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ORIGINAL ARTICLE

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Physical properties of food or bile redirection do not contribute to the intestinal adaptations after Roux-en-Y Gastric Bypass in rats

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Abstract

Objective: Metabolic and morphological adaptations of the intestine have been suggested to play a role in the various therapeutic benefits of Roux-en-Y Gastric Bypass (RYGB) surgery. However, the precise underlying mechanisms remain unclear. In this study, the effects of physical properties of ingested food and redirection of biliopancreatic secretions on intestinal remodeling were investigated in RYGB operated rats.

Methods: RYGB employing two different Roux Limb (RL) lengths was performed on high fat diet induced obese rats. Post-operatively, rats were fed either Solid or isocaloric Liquid diets. Metabolic and morphological remodeling of intestine was compared across both diet forms (Solid and Liquid diets) and surgical models (Short RL and Long RL).

Results: RYGB surgery in rats induced weight loss and improved glucose tolerance which was independent of physical properties of ingested food and biliopancreatic secretions. Intestinal glucose utilization after RYGB was not determined by either food form or biliopancreatic secretions. The GLUT-1 expression in RL was not influenced by physical properties of food. Furthermore, both physical properties of food and biliopancreatic secretions showed no effects on intestinal morphological adaptations after RYGB.

Conclusion: Results of this study demonstrate that physical properties of food and bile redirection are not major determinants of intestinal remodeling after RYGB in rats.

KEYWORDS
bariatric surgery, food, GI tract, glucose metabolism, metabolic adaptation

1 | INTRODUCTION

Bariatric surgery, most commonly Roux-en-Y Gastric Bypass (RYGB) and Sleeve Gastrectomy, is a cornerstone treatment modality in the management of clinically severe obesity.[1,2] As the number of individuals with obesity continue to rise globally, the prevalence in US is over 42.4% with alarming projections predicting every 1 in 2 adults to suffer from obesity, and every 1 in 4 adults with severe obesity (BMI $\geq$35 kg/m^2) by year 2030.[3] Currently established as the most effective long-term treatment,[4] RYGB surgery leads to substantial weight loss of 50%–60% excess body weight over 10 years and a remission of Type 2 diabetes mellitus.[5–7]

While it was historically believed that therapeutic benefits of RYGB were a consequence of either the restrictive or malabsorptive effects of the surgery,[8] research over past few decades has identified changes in gastrointestinal enzymes, enteroendocrine signaling pathways and gut microbiota, as some of the critical underlying processes contributing to benefits of RYGB.[9–12] Furthermore, RYGB causes significant intestinal morphological adaptations like intestinal hypertrophy and hyperplasia manifested as increased villus height, crypt depth, and rates of cellular growth and proliferation.[10,13–16] Concomitantly, there is a noteworthy increase in intestinal glucose utilization and metabolism, which has been suggested to play a role in the resolution of diabetes mellitus.[17–20] Despite several pre-clinical and clinical studies investigating these morphological and functional adaptations in RYGB, the precise underlying mechanisms remain ambiguous.

The main premise of RYGB surgery is an alteration in the anatomy of gastrointestinal tract, wherein the jejunum is transected and connected to a newly created gastric pouch. Following this procedure, undigested food travels down the esophagus, bypassing stomach and duodenum, and directly enters into jejunum, forming the Roux Limb (RL). The Biliopancreatic Limb (BPL), comprising of bypassed duodenum and various accompanying secretions, remains unexposed to nutrients, and meets the distal end of RL to continue distally as the Common Limb (CL), which carries both partially digested nutrients and biliopancreatic secretions.[21] The intestinal reprogramming seen following RYGB is a consequence of this surgical anatomical reconfiguration. This is further supported by the fact that these changes are absent/minimal after Sleeve Gastrectomy, wherein the gastrointestinal anatomy remains largely unmodified.[22,23] Additionally, the observed intestinal metabolic remodeling occurs in a segment-dependent pattern, such that it is most pronounced in RL followed by CL. This gradient suggests that a local factor, rather than a systemic one such as a humoral effector, may mediate these intestinal changes. Interestingly, these morphological and metabolic adaptations occur exclusively in the segments that are exposed to flow of ingesta, suggesting that the interaction between food and intestine may play a contributing role in intestinal remodeling in RYGB.

Given that a major outcome of RYGB surgery is direct delivery of undigested, mechanically coarse nutrients into the RL (physiologically receiving only digested nutrients as chyme), for this study it was hypothesized that mechanical stimulation owing to undigested food in the RL triggers its hypertrophy and cellular hyperplasia. Evidence of mammalian tissue undergoing hypertrophy in response to mechanical stimulation has been documented in intestinal tissue, bladder, and muscles.[24–26] Both in vivo and in vitro studies have shown evidence of mechanical load leading to cellular proliferation and accelerated glucose metabolism through induction of GLUT-1 as a possible mechanism of the increased uptake.[27,28] Moreover, physical properties of ingested food such as consistency, form and size have been previously studied and are known to influence satiety, digestion, absorption, and gut hormonal responses.[29–31]

Another notable consequence of RYGB is the modified interaction of food with biliopancreatic secretions. Owing to the duodenal bypass, there is a delay in exposure of the food bolus to biliary secretions and pancreatic enzymes, leading to persistence of undigested or partially digested food in the distal intestine, a segment physiologically never exposed to coarse nutrients. Several studies have shown that presence of undigested food in the intestine, triggers an altered secretion of various gut hormones like GLP-1, cholecystokinin and peptide YY.[32–34]

While effects of variable lengths of Roux-en-Y anatomy on the physiological outcomes of RYGB surgery have been studied, its effects on morphological and metabolic changes seen in intestine have not been investigated. To test these hypotheses, the role of physical properties of ingested food in intestinal remodeling was examined by subjecting RYGB-operated rats to either regular Solid chow diets or isocaloric Liquid diets. Furthermore, the contribution of biliary secretions on the morphology and metabolism of RL was investigated by employing two modified RYGB surgical models in rats, with a variation in anastomosis of BPL and RL, thereby creating Short RL (Short Limb) and Long RL (Long Limb) RYGB surgical models.

2 | METHODS

2.1 | Animals

Animals were housed at Comparative Center for Medicine at Massachusetts General Hospital (MGH), Boston, MA in compliance with Institutional Animal Care and Use Committee guidelines. Male Sprague Dawley rats (3–6 rats/group) were used for the experiments and fed high fat diet for 4 months prior to undergoing RYGB surgery. Rats were individually housed and maintained on a 12/12 h light-dark cycle in specific pathogen free rooms at an ambient temperature of 19–22 °C with 40%–60% humidity.

2.2 | Surgical procedure

A modified RYGB procedure, Roux-en-Y esophago-jejunal bypass was used as the surgical model in the experiment.[35,36] With the rat placed on a heating pad throughout the operation, anesthesia was induced with Isoflurane and Meloxicam given subcutaneously (5 mg/kg) immediately prior to the procedure. A 4 cm midline upper

abdominal incision was made and Enrofloxacin (10 mg/kg) was injected intraperitoneally. Ligament of Treitz was identified and total small intestinal length measured to create different limbs of the anastomosis. The BPL measured 4 cm based on the arch of the mesenteric vessels. The distal jejunum was then ligated with a 4-0 silk suture with electro-cauterization of proximal peri-jejunal vessels at the level of transection. Next, proximal jejunum was transected, and dissection continued along the avascular plain to divide mesentery. The RL was created with a longitudinal incision along the anti-mesenteric side of jejunum, measuring 15 cm in Long Limb model and 2.5 cm in Short Limb model, through a jejuno-jejunal anastomosis with running 6-0 silk suture. Following this, the esophago-gastric junction was carefully dissected to ensure that Vagus nerve and surrounding vessels remained intact. The distal end of esophagus was ligated with a 4-0 silk and then transected. An end-to-side esophago-jejunal anastomosis was performed with 12 interrupted 6-0 silk sutures. Finally, after injection of 3–5 ml normal saline into the abdominal cavity, abdominal fascia and muscle layers were closed with continuous 5-0 silk suturing followed by interrupted 5-0 silk for skin closure. We used two different animal cohorts for the various experiments in this study (n = 5/group/cohort). One cohort was used for the body weight, glucose tolerance test (GTT) and positron emission tomography–computed tomography (PET/CT) experiments and another cohort was used for histomorphology and protein analyses.

2.3 | Operative and post-operative care

Pre-operatively, animals were fasted overnight. For the surgery, inhalational Isoflurane 1%–4% was used for anesthesia. Post-operatively, rats were placed on heating pads for recovery and kept nothing by mouth for 24 h. Subcutaneous Meloxicam (5 mg/kg) was administered from days 1–3 postoperatively for analgesia. Meloxicam was the preferred analgesic of choice owing to low risk of complications like gastrointestinal obstruction. After evaluating individual animal health and behavior, LD with Vital 1.0 Cal Vanilla (Abbott #64832) was introduced at approximately 60 ml/day for 48–72 h. Subsequently, on postoperative day 4, the rats were started on their special liquid or solid diets according to their cohorts. Mortality rates were at 10%–20% across the various cohorts with time under anesthesia and vascular bleeding being the biggest determinants of post-operative complications and survival.

2.4 | Diet

Two types of special diets—Solid and Liquid were used with similar total energy content and nutrients composition. The Solid diet cohort was provided special solid food ad libitum (Research diets D12492) and LD cohort was given same diet in liquid form, volume matched with amount for solid diet group, at approximately 60–90 ml every day.

2.5 | FBG and IPGTT

Rats were fasted overnight. The following morning, rats were weighed followed by an intraperitoneal injection of 50% glucose (1gm/kg dose). Blood glucose levels were measured at 0 (baseline) and 10, 20, 30, 45, 60, and 120 min after glucose administration, using blood glucose meter (LifeScan Inc). The tail of each rat was cut and gently massaged to get 35–50 μL of blood onto a glucose test strip for measurement.

2.6 | Positron emission tomography–computed tomography scan

Imaging was performed with microPET Focus 220 (Siemens USA) coupled with portable CereTom CT scanner (NeuroLogica Inc). Rats were fasted overnight and imaged the following morning 50 min after receiving a tail vein injection of 1 mCi of 2-deoxy- 2-(^{18}F) fluoro-D-glucose (^{18}F FDG). Individual rats were positioned in a rat head holder in PET camera gantry, under isoflurane anesthesia; and imaged in different positions for 5 min per bed position. Additionally, whole-body CT for each rat was performed. Corresponding CT data were used for attenuation correction along with dead time and random corrections for each position. Finally, data from all different bed positions were assembled to a whole-body format and analyzed using Siemens Inveon Research Workplace (IRW 4.1).

2.7 | Tissue collection and histology

Rats were euthanized with CO2 chamber and tissues immediately collected and snap frozen in liquid nitrogen, followed by storage in −80°C. Intestinal segments for different surgical limbs RL, CL and BPL were collected to ensure comparable regions in both Limb models. Thereby, RL measuring 2.5 cm (in Short Limb) and 15 cm (in Long Limb), and CL measuring 15 cm (in Short Limb) and 2.5 cm (in Long Limb) were collected for experiments. For histological evaluation, segments were flushed with formalin and placed in 10% Formalin at 25°C for 2 days, followed by transfer to 70% ethanol at 4°C. Tissues were sent to MGH Histology Core for embedding in Tissue-Tek optimum cutting temperature and sectioning. Hematoxylin and Eosin staining was done for the different segments from rats of all experimental cohorts. Morphological analysis was performed using ImageJ software from National Institute of Health (NIH).

2.8 | Western blotting

Frozen full-thickness intestinal tissues from rats were crushed using liquid nitrogen cooled mortar and pestles. Frozen powder so formed was then transferred to conical tubes with addition of 1.5 volumes

of ice cold radio-immunoprecipitation assay buffer (Sigma Aldrich) containing 1X protease inhibitors (Roche Diagnostics) for each sample. Next, samples were homogenized for 40 s on ice, using homogenizer LabGEN 700 (Cole Parmer). Samples were then rotated at 4°C for 15 min followed by centrifugation at 11,000 rpm for 20 min. Extracted proteins were then quantified using Bradford protein assay (BioRad). To check for different genes, extracted protein lysates were heated at 60°C (for GLUT-1) or 95°C (for other proteins of interest) for 5 min with Laemmli buffer and then separated by polyacrylamide gel electrophoresis through 12.5% sodium dodecyl sulfate or 4%–20% gradient gels (PreciseTM). Subsequently, proteins were transferred to polyvinylidene difluoride membranes (Immobilon-P, EMD Millipore) at 400 mA for 75 min at 4°C, using wet transfer system (Hoefer Inc). Membrane blocking was done in 5% milk dissolved in phosphate buffered solution-Tween (PBS-T) (1X PBS +0.1% Tween20) for 1 h at 25°C. Primary antibodies were diluted in 5% milk or 5% bovine serum albumin in PBS-T per manufacturer's recommendations. The following primary antibodies were used from Cell Signaling Technology - GLUT-1 (1:1000, #12939), phosphoinositide 3-kinase (PI3K) p110α (1:1000, #4249), phospho-protein kinase B (phospho-Akt) (1:2000, #4060), Akt (1:1000, #4691) and extracellular signal-related kinase (ERK) (1:1000, #9102). Blots were incubated with primary antibodies overnight at 4°C. After washing with PBS-T, blots were incubated with goat anti-rabbit IgG horseradish peroxidase (HRP) (Abcam) or goat anti-mouse IgG HRP (Santa Cruz) in PBS-T for 1 h at 25°C. Membranes were developed using Pierce ECL Western Blotting Substrate (Thermo Scientific). ERK was chosen as the most appropriate loading control as both β-actin and glyceraldehyde-3-phosphate dehydrogenase are regulated and modified in intestine after RYGB.

2.9 | Statistical analyses

Results were analyzed, and statistics performed using Prism 9 (GraphPad software). Comparison of means across experimental cohorts was performed using unpaired two-tailed Student's *t* test and two-way anlaysis of variance when appropriate. Data for all figures is presented as mean ± SD unless otherwise indicated. *p*-values <0.05 were considered as significant.

3 | RESULTS

3.1 | Weight loss and glucose tolerance after Roux-en-Y Gastric Bypass

Roux-en-Y Gastric Bypass surgery was performed on diet-induced obese Sprague Dawley rats. On post-operative day 3, rats were divided into two cohorts based on their diets. They were fed either Solid chow diet (SD) or an isocaloric LD. Additionally, two different surgical approaches were utilized to create Short Limb and Long Limb RYGB models, based on the site of BPL to RL anastomosis. In the Short Limb model (Figure 1A), BPL measuring 4 cm was anastomosed more proximally along the intestine such that a shorter RL was created measuring 2.5 cm, followed by a longer CL. For the Long Limb model (Figure 1B), BPL measuring 4 cm was anastomosed more distally along the intestine, creating a longer RL measuring 15 cm, followed by a shorter CL.

Intestinal segments of equal lengths were collected from comparable regions of both Short and Long limb models. For the Long Limb model, RL was divided into proximal RL (PRL) measuring 2.5 cm, and distal RL (DRL) of 12.5 cm; followed by CL measuring 2.5 cm. Similarly, in the Short Limb model, RL measured 2.5 cm, but CL was divided into proximal CL (PCL) of 12.5 cm and distal CL (DCL) of 2.5 cm, to ensure comparable regions for assessment across both models. Thereby, in this study there were four experimental cohorts, based on type of surgical procedure performed and post-operative physical form of supplemented diet—Short Limb on Solid diet (SL/SD), Short Limb on Liquid diet (SL/LD), Long Limb on Solid diet (LL/SD) and Long Limb on Liquid diet (LL/LD).

Following RYGB, all rats showed significant weight loss recorded until 5 weeks post-operatively with no differences in percentage of body weight lost across all rat cohorts (Figure 1C). Further, intraperitoneal Glucose tolerance tests (ipGTT) were administered and the improvements in glucose tolerance after ipGTT were similar across all four groups (Figure 1D). Fasting blood glucose levels were also recorded and showed no variation between cohorts (Figure 1E). Similar to other assessments, caloric intake for individual rats from each of the cohorts recorded no significant differences (Figure 1F).

3.2 | Roux-en-Y Gastric Bypass-induced intestinal glucose uptake

To characterize the intestinal metabolic changes seen after RYGB, PET/CT scanning with 2-deoxy-2-18F fluoro-D-glucose (^{18}F FDG) was performed at 5 weeks post-operatively. Rats from both Short Limb and Long Limb models on either Solid or Liquid diets were injected with ^{18}F FDG to evaluate intestinal glucose uptake using Positron emission tomography (PET). ^{18}F FDG uptake is indicative of glucose utilization within a specific tissue. Accordingly, a comparison of uptake in the RL in all four RYGB cohorts was performed showing no differences in ^{18}F FDG uptake on PET scanning across the groups (Figure 2A).

Next, the biodistribution of glucose across different limbs was evaluated in rats from both models by specifically determining ^{18}F FDG dose per gram of tissue from each intestinal segment. Similar to PET analysis, no statistically significant variations were noted between Solid and LD cohorts in both Short Limb and Long Limb models. Additionally, there were no significant differences between RL and PCL segments of Short Limb model on both forms of diet. These were comparable with PRL and DRL segment findings from Long Limb model rats (Figure 2B).

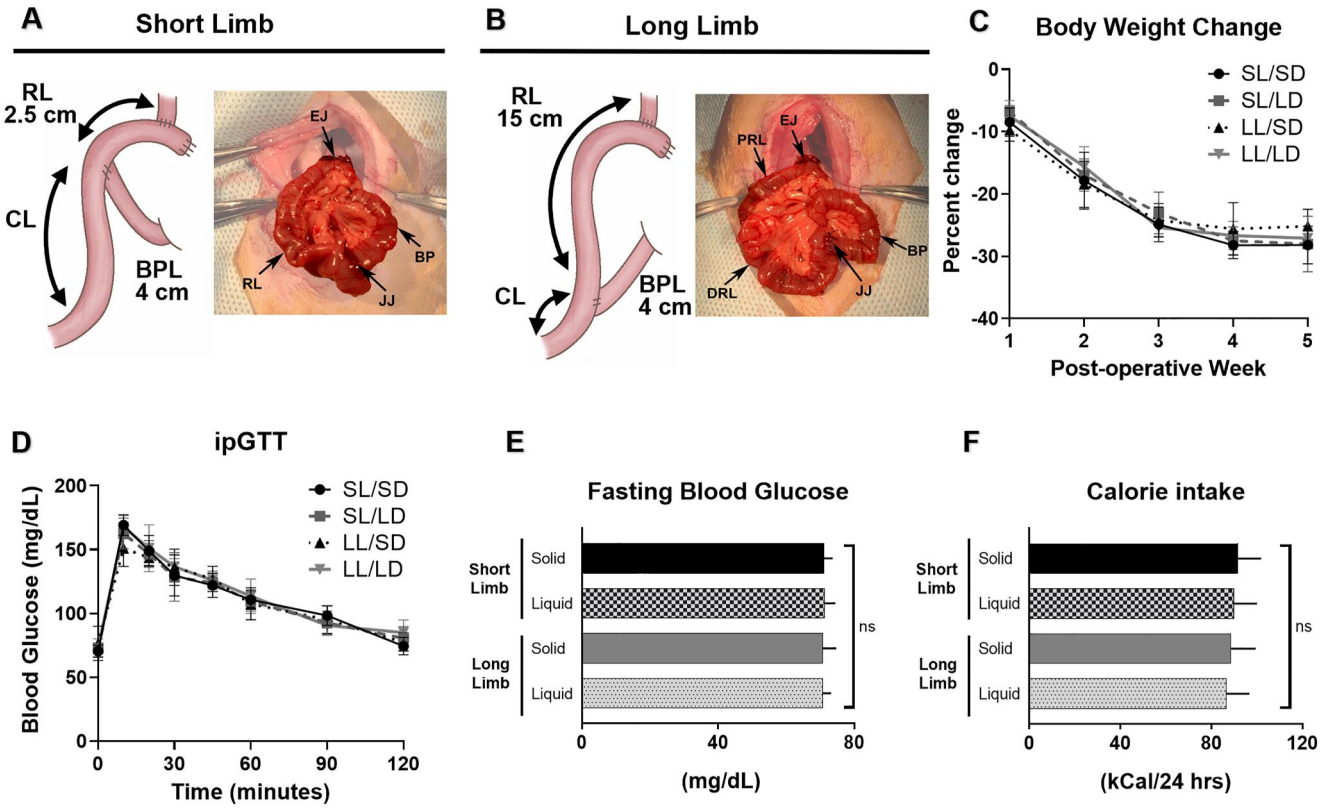

FIGURE 1 Roux-en-Y Gastric Bypass (RYGB) induces weight loss and improves glucose tolerance independent of physical properties of food and biliopancreatic secretions. 1A: Schematic drawing and representative image for Short Limb surgical model. 1B: Schematic drawing and representative image for Long Limb surgical model. 1C: Body weight changes post RYGB surgery recorded as percent weight loss at 1, 2, 3, 4, 5 weeks postoperatively. 1D: Glucose Tolerance Test performed with an intraperitoneal (IP) glucose injection plotted as blood glucose levels at 0, 10, 20, 30, 45, 60, 90 and 120 min. 1E: Fasting blood glucose levels (mg/dl) for rats on solid and liquid diets from both Short and Long Limb models. 1F: Daily calorie intake for rats on Solid and Liquid diets from both Short and Long Limb models. LL/LD, Long limb model on Liquid diet; LL/SD, Long limb model on Solid diet; SL/LD, Short limb model on Liquid diet; SL/SD, Short limb model on Solid diet. $n = 3–5$ rats/group

3.3 | Intestinal metabolic remodeling following Roux-en-Y Gastric Bypass

As previously established, RYGB surgery enhances intestinal glucose uptake and utilization, primarily through induction of Glucose transporter-1 (GLUT-1) and leads to activation of PI3K/AKT signaling pathway. Furthermore, the GLUT-1 upregulation was examined and observed to occur in a gradient-dependent manner, with more pronounced changes in proximal segments like RL, followed by CL (Figure 3A). Next, the protein expressions of GLUT-1 and PI3K/AKT pathway genes like PI3Kα and AKT, including phosphorylated AKT (p-AKT) were assessed using Western Blots at 5 weeks after RYGB surgery in different intestinal segments across both Short and Long Limb models being fed Solid and Liquid diets. The RL segment of Short Limb model and PRL segment of Long Limb model are representative of corresponding intestinal regions in each of the models respectively. Results found comparable GLUT-1 expression for both these segments across the two models, which was independent of diet form as Solid or LD. Likewise, there were no significant differences in expression of PI3K/AKT pathway genes between Solid and LD groups in comparable segments for both models (Figure 3B).

To investigate effects of biliopancreatic secretions on glucose metabolism after RYGB surgery, protein expressions for different genes between PCL segment of Short Limb and DRL segment of Long Limb model (representing corresponding intestinal lengths) were compared 5 weeks post-operatively. The PCL and DRL segments from their respective limb models showed no differences in GLUT-1 expression. On evaluation of PI3K/AKT pathway genes, it was found that DRL segment of Long limb model had relatively higher expression of PI3Kα and AKT genes when compared to PCL from Short limb model, indicative of potential effect of biliopancreatic secretions on PI3K/AKT genes. Interestingly, p-AKT showed no difference in expression across both models (Figure 3C).

3.4 | Intestinal morphological adaptations post Roux-en-Y Gastric Bypass

In order to investigate morphological adaptations in RYGB, intestinal segments from both Solid and LD groups of Short and Long Limb models were assessed and compared. As observed with metabolic adaptations, intestinal morphological remodeling demonstrated a

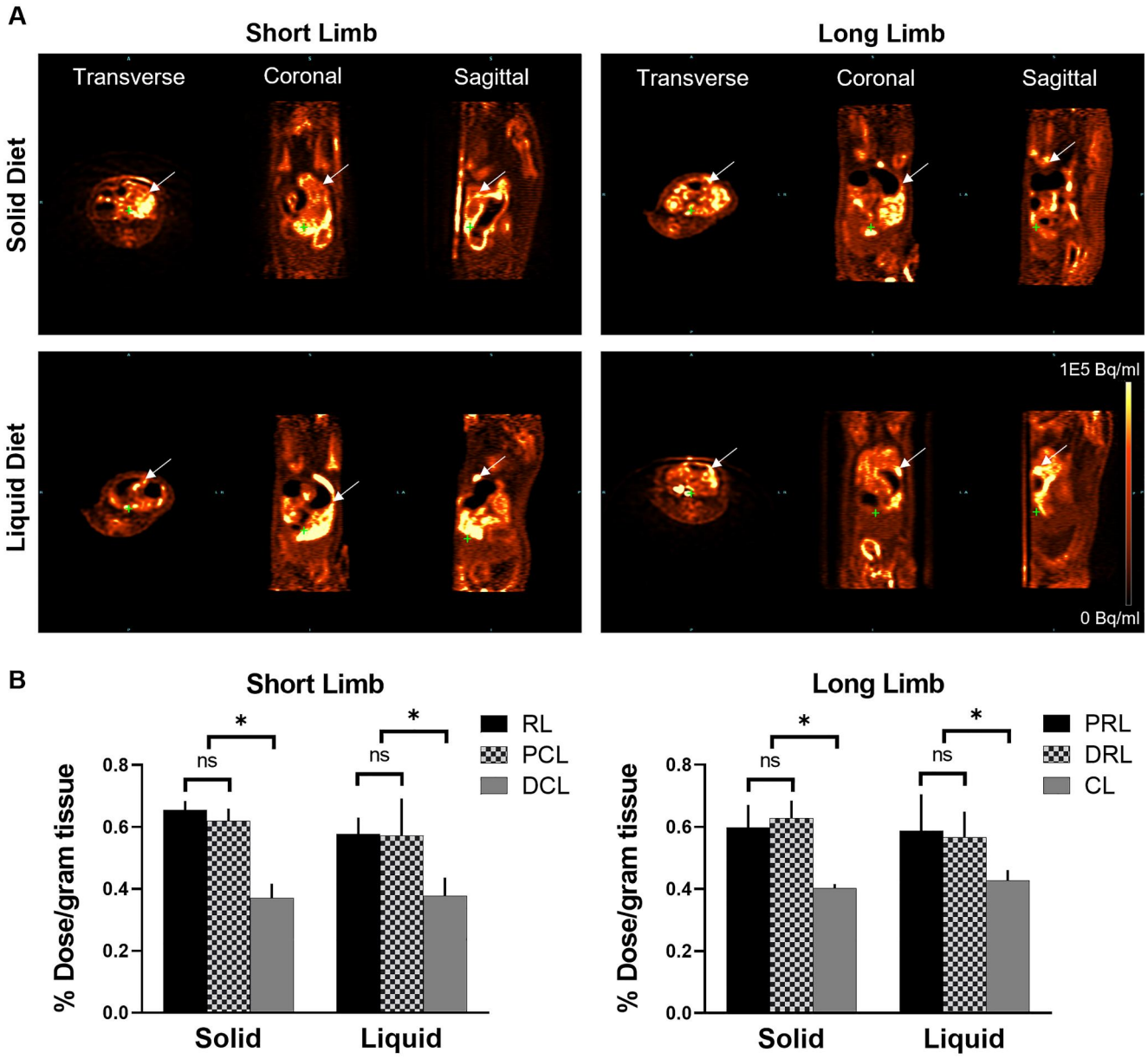

FIGURE 2 Post Roux-en-Y Gastric Bypass (RYGB) regulation of intestinal glucose uptake is independent of food form and biliopancreatic secretions. 2A: Representative images for whole-body ^{18}F fluoro-D-glucose (FDG) positron emission tomography–computed tomography (PET/CT) scanning done in RYGB treated rats across different experimental groups. ^{18}F FDG uptake is color-coded with areas of highest uptake exhibiting yellow-orange color. The white arrows show ^{18}F FDG uptake in Roux Limb (RL) in each rat across different scanned views. Green cross indicates same point of intestine in all images. 2B: ^{18}F FDG biodistribution analysis across different intestinal limbs of RYGB rats from Short and Long Limb models for both diet forms. n = 3–4 rats/group

segment dependent gradient showing PRL with more prominent villus density and cellular hyperplasia compared to DRL and CL. However, there were no remarkable differences between corresponding segments of RL and CL from Solid and Liquid groups (Figure 4A).

Additionally, total intestinal weights were compared, and as expected, Long limb model rats recorded significantly heavier weights when compared with Short limb rats. Interestingly, there were no differences in total intestinal weights between Solid and LD groups within each limb model, providing evidence for lack of effect due to physical properties of ingested food (Figure 4B). Next, the weights of RL and CL segments were measured and compared across both diet form and limb model groups and found no significant differences in weights of either RL or CL segments across all cohorts (Figure 4C).

4 | DISCUSSION

The aim of this study was to investigate effects of physical properties of food and biliopancreatic secretions on intestinal metabolic and morphologic adaptations in RYGB surgery in rats. We tested our

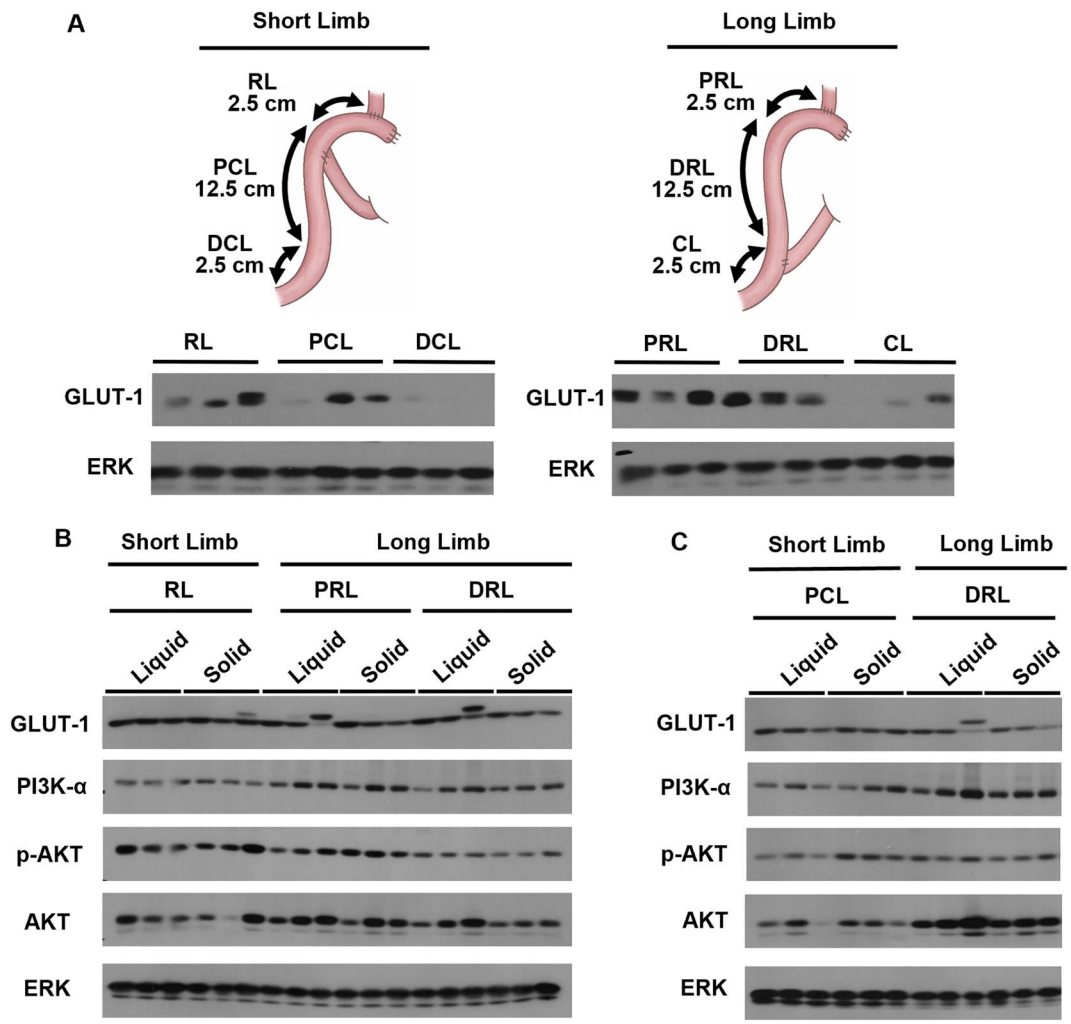

FIGURE 3 Intestinal metabolic remodeling is not influenced by physical properties of food. 3A: Graphical representation of different intestinal segments in Short and Long limb models. GLUT-1 protein expression after Roux-en-Y Gastric Bypass (RYGB) determined by Western Blotting, demonstrating gradient for higher expression in proximal segments over distal parts in both Short and Long limb models. 3B and 3C: Protein expression of GLUT-1, PI3K- α, phosphorylated AKT (p-AKT) and AKT, with ERK as loading control on Western blots across different experimental cohorts. In Long limb model, BPL, Biliopancreatic limb; CL, Common limb; DRL, Distal Roux limb; PRL, Proximal Roux limb. In Short limb model, BPL, Biliopancreatic limb; DCL, Distal Common limb; PCL, Proximal Common limb; RL, Roux limb. RL and PRL are corresponding regions in Short and Long limb model respectively. Similarly, PCL and DRL are corresponding segments in Short and Long limb models respectively. $n = 3–5$ rats/group.

hypotheses by feeding isocaloric Solid and Liquid form diets and by performing two modified RYGB procedures, with a variation in RL length, to create Short RL and Long RL models. Interestingly, the results from this study found no substantial effects of either physical form of ingested food or biliopancreatic secretions on the extent of intestinal remodeling in RYGB operated rats.

Postoperative weight loss and improved glucose tolerance are standardized measures commonly used to assess RYGB surgery outcomes.[37,38] This study reports similar trends in caloric intake and percent change in body weight of rats from all experimental groups, independent of food form and surgical Limb model, suggesting absence of any consequential effects due to either of the two factors. Further, adding to the evidence are comparable levels for fasting blood glucose and glycemic control (as indicated by GTTs) seen across all cohorts (Figure 1). Another critical finding is that intestinal metabolic alterations seen after RYGB are influenced nether by food form nor by biliopancreatic secretions. This was verified twice, first by determining glucose uptake and utilization in the intestine (Figure 2) and second with quantification of protein levels for GLUT-1 transporter and integral PI3K/AKT pathway genes (Figure 3). Furthermore, evaluation of intestinal morphological adaptations across both Solid and LD groups in Short and Long Limb models found no differences in degree of intestinal remodeling (Figure 4).

Intestinal adaptations after RYGB have been suggested to play a role in the improvement of glycemic control after surgery.[17,39] Our previous study established that RL undergoes significant morphologic adaptations observed as intestinal hypertrophy, along with an

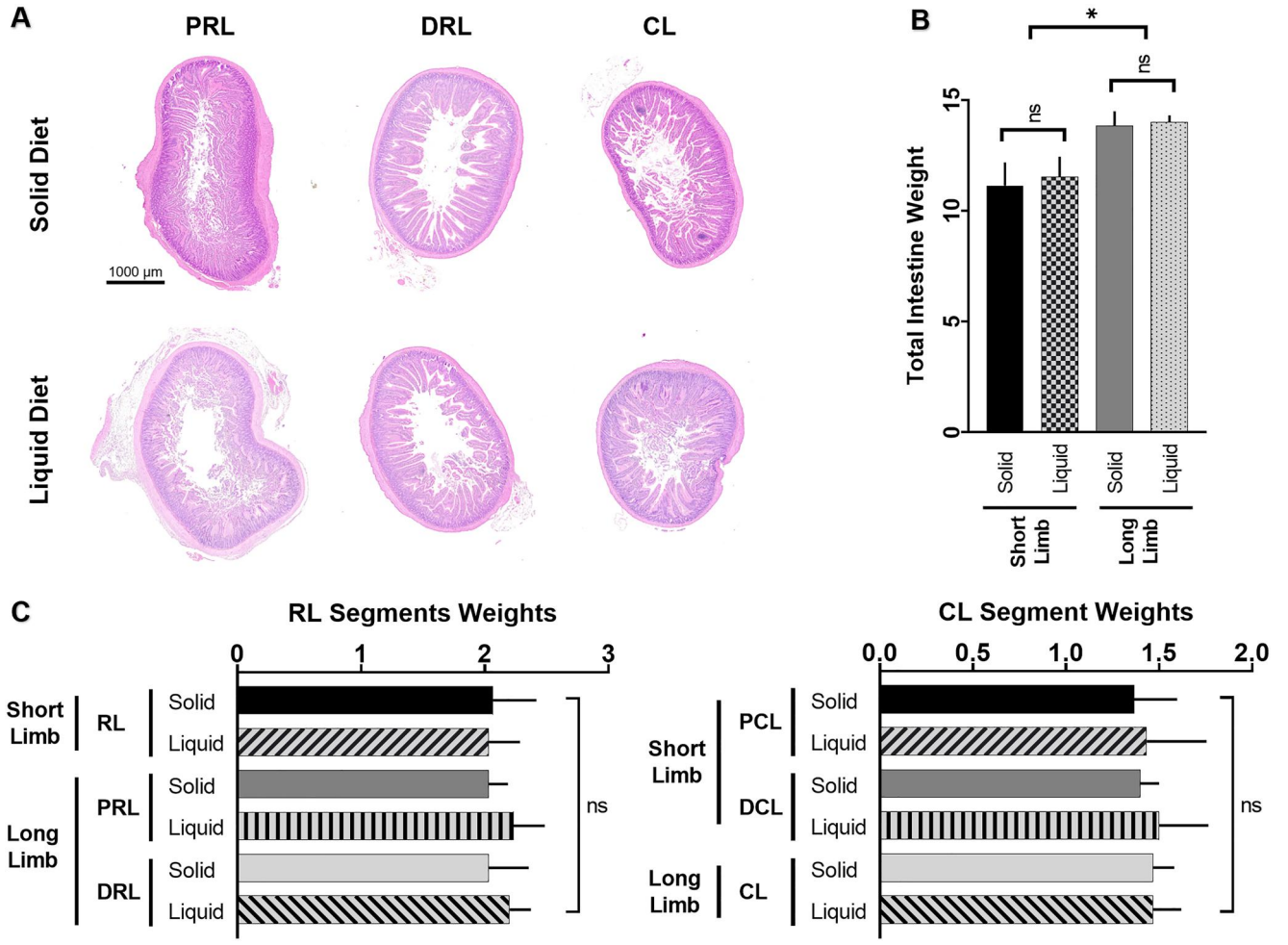

FIGURE 4 Intestinal morphological adaptations are unaffected by physical form of ingested food. 4A: Representative images of Hematoxylin and Eosin-stained sections for corresponding intestinal segments of Solid and Liquid diet (LD) Roux-en-Y Gastric Bypass (RYGB) models. $n = 3$-6 rats/group. 4B: Comparison of total intestinal weight across Solid and Liquid groups in both Short and Long Roux Limb (RL) models. 4C: Comparison of RL and Common Limb (CL) segments weights across Solid and Liquid groups in both Short and Long RL models In Long limb model, BPL, Biliopancreatic limb; CL, Common limb; DRL, Distal Roux limb; PRL, Proximal Roux limb. In Short limb model, BPL, Biliopancreatic limb; DCL, Distal Common limb; PCL, Proximal Common limb; RL, Roux limb.

upregulation of glucose transporter GLUT-1 to meet with the increased intestinal glucose demands of the hypertrophic tissue. Furthermore, several studies have observed a proximal to distal gradient in extent of intestinal adaptations seen after RYGB.[14,17,40] An explanation for this is based on the role played by enteral nutrition in maintenance of intestinal integrity and morphology.

Physiologically, as the intestine continues distally, there is a progressive decrease in intestinal wall thickness and villi length accompanied with a decline in function, seen as reduced number of transporters and digestive enzymes.[41] After RYGB surgery, owing to the anatomical reconfiguration of gastrointestinal tract, PRL is first to encounter the food bolus followed by DCL. In parallel to this, intestinal remodeling is manifested most prominently in RL, followed by CL with minimal to absent changes in BPL, strongly suggesting evidence of effects of ingested food on the intestinal adaptations in RYGB. These trophic effects seen across the different limbs of Roux-en-Y anatomy could either be a direct consequence of local mechanical stimulation of intestinal cells determined by the size, form and consistency of the ingested food, or be an indirect result, potentially mediated through endocrine and paracrine signaling pathways of the gut, or from a complex interaction of both local and systemic factors.[14,17,32,42] A few studies have previously investigated effects of food composition on intestinal transit time and glucose metabolism after RYGB.[43,44] However to the best of our knowledge, the effects of physical form of food on intestinal adaptations after RYGB have not been studied. In this study, the assessment of GLUT-1 expression and ^{18}F FDG glucose uptake on PET scanning (in parallel with body weight findings) showed no notable differences in metabolic adaptations seen in RYGB-operated rats that were fed either Solid or Liquid diets. Additionally, these results were corroborated with similar findings for intestinal morphological changes across different limbs for both diet forms. Thereby, the findings from this study provide evidence of lack of any significant effects of physical properties of food on the intestinal remodeling in RYGB and suggest

the possibility of other underlying mechanisms as major determinants.

Aside from examining physical properties of food, the effects of biliopancreatic secretions and their redirection (owing to Roux-en-Y anatomy) on intestinal adaptations of RYGB was also investigated. By employing two different surgical models with an alteration in site of anastomosis of BPL to RL, Short and Long RL models were created, to systematically investigate effects of bile redirection on intestinal metabolic and morphological adaptations. Given the essential role of biliopancreatic secretions in nutrient absorption and digestion, various studies have investigated role of bile acids in mediating intestinal adaptations following RYGB. Bile acids are known to be increased after RYGB and have been studied as potential regulators of glucose metabolism after surgery.[42,45] It is postulated that the regulation occurs through action of bile acids on nuclear receptor farnesoid X receptor and/or cell surface G-protein bile acid 1 receptor (TGR5).[46,47] Recently, a study demonstrated an inter-relationship between bile acids, gut microbiota and GLP-1 as the driving force for metabolic changes seen after RYGB.[48] Despite multiple studies examining role of bile acids in RYGB, the debate continues failing to reach a consensus about the extent and precise mechanism of biliopancreatic secretions on metabolic changes in RYGB surgery.[39,49]

Over the past 2 decades, multiple studies have focused on determining the whether the lengths of RL and/or BPL influence surgical outcomes to determine optimal length for each of these limbs. Several studies found that a longer RL in patients with severe obesity led to increased weight loss and resolution of Type 2 diabetes mellitus.[50,51] On the contrary, evidence from multiple studies has shown that altering the length of RL has limited to negligible benefits in post-RYGB patient outcomes.[50,52,53] Similarly, a few studies concluded that longer BPL led to significant resolution of diabetes mellitus with enhanced glycemic control.[54,55] However, a recent meta-analysis found no strong evidence supporting improved outcomes with BPL length modifications in RYGB procedures.[56] Given the wide disparity in literature examining variable RL and BPL lengths, there remain no consensus on this matter. In this study both metabolic and morphological adaptations in different intestinal limbs were evaluated using Short and Long RL experimental models, and it was found that no substantial differences occurred as a consequence of variation between the two groups. Furthermore, the absence of differential effects of physical form of food was verified within each of the two surgical Limb models.

This study's findings demonstrate that neither food form nor biliopancreatic secretions are key determinants of intestinal adaptations in RYGB surgery. A potential limitation is that discrepancies between human and rodent model studies are commonly observed in RYGB studies, necessitating the need for additional pre-clinical and clinical studies to fully verify the effects or lack thereof of physical properties of food and biliopancreatic secretions on intestinal remodeling. Nevertheless, these experimental findings are introduced to the literature to aid toward a better understanding of the precise underlying mechanisms responsible for therapeutic benefits of RYGB surgery.

AUTHOR CONTRIBUTION

Prabh R. Pannu, Po-Jen Yang and Nima Saeidi conceptualized the study and experimental design. Data analysis was performed by Prabh R. Pannu, Chijioke Chukwudi and Jianxun Wang Farid Nasr Esfahani and Po-Jen Yang performed the surgical procedures. The manuscript was written by P.P. and N.S. and all authors reviewed and approved the final manuscript. N.S. supervised and provided funding for the project.

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CONFLICT OF INTEREST

There are no disclosures or conflicts of interest for this study.

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