OBES SURG (2009) 19:484–489
DOI 10.1007/s11695-008-9710-9

RESEARCH ARTICLE

Larrad Biliopancreatic Diversion in Sprague-Dawley Rats. Analysis of Weight Loss Related to Food Intake

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Received: 8 August 2008 / Accepted: 11 September 2008 / Published online: 15 October 2008

Abstract
Background Existing medical therapeutic strategies to achieve and maintain clinically significant weight loss in morbid obesity remain limited and the biliopancreatic diversion (BPD) is still the most effective among the bariatric surgical procedures. Our objective was to evaluate the weight and food intake after this procedure in a rat model.
Methods Rats randomly underwent one of the following protocols (1) BPD (n=12) versus sham (n=12) with a follow-up period of 30 days and (2) BPD (n=4) versus pair-fed (PF; n=4) with a follow-up period of 50 days. Under intraperitoneal anesthesia with ketamine–xilacine, a subcardinal 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 ligament of Treitz to form the alimentary limb. The biliopancreatic limb was terminolaterally anastomosed to the distal ileum 5 cm above the ileocecal valve to form the common limb. Sham animals underwent only abdominal incision. Weight and food intake were measured every day.
Results In protocol 1, after postoperative day 30, BPD rats exhibited a mean weight reduction of 17.9% while shams increased 12.4%. There was no difference in food intake adjusted per 100 g of body weight. In protocol 2, after postoperative day 50, BPD rats had a mean weight reduction of 22.6% and, despite increasing their caloric intake from a mean of 42.6 after 6 days to 65.8 kcal/day after 50 days, they kept a similar mean weight of 344.0 and

The authors disclose that there is no commercial interest in the subject of study.

Source of financial support This work was supported by grants from the Spanish Ministry of Education, Xunta de Galicia, PGIDIT (02PXIB 2080 1PR) and the European Union (LSHM-CT-2003-503041).

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340.2 g, respectively; on the contrary, PF rats exhibited a 30.8% body weight gain.
Conclusions After the BPD, body weight is maintained independently of changes in food and energy intake.

Keywords Biliopancreatic diversion · Food intake · Weight

Introduction

The World Health Organization currently estimates that obesity and type 2 diabetes affect 300 million and 171 million persons, respectively, worldwide, with an alarming increase in the numbers of obese children presenting with diabetes [1, 2].

Existing medical therapeutic strategies to achieve and maintain clinically significant weight loss remain limited [3–5]. Surgical procedures for the treatment of obesity are, however, highly effective in achieving substantial and sustained weight loss [6, 7], but they are technically demanding and costly and carry small but significant morbidity and mortality rates [8]. In this regard, meta-analysis suggests that Roux-en-Y gastric bypass (RYGBP) results in greater weight loss than purely restrictive procedures [9, 10], perhaps due to adaptation of innate physiological mechanisms regulating energy balance [11]. This technique leads to an excess weight loss of 45% in a period of 8 years [12] and 35–40% after 15 years [13].

In 1979, Scopinaro et al. [14] described the biliopancreatic diversion (BPD) in dogs, having been confirmed 20 years later as the most effective technique for a good and sustained weight loss [15]. This technique, initially implicated in some complications, has been modified until reaching a minimum percentage of hypoproteinemias; in fact, now the “ad hoc stomach ad hoc alimentary limb” of Scopinaro and the Larrad modification is performed with similar results [16, 17]. The American variant of the BPD is the duodenal switch that, even eliminating the gastric fundus, gives very good results, similar to those original reported by Scopinaro technique [18]. However, in both models of intervention, the physiopathological mechanisms that control the weight loss and maintenance are not well understood, especially with respect to the neuroendocrine level. The main objective of this work was to evaluate the weight evolution and food intake behavior in an experimentally reproduced technique of Larrad BPD in rats [15].

Materials and Methods

Animals

Male Sprague-Dawley rats 387.0±11.7 g (Animalario General, University of Santiago de Compostela, USC, Spain) with free access to standard laboratory pellets of rat chow (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 (08:00 to 20:00) and 12-h dark cycle.

Rats were stratified according to body weight to ensure similar average starting body weight before the following surgical protocols: (1) BPD (n=12) versus sham (n=12) with a follow-up period of 30 days and (2) pair-fed (PF; n=4), by being given the same amount of rat chow to eat as consumed the previous day by paired BPD rats (n=4) with a follow-up period of 50 days. In all cases, rats were housed in metabolic cages 3 days before surgery to avoid stress.

Biliopancreatic Diversion

Our technique has been published previously [15]. Briefly, after 8–10 h of fasting and under intraperitoneal anesthesia levels II–III with ketamine–xilacine (Rompún®, Bayer Lever Kusen, RFA, 2 mg/ml; 200:5 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 (Fig. 1). Sham animals underwent only abdominal incision. Immediately after the intervention, rats were put for 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 6 days of oral diet with saline 0.9% and glucose solution 5%, animals were returned to standard chow neither supplemented with proteins nor vitamins. After the follow-up period for each set, animals were sacrificed by decapitation.

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

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) of each period (30 or 50 days) and was expressed as a percentage.

Statistical Analysis

We used the Mann–Whitney U test (nonparametric test, SPSS 10.0 statistics program, SPSS Inc, Chicago, IL, USA)

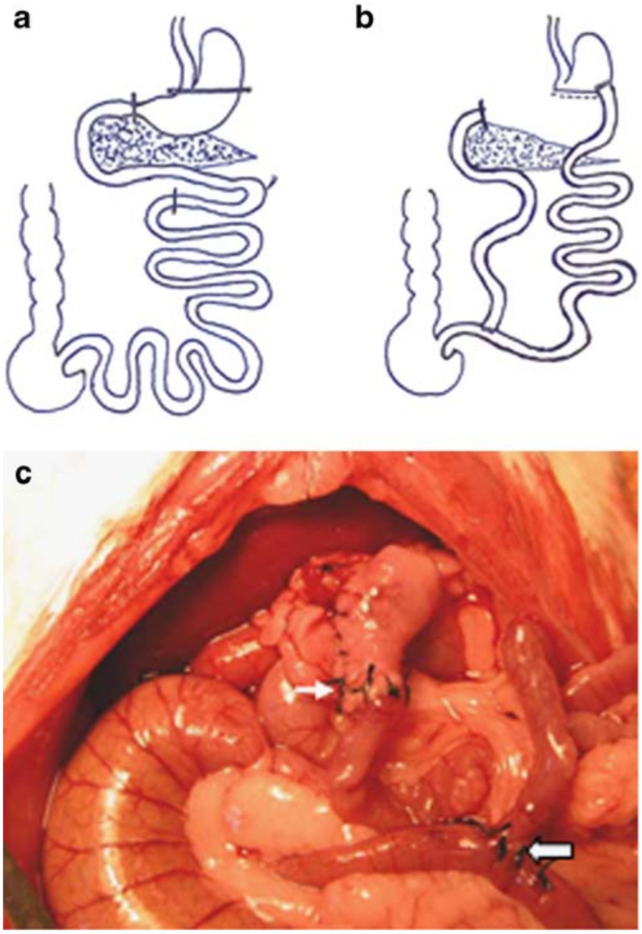

Fig. 1 Larrad biliopancreatic diversion in Sprague-Dawley rat. **a** Jejunal section 5 cm below the Treitz ligament. **b** Gastric resection and pouch formation for the gastrojejunal anastomosis. **c** Gastrojejunal (*narrow arrow*) and jejunoileal anastomosis (*wide arrow*). **a** and **b** were taken from Larrad-Jiménez et al. [15]

to compare the variables. All results were expressed as mean ± SEM. The level of probability was set at $p \leq 0.05$ as statistically significant.

Results

Surgical Protocol 1. BPD Versus Sham

The preoperative body weight was of 437.9±10.6 and 445.0±13.7 g in BPD and shams, respectively. After 6 days of liquid diet, BPD rats weighed 340.2±8.1 g and by the fourth week the weight was 359.1±14.2 g (mean reduction of 17.9%). Interestingly, three rats of this group (25%) exhibited a less than 7% weight reduction. As regards sham animals, after the first week (liquid diet), they weighed 398.2±19.7 g and by the end of the 30 days 500.2±12.7 g (increase of 12.4%). There was a statistically significant difference since the second week of follow-up (Fig. 2a).

The preoperative caloric intake in the BPD group was 73.1±3.3 kcal/day and after 1 month intake was 63.7±8.7 kcal/day; in the shams, these values were 79.1±4.1 kcal/day and 90.8±3.1 kcal/day, respectively. There were statistically significant differences on the first and second postoperative weeks (Fig. 2b) that were kept after adjusting for 100 g of body weight (Fig. 2c). Cumulative food intake was of 1,295.4 kcal in the BPD group and 1,904.8 kcal in shams. FE from weeks 1 to 4 was of 1.55 in bariatric rats and 5.59 in shams.

Surgical Protocol 2. BPD Versus PF

The preoperative body weight was of 439.9±17.9 and 459.7±10.9 g in BPD and PF, respectively. After 6 days of liquid diet BPD rats weighed 344.0±13.4 g and after 50 days they weighed 340.2±41.4 g (mean reduction of 22.6%), although one rat (25%) gained weight when restarting standard chow. Comparably, after the first week (liquid diet), PF rats weighed 360.5±15.9 g and by the end of the 50 days 521.0±26.4 g (increase of 30.8%; Fig. 2d).

The preoperative caloric intake in the BPD group was 114.4±10.4 kcal/day, decreasing to 42.6±4.5 kcal/day the first day with oral chow after 6 days of liquid diet and recovering to 65.8±9.4 kcal/day after 50 days; in the PF group, these values were 107.7±9.7 kcal/day, 91.8±1.7 kcal/day, and 66.5±9.9 kcal/day, respectively. Cumulative food intake was of 3,139.5 kcal in the BPD group and 2,887.4 kcal in the PF group. Considering the 50 days, FE in bariatric rats was of −0.12 and 5.74 in PF.

Discussion

Despite the favorable outcomes of bariatric surgery, about 10–40% of patients do not achieve successful long-term weight loss [19]. Even with the BPD, which is the most effective therapy for morbid obesity, there is an approximately 20% failure rate [15, 17]. Nonetheless, the BPD leads to excess weight reduction in relation to expected changes in fuel homeostasis without the compensatory increased appetite that usually leads to weight regain following most forms of intentional weight loss [20].

An early report of RYGBP in Zucker rats published in 1984 showed an early and short-term decrease in food intake and body weight loss after the surgical intervention [21]. The average weight loss after 4 weeks of RYGBP is 11% [22]. Another approach to reducing food intake includes the insertion of an intragastric balloon into Sprague-Dawley rats for a period of 8 weeks. This resulted in a prolonged 27% reduction of food intake and a 16% reduction in body weight [23]. The prolonged weight loss was attributed to decreased gastric volume and obstruction

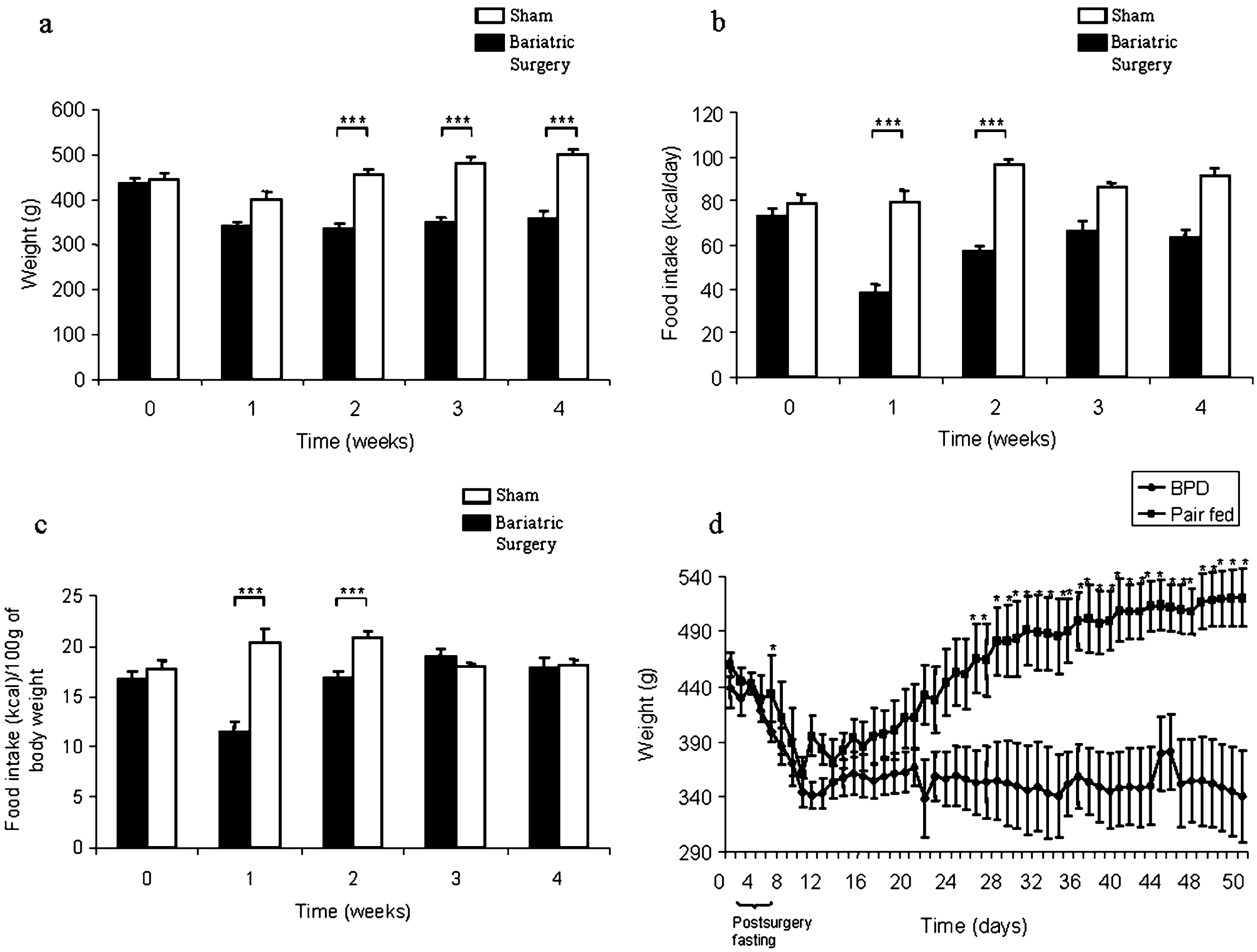

Fig. 2 Weight and food intake evolution. **a** Weight in BPD vs Sham, **b** food intake in BPD vs sham, **c** food intake in BPD vs sham adjusted for 100 g of body weight, **d** weight in BPD vs PF. *BPD*: biliopancreatic diversion + Roux-en-Y gastric bypass. *$p<0.05$; ***$p<0.001$

of the gastric outlet by the balloon. With our technique, weight loss after 1 month is 17.9% which even increases to 22.6% after 50 days. Strikingly, the weight loss at 28 and 50 days in a previous work [15] was higher (30.5±7.7% and 32.6±9.0%, respectively). This could be explained by methodological differences such as the type of cages used (longer in the first work that allowed greater animal mobility contrary to metabolic boxes in this second experiment with mobility limitation) and/or differences in the food palatability or genetic differences for nutrient metabolism depending on the animals used, Wistar strain in the first case and Sprague-Dawley in the second. Therefore, the type of animal used must be taken into account in assessing the results.

Xu et al. [24] published that the food intake diminished via a decrease in meal size due to the gastric volume reduction. Likewise, Furnes demonstrated a reduction in body weight and daily accumulated food intake measured during 3–8 weeks following gastric resection [25]. In this regard, our preliminary study confirms that BPD animals show diminished caloric ingestion, with similar findings to those of Borg et al. [26], and decreased FE, but we also noted that there were no differences in food intake when adjusted per 100 g of body weight. The second protocol shows that BPD rats, while increasing their caloric intake from a mean of 42.6 after 6 days to 65.8 kcal/day after 50 days, keep at the same postoperative time points a very similar mean weight of 344.0 and 340.2 g. This actually demonstrates that body weight after the BPD in these rats is maintained independent of changes in food and energy intake. Apparently, in rats, food intake after BPD is kept in a narrow margin of about 63–65 kcal/day (63.7±8.7 after 30 days and 65.8±9.4 after 50 days).

The successful and progressive decline in body weight and, thus, the weight loss pattern after BPD in rats is remarkably similar to the two-stage weight loss pattern defined in humans after successful BPD [15]. Following bypass surgery, it is likely that multiple mechanisms act in concert to achieve a 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 starch malabsorption. Besides, and in spite of the scarce knowledge, changes in the mechanisms of food intake and satiety derived from postsurgical gastrointestinal neuropeptide alterations should be taken into consideration. We also noted that 25% of the rats are "resistant" against the weight loss effect of the BPD, which is consistent to what has been published in animal models and humans [17, 27, 28]. It has been hypothesized that this outcome may depend on the differential adaptation characteristics of the diverse energy-related systems [27].

Several aspects deserve future research efforts such as the explanation of weight regain after bariatric surgery [15, 17, 19]. Undoubtedly, intestinal adaptation mechanisms are not identical in animals and humans undergoing bariatric surgery. Another fact of paramount importance is to understand the underlying mechanisms responsible for the recovery from type 2 diabetes of morbidly obese patients [29] following bariatric surgery, in particular, the changes in intestinal paracrine, autocrine, and peripheral hormonal actions. It is very likely that changes in gut peptides following specific bariatric procedures are related with the maintenance of weight loss and the improvement in metabolic comorbidities [30–32].

Acknowledgments Dr. Álvaro Larrad is funded by the Mutua Madrileña.

Dr. Hugo Mendieta Zerón is funded by the National Council of Science and Technology (CONACYT- Mexico) and belongs to the Youngs Talent Program of the Autonomous University of the State of Mexico (UAEMEX), Toluca, Mexico.

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