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Beta-cell mass adaptation to ileum nutrient flow. An experimental model

Jesús Salas-Alvarez[2], Francisco Javier Campos-Martínez[2], Alonso Camacho-Ramírez[1,3], Cristian Montero-Peña[4], David Almorza-Gomar[3,5], Antonio Ribelles-García[4], Gonzalo M. Pérez-Arana[3,4] and J. Arturo Prada-Oliveira[3,4]

[1]Surgery Unit, University Hospital Puerta del Mar Hospital, SAS, [2]Surgery Unit, University Hospital Puerto Real, SAS, [3]INIBICA, Institute for Biomedical Science Research and Innovation, [4]Department of Human Anatomy and Embryology, Faculty of Medicine and [5]Department of Operative Statistics and Research, University of Cádiz, Cádiz, Spain

Summary. The population with obesity has increased at an alarming rate during this century. Bariatric surgery has been demonstrated to be a good method to control weight and, most importantly, associated comorbidities, such as type 2 diabetes mellitus or high blood pressure. The reason why this happens even before losing significant weight remains unclear. Many authors believe that incretins play a main role, triggering special functions of the digestive tract. In reports, these hypotheses are known as foregut and hindgut theories. Initially, the theories were mutually exclusive; additionally, many other propositions have been analysed, according to different surgical techniques (e.g., bile acids and specific enterohormonal components). To elucidate the participation of the ileum, we developed a surgical technique to study the rapid response to nutrients in the ileum. Our goal was to study the stress functional test and histological changes in the pancreas that may explain the variations in glycaemic homeostasis in our rat model. After the oral glucose tolerance test, the experimental group presented an increased insulin release response with conserved glycaemia. We report an increasing beta-cell mass in the experimental group (+11.87 mg vs. +9.65 mg, respectively), while alpha-cell mass was not different. Based on transcription factors, the pathways that were increased were the proliferation process (as the number of PCNA-positive cells in the experimental group versus sham (+12.06 vs. +6.2 PCNA+ cells/mm^2)) and transdifferentiation (ARX; +2.67 ARX+ cells/mm^2 in the experimental group vs. +2.04 ARX+ cells/mm^2 in the controls). We report the consequences of the rapid arrival of nonprocessed nutrients to the ileum on the endocrine cellular pancreas. The ileum could be a principal effector in the enterohormonal axis, which conditions endocrine pancreas cellularity.

Key words: Beta-cell mass, Pancreas, Ileum, Type 2 diabetes mellitus, Incretins, Cellular differentiation

Introduction

Obesity rates continue to rise, along with the complications associated with this illness, making it a major concern in the 21st century. The population with obesity is expected to reach an incredible 439 million people by 2030, and obesity is considered one of the largest pandemics in the world (Chen et al., 2011; Finkelstein et al., 2012). Currently, 39% of adults are overweight, and 13% are clearly obese (Catalán et al., 2022; Yárnoz-Esquiroz et al., 2022).

Special consideration needs to be given to the multiple comorbidities that usually accompany this illness. Type 2 diabetes mellitus (T2DM) is one of those comorbidities that is closely associated with obesity (Maggio and Pi-Sunyer, 2003). This relation is due to different mechanisms, with the loss of tissue sensitivity to the effects of insulin being the most well-known.

The resolution of this side effect after bariatric surgery has led to increasing interest in understanding the mechanisms of the pathophysiology.

Although many hypotheses have been proposed, many reports have considered the portion of the intestine affected after surgery. The so-called hindgut or foregut hypothesis focuses on the mechanical and chemical stimulus of nutrients in every portion of the intestinal tube. The main reason for these changes is related to the precisely affected portion upon bariatric surgery (Duan et al., 2014; Camacho-Ramírez et al., 2017). Other

authors, in contrast, explain this phenomenon by the lack of transit of the alimentary bolus through the duodenum (foregut) (Hickey et al., 1998).

This controversy between the main importance of the terminal or proximal intestine in T2DM has been resolved. Other techniques exclusively affect the stomach (e.g., sleeve gastrectomy). Moreover, Roux-en-Y gastric bypass excluded the transit of the alimentary bolus through the duodenum. Some authors have focused on the jejunum as the key to the intestinal origin of paracrine signals over the pancreas (Camacho-Ramírez et al., 2017).

T2DM improvement exclusively originating from the intestine has been excluded. Other hypotheses, such as bile acids or the role of the microbiota, have been suggested in the resolution of T2DM and associated comorbidities.

We focused on this referenced mechanism regarding nonprocessed nutrients reaching the ileum rapidly. Therefore, we designed a new experimental surgical technique in which the ileum is moved to a preduodenal position (Salas-Álvarez et al., 2020). This technique was demonstrated to be an effective animal model for our purpose. The main goal is to achieve early passage through the distal ileum. However, we did not exclude or resect the duodenum. The expected results can be associated with the effects of the foregut on glycaemic metabolism.

This new surgical technique is called preduodenal ileal transposition (PDIT). We focused on the metabolic consequences of this ileal intervention. The consequences on the beta-cell mass are based on the demand for glycaemic changes related to absorption. Glucose absorption could be affected, and these glycaemic peaks could imply changes in beta-cell mass (Rubino and Marescaux, 2004).

We justify this hypothesis by the evidence that the changes in digestive tube conformation are related to changes in enterohormone release (Zhang et al., 2017; Prada-Oliveira et al., 2019). This hormonal synthesis and release are related to nutrient flow. Thus, this factor could be affected after PDIT, and beta-cell mass is the final consequence of these changes (Ramracheya et al., 2016). The PDIT experimental model leads to the rapid transport of nutrients to the ileum, which could stimulate the enterohormonal axes (Drucker et al., 2003a,b; Camacho-Ramírez et al., 2020).

In the current study, we focused on the pathophysiological changes in the endocrine pancreas after PDIT surgery and the early arrival of nutrients to the ileum. To this end, we used the PDIT model in healthy Wistar rats and provided a descriptive list of the medium-term effects on glucose metabolism homeostasis and the pancreatic beta-cell population.

Materials and methods

Animals

We used 30 nonobese Wistar rats and nondiabetic animals. The rats were supplied by the University of Cádiz Animal Production Service, which included the specific laboratories in which the surgeries were performed. We did not use female rats to avoid cyclic variations in the gonadotropin hormonal effect on glycaemic metabolism. The rats were 12-14 weeks old and weighed approximately 300-350 g when surgery was performed.

Every procedure on the animals was performed with the approval of the University of Cádiz Committee for the Ethical Use and Care of Experimental Animals. The specimens were maintained under constant temperature and humidity conditions in a 12-hour light/dark cycle, with *ad libitum* access to normal chow and water.

Research design

In this descriptive study, thirty Wistar rats were randomly divided into two groups: n=15 sham-operated rats (sham) and n=15 PDIT-operated rats (PDIT). All the animals were sacrificed twelve weeks after surgery. Glucose and insulin plasma levels were measured after the OGTT at the fourth and seventh weeks in both groups. Beta-cell mass, beta-cell proliferation, and neogenesis/transdifferentiation markers were tested in pancreatic samples from sham and PDIT animals after sacrifice.

Surgical techniques

The rats were randomly assigned to the experimental group, the preduodenal ileal transposition (PDIT) group, or to the control group (sham). Both techniques were performed once the rats were anaesthetized with isoflurane. The anaesthetic maintenance was obtained by the use of a continuous ventilation pump of isoflurane (between 1 and 3%).

In the PDIT group, the surgical procedure began with a bisubcostal incision after cleansing the abdominal wall with chlorhexidine. Then, the stomach was mobilized for better exposure after liberating the lesser omentum. We measured the last 10 cm of the terminal ileum and divided it, preserving the vascular supply and the distal edge 1 cm away from the ileocaecal valve. A transverse postpyloric division was made, 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 (Salas-Álvarez et al., 2020) (Fig. 1). The last anastomosis made was between the jejunum and terminal ileum, with interrupted sutures of PDS 5/0.

The control group (sham) animals received a bisubcostal incision, allowing exposure of the small bowel loops. A transversal enterotomy section was performed at 10 cm of the Treitz angle, without intestinal resection. Then, we made an end-to-end

anastomosis with interrupted sutures of PDS 5/0.

Oral glucose tolerance test (OGTT) and insulin measurement

Four and seven hours after surgery, an oral glucose tolerance test (OGTT) was performed in both groups after a 12-hour fast. A 2 g/kg, 20% w/v D-glucose solution was administered through gavage, and glycaemia was measured by a Glucocard G-Metre 1810 glucometer (Menarini Diagnostics, Italy) in blood samples obtained from the rats' tails at 0, 15, 30, 60, and 120 min after glucose solution administration. The results are expressed as milligrams of glucose per decilitre of plasma.

In addition, during the OGTT, insulin measurement was performed in blood samples obtained from the rat tails, at baseline and 15 min after glucose solution administration, 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.

Sacrifice and tissue preparation

Animals were sacrificed eight weeks after surgery by isoflurane inhalation overdose. The pancreases were immediately removed, weighed (precision scale Ohaus Pioneer Mod PA 3102), and fixed in Bouin's solution overnight at 4°C. Later, the samples were dehydrated, embedded in paraffin and cut into serial 10 μm-thick microtome sections for immunostaining.

Beta-cell mass quantification

To calculate the beta-cell mass, insulin-producing cells were stained using a monoclonal guinea pig anti-insulin antibody (Sigma-Aldrich, St Louis, MO, USA) and a secondary anti-GP IgG antibody conjugated to an Alexa 546® fluorophore (Molecular Probes Eugene, OR, USA). Finally, the cellular nuclei were stained using 4',6-diamidino-2-phenylindole (DAPI) in aqueous mounting medium (DABCO).

The insulin-positive areas were measured using a microscope equipped with a digital camera and analysed by ImageJ image analysis software. Those who performed the measurements were not aware of which experimental group the samples belonged to. Beta-cell mass was measured as the ratio of the insulin-positive area/total pancreatic area relative to the total pancreas weight, and it was expressed in milligrams.

Beta-cell proliferation assay

Proliferation was assessed by double immunostaining using polyclonal rabbit anti-PCNA (Abcam®, Cambridge, CB4 OFL, UK) and monoclonal mouse anti-insulin (Sigma-Aldrich, St Louis, MO, USA) antibodies according to the manufacturer's instructions. Prior to this, sections from the pancreas were incubated for 30 min with 0.1% Triton X-100 in PBS for tissue permeabilisation, washed with PBS, and then incubated for 30 min with 4% BSA blocking solution in PBS at room temperature. The results were expressed as the number of PCNA+/insulin+ cells/mm^2 per area of pancreatic islets.
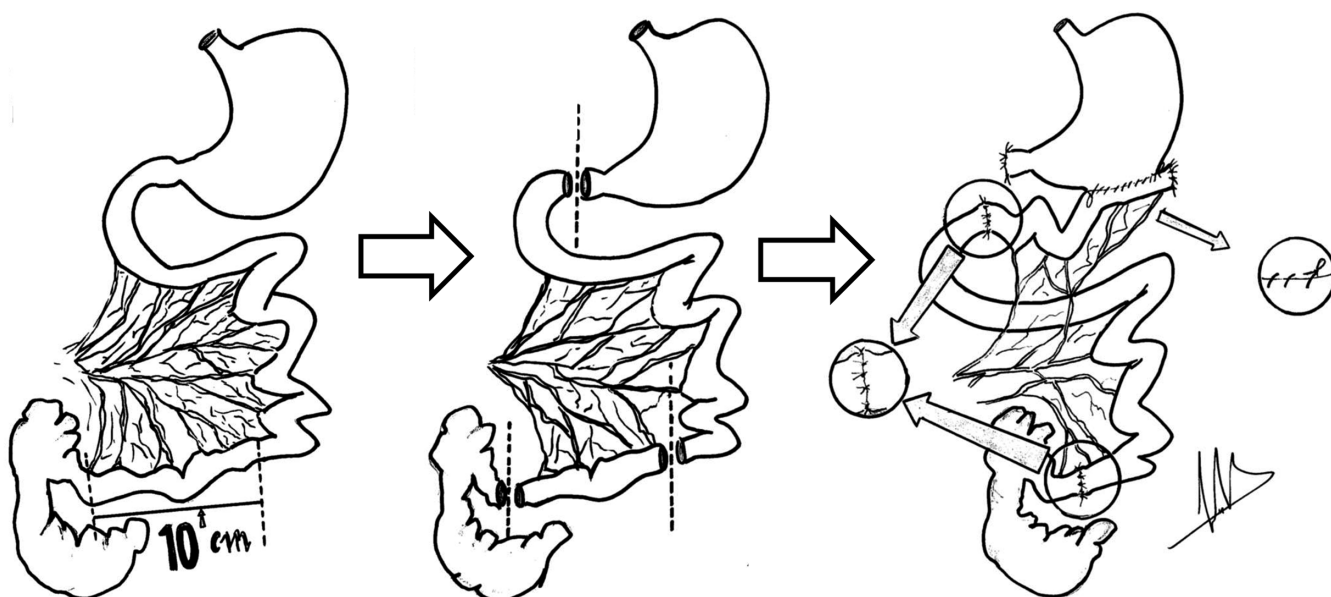

Fig. 1. The surgical technique as described in the Materials and methods section.

Beta-cell neogenesis study

To study PDX-1 expression, as a neogenesis marker, the pancreas sections were stained with monoclonal rabbit anti-PDX-1 antibody (Abcam ab 47267®, Cambridge, CB4 OFL, UK) and labelled using biotin-conjugated anti-rabbit IgG secondary antibody (Sigma-Aldrich, B8895, USA).

A secondary antibody conjugated to Alexa 488® and a mouse anti-IgG antibody conjugated to Alexa 546® fluorophore (Molecular probes Eugene, Oregon, USA) were employed. Nuclei were counterstained as mentioned before with DAPI. The results were expressed as the number of PDX-1+/insulin+ cells/mm^2 per area of pancreatic islets.

Beta-cell transdifferentiation study

The study of transdifferentiation processes consisted of the expression of ARX within the islet cell population, the actual alpha-cell mass and the expression of PCNA-positive cells within the glucagon-positive area.

To measure ARX expression, a monoclonal rabbit anti-ARX IgG antibody (Abcam® Cambridge, CB4 OFL, UK) was used, followed by labelling with a goat anti-rabbit IgG antibody conjugated to an Alexa 546® fluorophore. The results were expressed as the number of ARX+/insulin+ cells/mm^2 per area of pancreatic islets.

Alpha-cell mass quantification and alpha-cell mass proliferation assay

The total alpha-cell mass was measured by a monoclonal rabbit anti-glucagon antibody (Abcam®, Cambridge, CB4 OFL, UK) and a secondary anti-rabbit IgG antibody conjugated to an Alexa 488® fluorophore (Molecular Probes Eugene, OR, USA). Finally, the cellular nuclei were stained using 4′,6-diamidino-2-phenylindole (DAPI) in aqueous mounting medium (DABCO).

The expression of the proliferation marker (PCNA) within the glucagon-positive area was carried out by double immunostaining using polyclonal rabbit anti-PCNA (Abcam®, Cambridge, CB4 OFL, UK) and monoclonal IgG rabbit anti-glucagon (Abcam®, Cambridge, CB4 OFL, UK).

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

Statistical analysis

The measured data are expressed as the means ± SEMs. Initially, we examined the normality of distributions using the Shapiro-Wilk test. Comparisons between groups were performed using the nonparametric Mann-Whitney U test, and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS statistical software, version 24.0.

Results

After the described sacrifices, the pancreas was immunohistochemically processed as described. Regarding the immunohistochemistry study, we show the results of the relative cellular mass, proliferation, neogenesis and transdifferentiation processes.
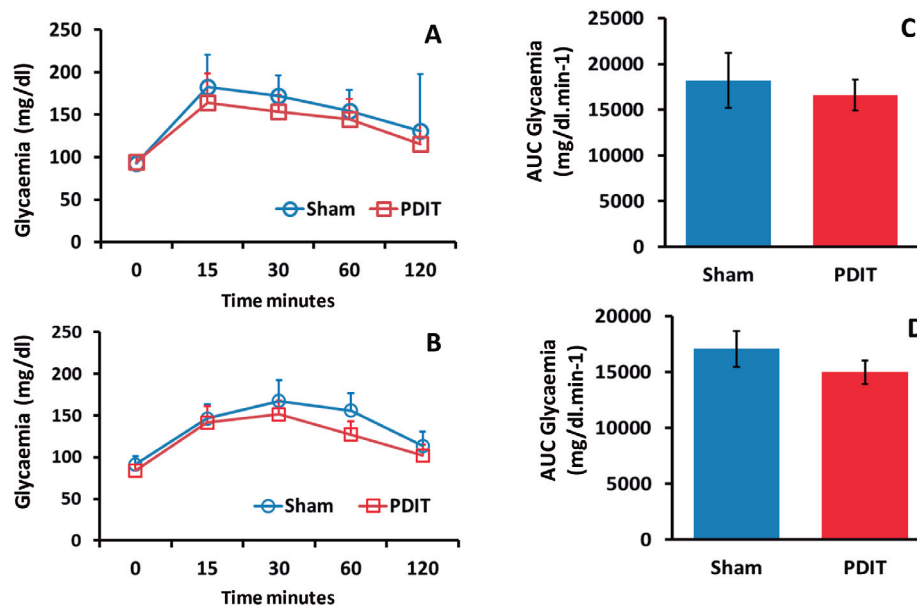

Fig. 2. Oral glucose tolerance test (OGTT) in the sham control (solid blue line with circles) versus the PDIT group (discontinuous red line with squares) at 4 **(A)** and 7 **(B)** weeks after surgery. Glycaemia is expressed as mg/dl on the Y axis, and time after glucose load is given on the X axis. Values are expressed as the means ± SEMs. Area under the corresponding curve (AUC) values at 4 **(C)** and 7 **(D)** weeks after surgery are expressed as mg/dl min-1 on the Y axis and represent the means ± SEMs in each group. Sham control (blue bar) and PDIT (red bar). Significant differences at $p < 0.05$ are marked as *.

OGTT and insulin secretion measurement

At four and seven weeks post-surgery, OGTTs were performed on rats. No differences appeared between the glucose tolerance patterns between the two groups or the two test time points (Fig. 2). The glucose area under the curve (AUC) was analysed for the two groups after OGTT. Similar values were observed between the experimental and sham groups.

Insulin secretion was also measured after the OGTT at four and seven weeks after surgery. Modified insulin secretion patterns appeared in PDIT rats with respect to surgical controls at 15 min after oral ingestion (Fig. 3A,B). In tests at both time points, insulin release showed a significantly elevated insulin secretion response by PDIT rats with respect to sham rats. Insulin secretion AUCs were calculated in each group after both tests. No differences were observed between the PDIT and sham groups at the fourth week (Fig. 3C). However, significantly higher AUC insulin values were found seven weeks after surgery in the PDIT group (Fig. 3D).

Beta-cell mass

Pancreatic beta-cell mass quantification was conducted for each group. Three months following surgery, the beta-cell mass showed a significant increase between the PDIT group and the sham group (11.98 ± 1.12 mg vs. 9.30 ± 0.41 mg, respectively) (Fig. 4).

Beta-cell proliferation assays

Beta-cell proliferation was analysed through the presence of the PCNA proliferation marker in the beta-cell nucleus. The data showed high rates of replication in the PDIT versus control groups ($p<0.05$) (Fig. 5). The proliferation rates of the sham group were lower

(6.7 ± 1.69 PCNA-positive cells/mm^2 insulin-positive area) than those of the PDIT group (12.44 ± 1.46 PCNA-positive cells/mm^2 insulin-positive area) ($p<0.05$).

Beta-cell neogenesis study

Neogenesis was analysed as the presence of the PDX-1 marker in the beta-cell population. The analysed data did not show any significant difference between the groups, although they did show a slight tendency towards an increase in the PDIT group (Fig. 6). The sham group had a lower value (4.45 ± 0.69 PDX-1-positive cells/mm^2 insulin-positive area) than the PDIT group (5.74 ± 0.82 PDX-1-positive cells/mm^2 insulin-positive area).

Beta-cell transdifferentiation study

This process was analysed by the study of ARX markers in the samples as well as the alpha-cell mass and proliferation processes within the islet area. The data collected showed a significant difference between the groups, with the PDIT group having a higher rate (2.80 ± 0.19 ARX-positive cells/mm^2 of islet) than the sham group (2.07 ± 0.21 ARX-positive cells/mm^2 of islet), as shown in Fig. 7 ($P<0.05$).

Alpha-cell mass quantification and alpha-cell proliferation assay

None of the other assays showed significant differences. The alpha-cell mass did not show differences between the groups, as revealed in Fig. 8. Additionally, we studied the alpha-cell proliferation ratio using a proliferation marker, that is, double immunostaining for PCNA and glucagon. However, as Fig. 9 shows, similar data were obtained for both groups.
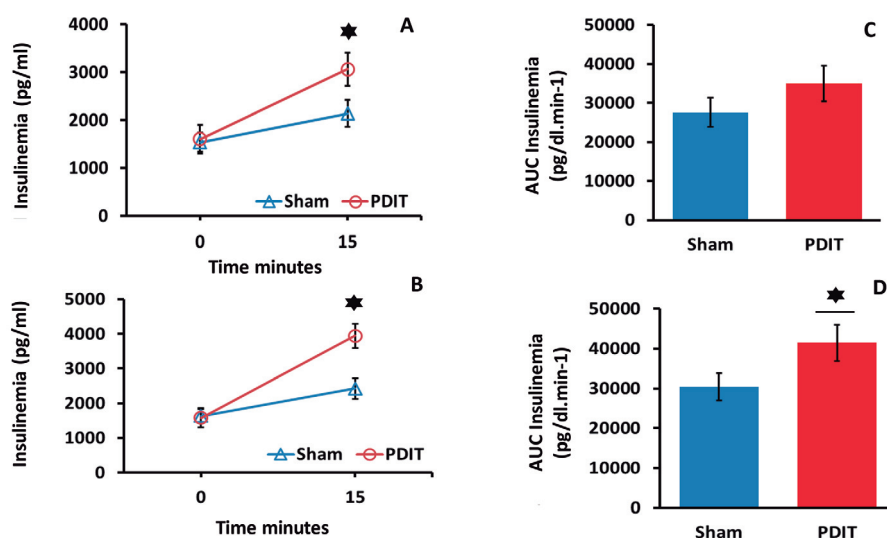

Fig. 3. Insulin measurement after the glucose tolerance test in the sham control (solid blue line with circles) versus the PDIT group (discontinuous red line with squares) at 4 **(A)** and 7 **(B)** weeks after surgery. Plasma insulin is expressed as pg/ml on the Y axis, and time after glucose load is given on the X axis. Values are expressed as the means ± SEMs. Area under the corresponding curve (AUC) values at 4 **(C)** and 7 **(D)** weeks after surgery are presented as pg/ml min-1 on the Y axis and are expressed as the mean ± SEM in each group. Sham control (blue bar) and PDIT (red bar). Significant differences at p<0.05 are marked as *.

Discussion

In this study, we developed a new surgical model focused on the ileum. Our interest was to identify the beta-cell changes related to the quick arrival of nutrients in the ileum. Therefore, we examined the morphological and histological changes in the endocrine pancreas. The functional differences in this PDIT model showed that there were no significant changes between the weight gain of the animals during the survival period between both groups (Salas-Álvarez et al., 2020). This result may be explained by the fact that our technique has no restrictive implications whatsoever (Boozer et al., 1990). For the basal glycaemia values, despite PDIT showing a moderate increase compared to the sham group, no significant differences were observed (Salas-Álvarez et al., 2020). The groups had similar values, probably due to the nonobese, nondiabetic condition of the rats.

An oral glucose tolerance test (OGTT) was performed in this animal experimental model twice during the survival period. Significant differences were found in the insulin serum levels after 15 min for the PDIT group. However, no differences were found regarding the glucose levels even though measurements were taken over 120 min.

The results of the OGTT did not reveal any significant differences. This result could have become significant if the study had been carried out for more than two months. The differences seen in the literature usually occur after the third month (Strader et al., 2005, 2009).

Another fact to be considered is the genetic difference that diverse animal breeds may display and the fact that Wistar rats are not as common as Long-Evans or Sprague-Dawley rats (Mencarelli et al., 2013). Technical differences may be a factor, as well as the use of OGTT versus IPGTT (Nausheen et al., 2013; Ramzy et al., 2014). This study presents a brand-new surgical technique with a simple and clear difference compared to previously described methods. The ileum is anastomosed to the stomach, providing direct drainage of the alimentary bolus, which will stimulate the ileal mucosa along with gastric secretions. In this case, the duodenum has a regulatory role with an anti-incretinic effect that has been shown in several studies (Rubino and Marescaux, 2004). This effect may not be enough
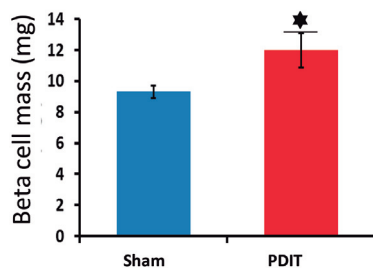

Fig. 4. Beta-cell mass quantification. Beta-cell mass expressed in milligrams (mg) on the Y axis as the mean ± SEM in n=15 sham-operated rats (sham, blue bar) and n=15 PDIT-operated rats (PDIT, redpbar) on the X axis. Statistical differences with p<0.05 are marked as *.
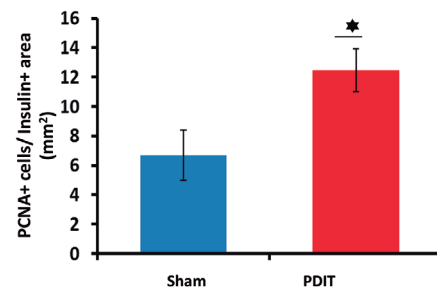

Fig. 5. Beta-cell proliferation assay. The number of proliferative beta-cells is presented on the Y axis as the mean ± SEM of PCNA and insulin+ cells/mm² of the insulin+ area in n=15 sham-operated rats (sham, blue bar) and n=15 PDIT-operated rats (PDIT, red bar) on the X axis. Statistical differences with p<0.05 are marked as *.
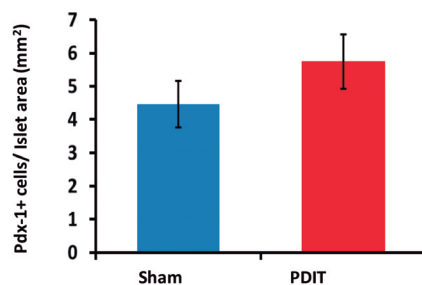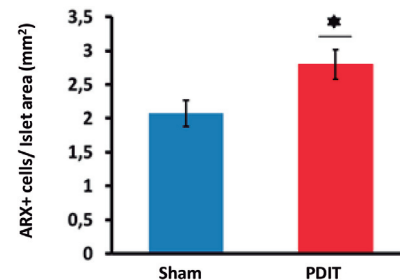

Fig. 6. Beta-cell neogenesis study. The beta-cell neogenesis ratio is presented on the Y axis as the mean ± SEM of PDX-1+ cells/mm² of islet area in n=15 sham-operated rats (sham, blue bar) and n=15 PDIT-operated rats (PDIT, red bar) on the X axis. Statistical differences with p<0.05 are marked as *.

Fig. 7. Beta-cell transdifferentiation study. The beta-cell transdifferentiation ratio is presented on the Y axis as the mean ± SEM of ARX+ cells/mm² of islet area in n=15 sham-operated rats (sham, blue bar) and n=15 PDIT-operated rats (PDIT, red bar) on the X axis. Statistical differences with p<0.05 are marked as *.

compared to such a direct stimulus as the transposed ileal mucosa. This phenomenon could explain why our subjects showed a significant difference in the insulinaemia measurements in the first 4 weeks after surgery that was confirmed at week 8 right before sacrifice. Similar results have been shown in other studies (Strader et al., 2005).

With regard to the morphological changes, the rats exposed to PDIT showed both a larger average area of the pancreatic islets plus a larger pancreatic beta-cell mass compared to the sham group, which is consistent with the findings of Camacho-Ramírez et al. (2017). This increase is related to the exposure of the ileal mucosa to a prompt pass of the alimentary bolus, resulting in a higher secretion of GLP-1 and PYY (Lindqvist et al., 2014; Camacho-Ramírez et al., 2020). The increased beta-cell mass is not absolutely related to an increase in the basal insulin response. However, after the OGTT, the insulin response appeared to be increased in the experimental group (Fig. 3). We can infer that there is a quicker insulin response after increased beta-cell mass.

These morphological changes can be explained by different mechanisms of adaptation revealed through immunohistochemistry techniques. Thus, three different techniques were performed to consider proliferation, neogenesis and transdifferentiation mechanisms.

Of these three mechanisms, the proliferation pathway seems to be the only one involved in the morphological changes of the pancreas. The GLP-1 levels are consistently high in subjects exposed to bariatric surgery, especially those techniques that impose a new anatomical configuration that exposes the ileal mucosa to the alimentary bolus in a more proximal location. This increase in GLP-1 levels induces beta-cell proliferation through different genes and growth factors (Drucker et al., 2003a,b; Lindqvist et al., 2014). In the surgical technique carried out in these subjects, not only is the ileum exposed promptly to the alimentary bolus, but the pyloric valve effect is also abolished by the anastomosis. This approach could lead to a more direct and consistent stimulus of the mucosae, which translates into a higher level of GLP-1 and therefore growth factor-induced proliferation. Unfortunately, we could not measure GLP-1 plasma in this study.

GLP-1 and PYY are not the only enterohormones related to the entero-pancreatic axis. Many other peptides have been described to participate at this level. Moreover, these peptides are synthesized at different portions of the digestive tract, such as GIP released by the duodenum or ghrelin and leptin released by the gastric mucosae. Interest in this idea stems from the fact that every surgical technique affects a defined intestinal portion. Only the peptides related to the portion affected by surgery would be implicated in the described homeostatic mechanism. Leptin has been described as a key factor in some consequences after surgery (Muruzábal et al., 2002). The embryonic releasing response of ghrelin is recovered after gastric surgery. These hormones cannot be related to our results. We did not alter gastric involvement in the PDIT model.

Regarding neogenesis, in this study, there were no significant differences, but there was a clear trend that can be compared to other studies (Camacho-Ramírez et al., 2017), which demonstrated significant differences in those subjects exposed to gastric bypass. This association between gastric bypass and neogenesis phenomena has been shown in other studies (Perez-Arana et al., 2015), where cells of different sizes, distributions and even locations within the pancreas have been shown to undergo neogenesis.

The cellular changes have been partially linked to the portion of the intestinal tube affected by surgery. In RYGB, many peptides released by the intestinal tube have been demonstrated, including GLP-1, GIP, GLP-2 and VIP. On the other hand, RYGB implies a drastic transection of gastric volume. The final gastric pouch has been implicated in these surgical mechanisms after bariatric surgeries, specifically related to the hormones ghrelin and leptin (Muruzabal et al., 2002). However, our experimental model did not include resectioning of the gastric-releasing surface, as in Roux-en-Y gastric bypass. The PDIT model only involved the sectioning and transpositioning of the ileum; thus, the gastric
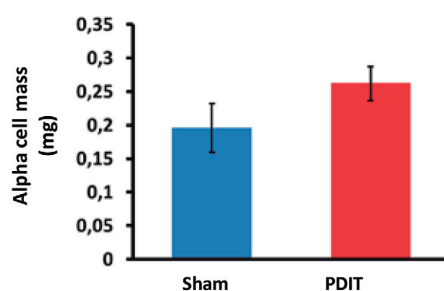

Fig. 8. Alpha-cell mass quantification. Alpha-cell mass expressed in milligrams (mg) on the Y axis as the mean ± SEM of n=15 sham-operated rats (sham, blue bar) and n=15 PDIT-operated rats (PDIT, red bar) on the X axis. Statistical differences with p<0.05 are marked as *.
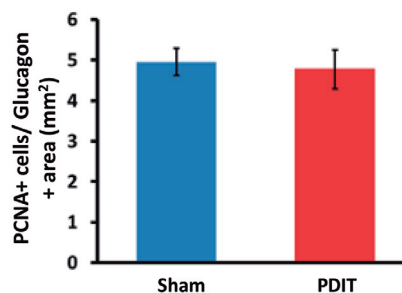

Fig. 9. Alpha-cell proliferation assay. The number of proliferative alpha cells is presented on the Y axis as the mean ± SEM of PCNA and glucagon+ cells/mm^2 of the glucagon+ area in n=15 sham-operated rats (sham, blue bar) and n=15 PDIT-operated rats (PDIT, red bar) on the X axis. Statistical differences with p<0.05 are marked as *.

hormonal component was not initially affected. Therefore, our study did not include multiplex analysis of enterohormones.

Neogenesis, which is strictly related to the expression of PDX-1 (pancreatic duodenal homeobox-1), seems to be especially triggered in techniques in which the duodenum (hindgut) is abolished, for example, in rats that undergo gastric bypass.

This novel technique respects the passage through every single portion of the tract, so PDX-1 is not overexpressed; therefore, neogenesis processes are not taken into account.

Regarding the transdifferentiation processes analysed in our study, the only lineage that showed significant differences among our samples was the one that expressed ARX. ARX may be involved in the determination of alpha cells, and its misexpression in adult beta cells induces the conversion into alpha or PP cells (Sosa-Pineda et al., 1997; Collombat et al., 2003, 2007). This conversion might occur as a control mechanism to prevent potential fatal effects in our rats, as they are neither obese nor diabetic but experience proliferative processes in their pancreas that are capable of causing nesidioblastosis or hypoglycaemia (Rumilla et al., 2009).

In this report, we employed a new surgical experimental method in which the ileum was located prior to the pylorus. The survival period was enough to develop a glycaemic homeostasis mechanism after the rapid arrival of nutrients to the ileum of the experimental animal groups. OGTT was analysed during this survival period. After sacrifice, the pancreas of the PDIT group showed cellular changes related to the activation of the enteropancreatic axis. On the basis of much evidence that has been reported, several hormones are implicated in these endocrine cellular consequences. In conclusion, we present an increase in beta-cell mass in a fast ileum nutrient arrival model of surgery in rats. This cellular increase was characterized by an increased expression of proliferation factors (PCNA), while transdifferentiation factors were expressed before alpha-cell levels were increased (ARX).

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