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Histologic assessment of the intestinal wall following duodenal mucosal resurfacing (DMR): a new procedure for the treatment of insulin-resistant metabolic disease

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ABSTRACT

Background and study aims Minimally invasive procedures that replicate aspects of bariatric surgery with more favorable safety and tolerability offer an attractive alternative in management of metabolic disease. Duodenal mucosal resurfacing (DMR), an endoscopic procedure using hydrothermal ablation, is designed to remove surface epithelium to allow subsequent epithelial regeneration and a reset to a more insulin-sensitive state.

Materials and methods DMR was performed on a healthy pig under general anethesia, approximating the procedure designed for use in humans. Immediately post-DMR, analysis of the histological landscape was conducted in distinct duodenal areas that received ablation treatment.

Results DMR submucosal lift and hydrothermal ablation elicited disruption of villous tips and partial disruption of the crypt base with no damage to deeper tissue. Excessive ablation (purposeful double ablation exposure) did incur damage to the underlying muscle layer.

Conclusion Our results confirmed that DMR elicits superficial ablation of duodenal villi and crypts. Defining the cellular consequences of ablation and regeneration of the epithelium will aid our understanding of how and why DMR affects metabolic homeostasis.

Introduction

Bariatric surgery elicits a potent improvement in the metabolic state of obese subjects with type 2 diabetes [1], and the effects on diabetes remission and long-term weight loss have been described in several randomized trial reports and systematic reviews [2–4]. However, bariatric surgery is associated with severe adverse events, rendering it unappealing for many patients [5–7]. Novel minimally invasive procedures with more favorable safety and tolerability profiles are being developed to elicit the metabolic benefits of bariatric surgery.

Duodenal mucosal resurfacing (DMR) is a minimally invasive endoscopic procedure using hydrothermal ablation. The duodenal mucosa layer is thought to play a key role in metabolic homeostasis. Results from animal and human studies have demonstrated that bypass of the duodenum elicits insulin-sensitizing effects that appear to be weight independent, and acute reintroduction of nutrients into the bypassed foregut can cause a lapse to the presurgery dysmetabolic state [8,9]. Preclinical and clinical effectiveness and safety of DMR were previously reported [10,11]. Herein we describe local tissue changes after acute hydrothermal mucosal ablation in the pig duodenum, which has many anatomical similarities to humans, but is not as thick and is more fragile.

Materials and methods

Duodenal hydrothermal ablation

DMR was conducted at the Heart Institute of São Paulo University experimental laboratory in a live healthy pig (Danish Landrace, male, ~176 lb) as part of animal laboratory training for the ongoing clinical protocol (NCT02879383).

Under general anaesthesia, the procedure was performed transorally using a pediatric colonoscope. The major duodenal papilla was identified, and the opposite wall was marked with a metal clip for fluoroscopic and endoscopic guidance to avoid ampullary ablation. The Ligament of Treitz was surpassed, and a stiff-shaft 0.035-inch guidewire was placed in the proximal jejunum. The scope was then removed with the wire left in place. A polyethylene terephthalate balloon catheter (Revita System, Fractyl, Lexington, Massachusetts, United States; ▶ **Fig. 1a**) was then introduced over the wire using fluoroscopic and endoscopic guidance.

DMR was performed by first injecting saline plus methylene blue solution into the submucosa layer to lift the mucosa away from the underlying tissue. Submucosal injection was administered by three auxiliary needles with vacuum devices that were oriented in a 120-degree angle toward the intestinal wall that allowed elevation of the mucosa circumferentially. Using fluoroscopic guidance, the balloon (length 2 cm) was then inflated with 82°C water for 10 seconds. Five longitudinal ablations were performed, totalling 10 cm of duodenal mucosal ablation. DMR was initiated in the descending portion 1 cm distal to the papilla and continued up to the fourth portion. Overlapping ablations were avoided. However, for training purposes, after the fifth ablation, the third portion of the duodenum was reablated, producing distinct duodenal areas for later examination.

These included:

1. control tissue that did not undergo lift or ablation;
2. lifted tissue that did not undergo ablation;
3. tissue that underwent lifting and ablation; and
4. an area where lifting and double ablation had occurred.

Histological analysis and immunohistochemistry

Shortly after the procedure, the pig was euthanized according to local animal ethics protocols [12], and the duodenum was resected. Tissue was processed and histologically evaluated. Tissues were fixed in 10 % buffered formalin solution for 24 hours, processed in crescent alcohol concentration series, diaphanized in xylol, and embedded in paraffin. Paraffin blocks were cut into 5- to 8-µm-thick sections using a microtome. Sections were stained with hematoxylin and eosin (HE) or Masson's trichrome stain to identify connective tissue and collagen fibers.

Sections were stained to evaluate the effect of DMR on the crypt stem cell and transit amplifying epithelial zones. Sox-9 protein was detected on formalin-fixed, paraffin-embedded tissue sections (5 µm) from all four regions. Sections were deparaffinized in two 5-minute xylene-incubations, then hydrated through a graded alcohol series (100 %, 95 %, 70 %, water). Heat-induced epitope retrieval with sodium citrate buffer for 20 minutes was applied before slides were incubated at room temperature with 5 µg/mL green-fluorescent Oregon Green 488 wheat germ agglutinin (WGA, Invitrogen, Carlsbad, California, United States) for 10 minutes. After blocking with 1 % BSA/5 % donkey serum (1 hour), sections were incubated with polyclonal goat anti-Sox-9 (1:100; R + D Systems, Minneapolis, MN) at 4°C overnight. Primary antibody was visualized using a donkey anti-goat AlexaFluor594 (1:200; Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania, United States) and counterstained with DAPI. Coverslips were mounted with Vectashield Vibrance Antifade Mounting Medium (Vector Laboratories, Burlingame, California, United States). Images were collected on an Eclipse Ti-E inverted microscope (Nikon Instruments, Inc., Melville, New York, United States).

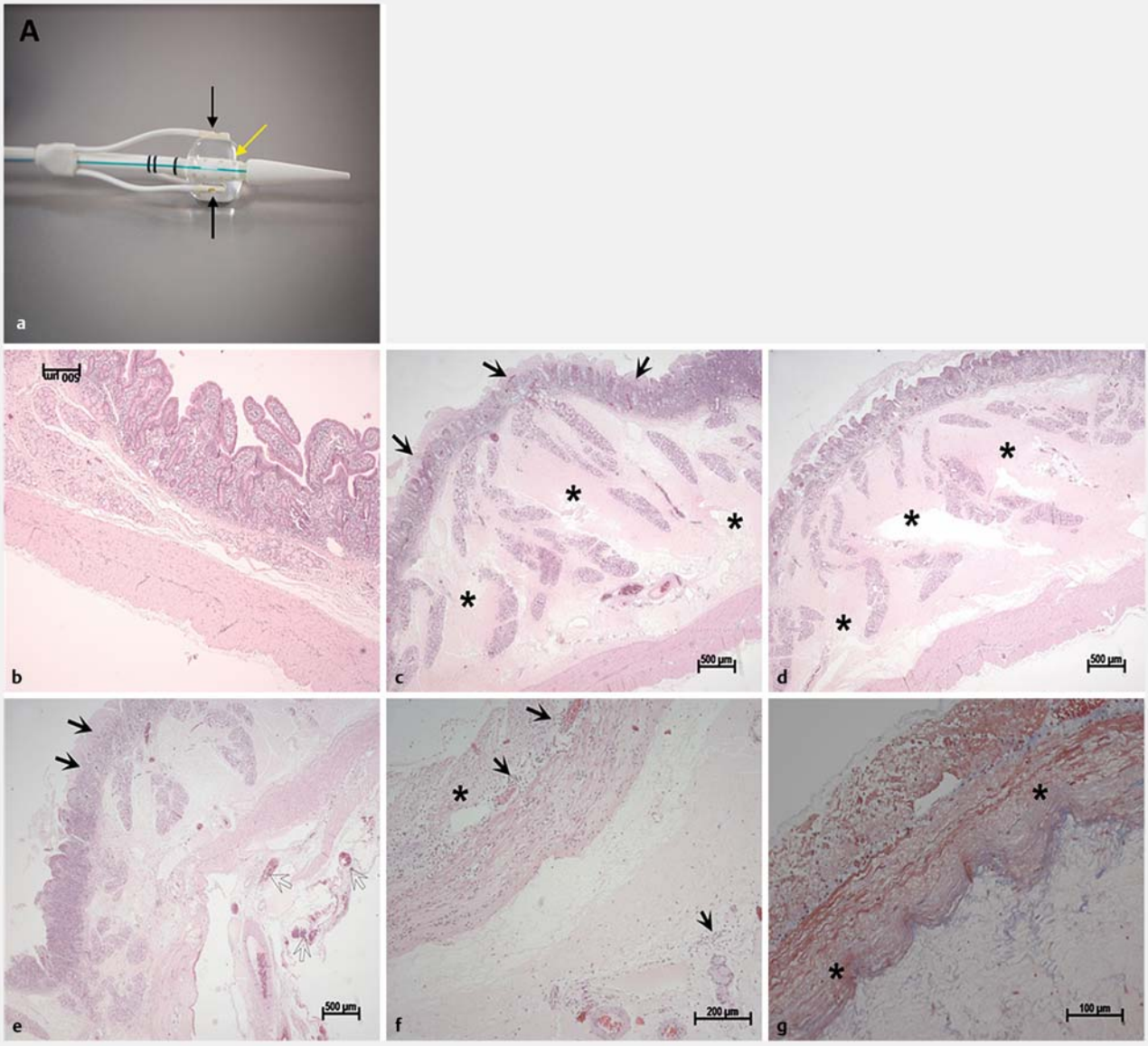
Results

In control sections, there were no gross signs of mucosal injury (▶ **Fig. 1b**). Defined villus structures with intact epithelial ribbons were clearly seen. Lectin WGA binds to sialic acid and *N*-acetylglucosaminyl residues, highlighting secretory granules and mucus, weakly staining cell membranes. Mucus-producing goblet cells were scattered throughout the intact epithelium of controls and mucus was also in the crypts (▶ **Fig. 2a**, ▶ **Fig. 2b**). SOX-9 is primarily expressed by intestinal stem cells and transit-amplifying (TA) progenitor cells, but it is also present in terminally differentiated endocrine cells. In controls, Sox-9 detected cells in the small intestine crypts base, consistent with the stem and TA zones with distinct nuclear localization in all positive cells (▶ **Fig. 2a**, ▶ **Fig. 2b**).

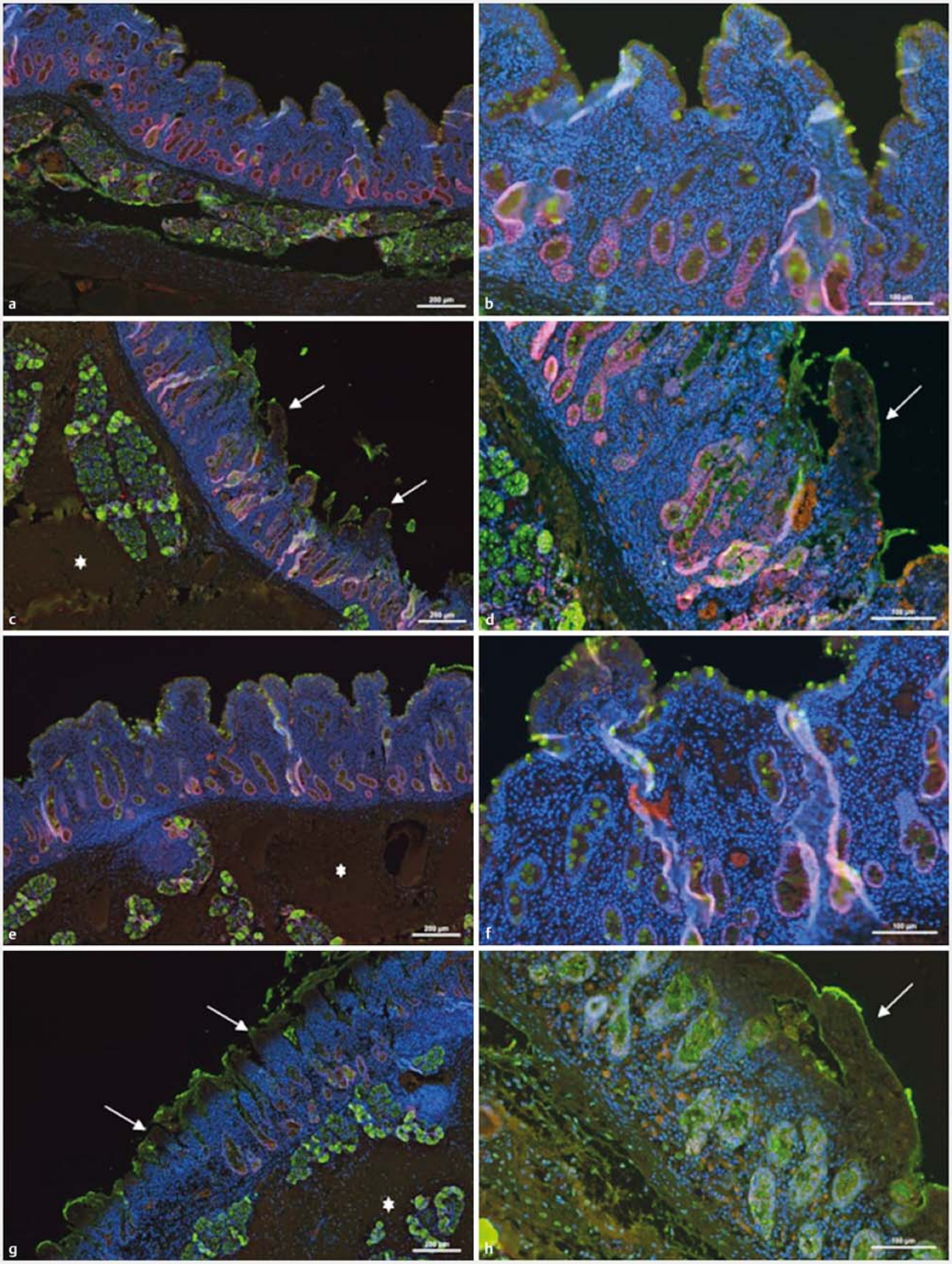
In areas where the mucosa was lifted and thermal ablation was applied, there was striking submucosal edema associated with superficial mucosal necrosis involving the tips of the villi (▶ **Fig. 1c**). WGA staining of epithelial mucus (associated with goblet cells) was absent in areas of ablation as was luminal staining of secreted mucus within the crypts (▶ **Fig. 2c**, ▶ **Fig. 2d**). Sox-9 staining generally appeared weaker than in control sections, suggesting some disruption of the crypt base, but the stem and TA zones were still identifiable. Architecturally, the base of the crypts seemed relatively intact but with areas of disruption of the morphology in lower portions of some crypts (▶ **Fig. 2c**, ▶ **Fig. 2d**).

In sections where the epithelium had been lifted but not ablated, the submucosal edema was obvious and the mucosal layer appeared indistinguishable from control section (▶ **Fig. 1 d**). In WGA-stained mucus and mucus-containing goblet cells, Sox-9 highlighted intact and strong staining of the stem and TA zones (▶ **Fig. 2e**, ▶ **Fig. 2f**).

In areas where double ablation was performed, we observed an increased level of damage compared with areas where single ablation was performed. Besides the mucosal injury, we found submucosal edema and severe congestion of the serosal vessels, which was associated with segmental necrosis of the muscularis propria (▶ **Fig. 1e**, ▶ **Fig. 1f**), with neutrophilic infiltration reaching the serosa. Necrosis of the muscular layer accompanied by edema of the submucosa and hemorrhage of the serosa was also visible (▶ **Fig. 1g**). WGA staining of goblet-like cells and mucus was absent and Sox-9 staining was weak or absent.



▶ **Fig. 1** Effect of duodenal mucosal resurfacing on the epithelium. **a** Revita balloon catheter used for duodenal mucosal resurfacing. Auxiliary needles vacuum devices (black arrows) and balloon (yellow arrow) (Fractyl Laboratories, Inc., Waltham, Massachusetts, United States). A series of photomicrographs depicting the gross histology by H&E staining of the four experimental regions is presented. **b** Control duodenal wall from a nonablated segment. The mucosa, submucosa, and muscular layers are preserved. **c** Lifted and ablated mucosa showing recent necrosis of the cells at the tips of the villi (arrows) and severe edema of the submucosal layer (asterisks). **d** Lifted and nonablated duodenal wall showing preserved epithelium and edema of the submucosal layer (asterisks). **e** Overlap ablation showing mucosal injury (necrosis, arrows), and submucosal edema and severe congestion of the serosal vessels (open arrows). **f** The external muscular layer of the duodenal wall from overlap ablation showing areas of necrosis of the muscular layer (asterisk), vascular congestion and neutrophilic infiltration (arrows). **g** The external muscular layer following overlap ablation showing focal necrosis (discontinuity of the smooth-muscle cell fibers [asterisks]) using Masson's trichrome stain. Edema and hemorrhage of the serosa are evident.



▶ **Fig. 2** Effect of duodenal mucosal resurfacing on the stem and transit-amplifying zones of the epithelium.

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▶ **Fig. 2** *(Continuation)* Sections from all four experimental regions were stained using Sox-9 (red) stain and counterstained with wheat germ agglutinin (WGA; green) and DAPI (blue) stains. In images on the left side of the panel (**a**, **c**, **e**, **g**) the scale bar shows 200 µm. The right side of the panel shows higher-magnification images (**b**, **d**, **f**, **h**) with the scale bar being 100 µm. **a, b** Control mucosal; Sox-9 nuclear staining is observed in the lower half of the intestinal crypt while WGA marks epithelial mucus and weakly all cell membranes. **c, d** Lifted and ablated mucosa; necrosis of the cells at the tips of the villi was evident (arrows), edema of the submucosal layer (asterisk) and absence of goblet-like WGA staining in the epithelium, Sox-9 staining identified relatively intact crypt base architecture with occasional disruption. **e, f** Mucosal lifting with no ablation; presence of submucosal edema (asterisk) but Sox-9 and WGA staining is similar to control mucosa. **g, h** Mucosal lifting and overlap/double ablation; presence of mucosal injury (arrows) and edema of the submucosal layer (asterisk) with absence of WGA staining and very weak or absent Sox-9 staining.

There was obvious disruption of the stem and TA zones in most of the areas receiving overlap ablation, which equated to the increased damage observed in H&E-stained sections (▶ **Fig. 2g**, ▶ **Fig. 2h**).

Discussion

Procedures that bypass the duodenum promote an immediate and long-term anti-diabetic effect before significant weight loss occurs [13 – 15], placing the gut front and center in diabetes pathology and treatment. The mechanisms by which bypassing the duodenum leads to metabolic improvements are not fully understood. Uncovering the mechanisms leading to favorable metabolic changes observed could lead to novel treatments for type 2 diabetes and related metabolic disease.

Despite the effectiveness of bariatric surgery, less invasive alternatives that recapitulate some metabolic benefits of surgery are highly sought after. DMR is a promising alternative. Recent studies report glycemic improvement with an average reduction in HbA1c of 1.0 % to 1.2 % at 6 months and 1-year post-DMR, respectively [10, 11]. How DMR elicits glycemic improvement is currently unknown. The working hypothesis proposes that DMR ablation of the superficial mucosa elicits epithelial regeneration, resetting to a more insulin-sensitive state [8].

Histologic assessment of the duodenal epithelium following DMR is an important step to linking superficial epithelial ablation, its subsequent regeneration, and the beneficial effect on glucose homeostasis. We confirmed that DMR promotes superficial mucosal ablation of the villus tips to the crypt base while also observing that the submucosal lift creates a submucosal barrier protection against deeper tissue injury. Results from the first-in-human DMR study found a positive correlation between ablation length and degree of improvement in HbA1c [10]. The standard DMR procedure likely leaves small gaps of preserved mucosa between the ablation sites produced by the catheter design, but this does not appear to negatively impact DMR's outcomes.

Conclusion

Our results suggest that in the pig duodenum, thermal injury could reach the muscularis propria if overlap ablation is applied, increasing risk of duodenal stricture. Therefore, overlaps should be avoided.

Future studies on effect of DMR on the duodenal stem cell population, which seeds the rest of the epithelium, are needed. Understanding the DMR mechanism of action will shed new light on intestinal cell biology and its pathology in diabetes and obesity. A large sham-controlled trial is ongoing to assess efficacy and safety of DMR in humans.

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Competing interests

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References

[1] Pories WJ, Swanson MS, MacDonald KG et al. Who would have thought it? An operation proves to be most effective therapy for adult onset diabetes mellitus Ann Surg 1995; 222: 339 – 350; Discussion 350 – 352

[2] Ikramuddin S, Korner J, Lee W-J et al. Roux-en-Y gastric bypass vs intensive medical management for the control of type 2 diabetes, hypertension, and hyperlipidemia: the Diabetes Surgery Study randomized clinical trial. JAMA 2013; 309: 2240 – 2249

[3] Buchwald H, Estok R, Fahrbach K et al. Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. Am J Med 2009; 122: 248 – 256.e5

[4] Schauer PR, Kashyap SR, Wolski K et al. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. N Engl J Med 2012; 366: 1567 – 1576

[5] Flum DR, Belle SH, King WC et al. Perioperative safety in the longitudinal assessment of bariatric surgery. N Engl J Med 2009; 361: 445 – 454

[6] Obeid NR, Malick W, Concors SJ et al. Long-term outcomes after Roux-en-Y gastric bypass: 10- to 13-year data. Surg Obes Relat Dis 2016; 12: 11 – 20

[7] Yan Y, Sha Y, Yao G et al. Roux-en-Y gastric bypass versus medical treatment for type 2 diabetes mellitus in obese patients: a systematic review and meta-analysis of randomized controlled trials. Medicine (Baltimore) 2016; 95: e3462

[8] Kindel TL, Yoder SM, Seeley RJ et al. Duodenal-jejunal exclusion improves glucose tolerance in the diabetic, Goto-Kakizaki rat by a GLP-1 receptor-mediated mechanism ligament of Treitz. J Gastrointest Surg 2009; 10: 1762 – 1772

[9] De Jonge C, Rensen SS, Verdam FJ et al. Endoscopic duodenal-jejunal bypass liner rapidly improves type 2 diabetes. Obes Surg 2013; 9: 1354 – 1360

[10] Rajagopalan H, Cherrington AD, Thompson CC et al. Endoscopic duodenal mucosal resurfacing for the treatment of type 2 diabetes: 6-month interim analysis from the first-in-human proof-of-concept study. Diabetes Care 2016; 39: 2254 – 2261

[11] Van Baar ACG, Nieuwdorp M, Holleman F et al. Duodenal mucosal resurfacing elicits improvement in glycemic and hepatic parameters in type 2 diabetes – one-year multicenter study results. Diabetes 2018; 67: [http://diabetes.diabetesjournals.org/content/67/Supplement_1/1137-P.abstract]. doi:10.2337/db18-1137-P

[12] Machado CJS, Tereza A, Filipecki P et al. Current Brazilian law on animal experimentation. Science 2009; 324: 10 – 12

[13] Cummings DE, Rubino F. Metabolic surgery for the treatment of type 2 diabetes in obese individuals. Diabetologia 2018; 61: 257 – 264

[14] Cummings DE, Bloom SR. Community corner at the heart of the benefits of bariatric surgery Francesco Rubino. 2012; 18: 2011 – 2012

[15] Rubino F, Forgione A, Cummings DE et al. The mechanism of diabetes control after gastrointestinal bypass surgery reveals a role of the proximal small intestine in the pathophysiology of type 2 diabetes. Ann Surg 2006; 244: 741 – 749