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A surgical study about jejunum: the exclusion to nutrients flow. A novel strategical technique

Alejandra Moreno-Arciniegas[2], Marcos Vilches-Aguilera[4], Alonso Camacho-Ramírez[1,3,6], David Almorza-Gomar[5], Gonzalo M. Pérez-Arana[3,4,6], J. Arturo Prada-Oliveira[3,4,6]

[1]Surgery Unit, Puerta del Mar Hospital, University of Cádiz
[2]Surgery Unit, Ceuta Hospital, INSALUD
[3]INIBICA, Research Biomedical Institute of Cadiz, University of Cadiz
[4]Department of Human Anatomy and Embryology, Faculty of Medicine, University of Cádiz
[5]Department of Operative Statistic and Research. University of Cádiz.
[6]AGAI. Cadiz Researcher Support Association. Cádiz

SUMMARY

The bariatric surgery techniques applied in patients with obesity have reported a great ability to improve Type 2 Diabetes mellitus (T2DM). Some published data report an increasing beta-cell mass in some surgical processes. This mechanism was specially seen in the bariatric surgeries which affect the length of the small bowel. The intrinsic mechanism that links both phenomena seemed to be related to the enterohormonal secretion pattern. Many enteral hormones have been invoked as the effector of these mechanisms. Previous reports focused on the medial portion of jejunum, as the precise place in which some particular enterohormones determine the homeostatic glycemic improvement. Goto-Kakizaki diabetic male rats underwent surgery to exclude the 50% medial jejunum from the normal nutrients flow. This medial portion of jejunum was not resected, but anastomosed by both extremes to the abdominal wall, and a stoma was performed. This surgery was named as Medial Jejunal Exclusion (MJE). We studied the functional parameters in a three-month survival period. In this sense basal glycaemia, weight increase and food intake were not modified between the surgical and control groups. The study presented a mortality of the 24%. This model was designed for the late study of serum and enterohormones release in this jejunal portion, excluded of nutrients flow. We report a new surgical technique, which appears to balance the homeostatic processes in order to maintain the survival of diabetic rats. Thus, this mechanism could be in the basis of T2DM improvement, and this novel surgical model will help study this precise portion of jejunum.

Key words: Jejunum – Type 2 Diabetes mellitus – Malabsorption syndrome – Animal models – Gastrointestinal-hormones

INTRODUCTION

Many surgical techniques have been widely used during the past years for the purposes of weight

Corresponding author: Dr. G. Pérez-Arana and Dr. J.A. Prada-Oliveira. Both authors are equally co-responsible of the corresponding authority. Department of Human Anatomy and Embryology, Faculty of Medicine, Plaza Fragela s/n, University of Cádiz, Cádiz 11009, Spain. .
E-mail: arturo.prada@uca.es

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loss. Many of them have proved to be safe and effective for their goals and are currently used in bariatric surgery (Schauer et al., 2014). Some positive side effects have been reported, remarkably the remission of type 2 Diabetes Mellitus (T2DM) in non-insulin-dependent patients (Patriti et al., 2004; Frühbeck, 2015; Batterham and Cummings, 2016)).

The physiopathological mechanisms involved have not yet been clarified (Stefater et al., 2012). One main theory involves incretins, hormones secreted by the gastrointestinal (GI) tract (Rubino et al., 2010; Meek et al., 2016). They are usually released in response to various stimuli, including the presence of nutrients in the GI lumen, psychical pressure, chemical substances, pH and other hormones (Moreno-Arciniegas et al., 2019).

Many of these parameters change substantially after bariatric procedures due to the characteristics of the surgical technique. There is no surgical technique that can be used as a universal model for the study of incretins, because there is no way to control every known factor at once. We should rather use different techniques depending on the purpose of our study.

Two of the main theories regarding incretins and T2DM are the foregut theory and hindgut theory (Rubino et al., 2010). The hindgut theory suggests that the presence of the alimentary bolus promotes the release of regulatory hormones on the distal small intestine (Salinari et al., 2014). Many studies have been reported in this direction, describing hormonal releases after several surgical conditions. Moreover, the metabolic and pancreatic consequences of these hormonal changes have been also published (Camacho-Ramírez et al., 2017).

Some techniques have already been used in investigation on different parts of the intestine (Moreno-Arciniegas et al., 2019). The jejunum has been reported as the part with one of the most important effect on entero-pancreatic axe and peripheral insulin metabolism. The insular pancreas cellular adaptation has been related to enterohormonal feedback (Camacho-Ramírez et al., 2017; Prada-Oliveira et al., 2019).

This background encouraged us to surgically reproduce different situations in order to study in detail the function of every portion of the intestine in glucose metabolism. Thus, authors tried to elucidate the potential importance of the portion studied or affected after surgery. Regarding the ileum, some studies analyzed the consequences of the early passing of the alimentary bolus by removing a large portion of the jejunum (Moreno-Arciniegas et al., 2019). The hormonal release was affected after this massive jejunal resection, promoting significant endocrine changes.

We thought about some alternative techniques to those previously reported, when we resected the 50% of the jejunum. Thus, we eliminated this secreting portion of the jejunum although the nutrients reached the ileum early. Therefore, we designed a new surgical technique, promoting the early access of nutrients to the ileum while keeping the excluded portion of the jejunum with vascular support in order to maintain its capability of releasing jejunal hormones. We described this novel experimental surgical technique, with the objective of specifically studying the jejunum and the hindgut theory.

MATERIALS AND METHODS

Animals

This experiment was performed with the approval of the University of Cadiz Committee for the Ethical Use and Care of Experimental Animals, and following every due regulation. This Committee assured that the procedures were performed in accordance with international guidelines and regulations for animal welfare. For this study, we used 12 male Goto-Kakizaki (GK) rats provided and kept by University of Cadiz Animal Production Service (SEPA). The rats were randomly divided into two groups 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 to avoid the cyclic variations of gonadotropins and the effect on the glycaemic metabolism.

The two groups n=6 Medial Jejunal Exclusion (MJE) and n=6 control (Sham) followed a standard care protocol. The rats were grown until they were 12-14 weeks old, so animals tolerated the surgical operation. The genetically modified GK strain usually debuts with chronic hyperglycemia around their tenth week after birth. Our rats presented an increasing glycaemia since the ninth week, which showed the diabetic status.

Surgical techniques

The techniques were performed by means of an open laparotomy under anesthesia by continuous infusion of Isofluorane 3% V/V (Isoflo, Abbott 571329.8). Both experimental and control groups underwent identical preoperative and postoperative conditions, with a 12 hour fast pre- and post-surgical procedure. After surgery, all animals went through a re-adaptation period to normalize fasting.

For the experimental group (MJE) (n=6), once we gained access to the abdomen, we identified the duodenojejunal flexure and ileocecal valve. We measured the whole small bowel, which is approximately 80cm. We extended the small bowel to identify the 50% medium portion of the small bowel, which included mostly jejunum. After that, at the proximal and distal 25% of the small bowel, an enterotomy was performed. The proximal jejunum and the distal ileum were termino-terminal anastomosed. We made an end-to-end anastomosis with interrupted sutures (polypropylene 4/0, Ethicon

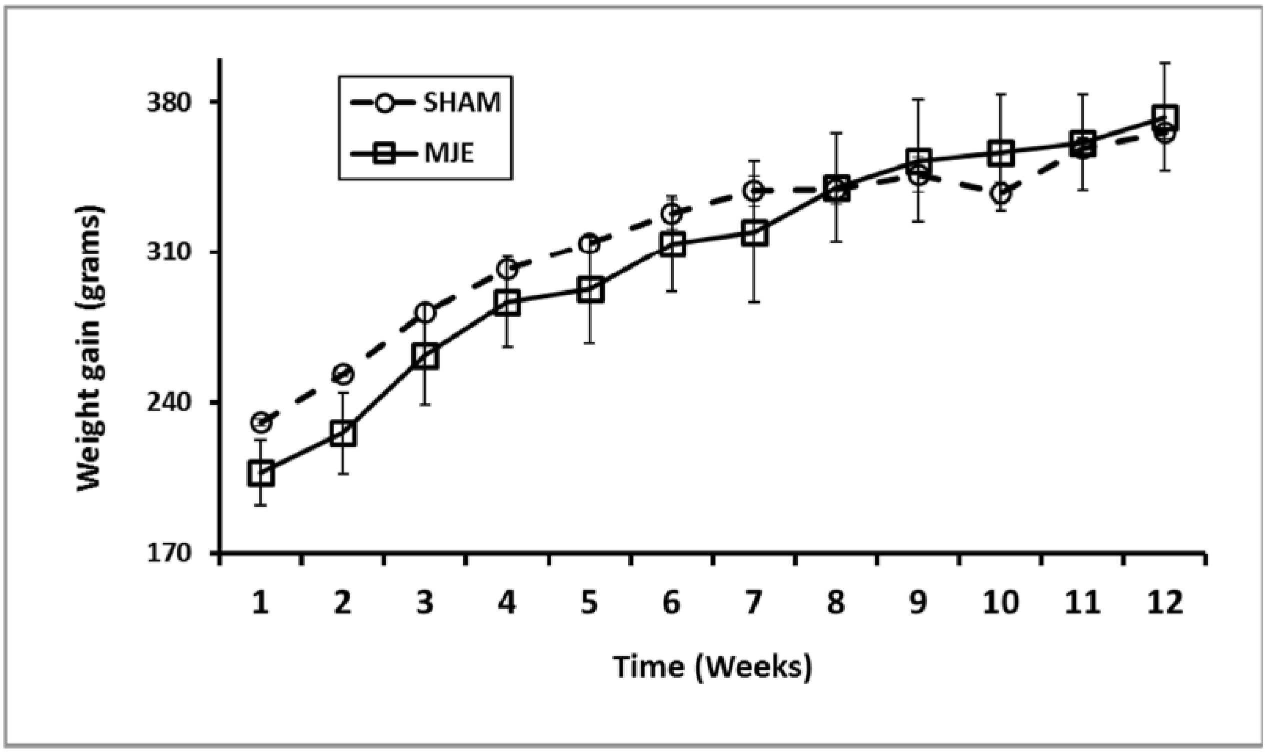

Fig 1. Graphic showing the mean weight gained per week for each group, expressed in grams.

Prolene). Thus, the medial 50% was conserved with the mesenteric vessels to preserve vascularization, but the lumen of both extremes was open forming a stoma in the abdominal wall with interrupted sutures (polypropylene PDS 3/0, Ethicon Prolene).

While the proximal and distal jejunum were anastomosed bypassing the normal flow of nutrients, the excluded medial portion of the jejunum reproduced a blind convolution with the lumen opened to the exterior. This way the secretions of the excluded medium jejunum can be evacuated through the stoma. This stoma was located on the left flank of the animal due to the best anatomical location of the excluded jejunum.

For the surgical control group (Sham) (n=6), we did a laparotomy. The bowel was exposed and measured. At the middle portion, a section and termino-terminal anastomosis - without resection- was performed.

In both groups, an anesthetic infiltration was performed with 0.2% mepivacaine before closing the abdominal wall with Vicryl 2/0 running suture. The remnant intestinal tube was introduced inside the cavity with warm physiological saline solution (36º Celsius). This surgical technique was always performed by the same main surgical team.

Weight gaining, food intake and basal glycemia

In order to ensure our technique was viable and evaluate its effect on animals, we followed several functional parameters. The weight gaining and food ingested were quantified weekly since the surgeries until sacrifice and expressed in grams (gr). Once a week, since surgery until sacrifice, basal glycaemia was measured with a glucometer (Glucocard G-Meter 1810, Menarini diagnostics, Italy) and values obtained were expressed as milligrams of glucose/deciliter of blood.

Animals were sacrificed three months after surgery using an isoflurane overdose to avoid any unnecessary pain.

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 21.0 statistical software.

RESULTS

In order to analyze the effect of this technique in our animals we measured the weight gain, basal glycaemia and chow intake as functional ascertainment. These were monitored weekly from postoperative until the sacrifice.

The mean weight gain per week for each group was expressed in grams (Fig. 1). There were no significant differences between groups at any point. The body weight decreased slightly more in MJE group versus Sham after the surgical procedure. In both groups, the body weight increased slowly until eight weeks after surgery. In the last month of monitoring, the experimental group even increased the mean body weight versus the Sham group, but these data did not reach statistical significance.

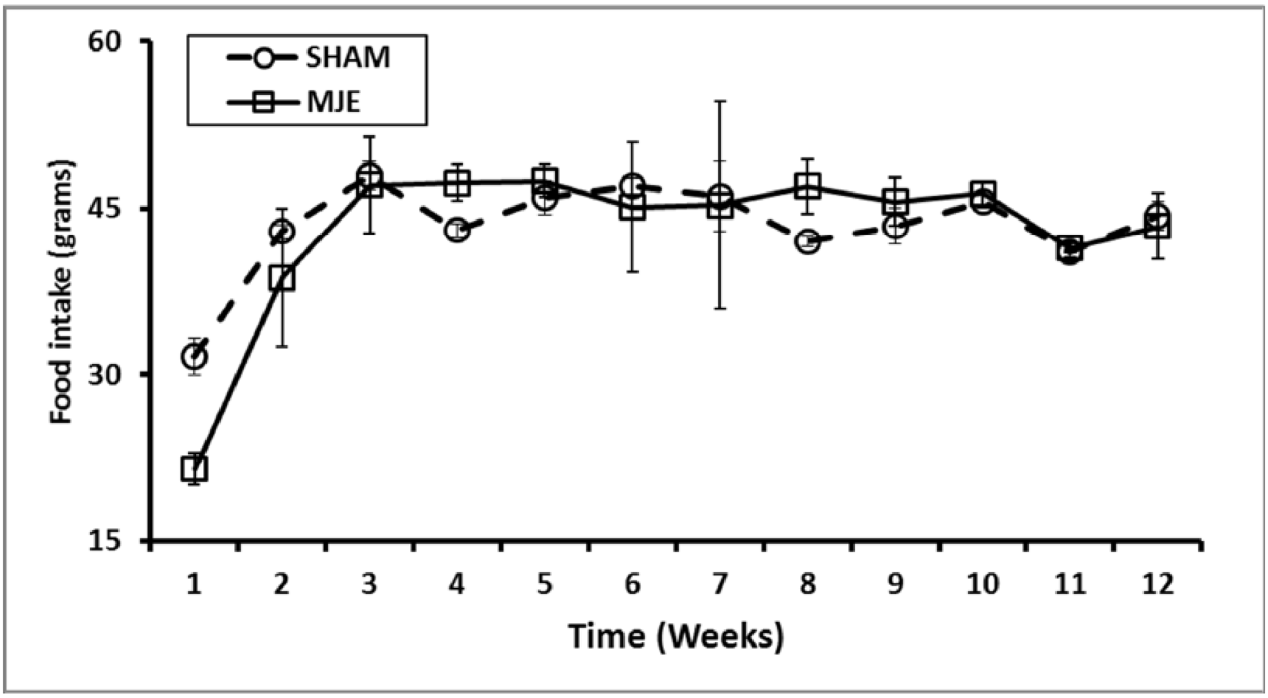

Fig 2. Food intake in both experimental groups.

On the other hand, the food intake did not show any difference between both groups (Fig. 2). The food consumption was reduced initially in the MJE group, after surgery. Several days ago, animals from both groups reached a similar food consumption, and in comparison to the normal intake measured in the animal laboratory (SEPA).

Medium basal glycaemia per week, expressed in mg/dl, were obtained (Fig. 3). The data of both groups showed no significant differences between them during the period of the study. The Sham group presented a lower insignificant glycaemia versus the experimental group, with similar values during the survival period. Instead of this, MJE showed an increasing hyperglycemia. Both groups expressed the hyperglycemic status of this GK strain.

Mortality in both groups was significantly reduced. The Sham group showed a mortality rate of 16% (2 of 12 rats). In the MJE group, 4 of the 12

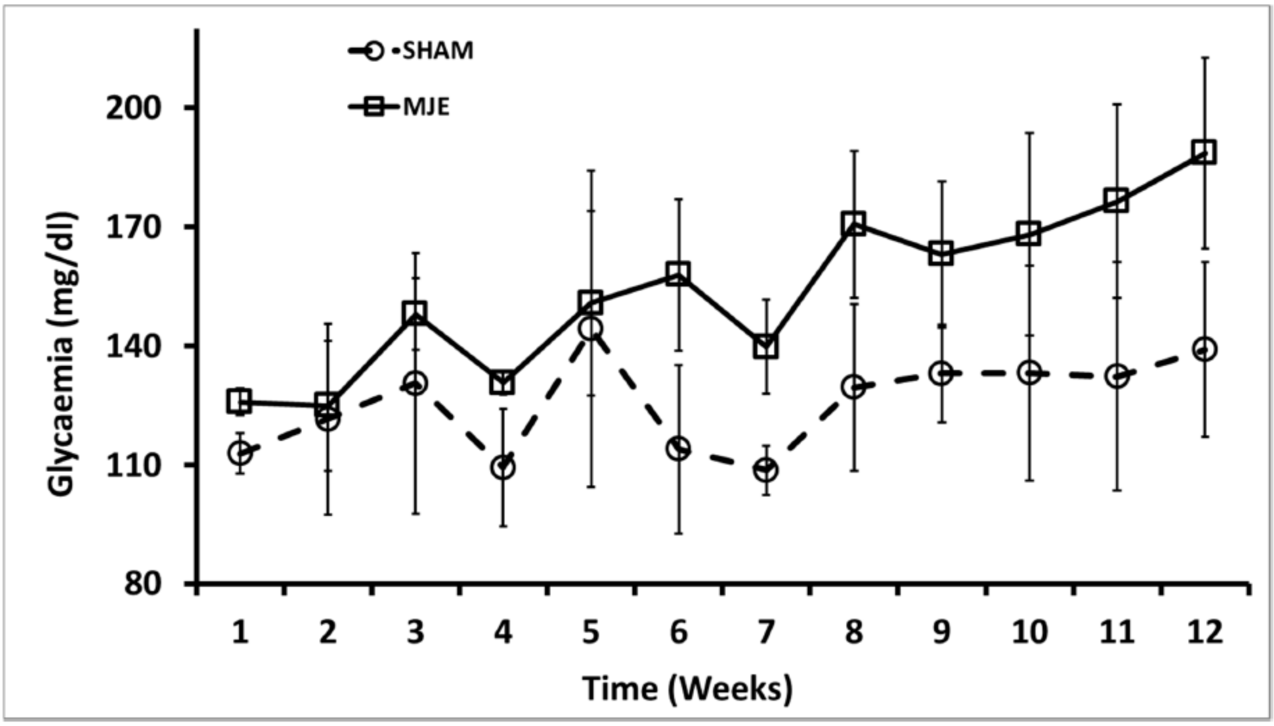

Fig 3. Graphic expressing the medium basal glycaemia per week along the experiment.

rats died, which constituted a death rate of 33%. The death was related to the postsurgical period in 5 cases, during the first 24 hours after surgeries (4 in MJE group and 1 in Sham group). One rat died during surgical process, due to isoflurane overdose.

DISCUSSION

Given the data previously shown, the surgical technique is perfectly viable for the purpose of animal experimentation. Weight gain, food intake and basal glycaemias are very similar to those of sham animals. Mortality is increased significantly in MJE group, which is perfectly understandable due to the nature of the procedure.

Weight gain is not affected, even when the surface available for absorption is severely decreased in MJE group. In this way, the fact that the rats were diabetic may be important considering that it is widely known that glucose metabolism is significantly different in diabetic rats and healthy animals.

Something similar happens with food intake. Some could think experimentation animals would eat more in order to compensate with the lack of absorptive tissue, but this has not been observed in our experiment. Microbiome changes can be expected, even though our investigation did not include this parameter. Taking into account that studies have shown that microbiome affects glucose metabolism, this should be studied further.

We must remember this is a procedure designed for animal experimentation only. On the other hand, every possible procedure results in a greater mortality than the one caused by sham surgery. It is also worth mentioning that mortality in both groups was slightly higher due to the fact that all rats were diabetic, with all due complications, especially in the post-operative period. Taking these matters into account, the mortality rate is fully acceptable.

The proven viability of this model is just a mere requirement for it to fulfil its purpose; this is, to study a segment of the digestive tube which has not yet been addressed entirely due to the lack of a suitable model.

Preserving the jejunum entirely, while excluding a large portion from the passing of nutrients is necessary in order to improve current knowledge about enterohormones and T2DM (amongst others). This new technique conserves all of the endocrine intestine while significantly reducing the area of jejunum in contact with the alimentary bolus. All previous models studying the jejunum require a portion to be resected (e.g. IR50) (Prada-Oliveira et al., 2019), therefore introducing an important bias.

We expect this technique to change the pattern of most enterohormones both in humans and animals. GIP and GLP-1 should be especially affected. Both of them are nowadays targeted as fundamental for the understanding and future treatment of T2DM (Rao and Kini, 2011; Moreno-Arciniegas et al., 2019), as it has been observed with other techniques. Conserving all of the intestine might be key to shed some light into this difficult and vital subject.

We understand that this technique is aggressive, but not significantly more than other ones used in human surgery. On the other hand, we consider this is a very useful model that allows us to study a portion of the small intestine (jejunum) with proven importance regarding glycaemic metabolism. The recently discovered and intense endocrine nature of the jejunum entails that further investigation is required. Moreover, the mortality and morbidity rates support our model while guaranteeing animal -welfare.

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Declaration of Interest Statement

The authors declare no conflict of interest. All the authors declare to have read and signed 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 the grant). We have no financial relationships (regardless of the amount of compensation) with entities that could be related to the aim of the study. We have no patents or manuscripts, 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 influence, what we have written in the submitted work. The authors state that the article is original, it has not been submitted for publication in another journal, and it has not yet been published either wholly or in part.

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