Obesity Surgery (2020) 30:697–706
https://doi.org/10.1007/s11695-019-04239-y

ORIGINAL CONTRIBUTIONS

The Leading Role of Peptide Tyrosine Tyrosine in Glycemic Control After Roux-en-Y Gastric Bypass in Rats

Alonso Camacho-Ramírez[1,2,3] · J. Arturo Prada-Oliveira[2,3,4] · Antonio Ribelles-García[4,5] · David Almorza-Gomar[3,6] · Gonzalo M. Pérez-Arana[2,3,4]

Published online: 8 November 2019

Abstract

Aims Roux-en-Y gastric bypass (RYGB) is one of the most effective surgical therapies for the rapid resolution of type 2 diabetes. However, the mechanisms underlying the entero-hormonal response after surgery and the role of peptide tyrosine tyrosine (PYY) in the restoration of normoglycemia are still not clear.

Methods We reproduced the RYGB technique in Wistar and Goto–Kakizaki rats and performed serum hormonal, histological, and hormonal-infusion test.

Results Using the diabetic Goto–Kakizaki (GK) rat model, we demonstrated that PYY plasma levels showed a remarkable peak approximately 30 min earlier than GLP-1 or GIP after mixed-meal administration in RYGB-operated rats with PYY. The GLP-1 and GIP areas under the curve (AUCs) increased after RYGB in GK rats. Additionally, the findings suggested that PYY (3-36) infusion led to increased GLP-1 and GIP plasma levels close to those obtained after a meal. Finally, the number of GLP-1-positive cells appeared to increase in the three segments of the small intestine in GK-RYGB-operated rats beyond the early presence of nutrient stimulation in the ileum. Nevertheless, PYY-positive cell numbers appeared to increase only in the ileum.

Conclusion At least in rats, these data demonstrate an earlier essential role for PYY in gut hormone regulation after RYGB. We understand that PYY contributes to GLP-1 and GIP release and there must be the existence of enteroendocrine communication routes between the distal and proximal small intestine.

Keywords Enteroinsular axis · Insulin secretion · Peptide hormones · Peptide tyrosine tyrosine · Bariatric surgery · Glucagon-like peptide-1

Introduction

The effect of bariatric surgery on type 2 diabetes (T2DM) has generated considerable attention from the research community. Roux-en-Y gastric bypass (RYGB) is known as one of the most effective methods for maintaining body weight and improving glycemic control compared to medical therapy alone [1], but the underlying mechanism that leads to T2DM resolution remains controversial.

We now know that RYGB promotes substantial changes in the secretion of gut hormones, such as gastric inhibitory polypeptide (GIP) or mainly glucagon-like peptide-1 (GLP-1), an incretin hormone released postprandially by L cells from the ileum into the bloodstream. GLP-1 has been suggested to be an important contributor to insulin secretion and glucose tolerance and is key to explaining glycemic control improvement after RYGB [2–4].

However, some authors have reported enhanced glucose control after RYGB in a mouse model of functional GLP-1 and GLP-1 receptor deficiency, suggesting a GLP-1-independent mechanism for glycemic control improvement [5, 6]. In addition, notable increases in plasma peptide tyrosine tyrosine (PYY) levels have been reported after RYGB in rats

✉ Gonzalo M. Pérez-Arana
gonzalompp@hotmail.com

1 Puerta del Mar Hospital, University of Cadiz, Cadiz, Spain

2 Asociación Gaditana de Apoyo al Investigador, Cadiz, Spain

3 Biomedical Science Research and Innovation Institute (INIBICA), University of Cadiz, Cadiz, Spain

4 Department of Human Anatomy and Embryology, Faculty of Medicine, University of Cadiz, Plaza Fragela s/n, 11003 Cadiz, Spain

5 Sustainable Social Development Research Institute (INDESS), University of Cadiz, Cadiz, Spain

6 Operative Statistic and Research Department, University of Cadiz, Cadiz, Spain

and humans [7, 8]. Like GLP-1, PYY is an incretin hormone found in L cells in the mucosa of the ileum [9]. Total plasma PYY is a mix of PYY (1-36) and the active endocrine isoform PYY (3-36), obtained by NH_2-terminal PYY (1-36) residue cleavage by dipeptidyl peptidase-4 (DPP-4) [10]. It was recently demonstrated that PYY (3-36) can modulate the insulin secretory response to glucose in the pancreas and restore glucose-induced suppression of glucagon secretion [7, 11]. Furthermore, PYY (3-36) can improve insulin sensitivity by increasing glucose uptake in adipose tissue and muscle in rodents [12] and slowing gastric emptying [13]. It has also been reported to increase hepatoportal GLP-1 plasma levels after PYY (3-36) injection. This increase is probably due to neuropeptide Y2 receptor (NPY2R) activation in the small intestine [14]. NPY2R activation has also shown marked postoperative glucose tolerance improvement and increased PYY plasma levels in wild-type mice after enterogastric anastomosis (EGA) but not in PYY-null mice [14].

These data and the revelation of enteroendocrine communication routes between the distal and proximal small intestine by a serial block-face scanning electron microscopy technique have indicated the presence of neuropods from L cells. These axon-like basal processes ended in synapse-like appositions to the enteric nervous system [15]. These communication routes and the data reported previously led us to think about PYY as a possible new key to explaining glycemic control improvement after RYGB.

The aim of this study was to determine the contribution of PYY to glycemic control improvement after RYGB in a Goto–Kakizaki (GK) diabetic rat model by analyzing its ability to trigger changes in the gut hormone expression pattern.

Research Design and Methods

Animals

All animal procedures were approved by the Committee for Ethical Use and Care of Experimental Animals at Cadiz University. Thirty-six male GK rats and six male Wistar rats weighing 200–220 g, at an age of 10–11 weeks, were provided and kept at the Experimentation and Animal Production Service of University of Cadiz (SEPA). Female rats were not used to avoid the cyclic variations in gonadotropins and their effect on glycemic metabolism.

All animal procedures were performed with the approval of the University of Cadiz Committee for the Ethical Use and Care of Experimental Animals. This committee ensured that the procedures in all experiments were performed in accordance with international relevant guidelines and regulations of animal welfare. The internal Committee for the Ethical Use and Care of Experimental Animals followed the instructions marked for the Autonomous Andalusian Authority.

Experiment Protocol

For hormonal and histological studies, 18 GK rats and 6 Wistar rats were randomly divided into four groups: n = 6 Wistar (Wistar); n = 6 Goto–Kakizaki (GK); n = 6 GK sham-operated (GK-sham); n = 6 GK RYGB-operated (GK-RYGB). Animals were kept for 12 weeks from surgery to sacrifice.

For the PYY (3-36) infusion assay, another 18 male GK rats were kept for 4 weeks after RYGB surgery. Rats were randomly divided into three groups: n = 6 GK saline-infused rats (RYGB-GK + vehicle); n = 6 GK PYY (3-36)-infused rats (RYGB-GK + PYY (3-36)); n = 6 GK mixed-meal-fed rats (RYGB-GK + meal). Mixed meal was prepared combining 0.5 g dextrose/ml 0.092 g vegetable oil/ml, and 0.125 nitrogen casein/ml in purified water [16].

Surgical Procedures

The RYGB and sham groups were subjected to a pre- and postsurgical 12-hour fasting period. Briefly, to describe RYGB, animals were anesthetized with continuous infusion of 3% *v*/*v* isoflurane (Isoflo; Abbott 571329.8, Madrid). A laparotomy of 3 cm was made in the midline of the abdomen, and a gastric pouch with a volume of approximately 30% of the normal gastric volume was created. The remnant gastric fundus was anastomosed to the jejunum at 14 cm distal from the ligament of Treitz. The abdominal muscular and skin layers were closed in one layer using a continuous suturing technique [17, 18].

For the sham operation, animals were anesthetized, and a 3-cm incision was made in the midline of the abdomen. The jejunum was transected 40 cm distal to the angle of Treitz, and terminus–terminus anastomosis was performed. Abdominal layers were closed as above.

Sacrifice and Tissue Preparation

Animals were sacrificed 12 weeks after surgery by isoflurane inhalation overdose. Tissues were immediately removed, and 1 cm full-thickness segments of the duodenum, jejunum, and ileum were harvested and fixed in Bouin's solution overnight at 4 °C. Later, the samples were dehydrated, embedded in paraffin, and cut into serial 10 μm microtome sections for immunostaining.

Gut Histology

In rehydrated sections of intestine, GLP-1, PYY, and GIP release was analyzed by immunostaining using GLP-1 (1:100 rabbit Abcam ab22625, Cambridge, UK), PYY (1:500 rabbit Abcam ab22663, Cambridge, UK), and GIP (1:100 rabbit Abcam ab202792, Cambridge, UK) primary

antibodies. The secondary antibody used was Alexa 488 anti-rabbit (1:250; Molecular Probes Inc. USA). DAPI was used to counterstain nuclei. To determine the positive cell fraction, the number of GLP-1-, PYY-, or GIP-positive cells and intestinal total areas were quantified in 10 fields per condition. The results were noted under randomized conditions by a single investigator and expressed as the number of GLP-1-, PYY-, or GIP-positive cells/mm^2 of intestine.

Oral Glucose Tolerance Test (OGTT) and Insulin Measurement

Three weeks after surgery, an oral glucose tolerance test (OGTT) was performed in Wistar, GK-RYGB, GK, and GK-sham 12-h fasting rats. A 20% *w*/*v* D-glucose solution (2 g/kg) was administered through gavage, and glycemia was measured by a Glucocard G-Meter 1810 glucometer (Menarini Diagnostics, Italy) in blood samples obtained from the rat tails at 0, 30, 60, 90, and 120 min after glucose solution administration [19].

Three weeks after surgery, after a 12-hour fasting period, insulin measurement was performed in blood samples from the rat tails every 10 min for 60 min after glucose solution administration using an ELISA kit (ALPCO Diagnostics, Salem, NH).

Hormonal Study and PYY Infusion Assay

Four weeks after surgery, a 4 ml/kg, 13.9 KJ/ml mixed meal was administered to the 12-h fasting rats by oral gavage. Blood samples obtained from the rat tails, every 15 min for 120 min, were added to EDTA tubes containing dipeptidyl peptidase-4 (DPP-4) inhibitor (10 μl/ml blood; Millipore) and centrifuged at 4000 × *g* for 15 min at 4 °C; the plasma was then removed and frozen at −80 °C. GLP-1 and GIP were assessed by sandwich ELISA kits (Cloud-Clone Corp, USA). PYY was measured using an ELISA kit (ALPCO Diagnostics, Salem, NH) according to the manufacturer's instructions. The area under the curve (AUC) was calculated by the trapezoidal rule for every parameter in the study.

Three groups of 12-h fasting RYGB rats were included in the PYY intravenous infusions assay 4 weeks after surgery. Rat PYY (3-36) (Bachem, Bubendorf, Switzerland) was dissolved in saline (0.9% NaCl). A dose of 0.6 pmol kg^{-1} min^{-1} or vehicle (saline) was infused for 10 min in the first and second groups, and oral mixed meal was administered in the third group. GLP-1 and GIP plasma levels were quantified as above.

Statistical Analysis

Data are presented as the means ± SEM. For AUC, histological and weight gain data analysis, one-way ANOVA followed by Tukey's/Bonferroni's post hoc test was conducted using SPSS v21.0 software. Statistical significance was accepted at $P < 0.05$.

Results

Weight Gain

As Fig. 1 shows, weight gain was measured in the Wistar, GK, GK-sham, and GK-RYGB groups 40 days after surgery. No differences appeared between any groups from the first day to the 34th day, but a limited weight gain was shown in the GK-RYGB group from the 34th day to the end of the study ($P < 0.05$) compared to the Wistar GK or GK-sham groups.

OGTT and Insulin Secretion

Three weeks after surgery, OGTT was performed in the Wistar, GK, GK-sham, and GK-RYGB groups. Similar curves were obtained for the Wistar and GK-RYGB groups showing a glucose tolerance improvement compared to curves obtained for the GK or GK-sham groups (Fig. 2a). The AUC was also calculated for the four groups. Significant differences ($P < 0.05$) were found between Wistar and GK-RYGB AUCs and the GK and GK-sham AUCs (Fig. 2b). Glucose tolerance improvement was confirmed in the RYBG group 3 weeks after surgery.

Additionally, insulin secretion was analyzed in all the groups 3 weeks after surgery. Similar and higher insulin secretion patterns were shown by Wistar and GK-RYGB rats compared to GK or GK-sham rats (Fig. 2c). The GK-RYGB or Wistar AUCs were significantly higher ($P < 0.05$) than GK or GK-sham AUCs (Fig. 2d).

Hormonal Assay

To test whether the improved glucose tolerance was related to changes in incretin secretion, GLP-1 GIP and PYY plasma levels were assayed after mixed-meal administration in the four study groups 4 weeks after surgery. As Fig. 3a shows, similar higher PYY secretion patterns appeared in the Wistar and GK-RYGB groups with respect to the GK and GK-sham groups. In addition, Wistar and GK-RYGB data displayed an early remarkable secretion peak approximately 30 min after mixed-meal administration. Wistar and GK-RYGB PYY AUC values appeared to be increased compared to GK or GK-sham PYY AUC values ($P < 0.05$) (Fig. 3b).

We also found intense differences regarding the GLP-1 secretion pattern between Wistar versus GK-RYGB rats and between GK versus GK-sham rats. The maximum GLP-1 plasma values occurred at 60 min after meal administration in Wistar, GK, and GK-sham groups but appeared 15 min later

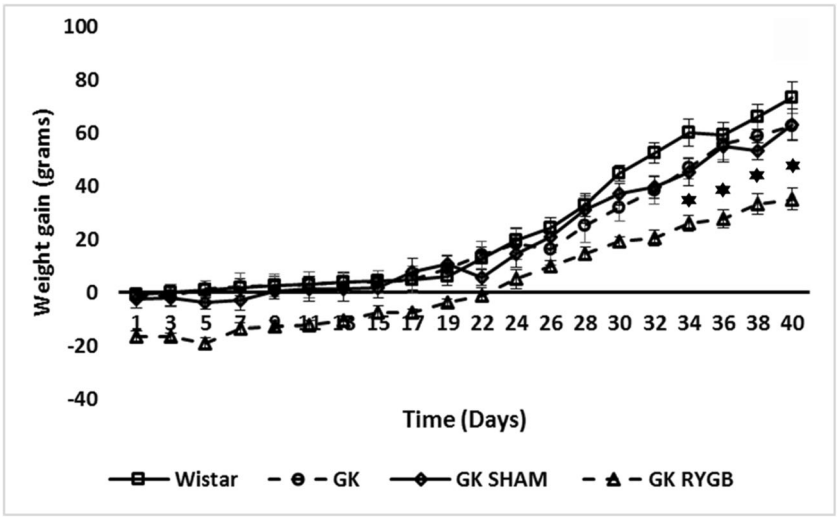

Fig. 1. Weight gain in $n = 6$ Wistar (solid black line with squares), $n = 6$ GK (discontinuous black line with circles), $n = 6$ GK-sham (solid black line with diamonds), and $n = 6$ GK-RYGB (discontinuous black line with triangles) is presented as grams on the *Y*-axis over 40 days following surgery represented on the *X*-axis. Values are expressed as the mean ± SEM. $^*P < 0.05$.

in GK-RYGB rats (Fig. 3c). GLP-1 AUCs were significantly increased in Wistar and GK-RYGB compared to GK and GK-sham groups ($P < 0.05$) (Fig. 3d).

Finally, GIP plasma levels were tested after mixed-meal administration. No differences were found between the groups in the first part of the assay, but higher GIP plasma levels

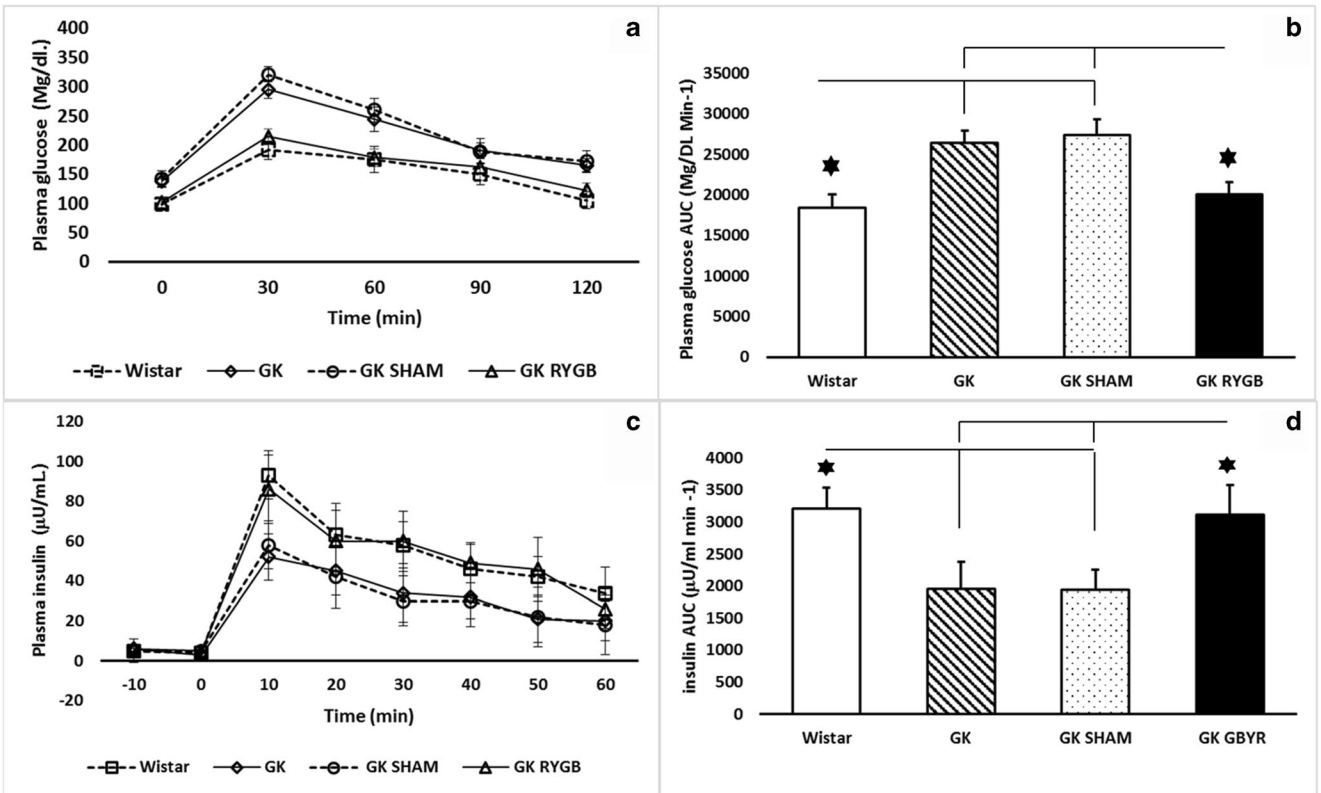

Fig. 2. **a** Oral glucose tolerance test (OGTT) in $n = 6$ Wistar (discontinuous black line with squares), $n = 6$ GK (solid black line with diamonds), $n = 6$ GK-sham (discontinuous black line with circles), and $n = 6$ GK-RYGB (solid black line with triangles). Glycemia is represented as mg/dl on the *Y*-axis and time after glucose load on the *X*-axis. Values are expressed as the mean ± SEM. **b** Area under the curve (AUC) values are presented as mg/dl min^{-1} on the *Y*-axis and expressed as the mean ± SEM. $^*P < 0.05$; Wistar: 18,420 ± 1640, GK: 26415 ± 1470, GK-sham: 27360 ± 1914, GK-RYGB: 19980 ± 1570. **c** Plasma insulin in $n = 6$ Wistar (discontinuous black line with squares), $n = 6$ GK (solid black line with diamonds), $n = 6$ GK-sham (discontinuous black line with circles), and $n = 6$ GK-RYGB (solid black line with triangles). Plasma insulin levels are represented as µU/ml on the *Y*-axis and minutes after glucose load in minutes on the *X*-axis. Values are expressed as the mean ± SEM. **d** Insulin area under the curve (AUC) values are presented as µU/ml min^{-1} on the *Y*-axis and expressed as the mean ± SEM. $^*P < 0.05$; Wistar: 3210 ± 327, GK: 1955 ± 424, GK-sham: 1935 ± 316, GK-RYGB: 3115 ± 462.

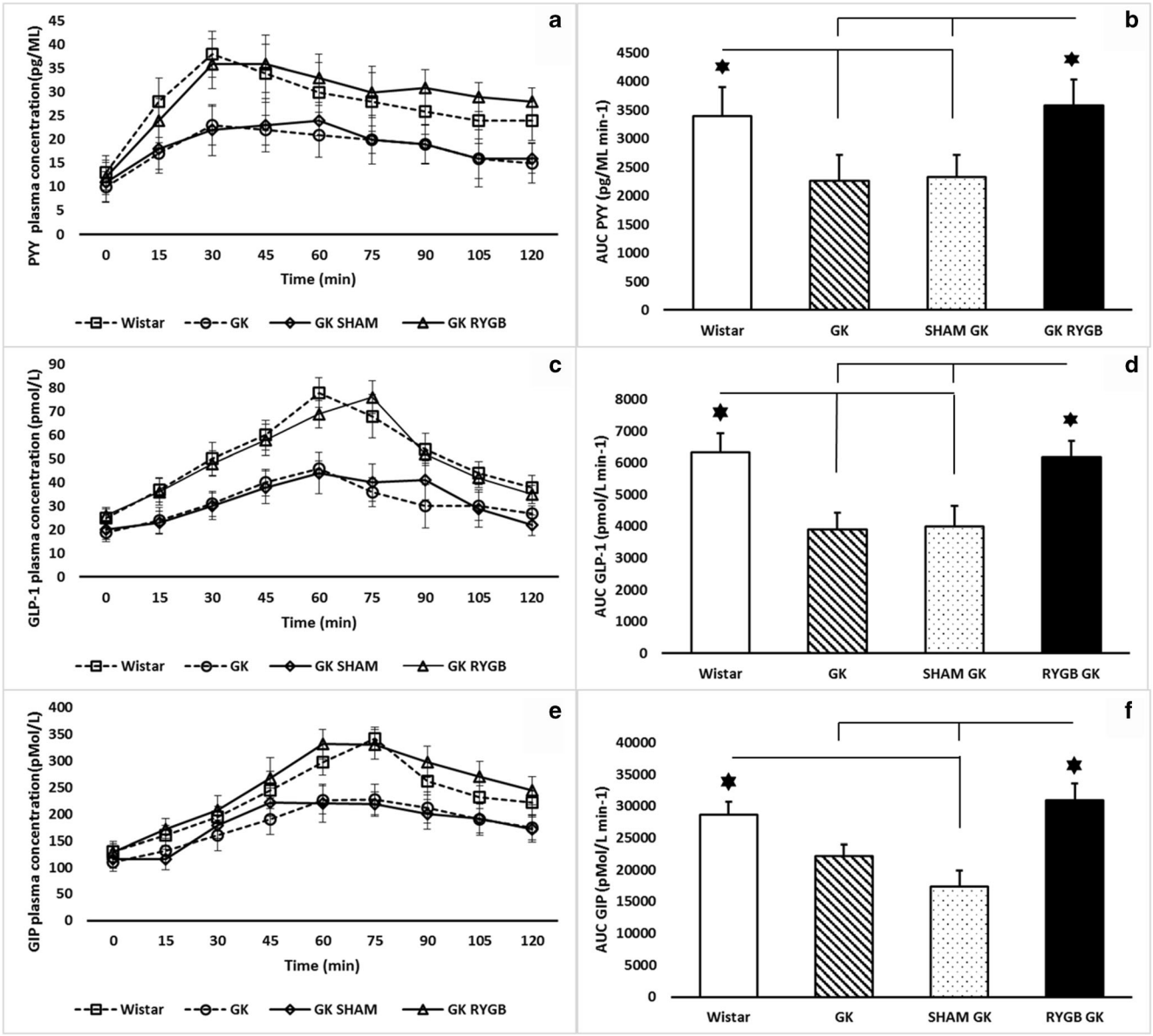

Fig. 3. **a** PYY plasma level after mixed-meal administration in $n = 6$ Wistar (discontinuous black line with squares), $n = 6$ GK (discontinuous black line with circles), $n = 6$ GK-sham (solid black line with diamonds), and $n = 6$ GK-RYGB (solid black line with triangles). PYY plasma levels are represented as pg/ml on the *Y*-axis and minutes after mixed meal in minutes on the *X*-axis. **b** PYY area under the curve (AUC) values are presented as pg/ml min^{-1} on the *Y*-axis and expressed as the mean ± SEM. $^*P < 0.05$; Wistar: 3397 ± 510, GK: 2257 ± 460, GK-sham: 2332 ± 381, GK-RYGB: 3585 ± 446. **c** GLP-1 plasma level after mixed-meal administration in $n = 6$ Wistar (discontinuous black line with squares), $n = 6$ GK (discontinuous black line with circles), $n = 6$ GK-sham (solid black line with diamonds), and $n = 6$ GK-RYGB (solid black line with triangles). GLP-1 plasma levels are represented as pmol/l on the *Y*-axis and minutes after mixed meal in minutes on the *X*-axis. **d** GLP-1 area under curve (AUC) values are presented as pmol/l min^{-1} on the *Y*-axis and expressed as the mean ± SEM. $^*P < 0.05$; Wistar: 6337 ± 600, GK: 3900 ± 536, GK-sham: 3990 ± 647, GK-RYGB: 6172 ± 504. **e** GIP plasma level after mixed-meal administration in $n = 6$ Wistar (discontinuous black line), $n = 6$ GK (discontinuous gray line), $n = 6$ GK-sham (solid gray line), and $n = 6$ GK-RYGB (solid black line) GLP-1 plasma levels are represented as pmol/l on the *Y*-axis and minutes after mixed meal in minutes on the *X*-axis. **f** GIP area under the curve (AUC) values are presented as pmol/l min^{-1} on the *Y*-axis and expressed as the mean ± SEM. $^*P < 0.05$; Wistar: 28,642 ± 2000, GK: 22,140 ± 1800, GK-sham: 17,362 ± 2500, GK-RYGB: 30,960 ± 2603.

appeared in the Wistar and GK-RYGB groups with respect to the GK and GK-sham groups in the second part of the assay (Fig. 3e). However, the AUC values of the Wistar and GK-RYGB groups were clearly different from the GK and GK-sham AUC values ($P < 0.05$) (Fig. 3f).

Histological Study

Twelve weeks after surgery, PYY, GLP-1, and GIP expression was studied in three intestinal segments—the duodenum, jejunum, and ileum of rats from each group—

and the obtained values were expressed as the number of incretin-positive cells per square millimeter of the intestinal area.

As Fig. 4a and b shows, a statistical increased number of PYY-positive cells appeared in the duodenum and jejunum from GK-RYGB rats compared to these segments from the Wistar, GK, and GK-sham rats ($P < 0.05$). However, GK-RYGB rats showed significantly higher numbers of PYY-positive cells in the ileum compared to GK and GK-sham ($P < 0.05$) (Fig. 4c) Representative photographs of PYY-immunostained ileum tissue in the experimental groups (Figure 5).

GLP-1 expression appeared to be increased in the three segments of the small intestine in GK-RYGB rats with respect to the Wistar, GK, and GK-sham groups ($P < 0.05$) (Fig. 4d–f). A remarkable decrease in GLP-1-positive cells in the ileum was observed in the GK and GK-sham groups of rats ($P < 0.01$) (Fig. 4f).

Low expression values of GIP-positive cells were found in the duodenum of the GK and GK-sham groups with respect to the Wistar and GK-RYGB groups ($P < 0.05$) (Fig. 4 g–i). However, no differences were found in the number of GIP-positive cells in the jejunum or ileum between any groups.

PYY (3-36) Infusion Assay

To test the role of PYY in stimulating GIP and GLP-1 secretion, a pool of GK-RYGB rats was randomly divided into three groups 4 weeks after surgery and fasted for 12 h. In this oral test, grouped animals were fed with mixed meal, infused

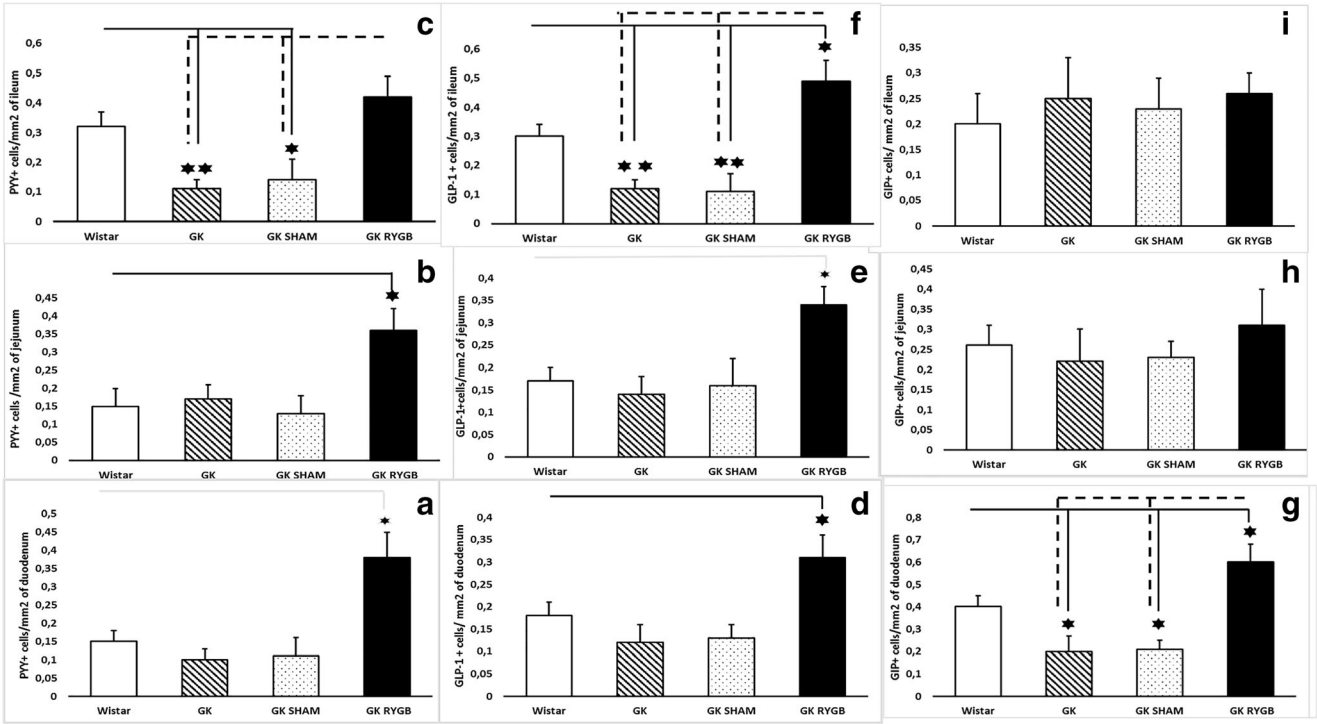

Fig. 4. PYY expression in the duodenum (**a**), the jejunum (**b**), and the ileum (**c**) presented as PYY-positive cells/mm^2 of intestinal area in the *Y*-axis. Wistar rats ($n = 6$), white bar; GK ($n = 6$) rats, striped bar; GK-sham ($n = 6$) rats, dotted bar and GK-RYGB ($n = 6$), black bar, are presented on the *X*-axis. Values are represented as the mean ± SEM. *$P < 0.05$, **$P < 0.01$. Data obtained in the duodenum were as follows: Wistar 0.15 ± 0.03; GK 0.10 ± 0.03; GK-sham 0.11 ± 0.05; GK-RYGB 0.38 ± 0.07. In the jejunum, the data were as follows: Wistar 0.15 ± 0.05; GK 0.17 ± 0.04; GK-sham 0.13 ± 0.05; GK-RYGB 0.36 ± 0.06. In the ileum, the data were as follows: Wistar 0.32 ± 0.05; GK 0.11 ± 0.03; GK-sham 0.14 ± 0.07; GK-RYGB 0.42 ± 0.07. In (**c**), discontinuous black line indicates significance respect to GK-RYGB group and solid black line indicates significance respect to Wistar group. **d**–**f** GLP-1 expression in the duodenum (**d**), jejunum (**e**), or the ileum (**f**) presented as GLP-1-positive cells/mm^2 of intestinal area in the *Y*-axis. Wistar rats ($n = 6$), white bar; GK rats ($n = 6$), striped bar; GK-sham rats ($n = 6$), dotted bar; and GK-RYGB ($n = 6$), black bar. The data are presented on the *X*-axis. Values are represented as the mean ± SEM. *$P < 0.05$. Data obtained in the duodenum were as follows: Wistar 0.18 ± 0.03; GK 0.12 ± 0.04; GK-sham 0.13 ± 0.06; GK-RYGB 0.31 ± 0.05. The jejunum data were as follows: Wistar 0.17 ± 0.03; GK 0.14 ± 0.04; GK-sham 0.16 ± 0.06; GK-RYGB 0.34 ± 0.04. The ileum data were as follows: Wistar 0.30 ± 0.04; GK 0.12 ± 0.03; GK-sham 0.11 ± 0.06; GK-RYGB 0.49 ± 0.07. In (**f**), discontinuous black line indicates significance respect to GK-RYGB group and solid black line indicates significance respect to Wistar group. **g**–**i** GIP expression in the duodenum, jejunum, or the ileum presented as GIP-positive cell/mm^2 of intestinal area on the *Y*-axis. Wistar rats ($n = 6$), white bar; GK rats ($n = 6$), striped bar; GK-sham rats ($n = 6$), dotted bar; and GK-RYGB ($n = 6$), black bar. Data are presented on the *X*-axis. Values are represented as the mean ± SEM. *$P < 0.05$. Data obtained in the duodenum were as follows: Wistar 0.4 ± 0.05; GK 0.2 ± 0.07; GK-sham 0.21 ± 0.04; GK-RYGB 0.6 ± 0.08. In the jejunum, the data were as follows: Wistar 0.26 ± 0.05; GK 0.22 ± 0.08; GK-sham 0.23 ± 0.04; GK-RYGB 0.31 ± 0.09. In the ileum, the data were as follows: Wistar 0.20 ± 0.06; GK 0.25 ± 0.08; GK-sham 0.23 ± 0.06; GK-RYGB 0.26 ± 0.04. In (**g**), discontinuous black line indicates significance respect to GK-RYGB group and solid black line indicates significance respect to Wistar group

with PYY (3-36), or infused with saline solution. The data showed a normal GLP-1 secretion pattern with a peak 75 min after meal administration in the GK-RYGB+meal group. A slight elevation in the GLP-1 plasma level, not as high as the GK-RYGB+meal group, appeared in the PYY (3-36)-infused rats. No increase in GLP-1 plasma levels was found in the saline-infused rats (Fig. 6a).

Significant differences were found between the GK-RYGB+vehicle AUC versus GK-RYGB+PYY (3-36) AUC ($P < 0.05$) and GK-RYGB+meal AUC ($P < 0.01$) (Fig. 6b).

A GIP secretion pattern similar to that described for the GK-RYGB group was found in the GK-RYGB+meal group. The described curve was similar to that described in Fig. 3e for the GK-RYGB group. A similar elevation in GIP plasma level was detected in the second part of the assay (from 60 min after infusion to the end of the test) in the GK-RYGB+PYY group (3-36), but no GIP plasma elevation was found in the GK-RYGB+vehicle rat group (Fig. 6c).

Statistical differences were determined between the AUC of the GK-RYGB+meal group versus the AUC of the GK-RYGB+vehicle group; no differences were found versus the AUC of the GK-RYGB+PYY (3-36) group ($P < 0.05$) (Fig. 6d).

Discussion

As presented in the data, a positive effect on glucose homeostasis was observed following RYGB in our model (Fig. 2a, b). This improvement can be attributed to increased insulin secretion (Fig. 2c, d), independent of body weight reduction (Fig. 1). A large number of authors reported variations in incretins GLP-1 and GIP secretion patterns after RYBG. They tried to explain this phenomenon, such as increased GLP-1 secretion, due to rapid delivery of nutrients to the distal bowel and L-cell stimulation [20–22] or/and suppression of GIP secretion by K cells due to a restricted delivery of nutrients in the duodenum [2, 23]. However, recently, a new actor may play an important role in glycemic regulation. Postprandial increased plasma levels of PYY have been reported in patients after RYGB [24]. PYY may indirectly stimulate postprandial insulin secretion in mice [14].

We investigated changes in plasma profiles of GLP-1, PYY, and GIP after meal administration in the RYGB group and controls 4 weeks after surgery. Our data revealed that PYY, GLP-1, and GIP AUC after a mixed-nutrient meal were increased postoperatively in the RYGB group, with values higher than or similar to those of the nondiabetic controls (Fig. 3d–f). These findings were consistent with several studies that reported increased postprandial PYY and GLP-1 plasma levels after RYGB [25, 26]. We also observed one peak in RYGB on PYY secretion 15 min after meal administration, while the peaks of GLP-1 and GIP secretion appeared 30 and 25 min later (Fig. 3a, b). As above, the data on GLP-1 and GIP secretion profiles have been supported for a long time by previous studies in rodents and patients, with similar responses to OGTT [27, 28]. However, the early PYY secretion profile in only the RYGB group was notably interesting. Specifically, attending to the effect of PYY (3-36) on neuropeptide Y2 receptor (NPY2R) leads to increased hepatoportal GLP-1 plasma levels and improved glucose tolerance [14].

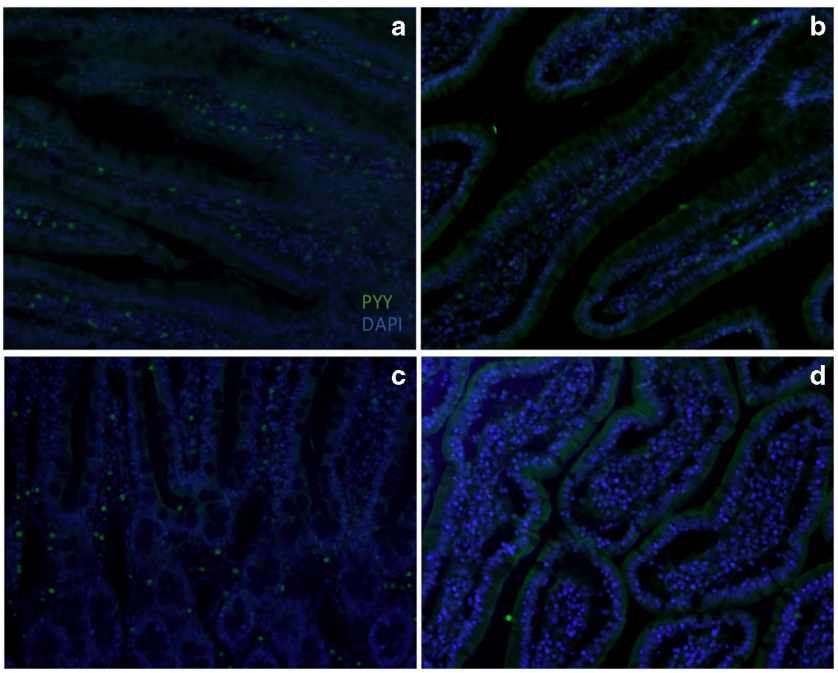

Fig. 5. Representative photographs of PYY-immunostained ileum tissue in the experimental groups. **a** An increased number of PYY-positive cells (green) appeared in the ileum of GK-RYGB rats compared to GK rats (**b**) and GK-sham (**d**). A high number of PYY-positive cells appear in Wistar rats too (**c**)

These findings lead us to think about PYY as a possible trigger that starts the gut hormone secretion chain postmeal in our model. We consider that PYY is probably activated in the early presence of nutrients in the ileum. However, some authors propose that PYY and GLP-1 postprandial secretion profiles are often dissimilar because DPP-4 inactivates GLP-1 and activates PYY [29, 30]. To confirm our hypothesis, we measured GLP-1 and GIP plasma levels in a RYGB group across several conditions (after meal, PYY (3-36) or vehicle infusion). Our data showed that GLP-1 and GIP AUCs after PYY (3-36) infusion were increased and similar to those obtained after meal (Fig. 5b), with similar secretion profiles (Fig. 5a). These data indicated a positive effect of PYY plasma levels on GLP-1 and GIP secretion, beyond the direct nutrient contact of the intestine (Fig. 6). This finding was supported in previous findings about the role of different stimuli, including paracrine, endocrine, and neural mechanisms, on GLP-1 secretion from L cells [31, 32]. Thus, the GLP-1 increase reduced GIP serum levels after feeding, as reported in RYGB-operated mice or type 2 diabetes patients [2, 33]. Moreover, additional rodent studies showed that PYY (3-36) reduced postprandial glycemia. This effect appeared to be mediated by GLP-1 because the GLP-1 antagonist exendin (9-39) blocked the glucose-lowering effects of PYY (3-36) [34].

In this way, the widespread distribution of NPY2R in the small intestine of rats [35] and its potential to increase hepatoportal GLP-1 plasma levels [14] are also acknowledged. Therefore, we analyzed GLP-1, GIP, and PYY expression in the small intestine of our model and found increased PYY expression in the ileum of the RYGB group similar to the nondiabetic controls, but not in the duodenum or jejunum, and increased expression of GIP in the jejunum. Nevertheless, GLP-1 expression appeared to increase in the three areas of the small intestine in the RYGB group (Fig. 4a–i). These data suggested widespread and increased expression of GLP-1, but not GIP, beyond the early presence of nutrient stimulation in the ileum of the RYGB group, which is probably due to other stimulation mechanisms. Some of these mechanisms could be

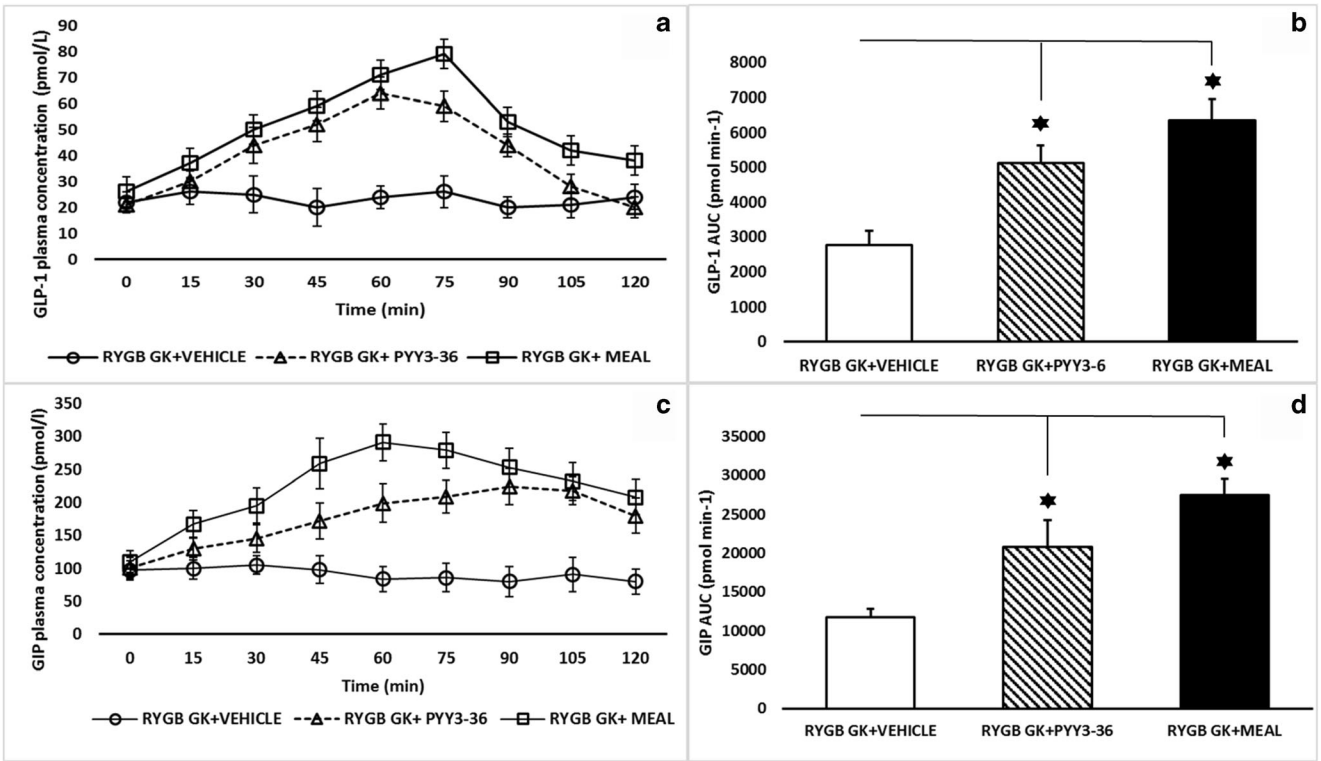

Fig. 6. **a** GLP-1 plasma level after meal administration in n = 6 GK-RYGB rats (solid black line with squares); after PYY (3-36) infusion in n = 6 GK-RYGB rats (discontinuous black line) or vehicle infusion in n = 6 GK-RYGB rats (solid black line with circles), GLP-1 plasma level expressed as pmol/ml is presented on the *Y*-axis, and time expressed as minutes on the *X*-axis. Values are represented as the mean ± SEM. **b** GLP-1 plasma level area under the curve (AUC) after meal administration (n = 6), black bar; PYY (3-36) infusion (n = 6), striped bar; or vehicle infusion (n = 6), white bar, in GK-RYGB rats. AUCs are represented as GLP-1 pmol/l min^{-1} on the *Y*-axis. Values are expressed as the mean ± SEM. *P < 0.05. Data found were GK-RYGB+vehicle: 2775 ± 405; GK-RYGB+PYY (3-36): 5122.5 ± 511; GK-RYGB+meal: 63,525 ± 590. **c** GIP plasma levels after meal administration in n = 6 GK-RYGB rats (solid black line with squares); after PYY (3-36) infusion in n = 6 GK-RYGB rats (discontinuous black line) or vehicle infusion in n = 6 GK-RYGB rats (solid black line with circles), GIP plasma levels are presented on the *Y*-axis expressed as pmol/ml. Time was expressed as minutes on the *X*-axis. Values are represented as the mean ± SEM. **d** GIP plasma level area under curve (AUC) after meal administration (n = 6), black bar; PYY (3-36) infusion (n = 6), striped bar; or vehicle infusion (n = 6), white bar, in GK-RYGB rats. AUCs are represented as GIP pmol/l min^{-1} on the *Y*-axis. Values are expressed as the mean ± SEM. *P < 0.05. Data found were GK-RYGB+vehicle: 11,715 ± 1123; GK-RYGB+PYY (3-36): 20,812.5 ± 3423.5; GK-RYGB+meal: 27,525 ± 2056.8

explained by the presence of neuropods found in mice [36]. These neuropods respond as an axon-like basal process containing peptide-secreting vesicles from the PYY-secreting enteroendocrine cells. One possibility is that neuropods contribute to trigger GLP-1 release in the jejunum and even GLP-1 and GIP release in the duodenum, linking the distal and proximal small intestine.

In summary, these data confirm a central role for PYY in glucose homeostasis improvement after RYGB in GK rats. Our experiments revealed a high peak in PYY plasma levels earlier than any other enterohormone after mixed-meal administration. This suggests the possibility that PYY is the first enterohormone to be secreted due to meal presence in the distal intestine. Recently, PYY was shown to trigger GLP-1 and GIP secretion. The PYY local immunohistochemical expression in the ileum and widespread expression of GLP-1 in the small intestine—beyond the place where the meal arrives early—support this thought. Additionally, the test of PYY (3-36) infusion in our model reports similar plasma profiles of GLP-1 and GIP after a meal or PYY (3-36) infusion and confirms our theory.

These findings allow us to think about PYY not only as an incretin that may mediate direct effect on insulin sensitivity [12] or gastric emptying [37]. As seen in our model, we propose that PYY is the trigger that allows secretion of other important enterohormones, such as GLP-1 or GIP.

Despite numerous efforts, further research is required to answer many more questions regarding PYY endocrine signals and function in a physiological context or after RYGB in animal models and humans.

Acknowledgments and Grant Support The authors desire to thank the *Asociacion Gaditana de Apoyo al Investigador (AGAI)* and Sustainable Social Development Research Institute (INDESS) University of Cadiz for economic support.

Authors' contribution statement All authors (A.C., G.M.P.A., and J.A.P.) designed the project. G.P.A. and D.A.G. analyzed the statistical procedures. A.C. and J.A.P. performed surgical techniques and followed the animal survival period. G.M.P.A. and A.R.G. performed the histological techniques. All authors participated in manuscript elaboration.

Data availability All the data supporting the results and critical resources will be available at the following institutional repository of the University of Cadiz http://hdl.handle.net/10498/21156.

Compliance with ethical standards

Disclosure and Conflict Statement The authors declare no conflict of interest. All the authors declare to have read and signed the disclosure and conflict statement. We did not receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted work (limited to the grant). We have no financial relationships (regardless of the amount of compensation) with entities that could be related to the aim of the study. We have no patents or manuscripts, pending or issued, broadly relevant to this work. There are no other relationships or activities that readers could perceive to have influenced, or that give the appearance of, or potentially influence, what we have written in the submitted work.

Prior Communications We, the authors, state that the article is original, it has not been submitted for publication in another journal, and it has not yet been published either wholly or in part.

Statement of Animal Welfare The authors declare that an ethical approval was obtained for our study. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted and ethical approval was obtained from the University of Cadiz Committee for the Ethical Use and Care of Experimental Animals. This committee ensured that the procedures in all experiments were performed according to international guidelines and regulations for the welfare of the animals. The internal Committee for the Ethical Use and Care of Experimental Animals followed the instructions marked for the Autonomous Andalusian Authority.

References

1. Schauer PR, Bhatt DL, Kirwan JP, et al. Bariatric surgery versus intensive medical therapy for diabetes—3-year outcomes. N Engl J Med. 2014;370(21):2002–13. https://doi.org/10.1056/NEJMoa1401329.
2. Zhou J, Hao Z, Irwin N, et al. Gastric inhibitory polypeptide (GIP) is selectively decreased in the roux-limb of dietary obese mice after RYGB surgery. PLoS One. 2015;10(8):e0134728. https://doi.org/10.1371/journal.pone.0134728.
3. Shah M, Law JH, Micheletto F, et al. Contribution of endogenous glucagon-like peptide 1 to glucose metabolism after Roux-en-Y gastric bypass. Diabetes. 2014;63(2):483–93. https://doi.org/10.2337/db13-0954.
4. Chambers AP, Jessen L, Ryan KK, et al. Weight-independent changes in blood glucose homeostasis after gastric bypass or vertical sleeve gastrectomy in rats. Gastroenterology. 2011;141(3):950–8. https://doi.org/10.1053/j.gastro.2011.05.050.
5. Mokadem M, Zechner JF, Margolskee RF, et al. Effects of Roux-en-Y gastric bypass on energy and glucose homeostasis are preserved in two mouse models of functional glucagon-like peptide-1 deficiency. Mol Metab. 2013;3(2):191–201. https://doi.org/10.1016/j.molmet.2013.11.010.
6. Ye J, Hao Z, Mumphrey MB, et al. GLP-1 receptor signaling is not required for reduced body weight after RYGB in rodents. Am J Physiol Regul Integr Comp Physiol. 2014;306(5):352–62. https://doi.org/10.1152/ajpregu.00491.2013.
7. Ramracheya RD, McCulloch LJ, Clark A, et al. PYY-dependent restoration of impaired insulin and glucagon secretion in type 2 diabetes following Roux-en-Y gastric bypass surgery. Cell Rep. 2016;15(3):944–50. https://doi.org/10.1016/j.celrep.2016.03.091.
8. Schmidt JB, Pedersen SD, Gregersen NT, et al. Effects of RYGB on energy expenditure, appetite and glycaemic control: a randomized controlled clinical trial. Int J Obes. 2016;40(2):281–90. https://doi.org/10.1038/ijo.2015.162.

9. Taylor IL. Distribution and release of peptide YY in dog measured by specific radioimmunoassay. Gastroenterology. 2008;88(3):731–7. https://doi.org/10.1016/0016-5085(85)90144-1.
10. Gu N, Tsuda M, Matsunaga T, et al. Glucose regulation of dipeptidyl peptidase IV gene expression is mediated by hepatocyte nuclear factor-1alpha in epithelial intestinal cells. Clin Exp Pharmacol Physiol. 2008;35(12):1433–9. https://doi.org/10.1111/j.1440-1681.2008.05015.x.
11. Guida C, McCulloch LJ, Godazgar M, et al. Sitagliptin and Roux-en-Y gastric bypass modulate insulin secretion via regulation of intra-islet PYY. Diabetes Obes Metab. 2018;20(3):571–81. https://doi.org/10.1111/dom.13113.
12. Van den Hoek AM, Heijboer AC, Corssmit EP, et al. PYY 3-36 reinforces insulin action on glucose disposal in mice fed a high-fat diet. Diabetes. 2004;53(8):1949–52. https://doi.org/10.2337/diabetes.53.8.1949.
13. Allen JM, Fitzpatrick ML, Yeats JC, et al. Effects of peptide YY and neuropeptide Y on gastric emptying in man. Digestion. 1984;30:255–62. https://doi.org/10.1159/000199117.
14. Chandarana K, Gelegen C, Elaine E, et al. Peripheral activation of the Y2-receptor promotes secretion of GLP-1 and improves glucose tolerance. Mol Metab. 2013;2(3):142–52. https://doi.org/10.1016/j.molmet.2013.03.001.
15. Bohorquez DV, Liddle RA. Axon-like basal processes in enteroendocrine cells: characteristics and potential targets. Clin Transl Sci. 2011;4(5):387–91. https://doi.org/10.1111/j.1752-8062.2011.00299.x.
16. Vannan DT, Bomhof MR, Reimer RA. Comparison of glucose and satiety hormone response to oral glucose vs two mixed-nutrient meals in rats. Front Nutr. 2018;5:89. https://doi.org/10.3389/fnut.2018.00089.
17. Yu H, Zheng X, Zhang Z. Mechanism of Roux-en-Y gastric bypass treatment for type 2 diabetes in rats. J Gastrointest Surg. 2013;17(6):1073–83. https://doi.org/10.1007/s11605-013-2188-3.
18. Camacho-Ramírez A, Almorza-Gomar D, Díaz-Gómez A, et al. The histomorphometric parameters of endocrine pancreas after bariatric surgery in healthy animal models. Tissue Cell. 2019;57:78–83. https://doi.org/10.1016/j.tice.2019.02.008.
19. Ndong M, Uehara M, Katsumata S, et al. Effects of oral administration of Moringa oleifera Lam on glucose tolerance in Goto-Kakizaki and Wistar rats. J Clin Biochem Nutr. 2007;40(3):229–33. https://doi.org/10.3164/jcbn.40.229.
20. Kashyap SR, Daud S, Kelly KR, et al. Acute effects of gastric bypass versus gastric restrictive surgery on beta-cell function and insulinotropic hormones in severely obese patients with type 2 diabetes. Int J Obes. 2010;34:462–71. https://doi.org/10.1038/ijo.2009.254.
21. Nannipieri M, Baldi S, Mari A, 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(11):4391–9. https://doi.org/10.1210/jc.2013-2538.
22. Le Roux CW, Aylwin SJ, Batterham RL, et al. Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. Ann Surg. 2006;243(1):108–14. https://doi.org/10.1097/01.sla.0000183349.16877.84.
23. Korner J, Bessler M, Inabnet W, et al. Exaggerated GLP-1 and blunted GIP secretion are associated with Roux-en-Y gastric bypass but not adjustable gastric banding. Surg Obes Relat Dis. 2007;3(6):597–601. https://doi.org/10.1016/j.soard.2007.08.004.
24. Yan W, Polidori D, Yieh L, et al. Effects of meal size on the release of GLP-1 and PYY after Roux-en-Y gastric bypass surgery in obese subjects with or without type 2 diabetes. Obes Surg. 2014;24(11):1969–74. https://doi.org/10.1007/s11695-014-1316-9.
25. Harvey EJ, Arroyo K, Korner J, et al. Hormone changes affecting energy homeostasis after metabolic surgery. Mt Sinai J Med. 2010;77(5):446–65. https://doi.org/10.1002/msj.20203.
26. Peterli R, Steinert RE, Woelnerhanssen B, et al. Metabolic and hormonal changes after laparoscopic Roux-en-Y gastric bypass and sleeve gastrectomy: a randomized, prospective trial. Obes Surg. 2012;22(5):740–8. https://doi.org/10.1007/s11695-012-0622-3.
27. Meirelles K, Ahmed T, Culnan DM, et al. Mechanisms of glucose homeostasis after Roux-en-Y gastric bypass surgery in the obese, insulin-resistant Zucker rat. Ann Surg. 2009;249(2):277–85. https://doi.org/10.1097/SLA.0b013e3181904af0.
28. Østoft SH, Bagger JI, Hansen T, et al. Incretin effect and glucagon responses to oral and intravenous glucose in patients with maturity-onset diabetes of the young—type 2 and type 3. Diabetes. 2014;63(8):2838–44. https://doi.org/10.2337/db13-1878.
29. Eissele R, Goke R, Willemer S, et al. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Investig. 1992;122(4):283–91. https://doi.org/10.1111/j.1365-2362.1992.tb01464.x.
30. Mortensen K, Christensen LL, Holst JJ, et al. GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. Regul Pept. 2003;114(2-3):189–96. https://doi.org/10.1016/S0167-0115(03)00125-3.
31. Tolhurst G, Reimann F, Gribble FM. Nutritional regulation of glucagon-like peptide-1 secretion. J Physiol. 2009;587:27–32. https://doi.org/10.1113/jphysiol.2008.164012.
32. Holst JJ. The physiology of glucagon-like peptide 1. Physiol Rev. 2007;87:1409–39. https://doi.org/10.1152/physrev.00034.2006.
33. Wu Q, Xiao Z, Cheng Z, et al. Changes of blood glucose and gastrointestinal hormones 4 months after Roux-en-Y gastric bypass surgery in Chinese obese type 2 diabetes patients with lower body mass index. J Diabetes Investig. 2012;4(2):214–21. https://doi.org/10.1111/jdi.12005.
34. Manning S, Batterham RL. The role of gut hormone peptide YY in energy and glucose homeostasis: twelve years on. Annu Rev Physiol. 2014;76:585–608.
35. Goumain M, Voisin T, Lorinet AM, et al. Identification and distribution of mRNA encoding the Y1, Y2, Y4, and Y5 receptors for peptides of the PP-fold family in the rat intestine and colon. Biochem Biophys Res Commun. 1998;247:52–6. https://doi.org/10.1006/bbrc.1998.8647.
36. Bohórquez DV, Samsa LA, Roholt A, et al. An enteroendocrine cell–enteric glia connection revealed by 3D electron microscopy. PLoS One. 2014;9(2):e89881. https://doi.org/10.1371/journal.pone.0089881.
37. Pironi L, Stanghellini V, Miglioli M, et al. Fat-induced ileal brake in humans: a dose-dependent phenomenon correlated to the plasma levels of peptide YY. Gastroenterology. 1993;105(3):733–9. https://doi.org/10.1016/0016-5085(93)90890-O.