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From Cell Biology to Tissue Engineering

Bariatric surgery influences β-Cell turnover in non obese rats

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Summary. Background. The aim of this study was to investigate the relation between the different bariatric surgeries and pancreatic β-cell turnover. Material and Methods. We used healthy adult male Wistar rats to undergo the different techniques. Three surgical techniques were developed (malabsorptive, Sleeve gastrectomy and Roux-Y Gastric Bypass-), together with two control groups (Sham and fasting control). Pancreatic β-cell mass was measured, as well as apoptosis, proliferation and neogenesis related to cellular turnover. Otherwise, we measured the functional issues to elucidate the physiological role that these surgical techniques trigger in the carbohydrate metabolism (e.g. food intake, weight gain, intraperitoneal glucose tolerance test, and basal glycaemia). Results included the differences in phenotypes of the rat after the surgery. The rats did not show important differences in glycaemic parameters between the surgical groups. The β-cell mass presented modifications related with proliferation processes. A significant increase of β-cell mass in the malabsorptive technique was reported. On the other hand, the peripheral resistance to insulin tended to be reduced in rats which underwent malabsorptive and mixed techniques. Conclusion. This work showed an increase in β-cell mass after the resection of an important portion of small bowel. The Roux-Y Gastric Bypass produced a non-significant increase in β-cell mass. We considered that these implications of surgery over the endocrine pancreas must be one of the mechanisms related to the improvement of type 2 Diabetes mellitus following bariatric surgery.

Key words: Pancreas, Diabetes, Bariatric-surgery, Insulin-Secreting Cells, Beta-cell mass.

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Introduction

The remission of type 2 diabetes (T2DM) has been observed as an additional outcome of surgical treatment for morbid obesity in humans (Pories et al., 1995). Induced caloric intake reduction, weight loss and malabsorption of carbohydrates and fats were suggested as explanations for this effect of bariatric surgery on T2DM (Ford et al., 1997; Cummings et al., 2004; Gumbs et al., 2005). More recently, the bile acids, lipoproteins (Pressler et al., 2015) and microbiota have been invoked as effectors in this entero-pancreatic axis (Stefater et al., 2012). Many mechanisms have been proposed but the real mechanism of this success remains unclear.

The glycaemic control often occurs prior to significant weight loss in surgical patients, and even glycaemic homeostasis is independent of the nonsurgical procedures (as gastric band) (Mingrone et al., 1997; Marion et al., 2009; Alejandro et al., 2015). Currently, it is assumed that glycaemic control might have a direct effect on secretion or sensitivity to insulin.

We inferred the idea that insulin secretion must be related to cellular changes in endocrine pancreas. Pancreatic β-cells adapt to stress situations, including obesity, pregnancy and surgery (Alejandro et al., 2015). We believe that bariatric surgery improvement creates changes in β-cell mass cellularity, which is at the basis of changes in insulin secretion. There must be some effectors acting as stimuli for these endocrine changes of the pancreas. Probably many substances have been implied in this enteral-axis, which finally will be the executers on β-cell turnover.

Several incretins have been stated in this mechanism of stimuli over the pancreatic cellularity. GLP-1 or PYY -both secreted by the L-cell in the ileum- or ghrelin in the antrum of the stomach; all of which have been involved in physiological (Thaler and Cummings, 2009; Batterham and Cummings, 2016) and physio-pathological (Nauck et al., 1993; Drucker, 2003) changes in the entero-insular axis and β-cell mass (Patriti et al., 2004; Hongwei et al., 2013). But not exclusively, since different peptides -GIP, leptin or CCK among theme- are already the object of studies in this sense (Meek et al., 2016).

However, the study of the relative degree of β-cell mass changes related to the portions and extension of digestive tube implied has not been reported. In this way, many studies reported that enhanced delivery of nutrients to the distal intestine and increased secretion of hindgut signals might affect the entero-insular axis (Duan et al., 2014). These explanations could be related to the pathophysiological consequences of some techniques (e.g. Roux-Y Gastric bypass, RYGB) (Rubino, 2008; Ochner et al., 2011; Yu et al., 2013), which exercise a massive distortion of the digestion and absorption processes. In any case, this hypothesis does not explain the positive behaviour of restrictive techniques (such as Sleeve Gastrectomy, SG), which are receiving progressively more attention in the clinical area and they are broadly employed.

Therefore, we hypothesised that the digestive tube must trigger an effect on the cellularity of the endocrine pancreas. All the entero-hormones, which have been related to a complex stability of homeostasis named as incretin-anti-incretin balance (LeRoux et al., 2006; Salinari et al., 2014), are involved in a reported glycaemic improvement of the T2DM, by an increase of β-cell turnover (Li et al., 2010; Portha et al., 2011; Alejandro et al., 2015).

This β-cell mass is severely affected in long-term obesity and T2DM. Many reports showed that β-cell function is clearly improved after bariatric surgery (Nannipieri et al., 2011; Rabiee et al., 2011; Kodama et al., 2013). Around this, the purpose of the present study is to understand how different bariatric procedures could affect pancreatic β-cell mass homeostasis. For this study, we employed a healthy, non-obese, animal model, the Wistar rat, in order to reduce the interferences with concomitant pathologies.

The malabsorptive model was included by resecting 50% of the small bowel to the usual bariatric techniques related to human treatment (RYGB and SG). Thus, IR50 is considered as a purely malabsorptive surgical technique. Even though this technique was actually rejected as a bariatric surgery, it has been included here to complete the sequence of variation related to these surgical models.

We measured various functional issues to elucidate the carbohydrate metabolism for each surgical group. These physiological parameters were triggered as consequences to the surgical techniques. A sequence of morphometric parameters were completed in order to conclude the tendency of the β-cell, which included the measurements of pancreatic β-cell mass, apoptosis, proliferation and neogenesis. We hypothesised that the β-cell mass must be modified after bariatric surgery, and that β-cell mass modification could partially be the basis of the T2DM clinical amelioration.

Materials and methods

Animals

All animal procedures were performed under the approval of the University of Cadiz Committee for the Ethical Use and Care of Experimental Animals. The 30 male Wistar rats were stabled in randomized groups (n=6 each group), under constant temperature and humidity conditions in a 12-hour light/dark cycle, with *ad libitum* access to normal chow and water. Female rats were not used for this study to avoid the cyclic variations of gonadotropin hormonal effect on the glycaemic metabolism.

Weight gain and feed intake. Basal glycaemia

In order to evaluate the effect of bariatric surgery in animals, we controlled the weight increase of the animals, as well as the grams of feed ingested. The chow intake was quantified every two days for the first month after surgery. The weight increase was measured every two days for the first month after surgery, and every week for the last two months.

Once a week, the basal glycaemia was measured with a glucometer (Glucocard G-Meter 1810, Menarini diagnostics, Italy) and expressed as mg of glucose/ decilitre of blood.

Surgical interventions and fasting controls

The fasting control group (FC) was subjected to the same preoperative and postoperative conditions as the operated groups, with a 12 hour-fasting pre- and post-surgical period. All surgical procedures on the animals were performed under general anaesthetic with continuous infusion of Isofluorane 3% V/V (Isoflo, Abbott 571329.8). An intake re-adaptation period followed each surgery to normalize fasting.

IR50 as the malabsorptive bariatric surgery was

performed with the following steps (Pérez-Arana et al., 2015). A laparotomy of about 3 cm in the midline of the abdomen. We identified the angle of Treitz and the ileocecal valve as anatomical references. The bowel between these points was exposed and measured. A resection of the central 50% was made, followed by an end-to-end anastomosis with 5-0 monoplane silk suture (polypropylene, Ethicon Prolene), leaving the proximal half of the jejunum and the distal half of the digestive tube. The ileum was left untouched. Lastly, instillation of physiological saline at 37°C in the abdominal cavity and closure of the abdominal wall in one layer was done. These final steps were repeated in every surgical procedure.

The RYGB, mixed -malabsorptive and restrictive-bariatric surgery, involved the exclusion of the proximal intestine by the bypass of the duodenum and a part of the jejunum, as well as the reduction of stomach to the fore-stomach. The stomach was exposed and the gastric fundus was sectioned, while preserving approximately 20% of the original gastric volume. The jejunum was dissected at 8 cm from the ligament of Treitz, and the terminal jejunum of the section was connected via end-to-end anastomosis to the preserved fundus. The antro-jejunal loop (biliopancreatic loop) was continued with the alimentary loop at 10 cm of the fundus-jejunum anastomosis.

Sleeve Gastrectomy (SG) was performed with a laparotomy of 5 cm in the upper third of the abdomen through sectioning of the gastrosplenic ligament and exposing the stomach. Curved forceps were applied from the angle of Hiss to antrum, performing a cylindrical stomach of approximately 0.5 cm of diameter. The stomach section included the section of the most fundus, stomach-corpus at greater curvature level, and antrum; the pylorus was preserved. The SG reduced the initial stomach volume by approximately 20% and it reproduced the actual selective technique used in humans, as the restrictive model of bariatric surgery.

The Sham-technique (Sham) reproduced the surgical aggression over the digestive tract and the stress of both pre-surgery and post-surgery, but the integrity of the digestive tube was preserved. Sham was performed by an incision of about 3 cm in the middle area of the abdomen, exposing the small bowel loops. After we measured the size from the angle of Treitz to ileocecal valve, a transversal enterotomy section was performed, without intestinal resection, and end-to-end anastomosis.

Intra-peritoneal glucose tolerance test (IPGTT)

A blood sample of 0.5 ml was collected from the tail vein of each fasted animal. Then, an intraperitoneal injection of 40% solution of glucose was administered (2 gr/Kg body weight) followed by blood sampling from the tail vein at 15, 30, 60 and 120 minutes following glucose administration. We did the IPGTT monthly. Basal glycaemia was measured pre-operatory and weekly after surgery. Glycaemia was measured with a glucometer (Glucocard G-Meter 1810, Menarini diagnostics, Italy) and expressed as mg of glucose/decilitre of blood.

β-cell mass quantification

Three months after surgical intervention, the animals were sacrificed by an intraperitoneal Chloral Hydrate overdose and perfused with Bouin's solution (25% Formalin/75% H_2O saturated with Picric acid). After this, the pancreas was resected, weighed (precision scale Ohaus Pioneer Mod PA 3102), and post-fixed in Bouin's solution, 24h at 4°C. The fixed pancreas was dehydrated, paraffin embedded and longitudinal 10 μm microtome sections were obtained.

To calculate β-cell mass, insulin producing cells were stained using a monoclonal mouse anti-insulin antibody (Sigma-Aldrich, I-2018 USA); a secondary biotin conjugated goat anti-mouse IgG antibody and a streptavidin complex (Sigma, Mouse Extra-2). The peroxidase-ABC complex was revealed with solution of 0.3 mg/ml of 3,3'Diaminobenzidine (Sigma, D5905) in presence of 0.2 μl/ml of H_2O_2 under microscopic control and counterstained with Harris's haematoxylin.

The insulin-positive areas were measured using a microscope equipped with a digital camera and the Image J image analysis. Those who performed the measurements were not aware of which experimental group the samples belonged to. β-Cell mass was measured as an insulin-positive area/total pancreatic area ratio by the total pancreas weight, and it was expressed in mg. Positive, negative and blocking with BSA samples were carried out as controls to ensure the immunohistochemical techniques.

Apoptosis assays

To determine β-cell apoptosis, 10 μm tissue sections from the pancreas were mounted on microscope slides and they were rehydrated through graded ethanol to PBS. The Dead End Fluorometric Terminal Deoxi-nucleotidil-Transferase-mediated 2'-deoxyuridine 5'-Triphosphate nick end Labelling (TUNEL) system (Promega, USA) was used according to the manufacturer's instructions. Insulin was simultaneously counterstained using polyclonal mouse anti-insulin antibody (Sigma-Aldrich, USA) incubated overnight at 4°C, and then stained with a secondary anti-mouse IgG antibody conjugated (Alexa 546) (Molecular Probes Inc. Eugene, USA). To determine the apoptotic fraction, TUNEL+/Insulin+ cells and islet areas were quantified by 20 islets/per sample. An image analysis Cell D software (Olympus, Hamburg, Germany) was used. Results were noted under randomized conditions by a single investigator, and expressed as the number of TUNEL+/insulin+ cells/mm^2 of islet. Negative samples were carried out as controls to ensure the immunohistochemical techniques. Positive controls were carried out in neural tissue samples of hypoxic rat.

Proliferation assays

Proliferation was assessed by double immunostaining, using polyclonal rabbit anti-Ki67 (Ab-Cam, AB16667, UK) and monoclonal mouse anti-insulin (Sigma-Aldrich, I-2018 USA) antibodies, and according to the manufacturer instructions. Previous to this, sections from the pancreas were incubated for 30 min with 0.1% Triton x-100 in PBS for tissue permeabilization, washed with PBS, and then incubated for 30 min with 4% BSA blocking solution in PBS at room temperature. Sections were stained using anti-rabbit IgG Alexa 488 and anti-mouse IgG Alexa 546 conjugated antibodies (Molecular Probes Inc Eugene, USA). The proliferation ratio was quantified in 40 islets/per sample. The results were expressed as the number of Ki67+/Insulin+ cells/mm^2 per area of pancreatic islets. We used the image analysis Cell D software (Olympus, Hamburg, Germany). Positive, negative and blocking with BSA samples were carried out as controls to ensure the immunohistochemical techniques.

Neogenesis study

To study PDX-1 expression, as a neogenesis marker, the pancreas sections were obtained as described above from rats aged 3 months-old after surgical intervention. We retrieved sections for 10 min with heat in citrate buffer pH 6.7 solution, stained with monoclonal rabbit anti-PDX-1 antibody (Ab-Cam, 47267 UK) and labelled using biotin conjugated anti-rabbit IgG secondary antibody (Sigma-Aldrich, B8895, USA). A streptavidin complex (Sigma, Mouse Extra-3) was employed and the peroxidase was revealed with a solution of 0.3 mg/ml of 3,3'Diaminobenzine (Sigma, D5905) under microscopic control and counterstained with Harris's haematoxylin. The results were observed qualitatively in 12 pancreas areas/per animal group. Negative and blocking with BSA samples were carried out as controls to ensure the immunohistochemical techniques. Positive controls were carried out in rat small intestine samples.

Statistical analysis

Data measurement was expressed as mean±SEM. The data were analysed with the Mann Whitney-U test, and $p<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS statistical software.

Results

Weight measurement and food intake

Body weight gain in rats was monitored from the time of surgery to the time of sacrifice as described in methods. No differences appeared between RYGB, Sham and FC (Fig. 1A). However, there are differences between IR50 group and the Sham and FC groups ($p<0.05$) from the tenth week after surgery to sacrifice (Fig. 1B). There were also significant differences

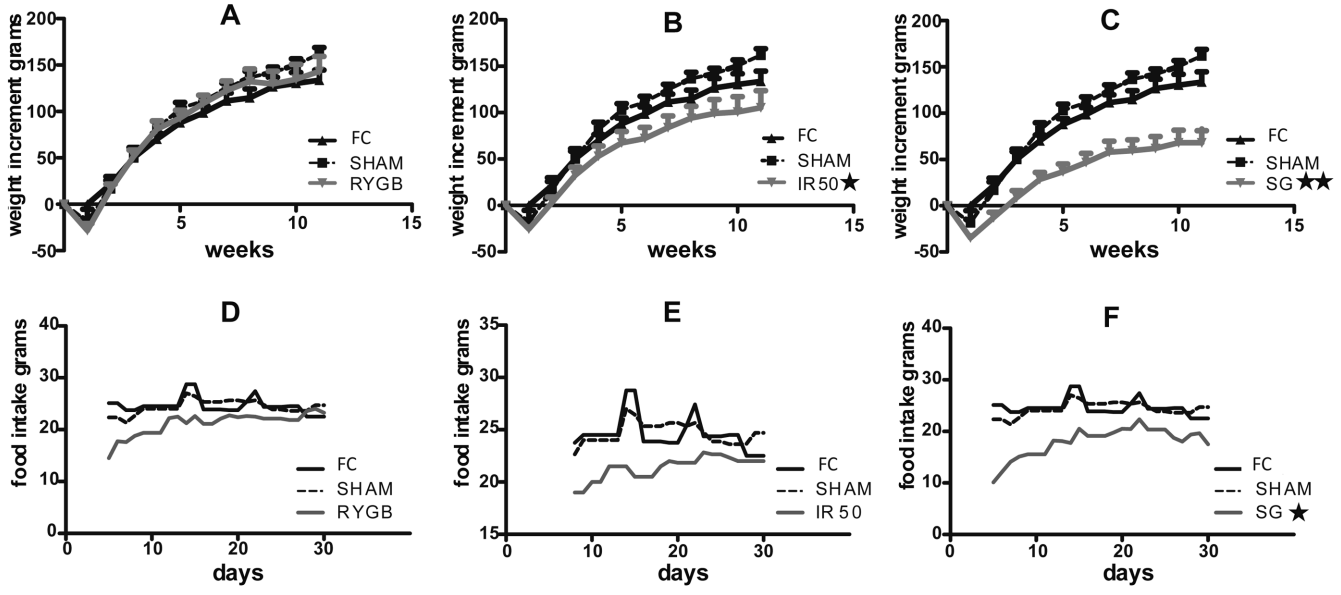

Fig. 1. Weight gain in groups RYGB **(A)**, IR50 **(B)** and SG **(C)** from surgery to sacrifice. Significant differences were found between IR50 group ($p<0.05$) and SG group ($p<0.01$) versus Sham and FC groups. Food intake in RYGB **(D)**, IR50 **(E)** and SG **(F)** versus control groups, for the first month after surgery. No food intake differences in IR50 and RYGB were reported **(D and E)**. Significant differences ($p<0.05$) between food intake values for SG group and FC group were seen. The Y-axis in weight gain of IR50 **(E)** had a different unit in order to facilitate the depiction.

between the SG group and the Sham and FC groups ($p<0.01$) from the time of surgery to the time of sacrifice. In the case of food intake, the graphs showed a lower intake in the SG group in relation to Sham and FC groups ($p<0.05$) (Fig. 1F) but not in RYBG or IR50 groups (Fig. 1D,E).

Other than the above, no changes were observed in the basal glycaemia during the period of the study. This data had no significant differences between groups.

Intra-peritoneal glucose tolerance test (IPGTT)

An intra-peritoneal glucose tolerance test (IPGTT) was performed in each group every four weeks from surgery to sacrifice. No differences appeared between the geometries of the curves along the study in RYGB and SG groups (Fig. 2A,E) versus the control groups. There were significant differences among the curves for the first month, for the second ($p<0.01$) month and third

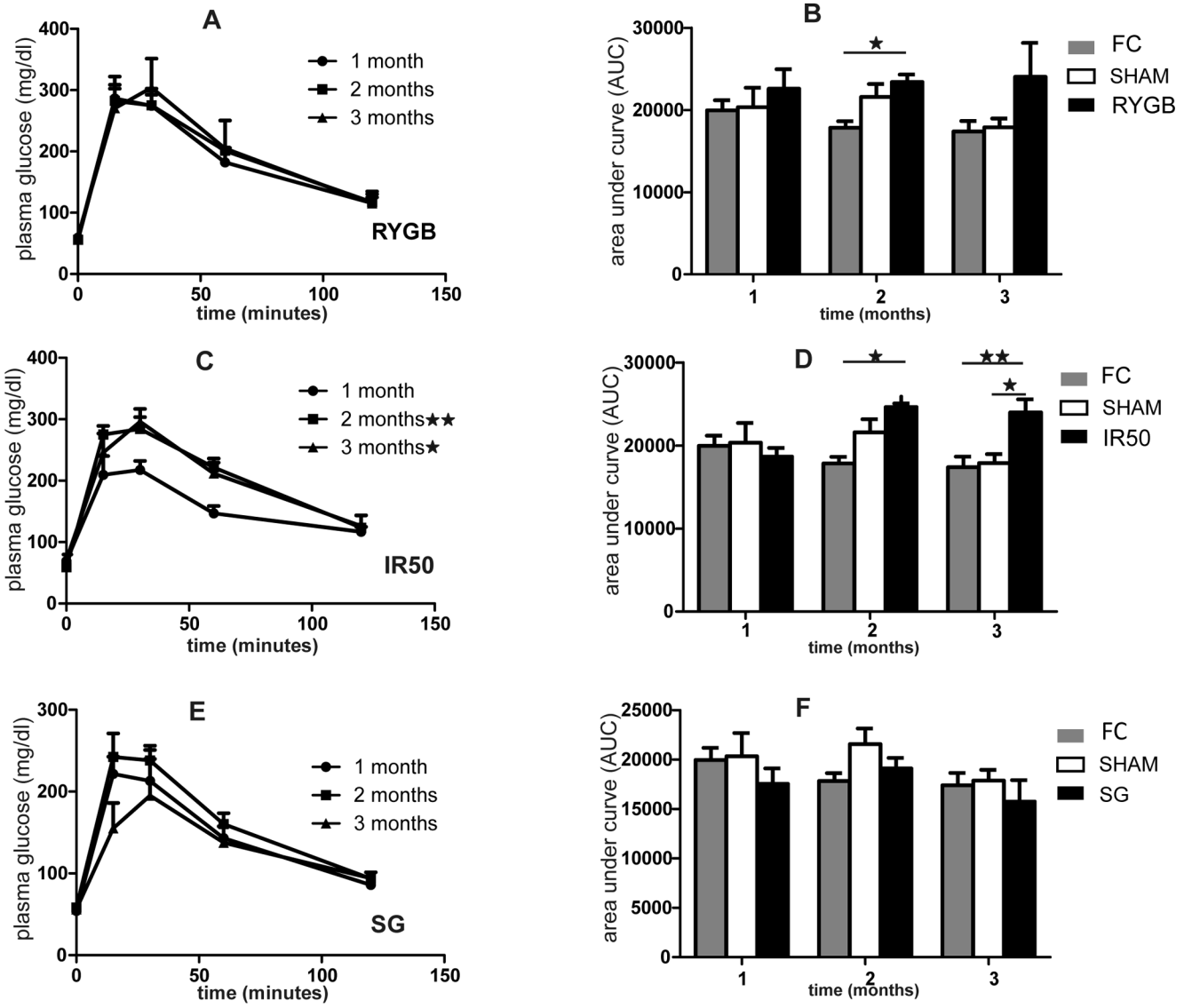

Fig. 2. IPTTG geometry curves development in surgery groups at the first, second and third months **(A, C, E)** and area under curve (AUC) in surgical, sham and fasting control groups, expressed as mg/dL/min-1 **(B, D, F).** Important differences appeared in IPTTG curves in the IR50 group between the first and the second months ($p<0.01$) and the first and the third months ($p<0.05$) **(C).** Also, significant differences were seen in the AUC at the second month between the IR50 surgery and fasting control group ($p<0.05$) and between the IR50 surgery and sham group ($p<0.05$) and the IR50 surgery and fasting control group at the third month ($p<0.01$) **(D).** There were no differences in IPTTG curve development in RYGB and SG groups **(A, E).** However, significant differences were seen in the AUC between the RYGB and fasting control groups at the second month ($p<0.05$) **(B)**. The Y-axis in the IPTTG and the AUC of SG **(E, F)** have a different unit in order to facilitate the depiction.

($p<0.05$) month in IR50 group (Fig. 2C).

We did not find any difference in the area under the curve (AUC) between the SG and Sham and FC groups during the study (Fig. 2F). However, in the RYGB group there were important differences ($p<0.01$) in AUC values (+15 mg dL/min) in comparison to the FC group AUC values (+10 mg dL min^{-1}) at eight weeks (Fig. 2B). Differences were also found among the FC group and IR50 group AUC values for the second month (+15 mg $dLmin^{-1}$ versus +19 mg dL/min) ($p<0.05$) (Fig. 2D). Finally, differences also persisted in the third month between the IR50 group AUC values (+15 mg dL min^{-1}) and the FC group AUC values (10 mg dL min^{-1}) ($p<0.01$) and also between the IR50 group AUC values and the Sham (10 mg dL/min) ($p<0.05$) (Fig. 2D).

B cell mass

Pancreatic β-cell mass quantification was accomplished for each group immediately after sacrifice. Three months following surgery, no β-cell mass significant differences appeared among the RYGB or SG groups versus the Sham and FC groups (Fig. 3). On the other hand, there were significant differences among the FC and the Sham control ($p<0.05$) β-cell mass values (+5 mg and +6 mg, respectively) versus the IR50 group β-cell mass value (Fig. 3).

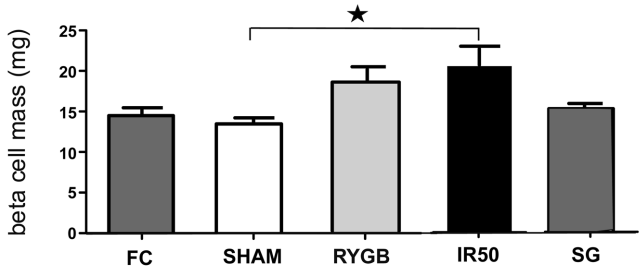

Fig. 3. β-cell mass quantification expressed as mg. The difference appeared in this study between Sham control group and IR50 group, with the IR50 group β-cell mass having roughly 0.6 times more β-cell mass ($p<0.05$) compared to the Sham control group.

Proliferation assays

β-cell proliferation was analysed through the presence of the Ki67 proliferation marker in the β-cell nucleus. The data showed high rates of replication in the IR50 (showed +9 Ki67 positive cells/mm^2 insulin positive area), and the RYGB (presented +11 Ki67 positive cells/mm^2 insulin positive area) over control groups ($p<0.05$) (Fig. 4). The SG group proliferation rates were decreased (+5 Ki67 positive cells/mm^2 insulin positive area) when compared to the Sham and FC group (+8 and +7 Ki67 positive β-cells/mm^2 insulin positive area, respectively) ($p<0.05$) (Fig. 4).

Apoptosis assay

Rate of β-cell apoptosis was performed using the TUNEL system in each group. No significant differences appeared among any of the surgical groups versus Sham or FC groups (Fig. 5).

PDX-1 analysis

Presence of new β-cells from non β-cells, known as differentiation, was assessed using PDX-1 transcription factor immunostaining in pancreas samples for each group. The study showed that no group expressed variations in the immunostained expression of PDX-1

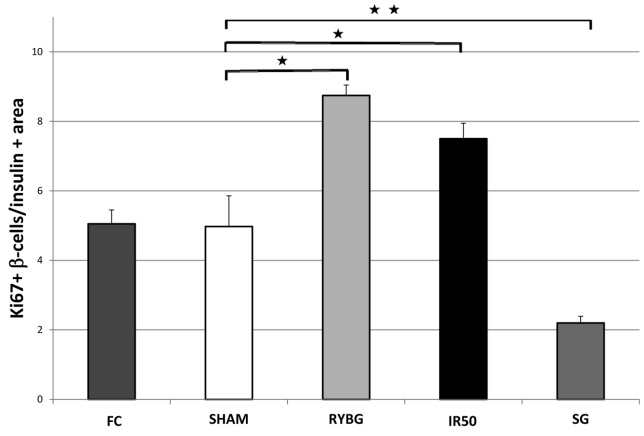

Fig. 4. Proliferation assays. Rates of β-cell proliferation expressed as the number of β-cells, which expressed Ki67 positive/ insulin positive area (mm^2). IR50 and RYGB groups show higher proliferation rates than Sham control group ($p<0.05$). However, the rate of β-cell proliferation appeared diminished in SG group, compared to Sham control ($p<0.05$).

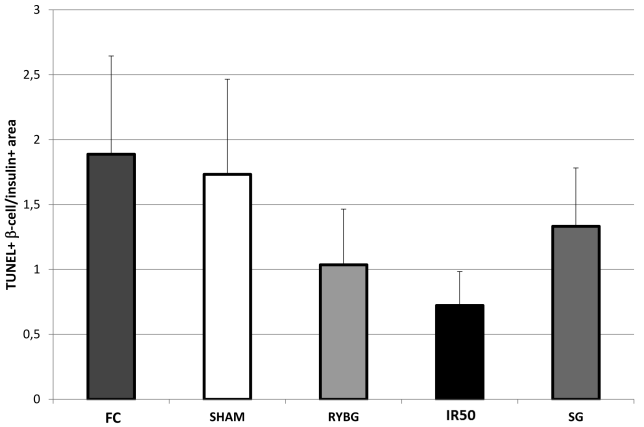

Fig. 5. Apoptosis assays. Rates of β-cell apoptosis expressed as the number of TUNEL positive β-cells/ insulin positive area (mm^2) for all of the groups. No group showed differences in β-cell apoptosis ratio between surgical groups and controls.

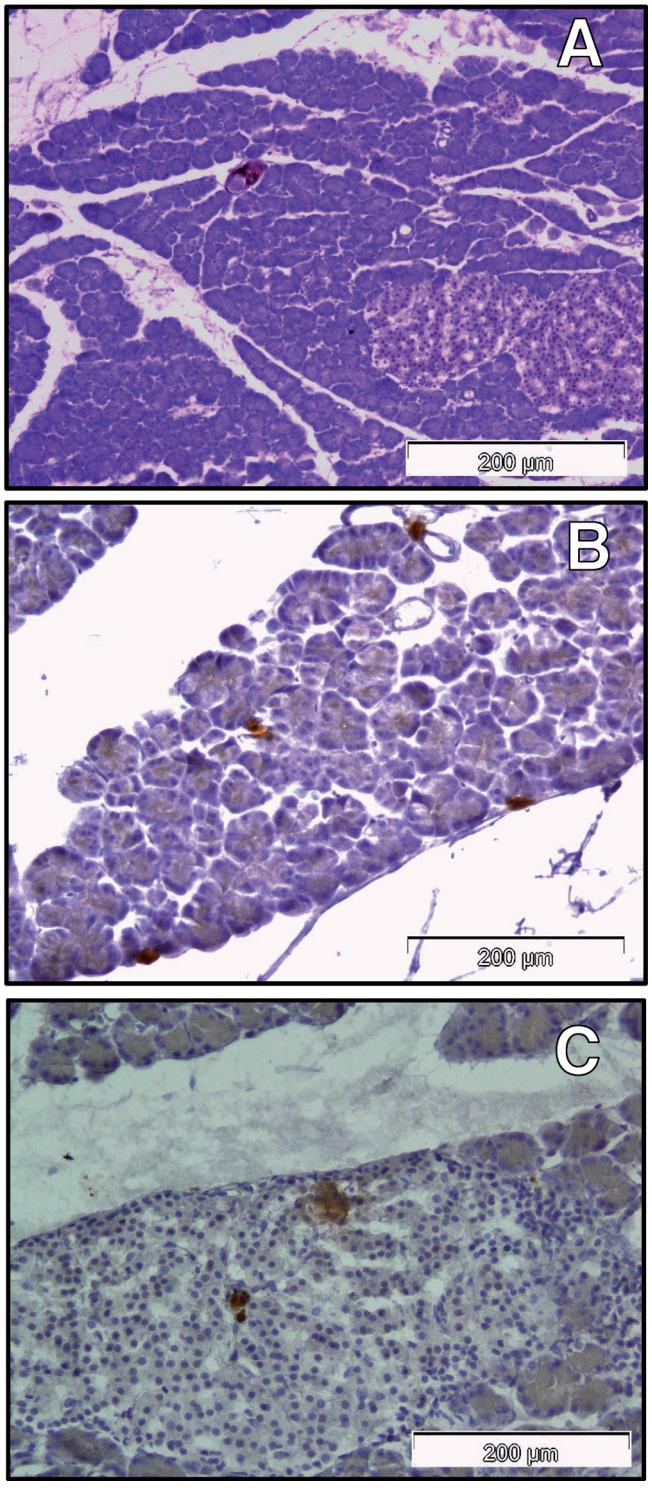

Fig. 6. Images showing pancreas expression of PDX-1. Fasting control group **(A)**, Sham control group **(B)** and RYGB group **(C)**. Enhanced expression of PDX-1 appeared in the RYGB group compared to the other groups.

(Fig. 6A -FC-and B -SHAM-), except in the RYGB group, where visually greater intensity and frequency immunostaining appeared as we can see in Fig. 6C.

Discussion

The endocrine pancreas has a significant remodelling capacity, attending not only to several physiological conditions, but also to pathological status such as obesity. This mechanism even prevents the development of T2DM in many patients (Prentki and Nolan et al., 2006; Alejandro et al., 2015). The changes in β-cell number and size, islet neogenesis, proliferation and apoptosis contribute to the remodelling of the endocrine pancreas. The balance among these cellular mechanisms determines the change in β-cell mass.

Therefore, the study of β-cell mass dynamic plays an important role in the adaptation to obesity, as well as in the pathogenesis of T2DM. Many studies had focused on bariatric surgery effect on pathologic models of T2DM and obesity. Rather than following this model, our experiments were designed to analyse the effect of bariatric surgery on glucose control and β-cell population behaviour in a non-obese and non-diabetic model. We promoted an altered transit of food across the digestive tube. The pancreas was not affected at the beginning of the study, and β-cell islets were able to accommodate to the special situation generated from the bariatric surgery. Then, the changes in the islets were observed on the basis of a healthy pancreas.

The hyperglycaemia have a reported toxic effect on glucose-sensing of β-cell. In obesity, this mechanism potentiates β-cell dysfunction and T2DM will lately occurred. So, we considered that the histological consequences observed and described in this paper could be diminished because these pancreas were able to stabilise the glucose homeostasis. These pancreas of healthy rats were not previously damaged for the mentioned factors, without the demand of workload on the β-cell population. The glycemic parameters showed a resultant equilibrium, which represented the histological changes reported in this paper.

To the best of our knowledge, not many previous studies have focused on the changes in pancreas islets related to surgical models, in order to evaluate parameters about the β-cell mass and the cellular turnover (Patriti et al., 2007). We report how these processes could be related to the maintenance of glucose homeostasis.

Firstly, the results related to the weight gain and food intake showed that surgical processes reproduced those applied in humans. The functional parameters were according to the expected alteration of digestive tube and the increased transit and absorption of nutrients. The animals of surgical groups tolerated the surgery; all surgical groups gained less weight and food intake was reduced in models that affected the stomach.

On the other hand, the basal glycaemia was not altered in all of the studied groups. For this reason, we

consider that the altered flow of feed or the swift absorption of nutrients were not enough stimuli to create homeostatic imbalance and that these must be related to the condition of the healthy animals, with healthy endocrine pancreases, which were able to adapt to the surgical conditions.

In this study, the different surgeries had various consequences on glucose tolerance. Through the study of the IPTTG test, the results showed significant differences between FC group versus IR50 and RYGB groups in some intervals of the study (Fig. 2). One common point in the IR50 and RYGB surgical groups was that both groups produced a shortening of food transit in the small intestine (specifically by the jejunum), unlike in the SG group. These new anatomical situations, according to the data collected, seemed to have caused a modification in their AUC. This effect could probably be due to the early arrival of food to the ileum. It is well known that the rapid influx of nutrients may trigger the release of several incretins (such as GLP-1, GIP, Oxyntomodulin, PYY, etc.) and this would lead to a normalization of the AUC in both groups (Rubino et al., 2010; Batterham and Cummings, 2016; Meek et al., 2016). In this sense, the AUC is marking the global capacity of the endocrine pancreas to resolve the glucose overload.

In addition, a normalization of the AUC in the RYGB group from the eighth week was observed. However, this normalization of the AUC did not occur in the IR50 group. This different behaviour in the IR50 group could be explained by the decreased transit of food through the duodenum, which results in less secretion of GIP by K-cells (Cummings et al., 2004; Rao and Kini, 2011). This idea was the etiological basis of the foregut hypothesis. This hypothesis argued that RYGB, and partially IR50, bypasses the foregut. So the GIP -secreted for the duodenum and proximal jejunum- inhibited the β-cell function (Salinari et al., 2014). These results could reinforce the role of the ileum.

We explored the effect of these bariatric surgeries on the pancreas because it was considered that islets must be the target of the entero-insular axis. We believe that the improvement of β-cell dysfunction must be related to changes in the cellularity of islets, and this could be a consequence of the surgically altered anatomy of the gastrointestinal system.

Some authors have reported that the surgery per se causes an inflammatory distress, which inhibits insulin sensitivity. This metabolic stress related to surgery could explain why our Sham control presented different parameters than FC. We reported results in which Sham group presented significant differences with surgical groups, which did not occur with FC (Lingvay et al., 2013).

First, this study showed that β-cell mass was slightly increased in the surgical groups following surgery, but only the IR50 group presented with significant differences versus the FC and the Sham groups (Fig. 3B). These results could be explained by the increased stimulation of the ileum due to earlier presence of food, which could lead to an elevated release of GLP-1 or PYY (Rubino, 2008; Rubino et al., 2010; Batterham and Cummings, 2016). This idea could be supported by the data of RYGB group. The behaviour of the AUC and the un-significant increase of β-cell mass in the RYGB group indicated a common aspect with IR50. Both groups, RYGB and IR50 (malabsorptive and mixed surgical groups) incited an early arrival of nutrients to the ileum. However, this mechanism occurred sooner in the IR50 when compared to the RYGB group, so the effect was greater and earlier in the IR50 group. Both groups, IR50 and RYGB, had significant proliferation rates versus the control groups (Fig. 4), which correlate with β-cell mass changes.

The possible role of GLP-1 is controversial. The proliferative effect of the release of GLP-1 on β-cell population has been well described (De Leon et al., 2003; Egan et al., 2003; Liu, et al., 2011; Yabe and Seino, 2011). However, some authors considered that GLP-1 does not have a direct effect on the pancreas, and it is related to satiety by acting on the central nervous system (Stefater et al., 2012). Otherwise, the GLP-1 receptor modifications have been excluded as a fact related to the T2DM improvement after SG (Wilson-Pérez et al., 2013).

The SG group showed a significant low proliferation ratio versus the control groups (Fig. 4); however, it did not show a decreased β-cell mass versus the control groups (Fig. 3). The explanation of this could be in a slight decrease in the β-cell apoptosis ratio in SG group. There were no significant differences in the β-cell apoptosis rates between any studied groups (Fig. 5). Thus, the explanation for this effect must be an increased phenomenon of cell differentiation from stem cell to β-cell (Juhl et al., 2010). This fact was supported by the normal presence of PDX-1 stained β-cells, as observed in SG samples (Fig. 6).

The cell differentiation from stem cell to β-cell after bariatric surgery, regarding the PDX-1 study, showed more data in the RYGB surgical group (Juhl et al., 2010; Bonner-Weir et al., 2012). In the RYGB samples, an increased presence of PDX-1 β-cell was observed (Fig. 6C). The increased β-cell differentiation is related to some stimulating factors (e.g. GLP-1 or PYY) on the pancreas (Rubino, 2008; Ochner et al., 2011; Yu et al., 2013).

It was of note that both surgical techniques (RYBG and IR50) have a common region, which is affected -the duodenum and first jejunal portion-, and that this increment could be related to a direct influence on the pancreas. There is an uncommon region to both surgical techniques. The exclusion of duodenum to the nutrients in the RYGB versus the IR50 could explain the different effect of both techniques on the pancreas. We believe that the so called anti-incretin effect, secreted in the duodenum (e. g. CCK or GIP) could be the basis of these differences (Le Roux et al., 2006; Salinari et al., 2014; Meek et al., 2016).

The complete observation of data leads us to suggest the presence of two possible mechanisms that could be the basis of the β-cell mass increase. The first proposed mechanism is the direct path due to the early presence of food in the ileum, as is the case of the IR50 and RYGB surgeries. The premature appearance of partially digested nutrients in the ileum seemed to show an earlier increase in proliferation. The proliferation mechanism could be related to ghrelin level changes (Patriti et al., 2004; Zhou et al., 2014). This direct pathway could also act on β-cell mass through a release of GLP-1, a recognized stimulatory of β-cell proliferation agent secreted by L-cells (De Leon et al., 2003; Egan et al., 2003; Thaler and Cummings, 2009). We consider that the entero-hormonal axis must have an effect on the final changes in the cellular conformation of endocrine pancreas.

A second path could be related to the restriction of the transit of food through the jejunum. The mechanism appeared also in the IR50 and RYGB surgical groups. This second mechanism would act indirectly through a transient modification of glucose tolerance. The surgical processes that induce a reduction of glucose tolerance could stimulate an increase in β-cell-mass, due to the high blood glucose level (Fleck et al., 1987; Steil et al., 2001). But in the case of the IR50 group, this mechanism seems to be more important than in the RYGB group (Fig. 2). This could be explained by the fact that the IR50 samples experienced a shorter transit through the jejunum than in the RYGB group. Therefore, the second proposed mechanism would reinforce the first path we described above, the enhanced growth of β-cell mass.

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