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Biliopancreatic diversion induces villi elongation and cholecystokinin and ghrelin increase

Hugo Mendieta-Zerón [a,1], Álvaro Larrad-Jiménez [b,*], María Angela Burrell [c], Marina Martín Rodríguez [c], Katia Da Boit [d], Gema Frühbeck [e], Carlos Diéguez [d]

[a] Medical Research Center (CICMED), Autonomous University of the State of Mexico (UAEMEx), Toluca, Mexico
[b] Endocrinometabolic Surgery Unit, Hospital Nuestra Señora del Rosario, Prince of Vergara 53, 28006 Madrid, Spain
[c] Department of Histology and Pathology, University of Navarra, Pamplona, Spain
[d] Department of Physiology, School of Medicine, University of Santiago de Compostela (USC),Spain
[e] Metabolic Research Laboratory, Clínica Universidad de Navarra, University of Navarra, Pamplona, Spain

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ABSTRACT

Introduction: Factors leading to weight loss and weight stabilization after bariatric surgery are not fully understood. Our aim was to evaluate, in Sprague–Dawley rats, the histological and gut hormonal changes after Larrad-biliopancreatic diversion (Larrad-BPD).

Materials and methods: Rats randomly underwent the following protocols: Larrad-BPD ($n = 4$) *versus* pair fed (PF) ($n = 4$). Weight and food intake were measured every day. By immunohistochemistry ghrelin was examined in the stomach, while cholecystokinin (CCK), glucagon-like-peptide-1 (GLP-1), peptide YY (PYY) and serotonin (5-HT) expression were analyzed in alimentary limb and ileum following or not the Larrad-BPD.

Results: Larrad-BPD rats exhibited significant ($P < 0.05$) weight loss compared to PF rats. Villi enlongation was observed in Larrad-BPD rats. In residual stomach, ghrelin was diminished. In the alimentary limb, ghrelin and CCK positive cells were detected more than in the ileum of PF rats. GLP-1 expression was decreased and PYY expression was absent after Larrad-BPD compared with PF rats.

Discussion: Larrad-BPD is followed by histological changes and a pleiotropic gut endocrine response aimed to compensate the reduction of intestinal area exposed to food. Until now, the hormones responsible for the intestinal hypertrophy have not been defined.

1. Introduction

Overweight and obesity are associated with an increased risk of acquiring type 2 diabetes (T2DM) and metabolic syndrome [1,2]. On the other hand, several conventional methods of bariatric surgery can induce long-term remission of T2DM. These procedures also dramatically improve other metabolic conditions, these metabolic effects are not simply the results of drastic weight loss and decreased caloric intake but might be attributable, in part, to gut endocrine changes [3].

Factors leading to weight loss and weight stabilization after bariatric surgery are not fully understood. What we know is that gut peptides, acting both peripherally and centrally, contribute to glycemic control and regulate food intake. Larrad-biliopancreatic diversion (Larrad-BPD) is an effective bariatric procedure. Our technique has offered excellent results in terms of weight loss, comorbility reduction and quality of life after 2, 5 and 10 years with a low rate of metabolic sequelae [4], nonetheless the intimal mechanisms responsible for these responses are ignored. We speculated that if the mechanism of action of this bariatric surgery involves an endocrine effect, then hormonal changes should occur in the intestinal epithelium.

Gut peptides, which mediate the enteroinsular axis, include cholecystokinin (CCK), ghrelin, the incretin glucagon-like peptide-1 (GLP-1), peptide YY (PYY) as well as serotonin (5-hydroxytryptamine; 5-HT) among others.

Most articles about bariatric surgery assess hormone changes in whole blood or serum but not in the intestinal tissue. The aim of this study was to analyze histopathological and gut hormonal changes after a biliopancreatic diversion model in rats, in an

* Corresponding author at: Endocrinometabolic Surgery Unit, Hospital Nuestra Sra del Rosario, Rafael Bergamín 12, ático C, escalera izquierda, 28043 Madrid, Spain.
E-mail addresses: mezh_74@yahoo.com (H. Mendieta-Zerón), alarradjimenez@gmail.com (Á. Larrad-Jiménez).
[1] Medical Research Center (CICMED), Autonomous University of the State of Mexico (UAEMEx); Toluca, State of Mexico. Paseo Tollocan s/n. Col. La Moderna. Toluca, Mexico. Tel.: +52 722 2194122.

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attempt to find explanations to the pathophysiological mechanism of action resulting from the intervention.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats 454.5 ± 10.4 g (median ± standard error, SE) (Animalario General, University of Santiago de Compostela, USC, Spain), with free access to standard laboratory rat chow pellets (Scientific Animal Food and Engineering, SAFE, France, A04; 2.9 kcal/g, 25 g of salt per 100 g) and tap water, were housed at 23 °C under a 12-h light-darkness alternating cycle (light from 0800 to 2000).

Rats were stratified according to body weight in order to ensure similar mean starting body weight before the following surgical protocols: pair-fed (PF) ($n = 4$), by being given the same amount of rat chow to eat as consumed the previous day by paired Larrad-BPD rats ($n = 4$) with a follow-up period of 50 days. In all the cases, rats were housed in metabolic cages three days before surgery to avoid stress.

2.2. Larrad-biliopancreatic diversion (Larrad-BPD)

Our technique has been published previously [5]. Briefly, after 8–10 h of fasting and under intraperitoneal anesthesia level II–III with ketamine/xilacine (Rompún®, Bayer Lever Kusen, RFA, 2 mg/ml), (200 mg:5 mg, 200 μl per 100 g of body weight), for a duration of 90 min, a subcardial corpo-antral gastrectomy was made, preserving the gastric fundus that was anastomosed to a jejunal limb after dissecting the proximal jejunum 5 cm below the Treitz ligament to form the alimentary limb. The biliopancreatic limb is end-to-side anastomosed to the distal ileum 5 cm above the ileocecal valve to form the common limb. PF animals underwent only abdominal incision. Immediately after the intervention, rats were put 1 h in a cage with sawdust to avoid body temperature loss. Oral enrofloxacin 8 mg/kg was administered as prophylaxis for postoperative infection. After six days of oral diet with saline 0.9% and glucose solution 5%, the animals were returned to standard rat chow supplemented neither with proteins nor vitamins.

All procedures were carried out in accordance with the European Community Council Directive (86/609/EEC); and in agreement with the rules of the Laboratory Animal Care and the International Law on Animal Experimentation and were approved by the Ethics Committee of USC.

2.3. Body weight, caloric intake, food efficiency

Postoperatively, daily body weight and food intake in calories were measured. Food efficiency (FE), an index of nutrient assimilation into body mass was calculated as a ratio of change in body weight [final weight (g) − initial weight (g)] to cumulative caloric intake (kcal) for each period of 50 days and was expressed as a percentage.

2.4. Sample collection

After the follow-up period, at the onset of the light circle rats were euthanized by decapitation in a fed state. Whole rat brains were rapidly removed and placed on dry ice for slow freezing and stored at −80 °C until their analysis. Resected as well as the pouch stomach and bowel biopsies of the alimentary limb and control ileum were placed on 10% neutral formalin.

2.5. Dissection of ileum

The dissection of ileum was carried out under a trans-illuminating stereomicroscope. Samples of the different regions were fixed for 24 h in 10% neutral formalin, and routinely processed for paraffin embedding. Some sections were stained with hematoxylin–eosin and periodic acid Schiff (PAS).

2.6. Immunohistochemistry

In the present work we have studied the frequency and distribution of several endocrine cell types in the resected as well as in the pouch stomach and intestines. The primary antisera used were CCK (Immunostar, 20078), ghrelin (Phoenix Pharmaceuticals, H-031-30), GLP-1(ABCAM, Ab26278), PYY (ABCAM, Ab22663) and 5-HT (INCSTAR, 20080).

Immunohistochemical staining was performed on conventional paraffin-embedded sections (5 μm thick). Paraffin sections were deparaffinized with xylene and rehydrated with graded ethanol to water. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 10 min. Background blocking was performed with 1:20 normal goat serum (Dako, Glostrup, Denmark) in Tris–HCl 0.05 M buffer, 0.5 M NaCl, pH 7.6 (TBS) prior to incubation with the specific antiserum. The tissue sections were incubated overnight at 4 °C with the specific antibody diluted 1:400 in TBS. The detection system used was the Envision® method (Dako, Glostrup, Denmark) consisting of a goat-anti-rabbit IgG secondary antibody coupled to a peroxidase labeled dextran polymer. The sections were incubated with this reagent for 30 min at room temperature, and subsequently washed in TBS for 5 min and in 0.1 M sodium acetate/acetic acid buffer, pH 6.0, for 5 min. The peroxidase activity was revealed using 0.03% 3,3′-diaminobenzidine (DAB, Sigma, St. Louis, MO) in 0.1 M sodium acetate/acetic buffer acid, pH 6.0, containing 2.5% nickel ammonium sulfate, 0.2% β-D-glucose, 0.04% ammonium chloride and 0.01% glucose oxidase [6]. The reaction was stopped by a wash in TBS. Finally, the sections were dehydrated and mounted with DPX. The frequency of immunoreactive endocrine cells was classified according a four-grade scale: +++: numerous cells, ++: moderate number of cells, +: few cells, −: absent.

2.7. Statistical analysis

We used the SPSS 10.0 statistics program to compare the variables which are presented as median ± SE. We developed pairwise comparison with the Mann Whitney U test. The level of probability was set at $P < 0.05$ as statistically significant.

3. Results

3.1. Body weight, caloric intake, food efficiency

The preoperative body weight (median ± SE) of Larrad-BPD rats was 444 ± 17.8 g while PF weighed 454.5 ± 10.8 g. After the postoperative period of six days with add libitum liquid diet with glucose (5%) and saline (0.9%) solutions, Larrad-BPD rats lost 15.4% (68 g/439 g) while PF lost 15.2% (70.2 g/459.7 g) of their initial body weights. In Larrad-BPD group, after change point day 10, the weight remained relatively stable. At the end of the study, despite the switch to solid chow, Larrad-BPD rats kept losing weight 22.5% (99 g/439 g) of their initial body weight. PF rats gained weight until the end of the study, when they weighed 13.3% (61.3 g/459.7 g) more than their initial body weight (Fig. 1). With the Mann Whitney U test the Larrad-BPD group had a significant weight reduction in days 8 ($P < 0.05$), 21 ($P < 0.05$), from day 26 to day 37 ($P < 0.05$) and from day 38 to day 50 ($P < 0.05$) compared to PF groups (Fig. 1).

The preoperative caloric intake (median ± SE) was of 118 ± 10.6 kcal/day for Larrad-BPD rats and 113 ± 9.7 kcal/day for PF rats. After the operation, when rats were started on a liquid diet, they ate 20 kcal/day, while PF ate 30 kcal/day. On day seven, standard

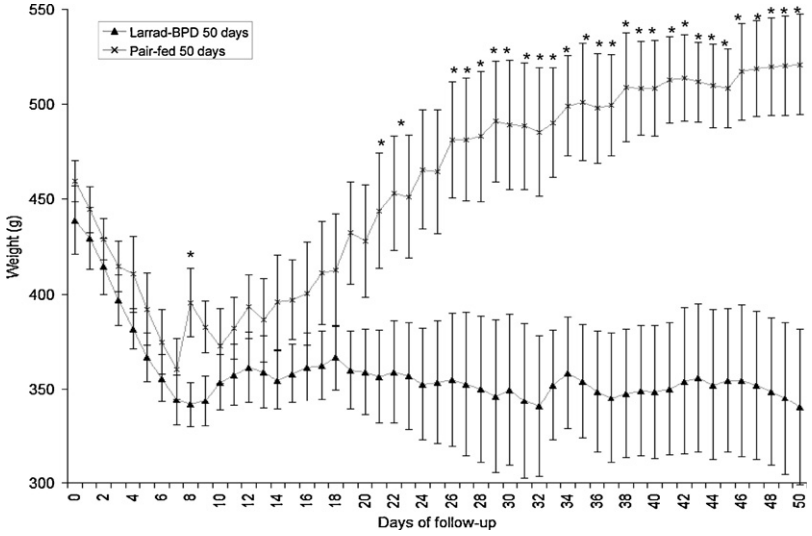

Fig. 1. Weight evolution. Larrad-BPD: Larrad-biliopancreatic diversion. $^{*}P < 0.05$.

laboratory rat chow was provided and caloric intake increased in all groups. This occurred during the same time that body weight was decreasing in Larrad-BPD rats. FE was of −3 (weight gain −98.7 g, cumulative caloric intake 3279 kcal) and 1.9 (weight gain 61.2 g, cumulative caloric intake 3095 kcal) for Larrad-BPD and PF rats respectively at the end of the study (Fig. 2).

3.2. Histology

Macroscopically (Fig. 3a), there was an increased diameter of the alimentary limbs in Larrad-BPD rats. Also, in many zones of these alimentary limbs there was a wider wall with villi elongation. There are some areas of thinner wall with broken villi observed in both PF and bariatric surgery rats (Fig. 3b and c).

3.3. Immunohistochemistry

In the alimentary limb of Larrad-BPD rats after 50 days, CCK-immunoreactive cells were expressed in a scale of moderate to numerous and, in PF rats, immunoreactivity against this antiserum was detected only in few cells in two rats. In Larrad-BPD rats after de follow-up period, ghrelin-producing cells were diminished in residual stomach (Fig. 4), but were detected in few to moderate number in the alimentary limb, while in ileum of PF rats were not detected. GLP-1 had an expression in PF rats from moderate to numerous and its expression was wider in Larrad-BPD rats, from few to numerous cells.

Contrary to the expression of ghrelin, PYY was absent in the alimentary limb of Larrad-BPD rats and was stratified in our scale as being expressed in moderate to numerous cells in PF ileum. 5-HT kept and expression in numerous cells in both groups.

4. Discussion

Surgery is the most effective treatment against obesity, which is associated with a high frequency of comorbidities. Following bypass surgery, it is likely that multiple mechanisms act in concert to achieve sustainable weight loss such as the discrete restrictive effect of the gastrectomy and especially, the greater fecal energy loss derived from the permanent lipid malabsorption and partial malabsorption of starch.

In a previous work it has been proved that bariatric rats initially consume less than the sham-operated counterparts, but when the rats are subsequently provided with the choice of chow and lard for

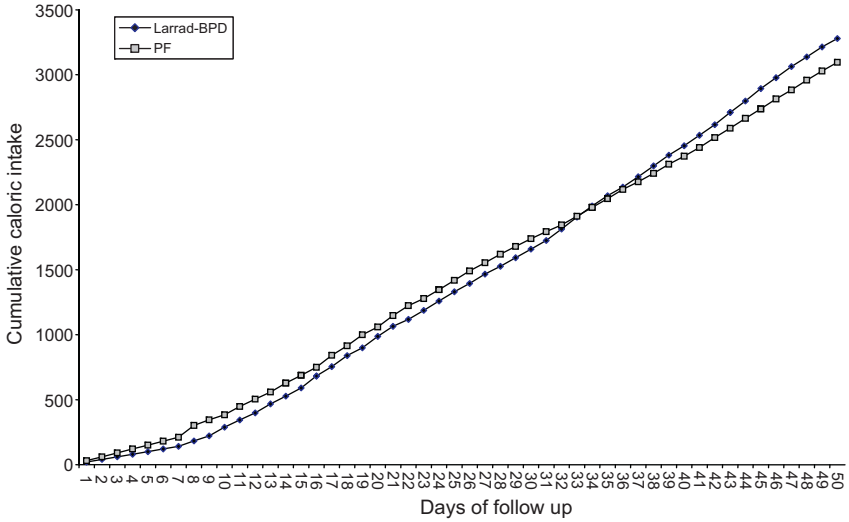

Fig. 2. Cumulative caloric intake. Larrad-BPD: Larrad-biliopancreatic diversion; PF: pair-fed.

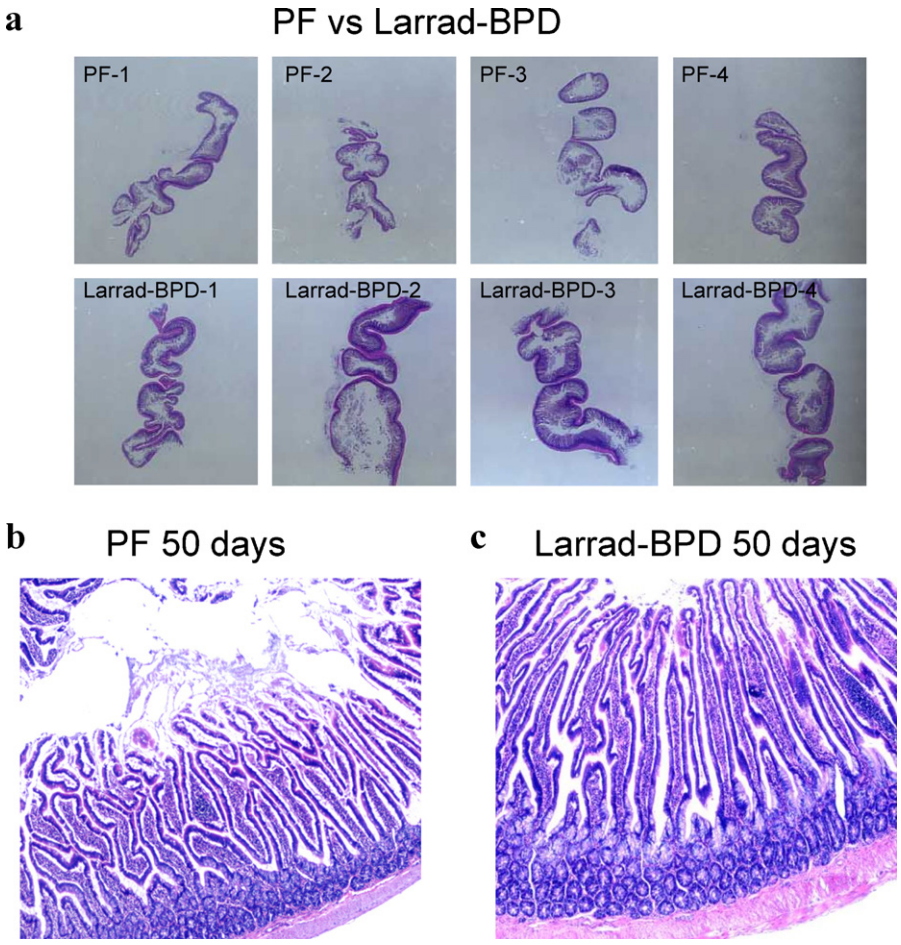

Fig. 3. Alimentary limb 50 days after surgical intervention. (a) Histological sections stained with hematoxylin–eosin at low magnification, (b) pair-fed ×10, (c) Larrad-biliopancreatic diversion ×10, Larrad-BPD: Larrad-biliopancreatic diversion; PF: pair-fed.

7 days, there are no differences in intake between the bariatric and sham-operated groups. Similarly, the majority of metabolic and neuroendocrine variables measured are unchanged [7]. In our work, interestingly, PF animals gained weight ($P < 0.01$) compared to operated rodents taking in the same amount of food, suggesting that there is an element of malabsorption in the operated animals. This is not unexpected considering the surgical model but the precise mechanisms whereby Larrad-BPD achieves sustained weight loss remain unknown. Current data suggest the involvement of mechanisms other than restrictive and malabsorptive factors [5,8], classically thought of as the mechanisms responsible for weight loss.

While some aspects of bariatric physiology such as malabsorption and early saciety are well understood, postsurgical gastrointestinal neuropeptide alterations remain unclear.

After a bariatric surgery there are several bowel adaptation mechanisms [5]. Duodenal exclusion significantly influences both intestinal structure and glucose transport function, with glucose absorptive capacity reduced after RYGB [9]. In an initial attempt, our results related to the histological changes are consistent with those showed by other authors [10,11], principally villous size increase in operated rats, expression of intestinal compensated hypertrophy originated as an adaptive intestinal mechanism [4]. As suggested by Dudrick et al. [12], these findings could be the response to a hormonal stimulus for intestinal epithelial growth.

Ghrelin expression assessed by immunohistochemistry is present in the fundus of obese patients and is significantly increased 1 year after laparoscopic adjustable gastric banding (LAGB), which would exclude a pathogenetic role of ghrelin in weight loss in some kinds of bariatric surgery techniques [13]. To gain more insight into the relationship between Larrad-BPD and ghrelin, we analyzed the expression of this hormone by immunohistochemistry finding that it was overexpressed in the alimentary limb of bariatric rats, suggesting a metabolic compensation as it was not detected in PF rats. Whether ghrelin is implicated in the intestinal hypertrophy compensation, should be a matter of deeper study. Likewise, some gastrointestinal peptides with a trophic effect over intestinal mucosa have been implicated in the food intake and weight control after bariatric surgery, but neither the intimal mechanism of action nor the interaction with other peptides such as those related to food intake (PYY) or insulin secretion (GLP-1) have been explained successfully [14,15], as well as weight regain, a theme of great therapeutic trascendence. In our study, by immunohistochemistry, PYY and CCK, showed also a marked tendency to be changed after surgical intervention. Expression of CCK was higher while PYY was decreased in Larrad-BPD rats compared to PF rats. These changes may be due to gut adaptation and hypertrophy [16,17]. 5-HT did not showed differences. It called our attention that two antagonist hormones showed the most notorious changes after the surgical intervention, ghrelin and CCK.

Other mechanisms involved in successful body weight loss after bariatric surgery in rats are determined *via* sympathetic nervous system activation, driven by increased PYY, corticotropin-releasing factor (CRF), and orexin signaling, decreasing FE and energy storage, demonstrated by reduced fat mass [18] as was observed in our study. In fact, we did not find visceral adipose tissue in Larrad-BPD after the period of 50 days.

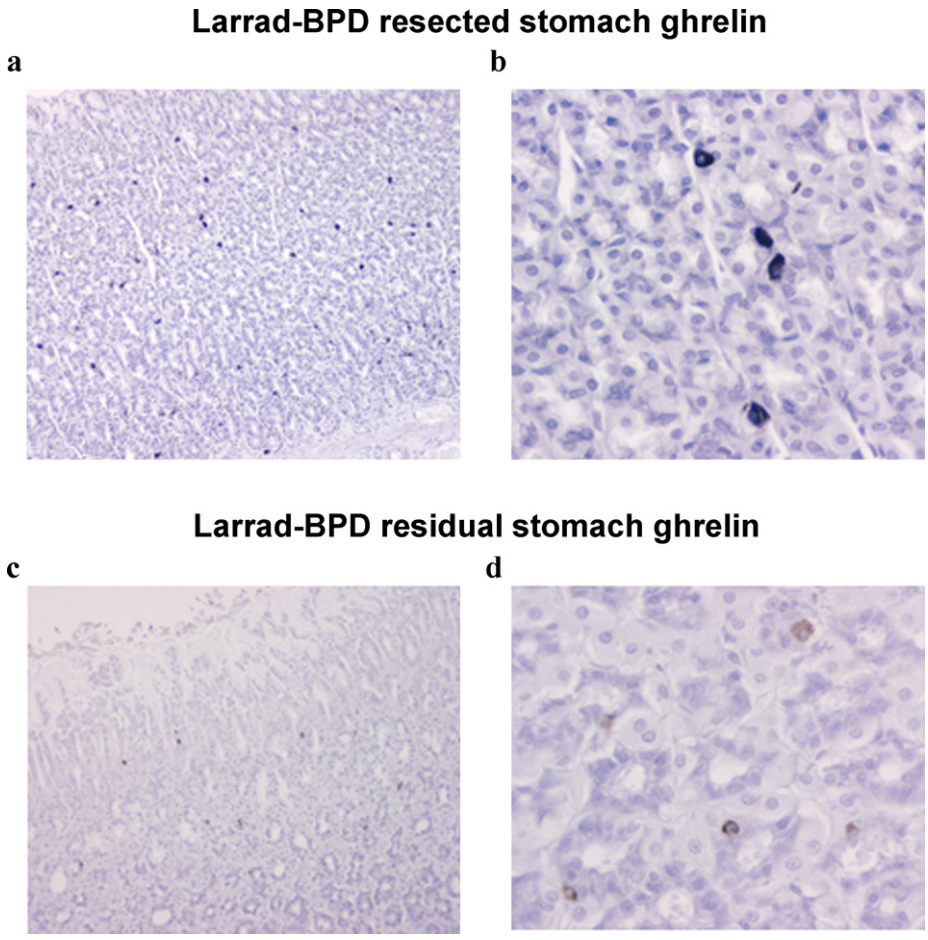

Fig. 4. Ghrelin expression in stomach. (a) Resected stomach ×10, (b) resected stomach ×40, (c) residual stomach ×10, and (d) residual stomach ×40. Larrad-BPD: Larrad-biliopancreatic diversion.

In our Larrad-BPD rat model many of the changes in gastrointestinal hormones, resemble the changes observed in the anorexia/cachexia rat model, suggesting that Larrad-BPD triggers a catabolic state responsible for appetite loss and prolonged body weight reduction [19], Larrad-BPD rats lose weight despite gut hormonal changes. Certainly, the number of rodents in each study group is small and therefore the statistical power of the study has to be considered as limited. Finally, we believe that the Larrad-BPD intervention produces intestinal histopathological changes aimed to compensate the reduction of intestinal area exposed to food but these changes are not strong enough to avoid weight loss. Until now, the hormones responsible for these histological changes have not been well defined.

Conflict of interest statement

Nil.

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