Annals of Anatomy 223 (2019) 1–7

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.com/locate/aanat

RESEARCH ARTICLE

GLP-1 mediated improvement of the glucose tolerance in the T2DM GK rat model after massive jejunal resection

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ARTICLE INFO

Article history:
Received 6 July 2018
Received in revised form 4 January 2019
Accepted 23 January 2019

Keywords:
GLP-1
Enterohormones
type 2 Diabetes mellitus
Malabsorption Syndromes
Insulin-Secreting Cells

ABSTRACT

Objective: The aim of this study was to clarify the role of the middle gut in the entero-pancreatic axis modification that leads to glucose improvement in the Goto-Kakizaki (GK) rat as a non-obese T2DM model.
Background: Bariatric surgery is considered an assured solution for type 2 Diabetes (T2DM). Enterohormones such as ghrelin, gastric inhibitory polypeptide and mainly glucagon-like peptide-1 (GLP-1) were recognized as key players in the physiophathological mechanisms associated with entero-pancreatic axis regulation and glucose tolerance improvement. However, the influence of anatomical arrangements post-bariatric surgery on this axis is still debatable.
Method: To this purpose, 50% of small intestine resections were performed on GK rats (n = 6), preserving the proximal half of the jejunum and the ileum (IR50). Phenotypic and functional changes, such as performance in oral glucose tolerance tests, ileal release of GLP-1, beta-cell sensitivity to GLP-1, beta-cell mass, and turnover were characterized in IR50 and the surgical control group (Sham).
Results: The glucose tolerance was improved and ileal release of GLP-1 was enhanced four weeks after IR50 versus the control group rats. Beta-cell mass, beta-cell proliferation, and beta-cell sensitivity to GLP-1 were also increased in the pancreas of IR50 versus the control group rats.
Conclusion: the jejunal exclusion increases beta-cell-mass and improves glucose tolerance by increasing in GLP-1 expression and number of receptors via the entero-pancreatic axis.
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1. Introduction

Obesity is a major public health problem in Western societies due to treatment costs, long-term effects on health, and high prevalence. An important obesity-associated pathology is Type 2 Diabetes (T2DM), characterized by a beta-cell failure along with peripheral and hepatic insulin resistance. The prevalence of T2DM has been estimated to affect 5–10% of the population, although trending upwards globally, especially in young people (Wild et al. 2004).

Since the 1990ś many authors have reported the effects of bariatric surgery on glucose control in T2DM subjects, with 70 to 90% of patients remaining euglycemic without pharmacological treatment for several years after the procedure (Pories et al., 1995; Smith et al. 1996). Many authors have suggested a relationship between digestive tract modification and glucose homeostasis as one of the main mechanisms implied in this euglycemic status (Alejandro et al. 2015). Many hypotheses were presented to explain these effects.

Some hypotheses focused on the gastrointestinal (GI) hormone suggesting that the functions of one or more GI hormones (e.g. GLP-1, GIP, PYY, Ghrelin, etc.) could be fundamental factors. Surgical techniques have altered the normal disposition of the intestinal tube. As a result of these surgeries, the normal flow and absorption of nutrients were altered in the different portion of intestine (Baggio & Drucker, 2007; Cummings and Shannon 2003). These

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https://doi.org/10.1016/j.aanat.2019.01.007
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changes in the intestinal mucosae would change the secretion pattern of the hormones (Batterham, 2016; Rubino 2008; Yousseif et al. 2014).

Other hypotheses focused on the potential role of biliary acids (BA) in regulating hepatic glucose (Penney et al. 2015), or on the existence of a control for a brain-gut-nervous system axis. In this axis, some gut-derived enterohormones could act as regulators of the central nervous system through a feedback mechanism of hepatic glucose production (Wang et al. 2008; Breen et al. 2012).

Nevertheless, the secretion of GI hormones, such as GIP, Ghrelin, or GLP-1, seems to be involved directly or indirectly in the antidiabetic effects of bariatric surgery. Focusing on GLP-1, it is known that this hormone has the capacity to induce β-cell proliferation in rodents (Drucker, 2003) and improves hepatic insulin sensitivity in mice after bariatric/metabolic surgery (Garibay et al. 2016). Although, these results have not always been reproduced employing Roux-en-Y Gastric Bypass (RYGB) or Sleeve gastrectomy (SG) techniques (Mokadem et al. 2013; Chambers et al. 2014). It is therefore difficult to discern which part of glucose tolerance improvement is due to enhanced GLP-1 secretion and which one is not, especially because these surgeries affect different anatomical elements.

In the present study we used a surgical experimental technique on a non-obese diabetic animal model as the Goto-Kakizaki rat (GK), which exclusively affects the gut but not the stomach. We conducted a resection of 50% of the small intestine with the aim to determine the role of GLP-1 in improving glucose tolerance. We then analyzed the effect of such resection on GLP-1 secretion in ileum, GLP-1 pancreatic β−cell sensitivity, and β-cell population turnover. Thus, our purpose was to confirm the relationship between the changes in the intestinal GLP-1 release and pancreatic sensitivity, as a possible consequence of the entero-pancreatic axis.

2. Material and methods

2.1. Animals

All animal procedures were performed with the approval of the University of Cadiz Committee for the Ethical Use and Care of Experimental Animals. This Committee controlled the procedures were performed in accordance with international relevant guidelines and regulations for animal welfare. The animals proceeded by the Animal Service and Production Unit (SEPA, at the University of Cádiz). The twelve male Goto-Kakizaki (GK) rats were randomly distributed to sham and IR50 surgical groups (n = 6). Animals were kept under constant temperature and humidity conditions in a 12-hour light/dark cycle, with ad libitum access to regular chow and water. GK rats weighed 220 g to 250 g when the surgical protocol was performed. Female rats were not used to avoid the cyclic variations of gonadotropins and its effect on the glycaemic metabolism.

2.2. Weight Gain and Food Intake - Basal Glycaemia

To evaluate the effect of the surgery on animals, we monitored several variables. The weight gain and food ingested were quantified daily after surgery and every 48 h from the second week until sacrifice. Once a day, after surgery until sacrifice, basal glycaemia was measured with a glucometer (Glucocard G-Meter 1810, Menarini diagnostics, Italy) and expressed in milligrams of glucose/deciliter of blood.

2.3. Oral Glucose Tolerance Test (OGTT)

A blood sample of 0.5 ml was collected from the tail vein of each animal after an overnight 12 hour fast to obtain basal glycaemia. Through an orogastric tube, a 40% glucose solution (2 gr/Kg body weight) was administered. The glycaemia was monitored by blood sampling from the tail vein at 15, 30, 60 and 120 minutes after glucose administration. We realized the OGTT four weeks after the surgical procedure.

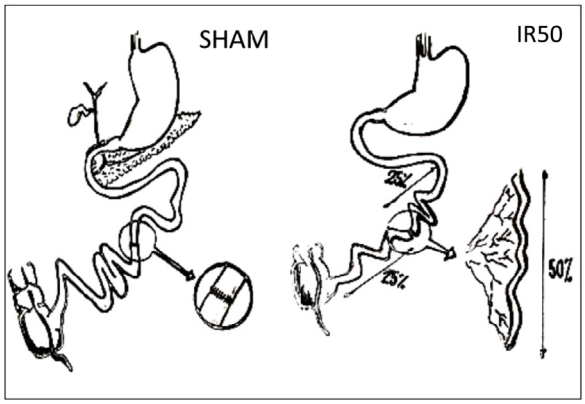

Image 1. XXX.

2.4. Surgical Interventions and Fasting Periods

Both experimental groups underwent identical preoperative and postoperative conditions, with a 12 h fast pre and post-surgical procedure. All surgical procedures were performed under anesthesia by continuous infusion of Isofluorane 3% V/V (Isoflo, Abbott 571329.8). Finally, they went through a re-adaptation period after surgery to normalize fasting.

The surgical IR50 group (n = 6) was performed in the following steps. Firstly, a 3 cm laparotomy was performed in the midline of the abdomen, and through the incision we identified Treitz's angle and the ileocecal valve as anatomical references. The bowel between these points was exposed and measured. We made a resection of the central 50%, followed by an end-to-end anastomosis with 4-0 monoplane suture (polypropylene, Ethicon Prolene), leaving the proximal half of the jejunum and most of the ileum (Image 1). Finally, the abdominal wall was closed in one layer. The control group (n = 6) followed the sham procedure. After measuring the gut, a transversal enterotomy section followed by an end-to-end anastomosis without intestinal resection was done.

2.5. Tissue Preparation

Five weeks after the surgical intervention, animals were sacrificed by an Isoflurane inhalation overdose. Pancreas and gut portions were perfused with Bouin's solution. After this, the pancreas and ileum were resected, weighed (precision scale Ohaus Pioneer Mod PA 3102), and post-fixed in Bouin's solution for 24 h at 4 °C. The fixed pieces were dehydrated, paraffin embedded, and 10 μm microtome sections were obtained.

2.6. Beta-cell Mass Quantification

To calculate β-cell mass, insulin producing cells were stained using a monoclonal mouse anti-insulin antibody (Sigma-Aldrich, St. Louis, MO, USA) and a secondary peroxidase conjugated goat anti-mouse IgG antibody (Sigma-Aldrich, St. Louis, MO, USA), and then revealed with solution of 0.3 mg/ml of 3,3'Diaminobenzidine.

The insulin-positive areas were measured by using Image J image analysis software. Those who performed the measurements in all immunohistochemical techniques were not aware of which experimental group the samples belonged to. Beta-cell mass was measured Insulin + area/total pancreatic area ratio by the total pan-

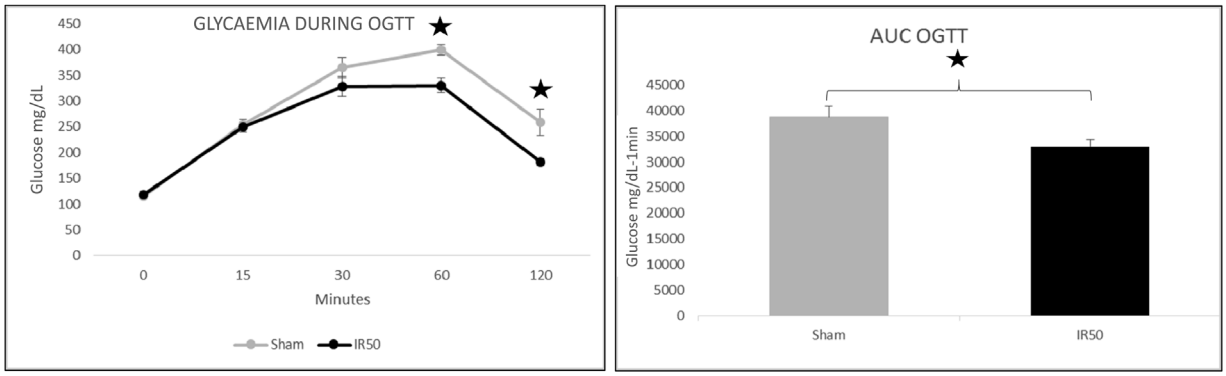

Fig. 1. A. **Oral glucose tolerance test (OGTT)**. Glycaemia was represented in the Y-axis as glucose mg/dl (mean±SEM) in both experimental groups along time, represented in minutes in the X-axis. *P ≤ 0.05. **1B. The area under curve (AUC)** of the graph showed statistical differences in glucose tolerance between groups. Blood glucose levels showed significant differences between Sham and IR50 at minute 60 and 120 of the OGTT.

creas weight, expressed in mg. Islet and insulin positive areas were quantified at 20 islets/per sample in two sample/per pancreas separated for 100 μm.

2.7. Apoptosis Assays

To determine β-cell apoptosis, 10 μm tissue sections from each pancreas were mounted on microscope slides and rehydrated with graded ethanol to PBS. The Dead End Fluorometric Terminal Deoxinucleotidil-Transferase-mediated 2ꞌdeoxyuridine 5ꞌTriphosphate nick end Labelling (TUNEL) system (Promega, Madison, WI, USA) was used to identify apoptotic cells, and the procedure was performed following the manufacturer's instructions. Insulin was simultaneously counterstained using polyclonal mouse anti-insulin antibody (Sigma-Aldrich, St. Louis, MO USA) incubated overnight at 4 °C, and then stained with a secondary anti-mouse IgG conjugated antibody (Alexa 546) (Molecular Probes Inc. Eugene, USA). To determine the apoptotic fraction, TUNEL+/Insulin + cells and islet areas were quantified in 20 islets/per sample, in two samples/per pancreas (TUNEL+/insulin + cells/mm^2 of islet). We used the image analysis Cell D software (Olympus, Hamburg, Germany). Negative samples were carried out as controls to ensure the immunohistochemical techniques. Positive controls were carried out in neural tissue samples of hypoxic rat.

2.8. Proliferation Assays

Proliferation was assessed by double immunostaining, using polyclonal rabbit anti-Ki67 (Abcam, Cambridge, CB4 OFL UK) and monoclonal mouse anti-insulin (Sigma-Aldrich, St. Louis, MO USA) antibodies. 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 animal. Results were expressed as the number of Ki67+/Insulin + cells/mm^2 per area of pancreatic islets, using Cell D for image analysis.

2.9. Neogenesis Study

PDX-1 expression was used as a neogenesis marker. We retrieved sections healed in citrate buffer pH 6.7 solution for 10 minutes. Samples were stained with monoclonal rabbit anti-PDX-1 antibody (Abcam, Cambridge, CB4 OFL UK). The we used a secondary biotin conjugated anti-rabbit IgG antibody (Sigma-Aldrich, St. Louis, MO USA), and finally revealed with a solution of 0.3 mg/ml of 3,3'Diaminobenzine. Results were observed qualitatively in 12 pancreatic areas/per animal group as number of PDX-1 positive cells/mm^2 of pancreas area.

2.10. Ileal GLP-1 Release

GLP-1 production in ileum was analyzed by immunostaining using rabbit anti GLP-1 (Abcam, Cambridge, CB4 OFL UK) antibodies. To determine GLP-1 positive cell fraction, the number of GLP-1 positive cells and Ileum total areas were quantified in 10 fields per animal. Results were noted by a single investigator and expressed as the number of GLP-1 positive cells/mm^2 of Ileum.

2.11. GLP-1 Receptor beta-cell Sensitivity

Beta-cell sensitivity to GLP-1 in pancreas was assessed by double immunostaining, using polyclonal rabbit anti-GLP-1 receptor (Abcam, Cambridge, CB4 OFL UK) and monoclonal mouse anti-insulin (Sigma-Aldrich, St. Louis, MO, USA) antibodies. 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 animal. The results were expressed as the ratio of GLP-1 r+/Insulin + cells/mm^2 of islet area between both groups. We used the image analysis Cell D software (Olympus, Hamburg, Germany).

2.12. Statistical Analysis

Data were expressed as mean ±SEM. Mann Whitney-U test was used to analyze differences between groups, and p≤0.05 was considered statistically significant. All statistical analyses were performed using SPSS statistical software.

3. RESULTS

3.1. Weight gaining, chow intake and basal glycaemia

Both groups of animal did not appear significant differences in these phenotypic parameters. Weight gain showed a decreased pattern from the first to the seventh day. It was probably due to surgical stress in both groups. After surgery until sacrifice, the difference of measured weight between both groups was fewer than 5%. About food intake, no significant differences were observed between Sham and IR50 groups along the study. Even both groups displayed a trend to increase slowly the volume of intake with a similar pattern. Both groups reached a usual daily intake at the end of the study.

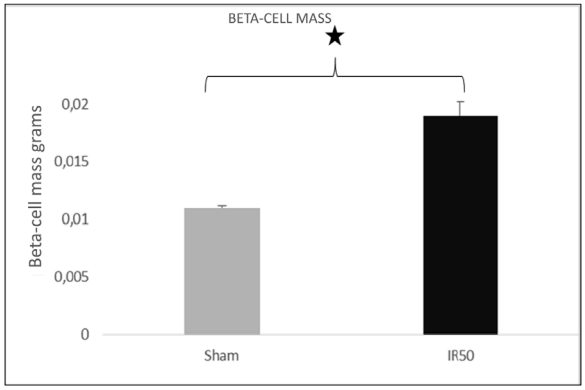

Fig. 2. β−cell mass was represented in the Y-axis in grams for both experimental groups as means values ±SEM expressed in grams. *P ≤ 0.05. Enhanced β-cell mass appeared in IR50 group (0.019±0.0001 vs. 0.011±0.0012 grams in Sham).

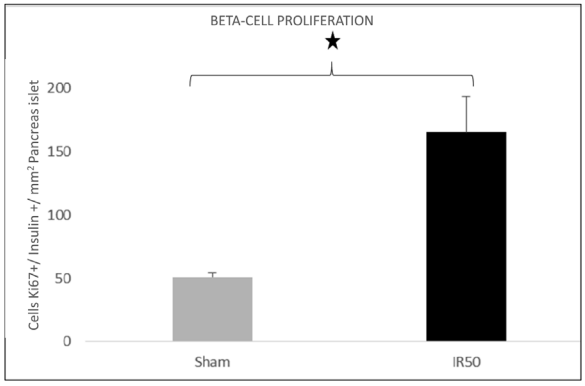

Fig. 3. Proliferating β-cells four weeks after surgery. B-cell proliferation rate was shown as means ±SEM where the Y-axis represents the number of Ki67+/insulin + cells per islet area expressed in mm^2. *P ≤ 0.05. Data showed a significant enhancement proliferation ratio in IR50 group compared to surgical controls (50.97±3.26 vs 165.60±28.07 Ki67+/insulin + cells/mm^2 of islet).

The basal glycaemia test, measured weekly along the survey period, represented no significant differences between Sham and IR50 groups along the study. Meanwhile, the diabetic status of GK rats was slightly reduced in the Sham group. The animals in Sham group showed a medium 7–10% lower glycaemia, with a high SEM.

3.2. IR50's effect on body weight and glucose homeostasis

To further characterize glucose homeostasis, we performed an OGTT during the last week before sacrifice. Statistical differences were shown in the final studied points of the OGTT. There were differences in glucose levels at 60 and 120 minutes time-points (Fig. 1A). The area under curve (AUC) for glucose showed significant differences in glucose tolerance between both groups (Fig. 1B).

3.3. Changes on β-cell population

B-cell mass was evaluated four weeks after the surgery in paraffin embedded pancreatic tissue sections using a mouse anti-insulin antibody. This study reported an increase in β-cell mass in the IR50 group compared with Sham group (Fig. 2).

3.4. Effect of IR50 on β-cell turnover

Apoptosis contribution to β-cell mass modification was analyzed. TUNEL and insulin simultaneous staining was used to evaluate the β-cell apoptosis ratio. No significant differences were found between both groups after the surgery.

To evaluate the role of cell proliferation and turnover in the observed β-cell mass increment after the surgery, we quantified the percentage of proliferating insulin positive cells in both groups. Proliferative β-cells were identified as double positive anti-insulin/Ki67 staining (Image 2). A significant increase in Ki67+/insulin + cell number was found in GK rats with intestinal resection in relation to sham animals (Fig. 3).

Study of PDX-1, as a neogenesis marker, showed no different expression pattern between GK rats with intestinal resection and Sham groups. No increased expression of PDX-1 positive cells was observed in IR50 group in relation to controls.

3.5. IR50 impact on L cells population

L cell population was measured in ileum sections from Sham and IR50 groups after the fourth week after surgery (Fig. 4). L cells were identified by staining with anti-GLP-1 antibody. GLP-1 positive cells were more than double in the IR50 group in relation to the Sham group.

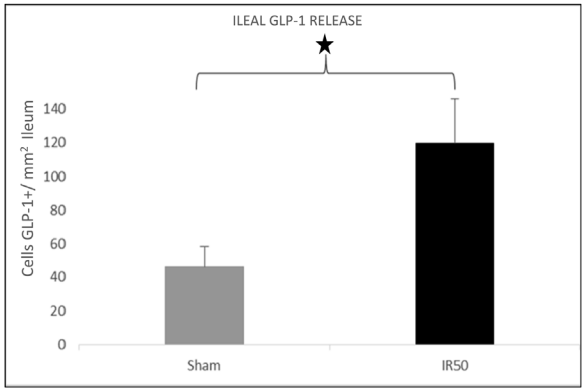

Fig. 4. Number of ileal GLP-1 positive cells is presented as means ±SEM where the Y-axis represents the number of GLP-1 positive cells per ileum area, expressed in mm^2. *P ≤ 0.05. Ileum from the Sham group presented significantly lower number of GLP-1 positive cells than RI50's (46. 33±11.67vs. 119.66±26.60 GLP-1+ cells/mm^2 of ileum, respectively).

3.6. B-cell sensitivity to GLP-1: expression of membrane receptor

The GLP-1 receptor expressed in β-cells was quantified in the pancreas sections from Sham and IR50 groups four weeks after surgery (Fig. 5). We identified a significant increased a β-cell area which co-labelled with GLP-1 r in the malabsorptive versus Sham group.

4. DISCUSSION

The effect of bariatric surgery on glucose homeostasis improvement is well known. Several hypotheses have been proposed to explain this fact, but the real underlying mechanisms to this improvement remain elusive. Moreover, T2DM remission could have various mechanisms implied. In order to gain insight in these mechanisms, our work used a pathogenic animal model as Goto-Kakizaki rat, which underwent resection of approximately 50% of the total length of the intestine (Collantes-Pérez et al.,2004).

Many authors have focused the explanation to this improvement of T2DM on the participation of different gastrointestinal profile. In this sense, our work might provoke a severe change in the pattern of these hormones related to the massive jejunal portion affected in the surgery. Two main hypotheses focused on diverse interpretations of the portions of the tube that are responsible for functional changes. The Foregut hypothesis, which suggested that

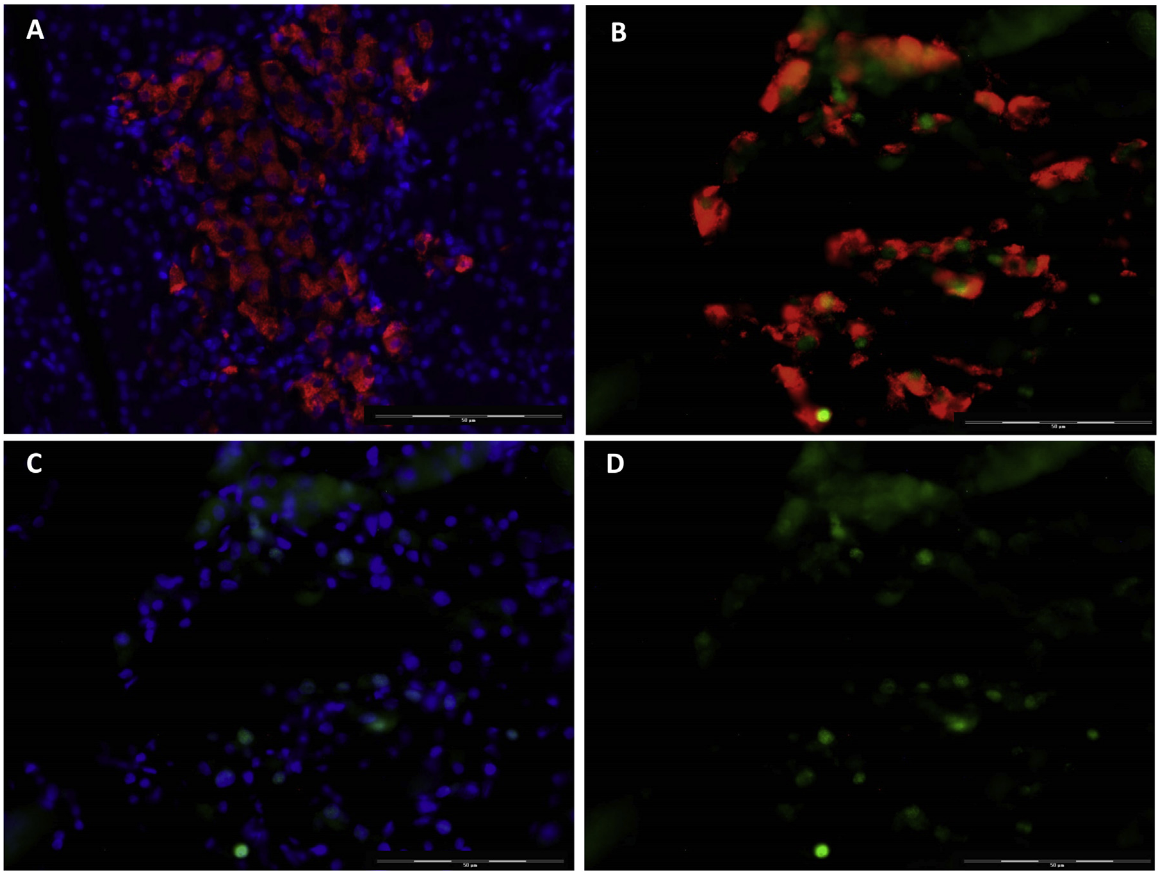

Image 2. A. Control Sham pancreas. The image showed a pancreatic islet, stained the cell nucleus in blue (4÷6Ďiamino fenil indol –DAPI-) and colored in red the insulin reserves in β-cells. **B. IR50 surgical pancreas**. An islet showed an irregular aspect and shape; these unusual and non-ovoid aspect is the regular aspect that is used to present the islets pancreas of GK specimens. The image showed Ki67+ (in green) expressed in some insulin + cells (red). **C. The image B islet.** The image showed Ki67+ (in green) expressed in some cells (nucleus in blue). **D. The image B islet** stained Ki67+ cells (in green) expressed in some cells (nucleus in blue).

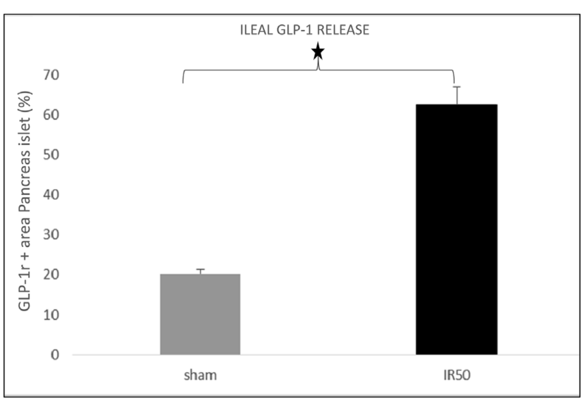

Fig. 5. GLP-1 receptor expression in β-cells was quantified in the pancreas sections from Sham and IR50 groups four weeks after surgery. GLP-1 receptor expression in β-cells was shown in bar graphs as means ±SEM where the Y-axis represents the GLP-1 r+/insulin + islet area expressed in percentage. *P ≤ 0.05. We found a significant increased islet area -β-cells co-labelled with GLP-1 r- in the IR50 group compared to Sham controls (61.33±5.23% vs 19.16 ± 19.14 GLP-1 r+/insulin + area of islet).

preventing contact of nutrients with the segment of the proximal small intestine, exerts direct antidiabetic effects by an unidentified mechanism (Rubino 2008). On the other hand, the Hindgut hypothesis, which proposed an accentuated GLP-1 release due to delivery of unabsorbed nutrients in the distal gut as antidiabetic stimulus (Yousseif et al. 2014). None of these hypotheses comprise our technique, which is related to the middle portion of small bowel.

This malabsorptive surgical technique is not used in humans as a bariatric surgery. There are other variations related to the human clinic (e.g. Scopinaro) which are purely malabsorptive. However, we focused on the participation of jejunum to elucidate the precise role of this portion in the pathophysiological mechanisms of T2DM improvement.

The phenotypic parameters after surgery (basal glycaemia, weight gaining and chow intake) showed that jejunal massive resection was tolerate for animal. These parameters did not express significate differences between both groups. Although the IR50 group showed a regular decrease in the animal weight, due to a maintained malnutrition after surgery. About basal glycaemia, both surgeries can not correct the initial condition of these diabetic animals. Thus, basal glycaemia showed a diabetic status in GK rats. Basal glycaemia were slightly reduced in Sham group, probably due to statistic reasons. The mechanical aggression and malabsorptive consequences in both surgeries did not support other explanations.

We were interested in the consequences on ileum cellularity. Many authors have focused on the enterohormones, which could be related to the physiological consequences of bariatric surgery. There are many involved hormones, but we analyzed according to the cellular secretions throughout many published reports. Some non-named anti-incretin factors secreted in duodenum and jejunum were described in foregut exclusion theory (Rubino 2008; Rao and Kini 2011). These hormones were responsible for the loss of insulin production. The resection of 50% of intestine that we performed did not affect the food transit through the duodenum or proximal jejunum. So, these factors might not be implied in our experience.

Secondly, GLP-1, an enterohormone secreted by L cells in ileum, capable of increasing insulin production and β-cell mass as proposed by Hindgut Hypothesis (Rubino and Gagner 2002). The IR50 caused an early traffic of nutrients into distal jejunum and ileum (Tian and Jin 2016). For this reason, we analyzed the GLP-1 expression in ileal samples from IR50 and Sham groups (Fig. 4) and we found an increased number of GLP-1-positive L cells in the ileum of the IR50 group. We believe that an enhanced expression of GLP-1 could be involved in the pathophysiological changes in glucose tolerance observed in our model, according to GLP-1's effects on insulin secretion (Patrick et al. 2002). However, this does not rule out that other agents may also be involved, as the Peptide YY secreted in the ileum too (Khan et al. 2016). Other route can be that GLP-1 is not direct responsible, but only an intermediary in a Neural-Gut-Brain axis (Scarlett and Schwartz 2015).

Then we examined the effect on β-cell population as one of the key elements around the possible pathophysiological changes generated by the surgery. We analyzed β-cell mass in Sham and IR50 groups. The β-cell mass appeared to increase in the IR50 group in relation to the Sham group (Fig. 2). Two possible explanations can be proposed to this phenomenon: an increase on β-cell proliferation in pancreas (Stewart et al. 2015); or the presence of neogenesis in the pancreas that leads to an expansion of β-cell population through differentiation of pancreatic stem cells (Sasaki et al., 2015). This second possibility has been proposed in response to stressful situations of β-cell population (Wu et al., 2013). Our data supports the former and finds no arguments for the latter, since we found an increased β-cell proliferation rate in the IR50 group (Fig. 3), but no significant differences in PDX-1 expression.

Previous studies report an enhanced expression of PDX-1 in pancreas from GK after a RYGB (Li et al. 2015). But, the resection of 50% of the small intestine we performed is a different surgical technique, with different anatomical and physiological implications. Our surgical model had previously been tested in normoglycemic non-obese rats. With similar results we could not find differences in PDX-1 expression either (Camacho-Ramírez et al., 2017).

Besides the finding of an increased cellular synthesis of GLP-1 in the ileum, we investigated a possible difference in β-cells' GLP-1 sensitivity. The GLP-1 contributes to the β-cell population expansion. To this end, we examined the number of β−cells showing GLP-1 receptors in both groups. We found an increased in the GLP-1 r in the islets of IR50 group compared to Sham per islet area (Fig. 5). This result was congruent with previous reports in hyperglycemic rats. The diabetic status reduced the GLP-1 receptors in β-cells; and successively the glycemic correction increased these receptors in response to GLP-1 serum increase (Xu et al. 2007).

All these findings in our model suggest a complex scenario. The resection of 50% of the small intestine was related to a significant β-cell expansion, an increased expression of GLP-1 in Ileum and increased levels of β-cells with GLP-1 receptors (which could be a sign of increased GLP-1 sensitivity). Nevertheless many studies support a GLP-1-independent improvement of glycaemia in mice with GLP-1 functional deletion (Rubino 2008) or GLP-1 receptor-null mice (Chambers et al. 2014) after bariatric surgeries as the SG or RYGB. However, these two techniques affect the stomach but do not exclude the jejunum. This difference suggested a physiopathological mechanism mediated by factors located at the jejunum in our model. Moreover, a recent study with Duodenal-jejunal bypass also found an improvement in diabetes control and increased L-cell expression in ileum of rats through increasing serum bile acids (Kashihara et al., 2015).

Regarding a possible mechanism to induce an increase of GLP-1 sensitivity in β−cell population, peptide YY (PYY) -also secreted by L cells of Ileum-, could be related. PYY has been recently reported as a regulatory peptide of metabolism from isolate mouse islets. This hormone was able to control islet adaptions to insulin deficiency/resistance through membrane receptor modifications among other mechanisms (Khan et al. 2016). A rise of PYY plasma concentrations after RYGB was documented in patients (Chronaiou et al. 2012). These facts lead us to think about PYY as the possible element to induce GLP-1 receptor overexpression in β−cell population after the massive resection of 50% of the small intestine. Even though, a direct effect of GLP-1 on the β-cells can not be discarded, as other authors reported in isolated treated islet (Xu et al. 2007).

Step by step, the physiophathological mechanisms and the controversial role of GLP-1 on diabetes improvement after bariatric surgery are being elucidated. In this way, the present study notes the important role of ileal GLP-1 production on the increased pancreatic β-cell mass -due to proliferation processes-, after resection of 50% of the small intestine. In addition, our data supports the importance of jejunum exclusion in the improvement of type 2 diabetes after bariatric surgery.

Grant support

This work was partially supported by the Research Vicerrectorado of the University of Cádiz. Ref. 20INPR0484.

Disclosure

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

Authorsćontribution statement

authors 1 and 7 designed the project and realized the histological techniques; authors 2, 3 and 4, underwent surgical techniques and followed the animal survival period; authors 1, 5 and 7 participated in manuscript elaboration; author 6 analyzed the statistical procedures.

Acknowledgements

The authors desire to thank the laboratory technical assistance of Mrs. Adelia Gramontell Lorenzo and Mrs. Cristina Martín Ateca in the histological processes of the samples. We thank Mr. Jonathan Stuart Arthur-Jimenez for critical discussions on the manuscript and for help in corrections in the text.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aanat.2019.01.007.

References

Alejandro, E.U., Gregg, B., Blandino-Rosano, M., Cras-Méneur, C., Bernal-Mizrachiet, E., 2015. Natural history of β-cell adaptation and failure in type 2 diabetes. Mol. Aspects. Med. 42, 19–41.

Baggio, L.L., Drucker, D.J., 2007. Biology of incretins: GLP-1 and GIP. Gastroenterology 132, 2131–2157.

Batterham, R.L., 2016. Cummings DE. Mechanisms of diabetes improvement following bariatric/metabolic surgery. Diabetes Care 39, 893–901, http://dx.doi.org/10.2337/dc16-0145.

Breen, D.M., Rasmussen, B.A., Kokorovic, A., Wang, R., Cheung, G.W., Lam, T.K., 2012. Jejunal nutrient sensing is required for duodenal-jejunal bypass surgery to rapidly lower glucose concentrations in uncontrolled diabetes. Nat. Med. 18, 950–955.

Camacho-Ramírez, A., Blandino-Rosano, M., Segundo-Iglesias, M.C., Lechuga-Sancho, A.M., Aguilar-Diosdado, M., Pérez-Arana, G.M., Prada-Oliveira, J.A., 2017. Bariatric surgery influences β-cell turnover in non-obese rats. Histol. Histopathol. 32, 1341–1350, http://dx.doi.org/10.14670/HH-11-909.

Chambers, A.P., Smith, E.P., Begg, D.P., Grayson, B.E., Sisley, S., Greer, T., 2014. Regulation of gastric emptying rate and its role in nutrient-induced GLP-1 secretion in rats after vertical sleeve gastrectomy. Am. J. Physiol. Endocrinol. Metab. 306, 424–432.

Chronaiou, A., Tsoli, M., Kehagias, I., Leotsinidis, M., Kalfarentzos, F., Alexandrides, T.K., 2012. Lower ghrelin levels and exaggerated postprandial peptide-YY, glucagon-like peptide-1, and insulin responses, after gastric fundus resection, in patients undergoing Roux-en-Y gastric bypass: a randomized clinical trial. Obes Surg. 11, 1761–1770.

Collantes-Pérez, J., Prada-Oliveira, J.A., Gomez, C., Vallo, J.J., Verastegui, C., 2004. A useful experimental model of short bowel syndrome. J. Invest. Surg. 17, 1–6.

Cummings, D.E., Shannon, M.H., 2003. Ghrelin and gastric bypass: is there a hormonal contribution to surgical weight loss? J. Clin. Endocrinol. Metab. 88, 2999–3002.

Drucker, D.J., 2003. Glucagon- like peptide 1 and the islet. B–cell: augmentation of cell proliferation and inhibition of apoptosis. Endocrinology 144, 5145–5148.

Garibay, D., McGavigan, A.K., Lee, S.A., Ficorilli, J.V., Cox, A.L., Michael, M.D., 2016. β-Cell glucagon-like peptide-1 receptor contributes to improved glucose tolerance after vertical sleeve gastrectomy. Endocrinology 157, 3405–3408.

Kashihara, H., Shimada, M., Kurita, N., Sato, H., Yoshikawa, K., Higashijima, J., Chikakiyo, M., Nishi, M., Takasu, C., 2015. Duodenal-jejunal bypass improves diabetes and liver steatosis via enhanced glucagon-like peptide-1 elicited by bile acids. J. Gastroenterol. Hepatol. 30, 308–315.

Khan, D., Vasu, S., Moffett, R.C., Irwin, N., Flatt, P.R., 2016. Islet distribution of Peptide YY and its regulatory role in primary mouse islets and immortalized rodent and human β-cell function and survival. Mol. Cell Endocrinol. 436, 102–113.

Li, Z., Zhang, H.Y., Lu, L.X., Li, D.F., Dai, J.X., Sha, O., Li, W.Q., Bai, Y., Yuan, L., 2015. Roux-en-Y gastric bypass promotes expression of PDX-1 and regeneration of β-cells in Goto-Kakizaki rats. World J. Gastroenterol. 16, 2244–2251.

Mokadem, M., Zechner, J.F., Margolskee, R.F., Drucker, D.J., Aguirre, V., 2013. Effects of Roux-en-Y gastric bypass on energy and glucose homeostasis are preserved in two mouse models of functional glucagon-like peptide-1 deficiency. Mol. Metab. 3, 191–201.

Penney, N.C., Kinross, J., Newton, R.C., Purkayastha, S., 2015. The role of bile acids in reducing the metabolic complications of obesity after bariatric surgery: a systematic review. Int. J. Obes. 39, 1565–1574.

Pories, W.J., Swanson, M., McDonald, K.G., 1995. Who would have thought it? An operation proves to be the most effective therapy for adult onset diabetes mellitus. Ann. Surg. 222, 339–350.

Rao, R.S., Kini, S., 2011. GIP and bariatric surgery. Obes. Surg. 21, 244–252.

Rubino, F., Gagner, M., 2002. Potential of surgery for curing type 2 diabetes mellitus. Ann. Surg. 236, 554–559.

Rubino, F., 2008. Is type 2 diabetes an operable intestinal disease? A provocative yet reasonable hypothesis. Diabetes Care. 31, 290–296.

Sasaki, S., Miyatsuka, T., Matsuoka, T.A., Takahara, M., Yamamoto, Y., Yasuda, T., Kaneto, H., Fujitani, Y., German, M.S., Akiyama, H., Watada, H., Shimomura, I., 2015. Activation of GLP-1 and gastrin signalling induces in vivo reprogramming of pancreatic exocrine cells into β− cells in mice. Diabetologia 58, 2582–2591.

Scarlett, J.M., Schwartz, M.W., 2015. Gut-brain mechanisms controlling glucose homeostasis. F1000 Prime Rep. 7, 12.

Smith, S.C., Edwards, C.B., Goodman, G.N., 1996. Changes in diabetic management after Roux-en-Y gastric bypass. Obes. Surg. 6, 345–348, http://dx.doi.org/10.1381/096089296765556674.

Stewart, A.F., Hussain, M.A., García-Ocaña, A., Vasavada, R.C., Bhushan, A., Bernal-Mizrachi, E., Kulkarni, R.N., 2015. Human β-cell proliferation and intracellular signaling: part 3. Diabetes (64), 1872–1885.

Tian, L., Jin, T., 2016. The incretin hormone GLP-1 and mechanisms underlying its secretion. J. Diabetes (8), 753–765, http://dx.doi.org/10.1111/1753-0407.12439.

Wang, P.Y., Caspi, L., Lam, C.K., Chari, M., Li, X., Light, P.E., 2008. Upper intestinal lipids trigger a gut-brain-liver axis to regulate glucose production. Nature 452, 1012–1016.

Wild, S., Roglic, G., Green, A., Sicree, R., King, H., 2004. Global prevalence of diabetes estimated for the year 2000 and projections for 2030. Diabetes Care 27, 1047–1053.

Wu, F., Guo, L., Jakubowski, A., Su, L., Li, W.C., Bonner-Weir, S., Burkly, L.C., 2013. TNF-like weak inducer of apoptosis (TWEAK) promotes β−cell neogenesis from pancreatic ductal epithelium in adult mice. PLoSOne 8, e72132.

Xu, G., Kaneto, H., Ross-Laybutt, D., Duvivier-Kali, V.F., Trivedi, N., Suzuma, K., King, G.L., Weir, G.C., Bonner-Weir, S., 2007. Downregulation of GLP-1 and GIP receptor expression by hyperglycemia. Possible contribution to impaired incretin effects in diabetes. Diabetes (56), 1551–1558.

Yousseif, A., Emmanuel, J., Karra, E., Millet, Q., Elkalaawy, M., Jenkinson, A.D., 2014. Differential effects of laparoscopic sleeve gastrectomy and laparoscopic gastric bypass on appetite, circulating acyl-ghrelin, peptideYY3-36 and active GLP-1 levels in non-diabetic humans. Obes. Surg. 24, 241–252.