n = 11$). Data are presented as means ± SD, *$p < 0.05$ vs classical BPL RYGB (unpaired *t*-test)

BPL biliopancreatic limb, *GLP-1* glucagon-like peptide-1, *GIP* glucose-dependent Insulinotropic peptide, *PP* pancreatic polypeptide, *PYY* peptide YY, *NT* neurotensin, *TBA* total bile acids

and NT levels which were significantly higher in the long BPL RYGB subjects (GLP-1: 24.8 ± 10.8 pmol/L vs classical RYGB group 12.9 ± 7.2, $p = 0.01$; NT: 16.6 ± 8.0 pmol/L vs classical RYGB group 8.7 ± 5.2, $p = 0.02$). TBA concentrations did not differ significantly between the groups (3.0 ± 1.3 μmol/L vs classical RYGB group 2.0 ± 0.5, $p = 0.06$) (Table 2).

Hormone response to the mixed meal

During the MMTT, no differences were found for plasma glucose levels between the two groups (Fig. 2a), which presented similar peak values and AUCs after the meal ($t =$ 30 min).

As compared to the classical RYGB procedure, both insulin and C-peptide levels were significantly lower at t = 30 min (insulin: 969 ± 464 pmol/L vs classical RYGB group 1726 ± 883, $p < 0.001$; C-peptide: 2702 ± 839 pmol/L vs classical RYGB group 4119 ± 1345, $p < 0.001$) after the meal (Fig. 2b and c, respectively) in the long BPL RYGB group. OGIS based on the carbohydrate content of the meal was significantly higher in the long BPL RYGB group (449 ± 12 mL × min^{-1} × m^2 vs classical RYGB group 408 ± 9, $p = 0.02$) (Table 1).

Glucagon secretion profiles were similar in the two groups, increasing up to 30 min followed by stabilization until 120 min after the meal (Fig. 2d).

GLP-1 levels were significantly higher at t = 45 min (153.5 ± 77.2 pmol/L vs classical RYGB group 95.3 ± 40.1, $p < 0.05$) (Fig. 2e), as well as for tAUC (11205 ± 3399 pmol/L × min vs classical RYGB group 7889 ± 1686, $p =$ 0.02) in the long BPL RYGB group (Table 3), while GIP levels were significantly lower at t = 15 min (43.9 ± 26.0 pmol/L vs classical RYGB group 72.3 ± 23.8, $p < 0.01$) in the long BPL RYGB group (Fig. 2f).

Neurotensin levels were significantly higher post-prandially at $t = 45$ min (223.2 ± 103.5 pmol/L vs classical RYGB group 138.9 ± 53.9, $p < 0.05$) and $t = 60$ min (197.8 ± 89.5 pmol/L vs classical RYGB group 95.3 ± 40.9, $p <$ 0.01) in the long BPL RYGB group (Fig. 2h), and tAUC values were also higher (18392 ± 7066 vs 11437 ± 3658 pmol/L × min, $p = 0.02$) (Table 3).

PYY levels were higher, albeit not significantly, from t = 30 to 120 min in the long BPL RYGB group when compared to the classical RYGB group (Fig. 2g).

There were no significant differences in PP or TBA levels between the two experimental groups (Fig. 2i and j, respectively).

Discussion

Our main goal was to explore the post-prandial GI hormone response elicited by increasing the RYGB BPL length, in order to assess its potential contribution to glucose homeostasis improvement observed with this surgical variant [3, 17]. To achieve this aim, the current study was performed in weight-stable, non-diabetic subjects previously submitted to RYGB with two different BPL lengths. By pairing the experimental groups for BMI both presurgical and post-surgical at the time of the MMTT, we sought to eliminate possible interferences of anthropometric features on the hormonal response.

Our results demonstrated that RYGB with a longer BPL is associated with higher fasting and post-prandial GLP-1 and NT levels and lower post-prandial insulin/C-peptide and GIP levels when compared the classical RYGB procedure.

RYGB surgery involves a rearrangement in the GI tract anatomy that yields sustained weight loss and significant improvement of glucose homeostasis, which could even result in T2D remission. The glycaemic effects of RYGB surgery were previously attributed to changes in enteroendocrine cells (EEC) GI hormone secretion elicited by the procedure [27], although it is widely debated whether the greater impact results from the upper or lower gut modifications.

RYGB surgery can be performed using a wide range of Roux-en-Y limb lengths. The choice between different limb lengths is usually empirical, largely based on the surgeon's personal preferences and patient's anatomical features. Although there is no current consensus on the ideal RYGB limb lengths, previous studies suggest that a longer BPL RYGB could be associated with improved weight loss and T2D control as compared to procedures with shorter BPL [3, 17, 28, 29].

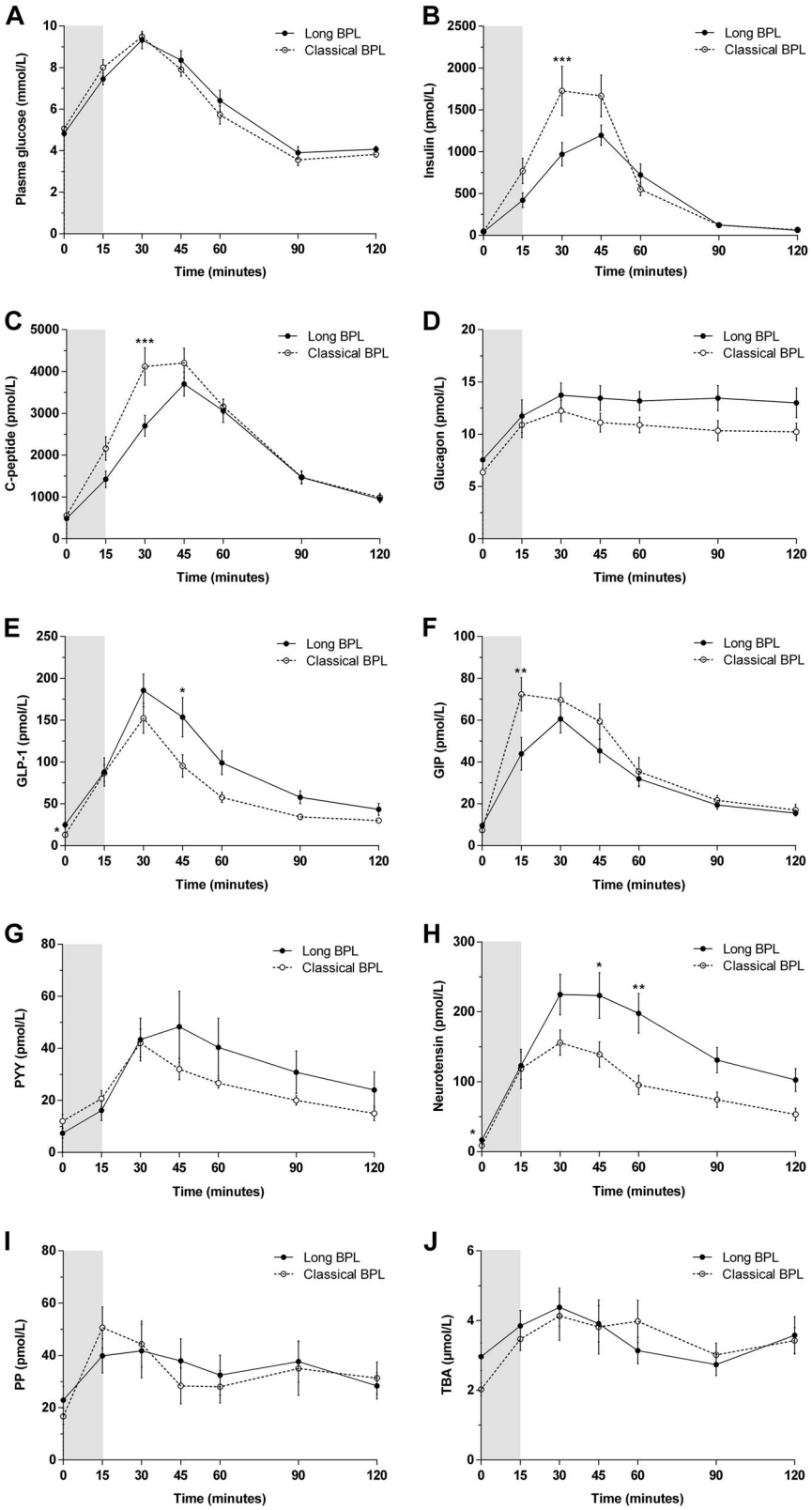

Fig. 2 Plasma levels of glucose (**a**), insulin (**b**), C-peptide (**c**), glucagon (**d**), GLP-1 (**e**), GIP (**f**), PYY (**g**), NT (**h**), PP (**i**) and TBA (**j**) in 20 non-diabetic weight-stable subjects previously submitted to classical RYGB ($n = 9$) or long BPL RYGB ($n = 11$) after ingestion of a standard mixed meal served at $t = 0$ min and consumed before $t = 15$ min. Data are presented as mean values ± SEM, $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ vs classical BPL RYGB (two-way ANOVA)

In the present study, subjects exhibited fasting and post-prandial plasma glucose levels within the normal ranges when challenged with a mixed meal, as it would be expected for individuals with normal endocrine pancreatic function [30]. Even though the two groups showed similar glucose excursion curves, a distinctive fasting and post-

Table 3 Glucose and enteropancreatic tAUC after the MMTT

tAUC		Classical BPL	Long BPL	*p*-Value
Glucose	(mmol/L × min)	711 ± 57	735 ± 91	0.50
GLP-1	(pmol/L × min)	7889 ± 1686	11,205 ± 3399	**0.02***
GIP	(pmol/L × min)	4775 ± 1237	3850 ± 1279	0.12
Glucagon	(pmol/L × min)	1269 ± 271	1536 ± 372	0.08
Insulin	(pmol/L × min)	79,829 ± 33,655	59,961 ± 17,064	0.10
C-peptide	(pmol/L × min)	291,550 ± 64,250	248,176 ± 50,928	0.11
PP	(pmol/L × min)	4125 ± 2220	4249 ± 2401	0.91
PYY	(pmol/L × min)	2935 ± 703	3864 ± 2968	0.37
NT	(pmol/L × min)	11,437 ± 3658	18,392 ± 7066	**0.02***
TBA	(μmol/L × min)	418 ± 109	412 ± 116	0.91

tAUC of glucose and enteropancreatic hormones. Data from subjects previously submitted to classical RYGB ($n = 9$) or long BPL RYGB ($n = 11$) after ingestion of a standard mixed meal served at $t = 0$ min and consumed until $t = 15$ min. Data are presented as means ± SD, *$p < 0.05$ vs classical BPL RYGB (unpaired *t*-test)

The p-values in bold are the ones considered as significantly different between the groups.

tAUC total area under the curve, *BPL* biliopancreatic limb, *GLP-1* glucagon-like peptide-1, *GIP* glucose-dependent insulinotropic peptide, *PP* pancreatic polypeptide, *PYY* peptide YY, *NT* neurotensin, *TBA* total bile acids

prandial GI hormones profile was disclosed, suggesting that a long BPL RYGB could result in additional benefits, particularly if applied to T2D patients [31, 32].

Despite similar glucose excursion curves and with no significant differences between the two groups in insulin resistance or pancreatic β-cell function, as assessed by HOMA-IR and HOMA-β, respectively, the post-prandial insulin and C-peptide levels were lower in the long BPL RYGB group when compared to the classical RYGB group. Overall, this suggests an acute increase in insulin sensitivity, as less insulin is needed to maintain the same glucose levels. Indeed, subjects submitted to the long BPL RYGB exhibited a higher OGIS index, suggesting improved post-prandial insulin sensitivity [26]. Similar findings were described in a study designed to assess the endocrine effects of nutrient direct delivery into different small intestinal locations by infusing a liquid formula diet through a naso-gastric tube in an experiment that simulates what occurs after different bariatric procedures. In that study, in both normoglycemic and diabetic obese subjects, insulin sensitivity was significantly increased after nutrient infusion in the mid-jejunum as compared to the duodenum [33].

Incretin-secreting cells are unevenly distributed along the human small gut [34]. The RYGB will inevitably result in activation of different cell populations depending on the location of (a) the division of the small intestine and (b) the entero-entero-anastomosis [34]. Therefore, depending on the anatomical modification different GI hormone secretion profiles are likely to be induced. A RYGB with a longer BPL allows the earlier stimulation of distal EEC cells, such as GLP-1-secreting cells, while a RYGB procedure with a shorter BPL will result in the early arrival of nutrients to the proximal small intestine, where GIP-producing cells are known to predominate [12]. In agreement with this hypothesis, both fasting and post-prandial GLP-1 levels were previously reported to increase after RYGB [14, 35], which are further corroborated by our own results, showing that RYGB with a longer BPL results in higher fasting and post-prandial GLP-1 levels as compared to the classical RYGB procedure.

Individuals submitted to RYGB with a long BPL also presented lower post-prandial GIP responses when compared to those that underwent a classical RYGB procedure, in which nutrients stimulate the proximal jejunum characterized by a greater proportion of K-cells [36]. Interestingly, previous reports on GIP changes after RYGB surgery were largely inconsistent, showing either increase [37], no change, or reduction of post-prandial GIP levels [38]. Given our own findings, these discrepancies could possibly be attributed to differences in the RYGB limb lengths used by different surgeons [39].

In accordance with the known distribution of incretin-secreting cells along the small intestine [34], different BPL lengths lead to different GIP and GLP-1 post-prandial profiles. Since GLP-1 and GIP provide equal contributions to the incretin effect in healthy subjects [40], the lower post-prandial insulin excursion after the long BPL RYGB is somewhat surprising. However, this observation suggests that additional mechanisms leading to decreased post-prandial insulin resistance, potentially mediated by the impact of GI hormones in the liver, may also occur leading to decreased insulin needs to sustain normoglycaemia [33].

Fasting and post-prandial NT levels were also found to be higher in the long BPL RYGB group, as compared to the

classical RYGB procedure, likely accountable to the early arrival of nutrients into the distal small intestine. NT is coexpressed in some L-cells with GLP-1 and PYY and is co-secreted in response to nutrient ingestion, particularly fat and other stimuli, such as bile acids. In experimental animals, NT acts synergistically with GLP-1 and PYY to decrease food intake, to inhibit gastric emptying, to decrease small bowel motility and to stimulate pancreatic secretion, which potentially contributes to maintain glucose homeostasis [41]. Thus, EEC may co-secrete NT and GLP-1, consistent with the similar secretion patterns found in our subjects, while enhanced NT may act synergistically with GLP-1 and PYY to decrease palatable food intake and inhibit gastric emptying [42]. Obese subjects have decreased NT levels that increase after RYGB, while these have been hypothesized to contribute to the weight loss observed after surgery [43, 44]. In further support of NT role in appetite regulation, NT antagonism results in a transient increase in food intake in rats following RYGB [45].

In physiological conditions, the expected endocrine response to a carbohydrate-rich meal would include the increase of GLP-1 and insulin levels accompanying the glucose excursions, along with the suppression of pancreatic glucagon. Yet, post-prandial glucagon levels increased in subjects previously submitted to RYGB and we also found a strong tendency for a greater response after long BPL RYGB. Recent evidence suggests that glucagon is not exclusively secreted by pancreatic α-cells and that intestinal L-cells may also secrete glucagon through "pancreatic type processing" of proglucagon [46, 47]. Glucagon physiological role includes, in addition to the well-known insulin antagonism effects, inhibition of food intake [48] that could also contribute to the negative energy balance induced by the surgery [49].

Other GI hormones, such as PYY, also secreted in the distal small intestine, were previously shown to increase early after RYGB and likely to contribute to the weight loss and anti-diabetic effects of the surgery [15]. PYY levels tended to be higher in the long BPL RYGB group, while PP levels that are largely regulated by parasympathetic activity were not different after the two operations, suggesting that the surgery has similar effects on the efferent vagal activity. Indeed, the effect of bariatric surgery on PP secretion is controversial and the available data do not suggest a major role for PP in mediating the outcomes of bariatric surgical procedures [44, 50, 51].

TBA levels were not significantly different between procedures, although trending higher in the long BPL RYGB group. Given the anatomical changes produced by RYGB, bile is delivered more or less distally, depending on the BPL length, in addition to promoting the early arrival of nutrients to the distal small intestine. In fact, a duodenal–jejunal bypass was shown to increase TBA levels in rats [52], while in humans higher TBA levels were demonstrated to occur after DS as compared to RYGB [53]. Bile acids, despite not being considered classical endocrine mediators, were recently demonstrated to participate in glucose homeostasis and to induce GLP-1 secretion through L-cells TGR5 receptors activation [54]. In addition, the increase in circulating bile acids levels after RYGB was suggested to contribute to the metabolic benefits of bariatric surgery, although the underlying mechanism remains to be fully defined [55].

Conclusion

The post-prandial gut hormone secretion in subjects previously submitted to RYGB is variable depending on the BPL length. The GI and pancreatic hormone secretion profile of subjects who underwent a long BPL RYGB is characterized by increased fasting and post-prandial GLP-1 and NT, along with decreased insulin and GIP, as compared to the classical procedure. The distinct hormonal profiles observed after the two surgical procedures suggest that the RYGB technique could be tailored to obtain a personalized response optimizing individual's clinical outcomes. Bariatric surgery could even contribute to design hormone-targeted therapies aiming to simulate the anticipated endocrine response.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Yan Y, Sha Y, Yao G, Wang S, Kong F, Liu H, et al. Roux-en-Y gastric bypass versus medical treatment for type 2 diabetes mellitus in obese patients: a systematic review and meta-analysis of randomized controlled trials. Medicine. 2016;95:e3462.
2. Kissler HJ, Settmacher U. Bariatric surgery to treat obesity. Semin Nephrol. 2013;33:75–89.
3. Nora M, Guimaraes M, Almeida R, Martins P, Goncalves G, Freire MJ, et al. Metabolic laparoscopic gastric bypass for obese patients with type 2 diabetes. Obes Surg. 2011;21:1643–9.
4. Madsbad S, Dirksen C, Holst JJ. Mechanisms of changes in glucose metabolism and bodyweight after bariatric surgery. Lancet Diabetes Endocrinol. 2014;2:152–64.
5. Berbiglia L, Zografakis JG, Dan AG. Laparoscopic Roux-en-Y gastric bypass: surgical technique and perioperative care. Surg Clin North Am. 2016;96:773–94.
6. Williams KV, Kelley DE. Metabolic consequences of weight loss on glucose metabolism and insulin action in type 2 diabetes. Diabetes Obes Metab. 2000;2:121–9.
7. Nelson DW, Blair KS, Martin MJ. Analysis of obesity-related outcomes and bariatric failure rates with the duodenal switch vs gastric bypass for morbid obesity. Arch Surg. 2012;147:847–54.
8. Sala PC, Torrinhas RS, Heymsfield SB, Waitzberg DL. Type 2 diabetes mellitus: a possible surgically reversible intestinal dysfunction. Obes Surg. 2012;22:167–76.
9. Rubino F, Forgione A, Cummings DE, Vix M, Gnuli D, Mingrone G, et al. The mechanism of diabetes control after gastrointestinal bypass surgery reveals a role of the proximal small intestine in the pathophysiology of type 2 diabetes. Ann Surg. 2006;244:741–9.
10. Cummings DE, Overduin J, Foster-Schubert KE. Gastric bypass for obesity: mechanisms of weight loss and diabetes resolution. J Clin Endocrinol Metab. 2004;89:2608–15.
11. Sala PC, Torrinhas RS, Giannella-Neto D, Waitzberg DL. Relationship between gut hormones and glucose homeostasis after bariatric surgery. Diabetol Metab Syndr. 2014;6:87.
12. Holst JJ. Gut hormones and gastric bypass. Cardiovasc Endocrinol. 2016;5:69–74.
13. Jacobsen SH, Olesen SC, Dirksen C, Jorgensen NB, Bojsen-Moller KN, Kielgast U, et al. Changes in gastrointestinal hormone responses, insulin sensitivity, and beta-cell function within 2 weeks after gastric bypass in non-diabetic subjects. Obes Surg. 2012;22:1084–96.
14. Dirksen C, Jorgensen NB, Bojsen-Moller KN, Kielgast U, Jacobsen SH, Clausen TR, et al. Gut hormones, early dumping and resting energy expenditure in patients with good and poor weight loss response after Roux-en-Y gastric bypass. Int J Obes (Lond). 2013;37:1452–9.
15. le Roux CW, Welbourn R, Werling M, Osborne A, Kokkinos A, Laurenius A, et al. Gut hormones as mediators of appetite and weight loss after Roux-en-Y gastric bypass. Ann Surg. 2007;246:780–5.
16. Nergaard BJ, Leifsson BG, Hedenbro J, Gislason H. Gastric bypass with long alimentary limb or long pancreato-biliary limb—long-term results on weight loss, resolution of co-morbidities and metabolic parameters. Obes Surg. 2014;24:1595–602.
17. Nora M, Morais T, Almeida R, Guimaraes M, Monteiro MP. Should Roux-en-Y gastric bypass biliopancreatic limb length be tailored to achieve improved diabetes outcomes? Medicine. 2017;96:e8859.
18. Fried M, Yumuk V, Oppert JM, Scopinaro N, Torres A, Weiner R, et al. Interdisciplinary European guidelines on metabolic and bariatric surgery. Obes Surg. 2014;24:42–55.
19. Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. Diabetes. 1994;43:535–9.
20. Krarup T, Holst JJ. The heterogeneity of gastric inhibitory polypeptide in porcine and human gastrointestinal mucosa evaluated with five different antisera. Regul Pept. 1984;9:35–46.
21. Orskov C, Jeppesen J, Madsbad S, Holst JJ. Proglucagon products in plasma of noninsulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. J Clin Invest. 1991;87:415–23.
22. Toräng S, Bojsen-Moller KN, Svane MS, Hartmann B, Rosenkilde MM, Madsbad S et al. In vivo and in vitro degradation of peptide YY3-363-36 to inactive peptide YY3-343-34 in humans. Am J Physiol Regul Integr Comp Physiol. 2016;310:R866-R874.
23. Kuhre RE, Bechmann LE, Wewer Albrechtsen NJ, Hartmann B, Holst JJ. Glucose stimulates neurotensin secretion from the rat small intestine by mechanisms involving SGLT1 and GLUT2, leading to cell depolarization and calcium influx. Am J Physiol Endocrinol Metab. 2015;308:E1123–30.
24. Pedersen JH, Stadil F, Fahrenkrug J. Preparation of 125I-(Tyr 3)- and 125I-(Tyr 11)- neurotensin for radioimmunoassay. Scand J Clin Lab Invest. 1983;43:483–91.
25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–9.
26. Bojsen-Moller KN, Dirksen C,Svane MS, Jorgensen NB, Holst JJ, Richter EA, et al. Variable reliability of surrogate measures of insulin sensitivity after Roux-en-Y gastric bypass. Am J Physiol Regul Integr Comp Physiol. 2017;312:R797–R805.
27. Meek CL, Lewis HB, Reimann F, Gribble FM, Park AJ. The effect of bariatric surgery on gastrointestinal and pancreatic peptide hormones. Peptides. 2016;77:28–37.
28. Stefanidis D, Kuwada TS, Gersin KS. The importance of the length of the limbs for gastric bypass patients--an evidence-based review. Obes Surg. 2011;21:119–24.
29. Kaska Ł, Kobiela J, Proczko M, Stefaniak T, Śledziński Z. Does the length of the biliary limb influence medium-term laboratory remission of type 2 diabetes mellitus after Roux-en-Y gastric bypass in morbidly obese patients? Wideochir Inne Tech Maloinwazyjne. 2014;9:31–9.
30. Freckmann G, Hagenlocher S, Baumstark A, Jendrike N, Gillen RC, Rössner K, et al. Continuous glucose profiles in healthy subjects under everyday life conditions and after different meals. J Diabetes Sci Technol. 2007;1:695–703.
31. Triplitt CL. Examining the mechanisms of glucose regulation. Am J Manag Care. 2012;18(1 Suppl):S4–10.
32. Rubino F, Gagner M, Gentileschi P, Kini S, Fukuyama S, Feng J, et al. The early effect of the Roux-en-Y gastric bypass on hormones involved in body weight regulation and glucose metabolism. Ann Surg. 2004;240:236–42.
33. Salinari S, Carr RD, Guidone C, Bertuzzi A, Cercone S, Riccioni ME, et al. Nutrient infusion bypassing duodenum-jejunum improves insulin sensitivity in glucose-tolerant and diabetic obese subjects. Am J Physiol Endocrinol Metab. 2013;305:E59–66.
34. Guedes TP, Martins S, Costa M, Pereira SS, Morais T, Santos A, et al. Detailed characterization of incretin cell distribution along the human small intestine. Surg Obes Relat Dis. 2015;11:1323–31.
35. Jorgensen NB, Jacobsen SH, Dirksen C, Bojsen-Moller KN, Naver L, Hvolris L, et al. Acute and long-term effects of Roux-en-Y gastric bypass on glucose metabolism in subjects with Type 2 diabetes and normal glucose tolerance. Am J Physiol Endocrinol Metab. 2012;303:E122–31.
36. McIntosh CH, Widenmaier S, Kim SJ. Glucose-dependent insulinotropic polypeptide (gastric inhibitory polypeptide; GIP). Vitam Horm. 2009;80:409–71.

37. Goldfine AB, Mun EC, Devine E, Bernier R, Baz-Hecht M, Jones DB, et al. Patients with neuroglycopenia after gastric bypass surgery have exaggerated incretin and insulin secretory responses to a mixed meal. J Clin Endocrinol Metab. 2007;92:4678–85.
38. Korner J, Bessler M, Inabnet W, Taveras C, Holst JJ. Exaggerated glucagon-like peptide-1 and blunted glucose-dependent insulinotropic peptide secretion are associated with Roux-en-Y gastric bypass but not adjustable gastric banding. Surg Obes Relat Dis. 2007;3:597–601.
39. Dirksen C, Jorgensen NB, Bojsen-Moller KN, Jacobsen SH, Hansen DL, Worm D, et al. Mechanisms of improved glycaemic control after Roux-en-Y gastric bypass. Diabetologia. 2012;55:1890–901.
40. Vilsboll T, Krarup T, Madsbad S, Holst JJ. Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. Regul Pept. 2003;114:115–21.
41. Monteiro MP, Batterham RL. The importance of the gastrointestinal tract in controlling food intake and regulating energy balance. Gastroenterology. 2017;152:1707–17 e2.
42. Grunddal KV, Ratner CF, Svendsen B, Sommer F, Engelstoft MS, Madsen AN, et al. Neurotensin is coexpressed, coreleased, and acts together with GLP-1 and PYY in enteroendocrine control of metabolism. Endocrinology. 2016;157:176–94.
43. Weiss HG, Nehoda H, Labeck B, Hourmont K, Marth C, Aigner F. Pregnancies after adjustable gastric banding. Obes Surg. 2001;11:303–6.
44. Holdstock C, Zethelius B, Sundbom M, Karlsson FA, Eden Engstrom B. Postprandial changes in gut regulatory peptides in gastric bypass patients. Int J Obes (Lond). 2008;32:1640–6.
45. Ratner C, Skov LJ, Raida Z, Bachler T, Bellmann-Sickert K, Le Foll C, et al. Effects of peripheral neurotensin on appetite regulation and its role in gastric bypass surgery. Endocrinology. 2016;157:3482–92.
46. Lund A, Bagger JI, Wewer Albrechtsen NJ, Christensen M, Grøndahl M, Hartmann B et al. Evidence of extrapancreatic glucagon secretion in man. Diabetes. 2016;65:585-597.
47. Holst JJ, Pedersen JH, Baldissera F, Stadil F. Circulating glucagon after total pancreatectomy in man. Diabetologia. 1983;25:396–9.
48. Geary N. Effects of glucagon, insulin, amylin and CGRP on feeding. Neuropeptides. 1999;33:400–5.
49. Salem V, Izzi-Engbeaya C, Coello C, Thomas DB, Chambers ES, Comninos AN, et al. Glucagon increases energy expenditure independently of brown adipose tissue activation in humans. Diabetes Obes Metab. 2016;18:72–81.
50. Nannipieri M, Baldi S, Mari A, Colligiani D, Guarino D, Camastra S, et al. Roux-en-Y gastric bypass and sleeve gastrectomy: mechanisms of diabetes remission and role of gut hormones. J Clin Endocrinol Metab. 2013;98:4391–9.
51. Schrumpf E, Linnestad P, Nygaard K, Giercksky KE, Fausa O. Pancreatic polypeptide secretion before and after gastric bypass surgery for morbid obesity. Scand J Gastroenterol. 1981;16:1009–14.
52. Miyachi T, Nagao M, Shibata C, Kitahara Y, Tanaka N, Watanabe K, et al. Biliopancreatic limb plays an important role in metabolic improvement after duodenal-jejunal bypass in a rat model of diabetes. Surgery. 2016;159:1360–71.
53. Risstad H, Kristinsson JA, Fagerland MW, le Roux CW, Birkeland KI, Gulseth HL, et al. Bile acid profiles over 5 years after gastric bypass and duodenal switch: results from a randomized clinical trial. Surg Obes Relat Dis. 2017;13:1544–53.
54. Brighton CA, Rievaj J, Kuhre RE, Glass LL, Schoonjans K, Holst JJ, et al. Bile acids trigger GLP-1 release predominantly by accessing basolaterally located G protein–coupled bile acid receptors. Endocrinology. 2015;156:3961–70.
55. Patti ME, Houten SM, Bianco AC, Bernier R, Larsen PR, Holst JJ, et al. Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. Obesity (Silver Spring). 2009;17:1671–7.