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Effects of preduodenal ileal surgical transposition on enteroendocrine intestinal cells in wistar rats: Histomorphological and serum changes

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Summary. In our study, we focused on the role of the distal ileum as a main endocrine actor in relation to the pancreas. We investigated the effects of intestinally released hormones on the pancreas in terms of type 2 diabetes mellitus (T2DM) improvement, as a main effect of bariatric surgeries. To specifically study the importance of the ileum, we used an experimental surgical model performed in healthy Wistar rats. After preduodenal transposition of the ileum, we analyzed the histology and enterohormonal cells of the intestine. We measured the plasma level of several hormones and effectors in this enteropancreatic axis. We used a surgical control (Sham) group and a surgical group, where ileum preduodenal transposition (PDIT) was performed. We measured basal glycemia and serum levels of several incretins, including GLP-1, PYY, and GIP, and we performed a glucose overdose test. After two test periods, the basal glycemia and glucose overdose results were not different between groups, however, the PDIT group had significantly increased expression of GLP-1, with increased cellular release in the ileum and duodenum compared with the Sham group. Both plasma GIP levels and GIP tissue expression were decreased in the PDIT group compared with the sham group. There were no differences in PPY hormone levels. The ileum crypts and villi of the PDIT group showed improvement in histological parameters. We concluded that model animals had an altered transposed ileum related to the enterohormonal adaptation of the ileum. Our results indicated that the ileum is important in the hormonal control of the enteropancreatic axis.

Key words: Ileum, Pancreas, Duodenum, Type 2 Diabetes mellitus, Incretins, Cellular differentiation

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Introduction

There is an increasing prevalence and incidence of obesity and type 2 diabetes mellitus (T2DM), and these conditions are considered a global public health epidemic (Oh et al., 2016a; Ng et al., 2021). Bariatric/metabolic surgery (B/MS) has become one of the most effective treatments to control T2DM (Yan et al., 2019; Somogyi et al., 2020) due to clinical improvement and even remission of the disease in a high percentage of surgical patients (Arterburn et al., 2020). However, the underlying pathophysiological mechanisms remain unclear (Batterham and Cummings, 2016). These mechanisms are thought to be related to the enteropancreatic axis and its surgical modification (Zhang et al., 2017; Prada-Oliveira et al., 2019).

This surgical manipulation and the subsequent changes in the secretion of different enteric hormones, such as glucagon-like peptide-1 (GLP-1), peptide tyrosine-tyrosine (PYY), and gastric inhibitory polypeptide (GIP), underlie the basis of the accepted theories in this field. The hindgut theory (Nausheen et al., 2013; Sawczyn et al., 2018) suggests that the quick passage of the alimentary bolus through the distal small bowel leads to overstimulation of L-cells, with a related increase in the secretion of incretins, especially GLP-1 and PYY (Duan et al., 2014; Camacho-Ramírez et al.,

2020). The increase in the serum levels of these enteric hormones would increase the secretion of insulin and its sensitivity in peripheral tissues, as well as having a protective effect against apoptosis and improving the proliferation rate of beta cells within the pancreatic islet (Oh et al., 2016b; Prada-Oliveira et al., 2019; Pérez-Arana et al., 2022b).

According to this hypothesis, we designed an experimental B/MS surgical procedure involving the transposition of a distal segment of the ileum to a preduodenal location (Zhu et al., 2018). Improved secretion of ileal incretins (GLP-1 and PYY) resulting from early passage through the transposed ileum leads to improved carbohydrate metabolism. Therefore, ileal transposition allowed us to determine the role of the distal small bowel in glycemic control and disease remission (Oh et al., 2016b; Sawcryn et al., 2018).

In this sense, we proposed the use of an experimental surgical technique that is both feasible and effective, consisting of ileal transposition to a preduodenal location (never described before) with no duodenal exclusion or resection. In our model, which was established in male Wistar rats with an observational period of eight weeks, the surgical group was subjected to a preduodenal ileal transposition (PDIT) technique based on three different anastomoses (gastric-ileal, ileal-duodenal, and jejunal-ileal); we also utilized an associated Sham group (Salas-Álvarez et al., 2020, 2023).

In this study, our aim was to determine, in healthy Wistar rats, the role of incretinic alterations in the ileum resulting from modifications to intestinal histology and cellular expression, and the implications of increased glucose tolerance in the resolution of T2DM. To achieve this goal, several functional tests were carried out and the serum levels of GLP-1, PYY, and GIP were measured. Afterward, an immunohistology study was conducted, including the analysis of ileal, duodenal, and jejunal tissue.

Materials and methods

Animals

We used 30 non-obese, non-diabetic Wistar rats. The 30 male Wistar rats were sorted into two randomized groups, an experimental and a surgical control (sham) group, with 15 rats each. The rats were 12-14 weeks old and weighed approximately 300-350 g at the time of surgery. The experimental period was four weeks after surgery.

The rats were provided by the University of Cadiz Animal Production Service, and the surgeries were performed in specific laboratories. All procedures on animals were performed with approval from the University of Cadiz Committee for the Ethical Use and Care of Experimental Animals. The specimens were housed under constant temperature and humidity with a 12-hour light/dark cycle and *ad libitum* access to normal chow and water. We did not use female rats to avoid cyclic variations in the gonadotropin hormonal effect on glycemic metabolism.

Surgical techniques

After a 12-hour fasting period, surgical techniques were performed once the rats were anesthetized with isoflurane. Anesthesia maintenance was obtained by the use of a continuous ventilation pump of isoflurane (between 1-3%). The rats were randomly assigned to the experimental group - undergoing preduodenal ileal transposition (PDIT) - or the control group (Sham).

The control group (Sham) technique involved a bi-subcostal incision allowing for exposure of the small bowel loops. A transverse enterotomy section was performed at 10 cm of the Treitz angle, without intestinal resection. Then, we performed an end-to-end anastomosis with interrupted sutures of PDS 5/0.

In the PDIT group, the surgical procedure was performed through a bi-subcostal incision (Fig. 1). The stomach was mobilized for better exposure after liberating the lesser omentum. We measured the last 10 centimeters of the terminal ileum and divided it, thereby preserving the vascular supply and the distal edge 1 centimeter away from the ileocecal valve. A transverse post-pyloric division was made, followed by closing the duodenal stump with a running suture with PDS 5/0. The distal duodenal stump was anastomosed to the distal edge of the transposed ileum with interrupted stitches of PDS 5/0, while the proximal edge of the ileum was anastomosed to the posterior gastric face with continuous suturing with PDS 5/0 after opening the greater omentum. The last anastomosis was made between the jejunum and terminal ileum, with interrupted sutures of PDS 5/0 (Fig. 2) (Salas-Álvarez et al., 2020).

Basal glycemia, oral glucose tolerance test (OGTT), and insulin measurement

Basal glycemia was measured weekly in both groups for five weeks after surgery. Then, in the fourth and eighth week after surgery, an oral glucose tolerance test (OGTT) was performed in fasted animals from the experimental and sham groups. To this end, a 2 g/kg (20% w/v) D-glucose solution was administered by gavage, and glycemia was measured using a glucometer (Glucocard G-Metre 1810, Menarini Diagnostics, Italy), and blood samples were obtained from the tail veins at 0, 30, 60, 90, and 120 minutes after glucose administration. There was a 12-hour fasting period before the OGTT.

The glucose values were expressed as mg/dl ± SEM. D-xylose was expressed as mmol/ml of plasma. Areas under the curve (AUCs) were calculated using the trapezoidal rule for each parameter in the study. The glucose AUC was calculated using the trapezoidal rule

and expressed as mg/dl.min^{-1}.

Hormonal measurement

Four weeks after surgery, during the OGTT, 10 µl/ml of blood was obtained at 0 and 15 minutes after glucose administration in fasted animals. Blood samples were added to EDTA tubes containing dipeptidyl peptidase-4 (DPP-4) inhibitor (10 µl/ml) (Millipore, USA) and centrifuged at 4000xg for 15 minutes at 4°C. The plasma fraction was removed and stored at -80°C. Plasma concentrations of GIP and GLP-1 were determined using an ELISA kit CEA882Mu/CEA804Mu (Cloud-Clone-Corp, USA), and PPY levels were determined with an ELISA kit 48-PYYRT-E01.1 (ALPCO Diagnostics, Salem, NH) according to the manufacturer's instructions and expressed as pmol/ml. The GIP, PPY, and GLP-1 AUCs were also determined by the trapezoidal rule and expressed as pmol/ml Min-1.

Sacrifice and tissue preparation

The animals were sacrificed eight weeks after surgery using an isoflurane inhalation overdose procedure. Gut samples were immediately removed, and 1 cm full-thickness segments of 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 8 µm microtome sections.

Tissue analysis

For histological analysis, samples of the three intestinal portions of the intestine from the PDIT and Sham groups were used. We measured villus height, crypt depth, and absorbent mucosae surface in 10 fields of eosin-hematoxylin stained slices. Values were expressed as lengths in µm.

In rehydrated sections of the three portions, GIP-, PYY-, and GLP-1-releasing cell populations were analyzed by immunostaining. We used rabbit primary antibodies against GLP-1 (ab111125), PYY (ab 22663), and GIP (ab209792) (Abcam, Cambridge, CB4 OFL, UK). For immunostaining, we incubated the samples with secondary antibodies (Alexa 488 anti-rabbit IgG) (Molecular Probes Inc.). DAPI was used to counterstain nuclei. To determine the positive cell fraction, the number of PYY-, GIP-, or GLP-1-positive cells and intestinal total areas were quantified in 10 fields per condition. The analyses were performed under randomized conditions and expressed as the number of PYY-, GLP-1-, or GIP-positive cells/mm^2 of the intestine.

Each histological parameter was measured and reported by a single investigator using a fluorescence microscope with a digital camera and image analysis Cell-D software (Olympus, GmbH. Hamburg, Germany).

Statistical analysis

Data are presented as the mean ± SEM for AUC, histological, and weight-gain data analysis, the U Mann-Witney test was conducted using SPSS V21.0 software. Statistical significance was accepted at $p<0.05$ or $p<0.01$.

Results

The global death rate was 20% and increased to 25% in the experimental arm of the study, which was acceptable considering its experimental nature. The greater weight loss among the PDIT group seemed to be associated with the anorectic effect of the surgical procedure itself.

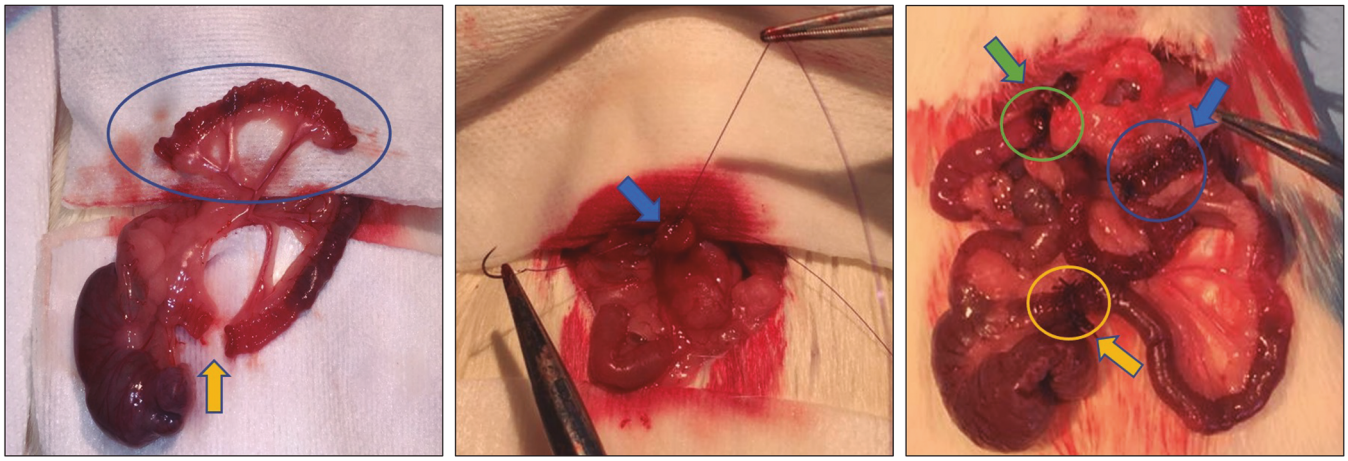


Fig. 1. A. Ileum portion sectioned with the vascular supply (red circle); preserving the ileocolic valve with the last centimeter of ileum. The yellow arrow signs next to anastomosis with terminal jejunum. **B.** Duodenum section proximal to pylorus (blue arrow). **C.** Final transit reconstruction with the gastro-ileum (blue circle) anastomosis, ileum-duodenum (green circle) anastomosis, and jejunum-terminal ileum (yellow circle) anastomosis.

Glycemia and OGT tests

Glycemia was measured from the time of surgery to sacrifice. The basal glycemia levels were not significantly different between the groups. Four and eight weeks after surgery, two OGTTs were performed on the Sham and PDIT groups. Similar curves were seen for the two groups in the two tests (Fig. 3A,C); no significant differences were observed in glucose tolerance patterns. The AUC was calculated for the two groups. Additionally, no significant differences between any group were found (Fig. 3B at four weeks and Fig. 3D at eight weeks).

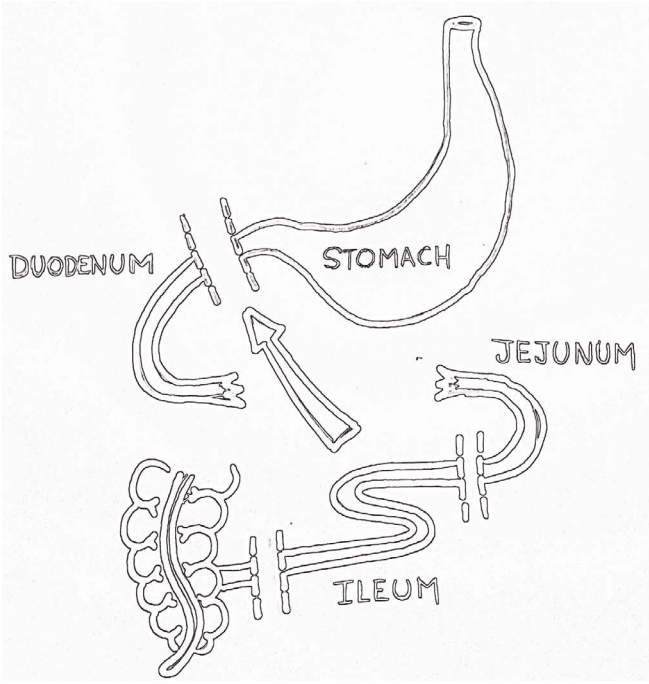


Fig. 2. Schematic image of the experimental surgery described as preduodenal ileal transposition (PDIT).

Hormone measurements

We measured the circulating plasma levels of hormones related to the ileum response, i.e., GIP, GLP-1, and PYY were measured during the OGTT in the fourth week after surgery. Two response points were measured at 0 and 15 minutes after glucose administration. As Figure 4A shows, there was increased GIP secretion 15 min after glucose overdose in Sham rats, in contrast to the PDIT group. Thus, the GIP AUC value was significantly decreased in the PDIT experimental group compared with the control group ($p<0.05$) (Fig. 4B).

Additionally, we analyzed plasma GLP-1 levels in the two groups. Paradoxically, an initial significant increase in the plasma GLP-1 level was detected in PDIT rats ($p<0.05$) at the beginning of the test. After 15 minutes, the GLP-1 level decreased in the PDIT group and increased in the Sham group. There was no significant difference in response after 15 minutes (Fig. 4C). Plasma GLP-1 AUC values showed high secretion of GLP-1 in the PDIT group (Fig. 4D) ($p<0.05$).

Finally, plasma PYY levels exhibited a slight crossed pattern between the two analysis points (Fig. 4D). Based on this result, there was a non-significant difference between the groups in terms of AUC (Fig. 4F).

Gut histology

We observed the villus height and crypt depth in samples of the three portions of the intestine obtained from both groups. No differences were observed in the

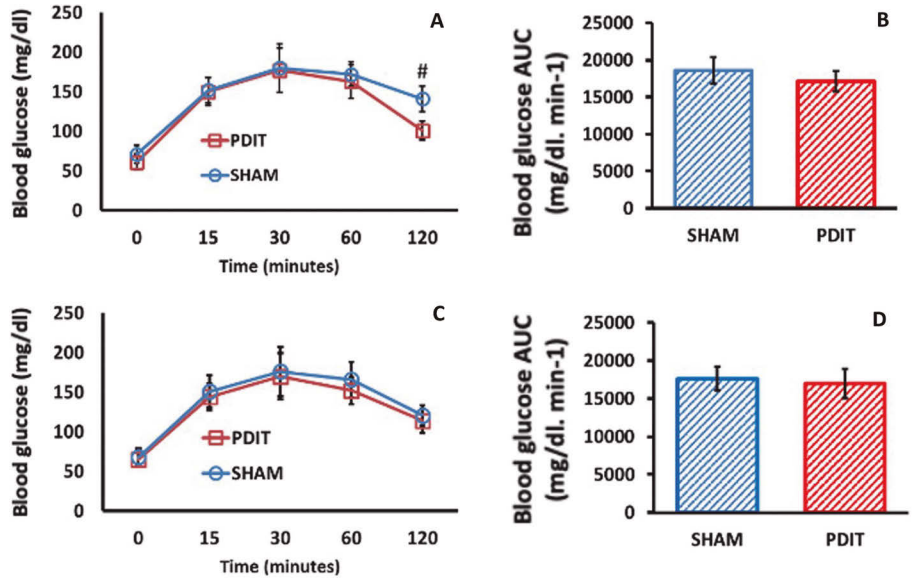


Fig. 3. Oral glucose tolerance test (OGTT) in n=15 Sham control rats (blue line with circles) and n=15 PDIT-operated rats (red line with squares), at four **(A)** and eight **(C)** weeks after surgery. Glycemia is represented as mg/dl in the Y axis and time after glucose load in the X axis. Values are expressed as mean +EEM ($^{\#}P<0.05$). OGTT area under curve (AUC) values are presented as mg/dl min-1 in the Y axis and expressed as mean +EEM for each group presented in the X axis. The Sham group is represented (blue striped bar) *versus* the PDIT group (red striped bar) at four **(B)** and eight **(D)** weeks after surgery.

histological characteristics of the duodenum and jejunum samples between the surgical PDIT and Sham groups (Fig. 5A,C,E,F). However, there was a significantly decreased villus height in ileum samples from the PDIT group compared with that of the Sham group ($p<0.05$) (Figs. 4D, 5A). As photomicrographs show, the absorption surface decreased significantly in the ileum of the PDIT group with epithelial atrophy (Fig. 5G).

Hormone production by cells in the intestine

In the duodenum, jejunum, and ileum samples from both groups, GIP-, PYY-, and GLP-1-positive cell numbers were determined using immunostaining. There was a significantly decreased number of GIP-positive cells in the PDIT *versus* Sham groups in duodenum samples ($p<0.05$) (Figs. 6B, 7). However, there were no differences in GIP-producing cells in the ileum and jejunum between the control and PDIT groups (Fig. 6A,C).

When we determined the number of GLP-1-positive cells in the three intestinal portions from animals of the two groups, we observed a significant increase in the number of GLP-1-producing cells in the ileum and duodenum samples from PDIT-operated rats with respect to that of sham-operated animals (as shown in Fig. 6D,E, 7) ($p<0.05$). The jejunum samples of both groups showed similar cellularity (Fig. 6F).

As Figure 6G,H,I show, there were no differences in the expression of PYY-releasing cells in the intestinal portions or the studied experimental groups.

Discussion

In our study, we evaluated the specific effect of the ileum in the enteropancreatic axis on the pathophysiological changes underlying the increase in glycemic metabolism after M/BS. With this background, we designed an experimental model involving a preduodenal ileal transposition (PDIT), in which an early passage of the alimentary bolus through the ileal lumen was performed without any duodenal exclusion. This model allowed us to identify the morphological alterations in the small bowel, as well as changes in hormonal expression in both tissue and serum samples.

Thus, this surgical model was both feasible and novel, although no significant differences were found (Salas-Álvarez et al., 2020).

Regarding the technique, a preduodenal ileal transposition has never been described before, and a jejunal location is usually performed. Furthermore, this study is the only one in the literature that compares serum incretin levels, morphological changes, and incretin expression, not only in the transposed ileum but also at three different locations along the small bowel.

Concerning functional metrics after surgery, no significant differences were observed in terms of glucose tolerance. Basal glycemia levels and those obtained after

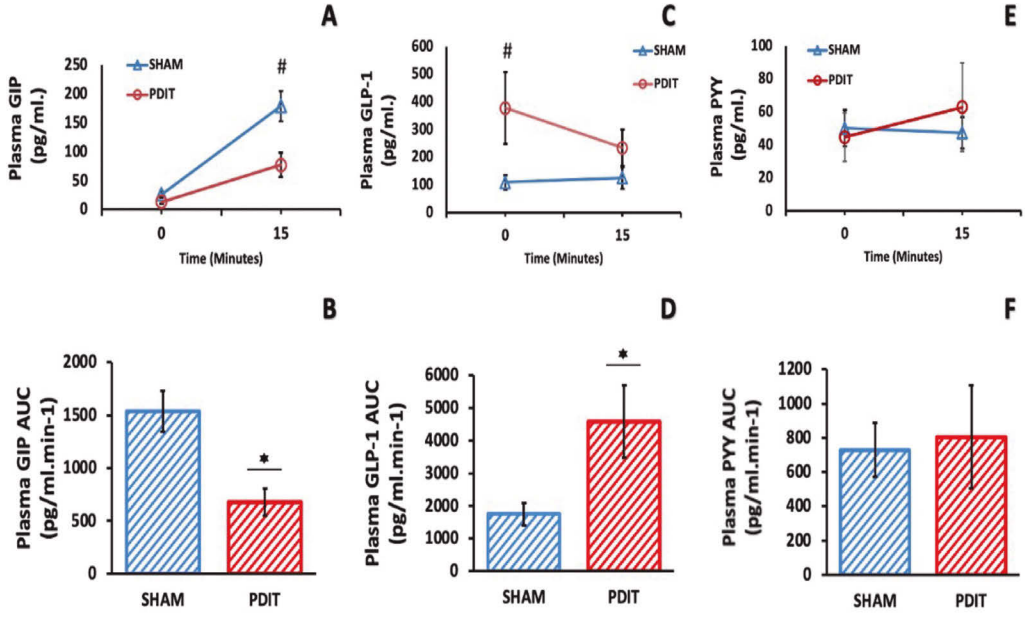
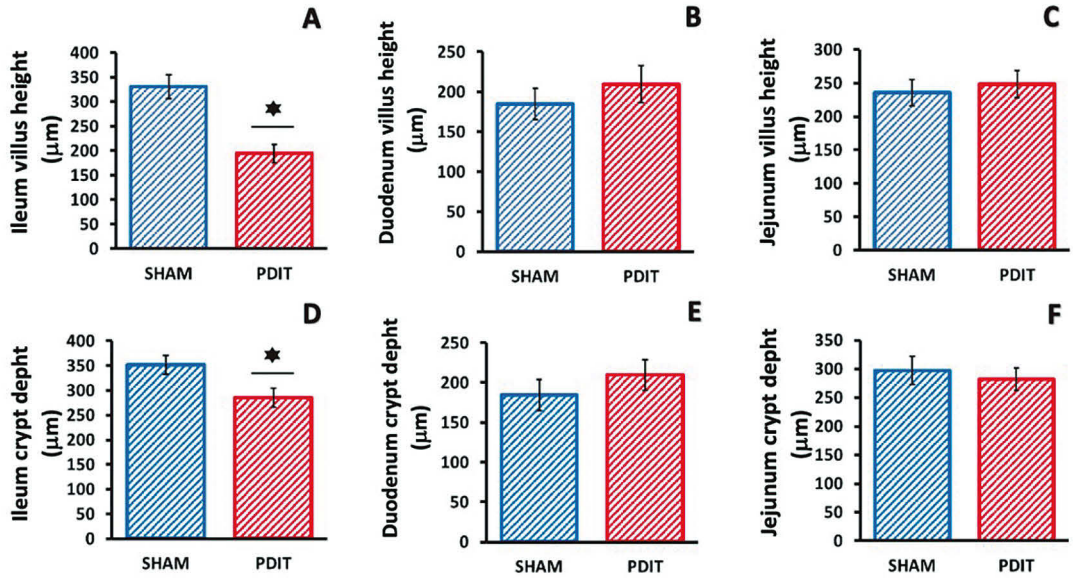


Fig. 4. Hormonal plasma study during OGTT, with two sample points at 0 and 15 minutes after ingestion. **A.** Plasma GIP secretion pattern in Sham-operated (blue line with triangles) *versus* the PDIT group (red line with circles). Plasma GIP levels are represented as pg/ml in the Y axis and time in minutes after glucose administration in the X axis. Values are expressed as mean +EEM ($^{\#}p<0.05$). **B.** Plasma GIP secretion area under curve (AUC) values are presented as pg/ml min-1 in the Y axis and expressed as mean ±EEM ($^{*}p<0.05$) for both groups presented in the X axis: Sham (blue striped bar), PDIT (red striped bar). **C.** Plasma GLP-1 secretion pattern in Sham-operated (blue line with triangles) *versus* the PDIT group (red line with circles). Plasma GLP-1 levels are represented as pg/ml in the Y axis and time in minutes after glucose administration in the X axis. Values are expressed as mean +EEM ($^{\#}p<0.05$). **D.** Plasma GLP-1 secretion area under curve (AUC) values are presented as pg./ml min-1 in the Y axis and expressed as mean ±EEM ($^{*}p<0.05$) for both groups presented in the X axis: Sham (blue striped bar), PDIT (red striped bar). **E.** Plasma PYY secretion pattern in Sham-operated (blue line with triangles) *versus* the PDIT group (red line with circles). Plasma PYY levels are represented as pg/ml in the Y axis and time in minutes after glucose administration in the X axis. Values are expressed as mean +EEM. **F.** Plasma PYY secretion area under curve (AUC) values are presented as pg/ml min^{-1} in the Y axis and expressed as mean ±EEM for both groups presented in the X axis: Sham (blue striped bar), PDIT (red striped bar).



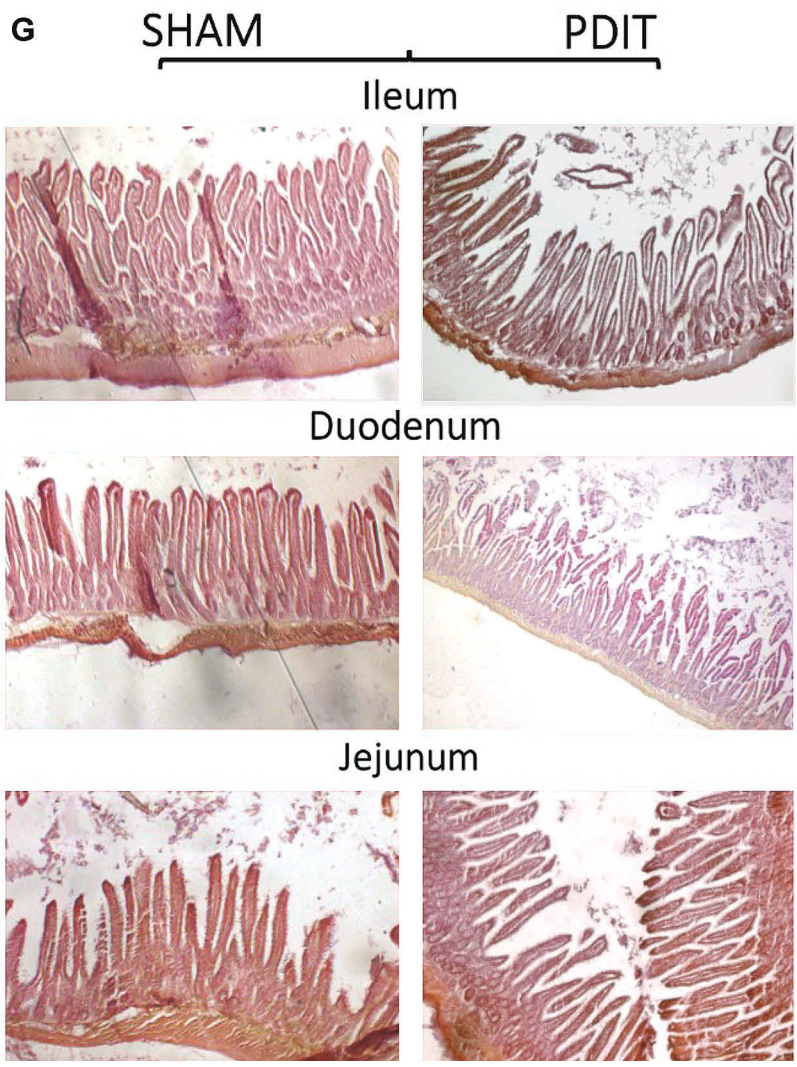


Fig. 5. A. Villus height in ileum samples, expressed as µm in the Y axis, as the mean ±EEM (**p*<0.05) for both groups: Sham (blue striped bar), PDIT (red striped bar). **B.** Villus height in duodenum samples, expressed as µm in the Y axis, as the mean ±EEM for both groups: Sham (blue striped bar), PDIT (red striped bar). **C.** Villus height in jejunum samples, expressed as µm in the Y axis, as the mean ±EEM for both groups: Sham (blue striped bar), PDIT (red striped bar). **D.** Crypt depth in ileum samples, expressed as µm in the Y axis, as the mean ±EEM (**p*<0.05) for both groups: Sham (blue striped bar), PDIT (red striped bar). **E.** Crypt depth in duodenum samples, expressed as µm in the Y axis, as the mean ±EEM for both groups: Sham (blue striped bar), PDIT (red striped bar). **F.** Crypt depth in jejunum samples, expressed as µm in the Y axis, as the mean ±EEM for both groups: Sham (blue striped bar), PDIT (red striped bar). **G.** Microphotographs of villus and crypts at the three intestinal portions of both rat groups. H&E, x 20.

OGTTs at weeks 4 and 8 after surgery (Fig. 2) were similar, despite the modest improvement in glycemic homeostasis among PDIT subjects. These results were expected considering that the model animals were non-obese, non-diabetic Wistar rats (Strader et al., 2005; Oh et al., 2016a) and the short period of observation of less than 12 weeks (Strader et al., 2005; Mencarelli et al., 2013).

The morphological metrics in the different small bowel sections (Fig. 4) only showed significant differences in the transposed ileum. Shorter intestinal villi along with a global decrease in the depth of the Lieberkühn crypts were observed (Fig. 4A,D, respectively), while no differences were detected in any of the other intestinal segments or parameters studied. These results were, at least, partially opposed to those obtained when a regular ileal transposition technique was performed, which leads to an increase in the number and length of intestinal villi (Trbojević-Stanković et al., 2010; Grunenberger et al., 2014; Hansen et al., 2014). This phenomenon is called the "intestinal jejunization" process. As regards the jejunum, an increase in the

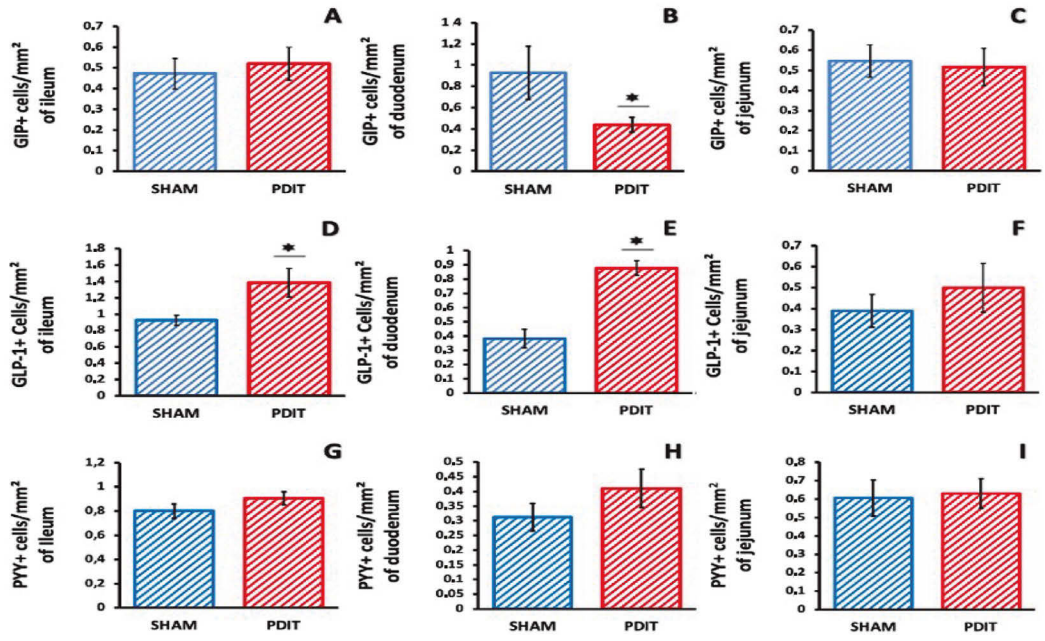


Fig. 6. Enterohormonal cellular immunostaining. **A-C.** GIP+ ileal, duodenal, and jejunal cells, respectively, the number of positive cells/mm^2 is expressed as mg. On the Y axis, values are noted as the mean ±EEM (*p<0.05) for each group: Sham (blue striped bar), PDIT (red striped bar). **D-F.** GLP-1+ ileal, duodenal, and jejunal cells, respectively, the number of positive cells/mm^2 is expressed as mg. On the Y axis, values are noted as mean ±EEM (*p<0.05) for each group: Sham (blue striped bar), PDIT (red striped bar). **G-I.** PYY+ ileal, duodenal, and jejunal cells, respectively, the number of positive cells/mm^2 is expressed as mg. On the Y axis, values are noted as the mean ±EEM (*p<0.05) for each group: Sham (blue striped bar), PDIT (red striped bar).

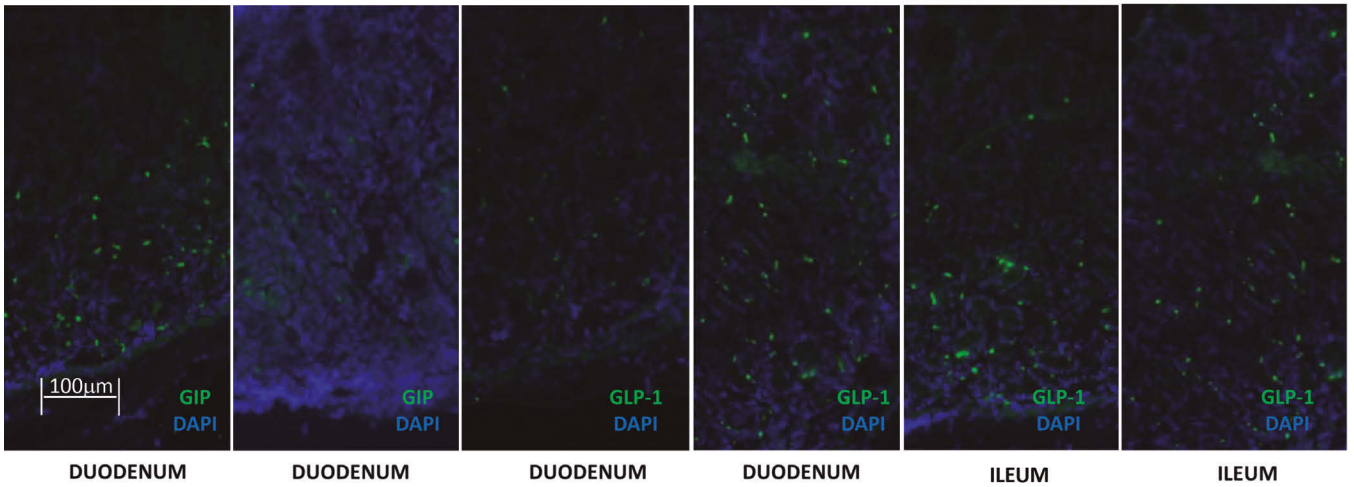


Fig. 7. Microphotographs of statistically significant results of intestinal immunohistochemical samples from Sham and PDIT rats, using anti-GIP and anti-GLP-1 antisera, stained with Alexa-488 (green), and DAPI to counterstain the nuclei (blue). x 20.

irregularity of the microvilli surface was described after performing an ileal transposition technique with no variation in number or length (Sawczyn et al., 2018). However, in the alimentary limb in a Roux-Y gastric bypass (RYGB), similar hypertrophy and hyperplasia phenomena have been described (Pérez-Arana et al., 2022a).

Nonetheless, in this study, ileal transposition in a preduodenal location in contact with both the stomach and duodenum and exposed to gastric juices, prompted ileal mucosa adaptation with a severe decrease in absorptive capacity (shorter villi with more superficial crypts), similar to that observed in the duodenum. There were no morphological modifications in the other studied sections; the duodenal segment was exposed continually to the intestinal acid content, which is not affected by biliary secretion, and the jejunum remained in the same location with no major changes in length.

In this sense, the morphological results in our model show a trend toward the "duodenization" of the transposed segment instead of the "jejunization" previously described, which could be due to the enteroplasticity of the small bowel (Oh et al., 2016b). This adaptation capacity, especially in the ileal segments, would not be related to the adaptive response of the enteroendocrine cell lineage or its serum secretion patterns after ileal transposition.

The role of GLP-1 in glucose metabolic improvement following ileum transposition (IT) surgery is widely accepted, mainly due to an increase in both insulin secretion and sensitivity, as well as an increase in pancreatic beta cell function (Sun et al., 2013; Oh et al., 2016b). These data are supported by our previous PDIT studies, which showed that higher insulin secretion was associated with an increase in beta cell mass (Pérez-Arana et al., 2023; Salas-Álvarez et al., 2023).

As the main IT studies reflect (Oh et al., 2016b), in our model, the PDIT technique led to a significant increase in serum GLP-1 levels compared with those in the Sham group (Fig. 3C,D). However, in our model, the increase in these levels was observed immediately after the OGTT in response to an early pattern of glucose-dependent GLP-1 secretion (Zhu et al., 2018). In this study, the secretory phase occurred even faster due to the location of the transposed ileum, which was proximal to the rest of the small bowel and received the alimentary bolus directly without digestion processes in the proximal bowel. This rise in GLP-1 secretion occurred because of the greater and accelerated nutrient exposure in the ileal mucosa, as well as the higher expression of ileal L cells, which are GLP-1 and PYY producers. Thus, there were significant differences between the two groups, with abundant tissue GLP-1 expression in the experimental group; these differences were seen not only in the ileum samples (Fig. 3D) but also in the duodenum sections. These observations agree with the limited literature analyzing the histology and serum expression of this hormone (MacDonald et al., 2002; Nausheen et al., 2013; Ramzy et al., 2014). Additionally, these data are in agreement with several studies performed by our group using similar surgical techniques that render a prompt nutrient delivery to the distal small bowel; these studies also showed a significant increase in the expression of GLP-1 after performing the classic RYGB technique (Moreno-Arciniegas et al., 2019; Camacho-Ramírez et al., 2020) and a large jejunal resection (Prada-Oliveira et al., 2019). In addition, these studies showed a greater number of GLP-1-marked cells in the duodenum and jejunum after RYGB.

Therefore, the early arrival of nutrients to the ileum could be responsible for the increased tissue expression of GLP-1 in the ileal segments. On the other hand, an explanation for the increase in L cells in the duodenum could be a possible adaptation of duodenal K cells, which could undergo transdifferentiation after anatomical reassignment. In this sense, the plasticity of endocrine lineage cells is known (Swisa et al., 2017) and some studies also propose that certain types of surgery, such as laparoscopic sleeve gastrectomy (LSG), can activate differentiation mechanisms that transform epithelial cells into enterohormone-producing cells in the digestive tract (Wölnerhanssen et al., 2017). So, the new GLP-1-producing cells could not just come from other cells of the endocrine lineage such as K cells but from the epithelial tissue itself.

These hypotheses would be consistent with the results obtained in the GIP study, as observed in Fig. 3A,B, there was a significant decrease in GIP serum levels in the PDIT group. Additionally, significantly lower levels of GIP-positive cells in the duodenal samples of PDIT subjects were observed (Fig. 3B), with no other major changes in the rest of the intestinal sections.

This finding is especially relevant and agrees with the conclusions of Oh et al. (2016b), however, further research is needed to confirm this.

The PDIT technique is characterized by the absence of duodenal exclusion and duodenum-jejunal disruption. The new anatomical location of the duodenum in a post-ileal position leads to the delayed stimulus of the K cells with the following cellular adaptation. The possible underlying trans-differentiation processes (K into L cells) could explain the decrease in GIP secretion. Nevertheless, as GIP can lead to GLP-1 secretion (Visbøll, 2004), the lower serum and tissue GIP expression could be related to an enterohormonal regulation between both incretins, which would counterbalance the increase in GLP-1 levels observed in our study. As far as the results regarding PYY secretion (Fig. 3E) and AUC (Fig. 3F), we found no significant differences between the groups, albeit there was a continuous increase in PYY levels in the PDIT group. No immunoreactivity increase in PYY levels was observed in either group. Our data differed from those of Ramzy et al. (2014), who observed healthy Sprague-Dawley rats. In that paper, the authors showed a significant increase in PYY serum levels and a higher number of immune-positive PYY-positive cells in the

transposed ileal segment.

However, these histological and serum PYY results, in association with those related to GLP-1 and GIP, support the enterohormonal regulation theory. These regulatory mechanisms are mediated by neuropods that communicate between different enteroendocrine cellular populations (Bohórquez et al., 2014).

In this sense, according to Camacho-Ramírez et al. (2020) and Moreno-Arciniegas et al. (2019), PYY could be a possible intermediary factor for GLP-1 and GIP secretion, which would be stimulated by prompt nutrient delivery to the transposed ileum. The mechanical stimulus would be prime and selective for GLP-1 secretion; however, it could have negative feedback in terms of PYY production. At the same time, PYY could induce a decrease in GIP as part of its contra-regulatory role over GLP-1. This hypothesis agrees with the immunohistology findings, which showed an increase in tissue GLP-1 expression in the ileum and duodenum, related to a decrease in the number of GIP-positive cells in the duodenal samples, without any changes in PYY expression or secretion in any of the segments.

Our model showed the importance of the ileum in the underlying pathophysiological role of the enteropancreatic axis in glucose metabolism, and these results reveal the adaptive histological changes (intestinal enteroplasticity) that are a result of ileal transposition. In this context, the expression and secretion of GLP-1 mediate negative feedback of PYY and trigger cellular trans-differentiation from duodenal K to ileal L cells. This process is only possible via a complex counter-regulation system among the different small bowel hormones.

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Statement of Animal Welfare. The authors declare that ethical approval was obtained for our study. All procedures involving animals were performed in accordance with the ethical standards of the institution at which the studies were performed, and ethical approval was obtained from the University of Cádiz 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.

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