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Malabsorption and Intestinal Adaptation After One Anastomosis Gastric Bypass compared to Roux-en-Y Gastric Bypass in Rats

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Author's contributions
J.-B.C., E.V., S.M., J.-P.M., M.L.G., and A.B. designed the experiments; J.-B.C., E.V., F.C., M.L.G., and A.B. performed experiments; E.V. performed animal surgeries; N.K. supervised stool analyses; J.-B.C., E.V., M.L.G., and A.B. analyzed and interpreted data; J.-B.C., M.L.G., and A.B. wrote the manuscript with comments from N.K., J.-M.C., and S.M.

Running head:

Protein malabsorption after Mini Gastric Bypass

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Abstract

The technically easier one-anastomosis (mini) gastric bypass (MGB) is associated with similar metabolic improvements and weight loss as the Roux-en-Y gastric bypass (RYGB). However, MGB is controversial and suspected to result in greater malabsorption than RYGB. In this study, we compared macronutrient absorption and intestinal adaptation after MGB or RYGB in rats. Body weight and food intake were monitored and glucose tolerance tests were performed in rats subjected to MGB, RYGB, or sham surgery. Carbohydrate, protein, and lipid absorption was determined by fecal analyses. Intestinal remodeling was evaluated by histology and immunohistochemistry. Peptide and amino acid transporter mRNA levels were measured in the remodeled intestinal mucosa and those of anorexigenic and orexigenic peptides in the hypothalamus. The MGB and RYGB surgeries both resulted in a reduction of body weight and an improvement of glucose tolerance relative to sham rats. Hypothalamic orexigenic neuropeptide gene expression was higher in MGB rats than in RYGB or sham rats. Fecal losses of calories and proteins were greater after MGB than RYGB or sham surgery. Intestinal hyperplasia occurred after MGB and RYGB with increased jejunum diameter, higher villi, and deeper crypts than in sham rats. Peptidase and peptide or amino acid transporter genes were overexpressed in jejunal mucosa from MGB rats but not RYGB rats. In rats, MGB led to greater protein malabsorption and energy loss than RYGB. This malabsorption was not compensated by intestinal overgrowth and increased expression of peptide transporters in the jejunum.

New and Noteworthy

Considered simpler and safer than the Roux-en-Y gastric bypass (RYGB), the mini-gastric bypass (MGB) is increasingly performed worldwide. Here we present the first rat model of MGB whose outcomes were compared with those of RYGB. MGB led to similar improvement of glucose tolerance but increased fecal nitrogen and energy loss in rats. These results suggest protein malabsorption after MGB despite intestinal overgrowth and higher expression of peptide transporters. Our study urges direct investigations in humans.

Keywords Bariatric surgery; mini-gastric bypass; Roux-en-Y gastric bypass, macronutrient absorption, intestinal adaptation

Introduction

Bariatric surgery groups several procedures that aim to cure obesity and its associated comorbidities. The success of bariatric surgery in promoting weight loss and resolving type 2 diabetes is now clear (28). However, despite the large number of different procedures, none appear to be an ideal choice. Each decade, new bariatric surgery models are established, showing improved efficiency but also introducing new attendant problems and complications (7). There is mounting pressure to find the best surgical treatment, leading surgeons to create and perform modifications of existing procedures without precise knowledge of the long-term consequences for the patients. Increasing efforts are being made to minimize the invasiveness of the procedures with simpler surgery, shorter operating times, and shortened hospital stays. Accordingly, in 1997, Robert Rutledge designed a new procedure called the mini gastric bypass (MGB) – also known as one-anastomosis or omega-loop gastric bypass – a variation of the Roux-en-Y gastric bypass (RYGB) with a single anastomosis (29). This surgical procedure provides similar results concerning weight loss and metabolic improvement while presenting the benefit of being more easily performable and revisable (30). Considered to be simpler, safer, and an easier procedure than the RYGB, the MGB is increasingly performed worldwide. However, this operation is still controversial because it results in the bile being in direct contact with the gastric mucosa; theoretically creating biliary reflux and possibly increasing the risk of developing gastric or esophageal cancers (2, 14, 22). In addition, clinical experience suggests that the mini gastric bypass results in greater malabsorption than RYGB but this has yet to be demonstrated in a published study. There are experimental models for RYGB or vertical sleeve gastrectomy, but there are no experimental models to investigate the short- and long-term consequences of MGB surgery on the physiology of the gastrointestinal tract. Here, we describe the development of a rat model of MGB and the intestinal adaptation

after this surgery. We compared weight loss, glucose tolerance, food intake, and the overall modifications of absorptive capacity after MGB, RYGB, or sham surgery.

Materials and Methods

Animal surgeries and post-surgery procedures

All experiments were performed in compliance with the European Community guidelines and approved by the Institutional Animal Care and Use Committee (*N° #2011-14/773-0030* Comité d'Ethique Paris-Nord). Male Wistar rats (Janvier Labs) weighing 450 ± 50 g were divided into MGB (n = 6), RYGB (n = 6), and sham-operated (n = 9) groups. They were fasted overnight before operation. Anesthesia was given by intraperitoneal injection of pentobarbital. After laparotomy, the stomach was isolated outside the abdominal cavity. Loose gastric connections to the spleen and liver were released along the greater curvature, and the suspensory ligament supporting the upper fundus was severed.

MGB: The forestomach was resected using an Echelon 45-mm staple gun with blue cartridge (Ethicon). The lesser curvature was then dissected and the vascular supply isolated in this region. A silastic tube was passed behind the esophagus to delimit the position of the stapler TA-DST 30 mm-3.5mm (Covidien). The retaining pin of the stapler was locked through the dissected lesser curvature, the stapler positioned in a parallel line with the transection line of the forestomach, and the gastric pouch created. The jejunum was then anastomosed to the gastric pouch 35 cm from the pylorus with 6-0 Polydioxanone (PDS) running sutures (Fig. 1A-B). The survival rate was 100% (6/6).

RYGB: After resection of the forestomach as above, the gastric pouch was created using a TA-DST 30-mm-3.5-mm stapler (Covidien) preserving the arterial and venous supply. The jejunum was transected 15 cm distally from the pylorus. The Roux limb was anastomosed to

the gastric pouch and the biliopancreatic limb was anastomosed 20 cm distal to the gastro-jejunal anastomosis with 6-0 PDS running sutures. The survival rate was 83% (5/6).

Sham: To mimic surgery, the stomach was tweaked with an unarmed staple gun and the jejunum was transected and repaired. The Survival rate was 100% (9/9).

For all procedures, the laparotomy was closed using 5.0 Polyglycolide (PGA) sutures in two layers and Xylocaine (10mg/kg) was infiltrated all along the sutures to reduce pain.

Post-operative care: Rats were maintained without food for 48 h after the surgery. They received subcutaneous injections of 12 mL Bionolyte G5 (Baxter) twice a day during this period and daily administration of 20,000 units/kg penicillin G (Panpharma). From day 3 to 4 after surgery, they had access to a liquid diet (Altromin C-0200, Genestil) corresponding to 50 Kcal/day (60% of preoperative intake). Free access to a normal solid diet (Altromin 1324, Genestil), was allowed from day 5. Sham-operated rats received the same post-operative care as the MGB and RYGB groups. Pain and distress were carefully monitored twice a day. Rats showing signs of pain or not eating were maintained on Buprenorphine (0.03mg/kg) and euthanized if there was no improvement after 24 h.

Rats were sacrificed after 20 days by lethal injection of pentobarbital and intestinal segments and the hypothalamus were rapidly collected in TRIzol reagent, frozen in liquid nitrogen, and stored at -80°C until RNA extraction. Some intestinal segments were also collected in formalin for histology and morphometric analyses.

Plasma analyses. Blood collected on day 20 post-surgery was used for the determination of albumin, triglycerides, cholesterol, and non-esterified fatty acids using an automatic analyzer AU400 (Olympus Diagnostics, Rungis, France).

Tomodensitometry (TDM) with oral opacification of the gastrointestinal tract

The surgical procedure was verified by tomodensitometry of the esophago-gastro-intestinal region using a CT scan (NanoSPECT/CT plus, Mediso medical imaging). Isoflurane-anesthetized rats received an oral load of Gastrografine (Bayer Santé). They were immediately placed in the scanner in a prone position and scanned for 15 min to obtain fine resolution images. ImageJ software was used to make 3D reconstructions.

Oral glucose tolerance test

Rats were fasted for 16 h before being subjected to an oral glucose tolerance test (OGTT) 16 days after the surgery. Blood was sampled from the tail vein before (t = 0) and 5, 15, 30, 60, 90 and 120 min after oral gavage of glucose (1g/kg body weight). Blood glucose levels were measured using the AccuChek System (Roche Diagnostics) and expressed in mg/dL.

Stool analyses

MGB, RYGB, or sham rats were maintained in metabolic cages from post-operative day 12 to 15. The stools were collected daily for two days and frozen at -20°C. After thawing, the 2-day stool samples were pooled and analyses were performed on homogenized samples. Nitrogen, lipid, and total energy content were determined by nitrogen elemental analysis (18) (Elemental Analyser CHN EA1112; Thermo Scientific), the method of van de Kamer (33), and bomb calorimetry (PARR 1351 Bomb Calorimeter; Parr Instrument Company), respectively. The energy derived from carbohydrates was calculated by subtracting the energy associated with the nitrogen and lipid components from the total energy. The calorie-conversion factors used were 4.2, 9.35, and 5.65 kcal/g for carbohydrates, lipids, and proteins, respectively. The conventional conversion factor of 6.25 was used to express elemental nitrogen content as protein content. The coefficient of net fecal loss, expressed as a percentage of total energy ingested of the three main energy sources (carbohydrates, lipids and proteins), represented the proportion of ingested energy recovered in the stool.

Histology and morphometric analyses

Intestinal segments were fixed overnight in formalin and embedded in paraffin. Three micrometer blank slices were cut from each block to perform hematoxylin phloxine saffron (HPS) staining. Each slide was scanned with an Aperio ScanScope® CS System (Leica Microsystemes SAS). Morphometric analyses were performed using the Calopix Software (TRIBVN) by measuring diameter, villus height, and crypt depth on three to four distant sections per rat sample. Averages were used for statistical analyses.

Reverse transcription and Quantitative Real-time PCR

Total RNA was extracted from frozen hypothalamus and intestinal mucosa scrapings with TRIzol reagent (Invitrogen). One microgram from each sample was converted to cDNA using the Verso cDNA Synthesis Kit (Thermo Scientific). Primers were designed using Roche assay design center or were based on previous studies; they were all synthesized by Eurofins. Real-time PCR was performed using the LightCycler 480 system (Roche Diagnostics) according to the manufacturer's instructions. Ct values of the genes of interest were normalized against three different reference genes (*L19*, *Hprt*, and *Rpl22*), which were chosen after multiple comparisons with numerous reference genes. The primers used in this study are presented in Table1.

Results

A rat model of MGB

In our rat model of MGB, the forestomach was resected and a small gastric pouch directed the food to flow from the esophagus into the jejunum (Fig. 1). The jejunum was anastomosed laterally to the gastric pouch 35 cm from the pylorus, excluding the duodenum and proximal jejunum from the food path (Fig. 1A and 1B). The survival rate after 20 days was 100% (6/6). The staple lines impede food from reaching the excluded distal stomach and avoid leakage as

verified by tomodensitometry analyses (Fig. 1C and movie S1). The contrast medium went indifferently through the bilio-pancreatic and alimentary limbs as expected. We compared this surgical procedure to our validated RYGB model (5, 10). We excluded the same length of intestine in RYGB rats, as the biliopancreatic limb was 15 cm and the Roux limb 20 cm, leaving 60 to 80 cm of common channel in both models.

MGB induces weight loss and better glucose tolerance similar to RYGB but increases orexigenic neuropeptides

All operated rats lost weight during the intensive postoperative care period and their weight stabilized seven days after the reintroduction of the normal solid diet, *i.e.* 12 days after the surgery (Fig. 2A). By that time, the sham rats returned to their preoperative bodyweight whereas the weight of the MGB and RYGB rats stabilized at approximately 6% and 12% less than their preoperative weight, respectively (Fig. 2A). We performed an oral glucose tolerance test on fasted rats 16 days after surgery. Both MGB and RYGB-operated rats had better glucose tolerance than sham rats (Fig. 2B). We also assayed the plasma of animals for different biochemical parameters 20 days after surgery (Table 2). Cholesterol was lower in RYGB rats but not in MGB rats relative to sham rats. Albumin and triglyceride levels were not significantly different between the three groups.

Caloric intake was recorded daily after the surgery (Fig. 2C). During the intensive post-operative period, food was provided as a liquid solution and restricted to 50Kcal/24h. After the reintroduction of a solid diet *ad libitum* (on the 5^{th} day), the daily caloric intake in the sham group rose to 100Kcal/day and remained stable until the end of the experiment. The increase in food intake occurred more rapidly in MGB than in RYGB rats. Additionally, the food intake of RYGB rats appeared to plateau at 80Kcal/day after nine days, whereas the

MGB-operated rats were eating approximately 100Kcal/day, 20% more than before the surgery (Fig. 2C).

Hypothalamic levels of mRNA encoding the orexigenic peptides, neuropeptide Y (*Npy*) and agouti-related polypeptide (*Agrp*), were 40 and 75% higher in MGB- than in sham-operated rats, respectively, whereas levels of mRNA encoding the anorexigenic peptides, Pro-opiomelanocortin (*Pomc*) and Cocaine- and amphetamine-regulated transcript (*Cart*) were similar between the two groups (Fig. 2D). In contrast, hypothalamic mRNA levels for the orexigenic peptides, *Npy* and *Agrp,* of RYGB rats were similar to those for sham rats (Fig. 2D). The levels of mRNA for the anorexigenic peptides, *Pomc* and *Cart,* were slightly lower in the hypothalamus of RYGB-operated rats than in sham or MGB-operated rats, but the difference was not statistically significant (Fig. 2D).

Fecal protein loss is higher after MGB than RYGB

MGB, RYGB, or sham surgery rats were kept in metabolic cages from post-operative day 12 to 15 to evaluate the intestinal absorptive capacity after the surgery. The experiment was set up so that daily food intake was not significantly different between the three groups during this analysis (Fig. 3A). Overall stool excretion (expressed as the percentage of food intake) was slightly, but not significantly, higher by MGB rats than by sham or RYGB rats (Fig. 3B). However, fecal caloric loss was 25% higher in MGB rats than in sham or RYGB rats (Fig. 3C). This higher overall caloric loss was due to greater fecal lipid loss (+ 40% in MGB-operated rats vs sham), and a doubling of fecal protein loss (+ 100% in MGB-operated rats vs sham) (Fig. 3D-E). We also noted a greater fecal lipid loss in RYGB-operated rats, although it was not significantly different from that of sham-operated rats. Finally, there was no difference in fecal carbohydrate loss (evaluated mathematically) between the three groups (Fig. 3F).

Intestinal morphological adaptation is comparable after MGB or RYGB

Intestinal remodeling was evaluated by morphometric analyses of different intestinal segments from MGB or RYGB-operated rats and compared to equivalent segments from sham rats (Fig. 4A). Intestinal regions excluded from the food path by MGB or RYGB surgery, *i.e.* duodenum and biliopancreatic limb (BPL), were not morphologically different from their corresponding segments in sham-operated rats (Fig. 4B quantified in 4C-E), except that crypts within the BPL of MGB rats were 25% deeper than those of sham rats (Fig 4E). The hyperplasia of the AL, previously reported in numerous models of RYGB and confirmed here, was even more pronounced in MGB-operated rats with a 40% greater diameter, 30% higher villi, and 100% deeper crypts than in sham rats (Fig. 4C-E). The distal ileum morphology was affected to a lesser extent, but the villi were 30% higher in MGB-operated rats than in sham animals (Fig. 4B and 4D).

The expression of genes involved in protein digestion and absorption is higher in the alimentary limb after MGB but not RYGB relative to sham-operated rats.

We evaluated the expression of genes encoding the peptidases Dipeptidyl peptidase-4 (*Dpp4*) and Leucine aminopeptidase 3 (*Lap3*) (Fig. 5A), Peptide transporter 1 (*Pept1*) with its associated sodium/hydrogen exchanger *Nhe3* (Fig. 5B), and amino acid transporters ASC amino-acid transporter (*Asct2*), Phosphoribosylanthranilate transferase (*Pat1*), and B(0,+)-type amino acid transporter 1 (*b(0,+)*) (Fig. 5C) by the alimentary limb and ileum mucosa 20 days after surgery.

None of these genes were differently expressed within the alimentary limb and ileum of RYGB rats relative to sham (Fig. 5). However, the alimentary limb of MGB rats had increased expression of genes encoding the peptidases DPP4 and LAP3 and the transporters

NHE3, PAT1, and B(0,$^{+}$)(Fig. 5). There were no differences in expression of these genes between the ileum mucosa from sham and MGB rats (Fig. 5).

Discussion

Considered to be simpler, safer, and easier than the Roux-en-Y gastric bypass, the single anastomosis (mini) gastric bypass is increasingly performed worldwide (3, 4, 11), despite a lack of knowledge about the consequences of this surgical procedure on intestinal function. There are few animal models of MGB surgery and the only published rat model was obtained by anastomosing the jejunum to the esophagus (32), making it impossible to investigate whether rerouting part of the bile flux through the gastric compartment could affect digestive functions. We developed a surgical model of MGB in rats that reflects the human surgery as closely as possible. A small gastric pouch was created and connected to the middle of the jejunum by its lateral side. We characterized the overall modifications induced by this surgery and directly compared them to a model of RYGB surgery.

Both bariatric operations led to significant weight loss and better oral glucose tolerance than in the sham group. The improvement in oral glucose tolerance was similar between MGB and RYGB rats in accordance with reports showing a similar response to oral glucose after MGB and RYGB in humans (16). Surprisingly, weight loss was less after MGB than after RYGB in rats, contrasting with the results in humans where MGB is equal to or even more effective than RYGB in reducing body weight (27).

A possible explanation for the reduced weight loss is the slightly higher (+10-20%) food intake by MGB rats than RYGB rats. In agreement, gene expression of the orexigenic peptides, NPY and AgRP, was higher in the hypothalamus of MGB rats than RYGB- or sham-operated rats, suggesting that the MGB rats were hungrier. This is the first study to

investigate orexigenic gene expression in rats subjected to MGB surgery and, to the best of our knowledge, a specific overeating pattern in MGB patients has not been reported. It is thus difficult to determine whether this adaptation is specific to our animal models or if it is a feature of human adaptation to MGB surgery as well. MGB surgery in animals may be less restrictive than RYGB because MGB lateral anastomosis is larger than RYGB terminal anastomosis. However, previous studies reported no correlation between the size/diameter of the gastrojejunal anastomosis and body weight loss in RYGB-operated rats (8). In addition, operated animals were able to significantly increase their food intake when metabolically challenged (23). The higher gene expression of orexigenic peptides only in the MGB group suggests that mechanisms distinct from mechanical restriction, and related to hunger, may be at play. RYGB rats displayed lower mRNA expression of anorexigenic genes than sham rats, although not statistically significant, whereas their food intake was similar, suggesting that lower anorexigenic signals *per se* were not sufficient to increase food intake.

An additional explanation for the reduced weight loss of MGB rats may involve energy expenditure and thermogenesis. An increase in energy expenditure has been demonstrated in RYGB rats (9) but it has never been studied after MGB. A specific effect of RYGB in rats is a resistance to decrease in energy expenditure and thermogenesis after body weight loss relative to food restricted animals (1). This resistance was not observed after vertical sleeve gastrectomy and it is possible that it did not appear after MGB either. In agreement, increased expression of orexigenic neuropeptides NPY and AgRP has been associated with decreased energy expenditure (19) and decreased NPY expression has been associated with increased thermogenesis and browning of white adipose tissue (31). Our observation that NPY and AgRP increase only after MGB, but not after RYGB, suggests that MGB rats may reduce their energy expenditure and thermogenesis and that these reductions contribute to the limited weigh loss after MGB. Of note, most human studies failed to reproduce findings on energy

303 expenditure and thermogenesis after bariatric surgery, probably because these studies were
304 performed at thermoneutral temperatures for humans but not for rodents.

305 More importantly, MGB surgery resulted in a greater degree of malabsorption than RYGB as
306 losses of fecal calories and proteins were higher in MGB-operated rats. This tendency has
307 often been reported in human studies (24, 34), but none have clearly demonstrated it.
308 Malabsorption leading to severe undernutrition was only observed in 0.4 to 1.3% of MGB
309 patients depending on the study (21, 25, 30). However, a recent report showed that
310 hypoalbuminemia was more frequent after MGB (13.1%) than RYGB (2%) or sleeve
311 gastrectomy (0%) (17). Malabsorption could be considered to be beneficial for patients who,
312 indeed, need to lose weight. However, if the protein malabsorption observed in our study is
313 confirmed in humans, it could be deleterious in the long term, leading to a higher risk of
314 sarcopenia and that could be difficult to manage in elderly patients. Increased protein
315 malabsorption could be responsible for the slightly higher food intake observed in MGB rats
316 as proteins are recognized to be satietogenic (26). By lowering the quantity of absorbed
317 proteins, MGB surgery could affect both protein-related satiety and diet induced
318 thermogenesis (35) and contribute to the lower weight loss observed in MGB rats than in
319 RYGB rats. The similar level of albumin observed in the three groups indicates that the rats
320 were not undernourished in the short term. The long-term consequences of protein
321 malabsorption in MGB rats remain to be evaluated.

322 We investigated the remodeling of gut epithelium after surgery to investigate the origin of the
323 malabsorption. The alimentary limb of MGB rats was hyperplasic with a bigger diameter,
324 longer intestinal villi, and deeper crypts than that of sham rats. This considerable hyperplasia
325 was limited to the new food path as the excluded duodenum was not histologically modified.
326 The distal portion of the bilio-pancreatic limb, that also received nutrient stimulation, as

shown by tomodensitometry analyses, was slightly modified with deeper crypts. This hyperplasia was less marked in RYGB-operated rats suggesting less pressure to increase the exchange surface to improve nutrient absorption. However, after MGB, intestinal overgrowth was insufficient to compensate for the malabsorption. These results were confirmed by the overexpression of genes related to the digestion and transport of proteins solely in the alimentary limb mucosa of MGB rats, which may be an additional adaptation of the reconfigured intestine to compensate for the malabsorption. Another possibility is that hyperplasia of the intestine is generally associated with an increase in epithelial cell shedding that could also contribute to protein loss in the feces. An in depth study of nitrogen metabolism will be required to evaluate the relative contribution of endogenous proteins in fecal protein losses.

It is still unclear why protein malabsorption occurred solely in the MGB-operated rats. Previous studies in rats suggested that gastric acid secretion and gastric pepsin may not be essential for protein digestion since complete gastrectomy does not cause severe protein malabsorption (6). In contrast, the absence of pancreatic secretion was shown to be responsible for severe protein malabsorption (12). After MGB and RYGB surgeries, protein digestion is more likely to occur in the common limb, where pancreatic secretions and food are mixed together. In this study, we made certain to exclude a similar length of intestine in both models (35cm); leaving the same intestinal fragment exposed to food and pancreatic secretions. Rerouting a part of the bile flux through the stomach pouch could affect digestive capacities by modifying the pH of the different digestive compartments. pH plays a crucial role in a normally functioning digestive tract and most digestive enzymes are sensitive to it. (13). Stomach proteolytic enzymes, such as pepsin, operate in an acidic environment (20), whereas the activity of pancreatic enzymes, such as trypsin, chymotrypsin, and carboxypeptidase, is optimal in a neutral/slightly basic environment (15). Rerouting the

352 biliopancreatic secretions into the gastric compartment, by adding bicarbonate and
353 neutralizing the acidic chyme, could lower the activity of both stomach and pancreatic
354 proteolytic enzymes and affect the digestibility of proteins. Studies investigating the
355 gastrointestinal pH profiles in patients who have had MGB or RYGB surgery are necessary to
356 confirm this hypothesis.

357 In conclusion, developing a rat model of MGB allowed us to characterize the consequences of
358 this surgical rearrangement on the physiology of the gastrointestinal tract. We observed a
359 greater degree of protein malabsorption induced by this surgery than by RYGB. This
360 malabsorption was not compensated by intestinal hyperplasia and transporter overexpression
361 in the jejunum. Studies investigating whether MGB surgery lead to undernourishment in the
362 long-term are needed. Moreover, the direct evaluation of absorptive capacity in humans who
363 have had MGB surgery are necessary to confirm these findings. The use of this less invasive
364 and revisable surgery as metabolic surgery for moderately obese patients is an attractive
365 option, but may be inappropriate if severe protein malabsorption is confirmed for patients
366 who have had MGB surgery. Finally, despite the growing popularity of this procedure, animal
367 models of MGB are scarce. This rat model of MGB will thus be useful to address the
368 controversy around the potential long-term risk of upper gastro-intestinal cancer after MGB,
369 by measuring bile concentrations in the gastric lumen, and exploring the expression of
370 carcinogenic markers in the gastric and esophageal mucosa.

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Disclosure

None of the authors have anything to disclose.

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Figure legends

Figure 1. MGB procedure

(A) Postmortem macroscopic views of rat stomach 20 days after sham (top) or MGB surgery (bottom). The MGB procedure results in ingested food flowing from the esophagus (es) to the gastric pouch (g. po) and then directly to the jejunum (je), bypassing the distal stomach (d. st), the duodenum (du), and part of the proximal jejunum.

(B) Postmortem view of rat gastrointestinal tract 20 days after MGB surgery, showing the lengths of the alimentary limb and biliopancreatic limb (draining gastric, hepatobiliary and pancreatic secretions) with, in continuity, the caecum and the colon. The red dotted line indicates the new path followed by food.

(C) Tomodensitometry of the thoraco-abdominal region in rat operated from MGB after oral opacification of the gastrointestinal tract. Note that the contrast medium goes from the esophagus through the gastric pouch and flows indifferently to both the biliopancreatic and alimentary limbs.

Figure 2. Weight loss, glucose homeostasis, caloric intake, and hunger signals after MGB or RYGB

(A) Loss of body weight after surgery in MGB-, RYGB- and sham-operated rats. The black box corresponds to the period of postoperative care (5 days) before the animals had free access to a normal solid diet. Data are expressed as the means ± SEM.

(B) Blood glucose levels after an oral load of glucose (1 g/kg) in rats, 16 days after MGB, RYGB, or sham surgery. Data are expressed as the means ± SEM. *$P < 0.05$, **$P < 0.01$, in MGB versus sham; $^{\#\#}P < 0.01$ in RYGB versus sham, in two-way ANOVA for repeated measures followed by Bonferroni correction for multiple comparisons.

(C) Changes in daily caloric intake in MGB-, RYGB- and sham-operated rats after surgery. The dotted line indicates mean caloric intake before surgery (85 Kcal/24 h). The data shown

are the means ± SEM. *P < 0.05, **P < 0.01 in MGB versus sham; $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001 in RYGB versus sham, in a two-way ANOVA for repeated measures followed by Bonferroni correction for multiple comparisons.

(D) Relative mRNA levels of orexigenic (left) and anorexigenic (right) peptides in the hypothalamus from MGB and RYGB rats compared to those from sham-operated rats. Data are expressed as the means ± SEM. *P < 0.05, versus sham-operated rats in a Krustal-Wallis test followed by Dunn's multiple comparisons test.

Npy: Neuropeptide Y; *Agrp*: Agouti-related peptide; *Pomc*: Pro-opiomelanocortin; *Cart*: Cocaine- and amphetamine-regulated transcript.

For all panels: sham n = 9, MGB n = 6 and RYGB n = 5.

Figure 3 Protein malabsorption after MGB or RYGB

(A) Food intake, (B) fecal output, (C) and caloric loss in sham-, RYGB- and MGB-operated rats, during a 3-day analysis in metabolic cages. Fecal outputs are expressed as the percentage of food intake and caloric loss as the percentage of caloric intake.

(D-E) Fecal losses of lipids (D), proteins (E), and carbohydrates (F) in sham-, RYGB- and MGB-operated rats. Protein and lipid loss were calculated by dividing the amount excreted in feces by the ingested amount. Carbohydrate loss was calculated from the difference between the total loss of calories and the loss of calories due to lipids and proteins.

Data are expressed as the means ± SEM. *P < 0.05, **P < 0.01 versus sham in a Kruskal-Wallis test followed by Dunn's multiple comparisons test.

For all panels: sham n = 6, RYGB n = 4 and MGB n = 5.

Figure 4. Intestinal remodeling after MGB or RYGB

(A) Localization of intestinal segment samplings in MGB-, RYGB- and sham-operated rats.

(B) Representative images of hematoxylin-phloxine-saffron (HPS)-stained sections of the duodenum (Duo), jejunum (Jej), biliopancreatic limb (BPL), alimentary limb (AL), and ileum of MGB-, RYGB-, and sham-operated rats 20 days post-surgery. Scale bar, 1 mm.

(C-E) Morphometric analyses showing the diameter (C), villus height (D), and crypt depth (E) in the intestine of MGB- (n = 6) RYGB- (n = 4) and sham-operated rats (n = 8). Data are expressed as the means ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 versus the sham corresponding segment in a Kruskal-Wallis test followed by Dunn's multiple comparisons test.

Figure 5. Expression of genes encoding enzymes and transporters involved in the final digestion and absorption of proteins after MGB or RYGB

Relative levels of mRNA coding for peptidases (A), peptide transporter *Pept1* and associated Na/H exchanger *Nhe3* (B), and amino acid transporters (C) in the alimentary limb mucosa (left panels) and ileum mucosa (right panels) from MGB- (n = 6) and RYGB- (n = 4) operated rats compared to mucosa from the corresponding segments in sham-operated rats (n = 8). Data are expressed as the means ± SEM. *P < 0.05 and **P < 0.01 versus sham-operated rats, in a Kruskal-Wallis test followed by Dunn's multiple comparisons test.

Dpp4: Dipeptidyl peptidase-4; *Lap3*: Leucine aminopeptidase 3; *Pept1*: Peptide transporter 1; *Nhe3*: Sodium–hydrogen exchanger 3; *Asct2*: ASC amino-acid transporter 2; *Pat1*: Phosphoribosylanthranilate transferase; *B(0,+)*: b(0,+)-type amino acid transporter 1.

547 **Table 1: Primers used in this study**

Gene	NCBI Accession #	Sequence
Agrp	NM_033650	CAGAGTTCTCAGGTCTAAGTC TTGAAGAAGCGGCAGTAGCAC
Asct2	NM_175758	TTCCCCTCCAATCTGGTGT CTCTGTGGACAGGCACCAC
B0+At	NM_053929	CAACGGAGCTCTTGCAGTC GATGCCGGATAGAGAACACG
Cart	NM_017110	TACGGCCAAGTCCCCATGTG GGGGAACGCAAACTTTATTGTTG
Dpp4	NM_012789	AGGCTGGTGCGGAAGATT CCATCTTTGTCACTGACGATTT
Hprt	NM_012583	GACCGGTTCTGTCATGTCG ACCTGGTTCATCATCACTAATCAC
L19	NM_031103	TGCCGGAAGAACACCTTG GCAGGATCCTCATCCTTCG
Lap3	NM_001011910	GCAGGAGAGAATTTTAATAAGTTGGT TGAGAGGAGGTCCCGATATG
Nhe3	NM_012654	CAGCTTGGCCAAAATCGT GCACTCTCCGGGACAACA
Npy	NM_012614	CCGCTCTGCGACACTACAT TGTCTCAGGGCTGGATCTCT
Pat1	NM_130415.1	CCTGGATTCGGAACCACTC TGAGTGACGACGAGGAAGAA
Pept1	NM_057121	AGGCATTTCCCAAGAGGAAC CATTATCTTAATCTGCGAGATGAGC
Pomc	NM_139326	AGGACCTCACCACGGAAAG CCGAGAGGTCGAGTCTGC
Rpl22	NM_031104	GCCGCCATGGCTCCTGTGAAAA ACAGGGTGAGTGCAGTCAAGGGT

548

549 **Table 2 Plasma parameters of operated rats**

	Sham	RYGB	MGB
Albumin (g/L)	29.9 ± 1.69	25.04 ± 3.72	29.73 ± 2.55
Triglycerides (mmol/L)	0.37 ± 0.08	0.46 ± 0.20	0.49 ± 0.23
Cholesterol (mmol/L)	2.25 ± 0.40	1.78 ± 0.10 *	2.41 ± 0.51
NEFA (mmol/L)	0.37 ± 0.10	0.44 ± 0.07	0.27 ± 0.10

550 Plasma levels of albumin, triglycerides, cholesterol, and non-esterified fatty acids (NEFA) 20
551 days after surgery RYGB (n = 5), MGB (n = 6), sham (n = 7).
552 Results are expressed as the means ± SD. *P < .05, vs sham-operated rats in a Kruskal-Wallis
553 with Dunn's multiple comparison test.

Figure 1

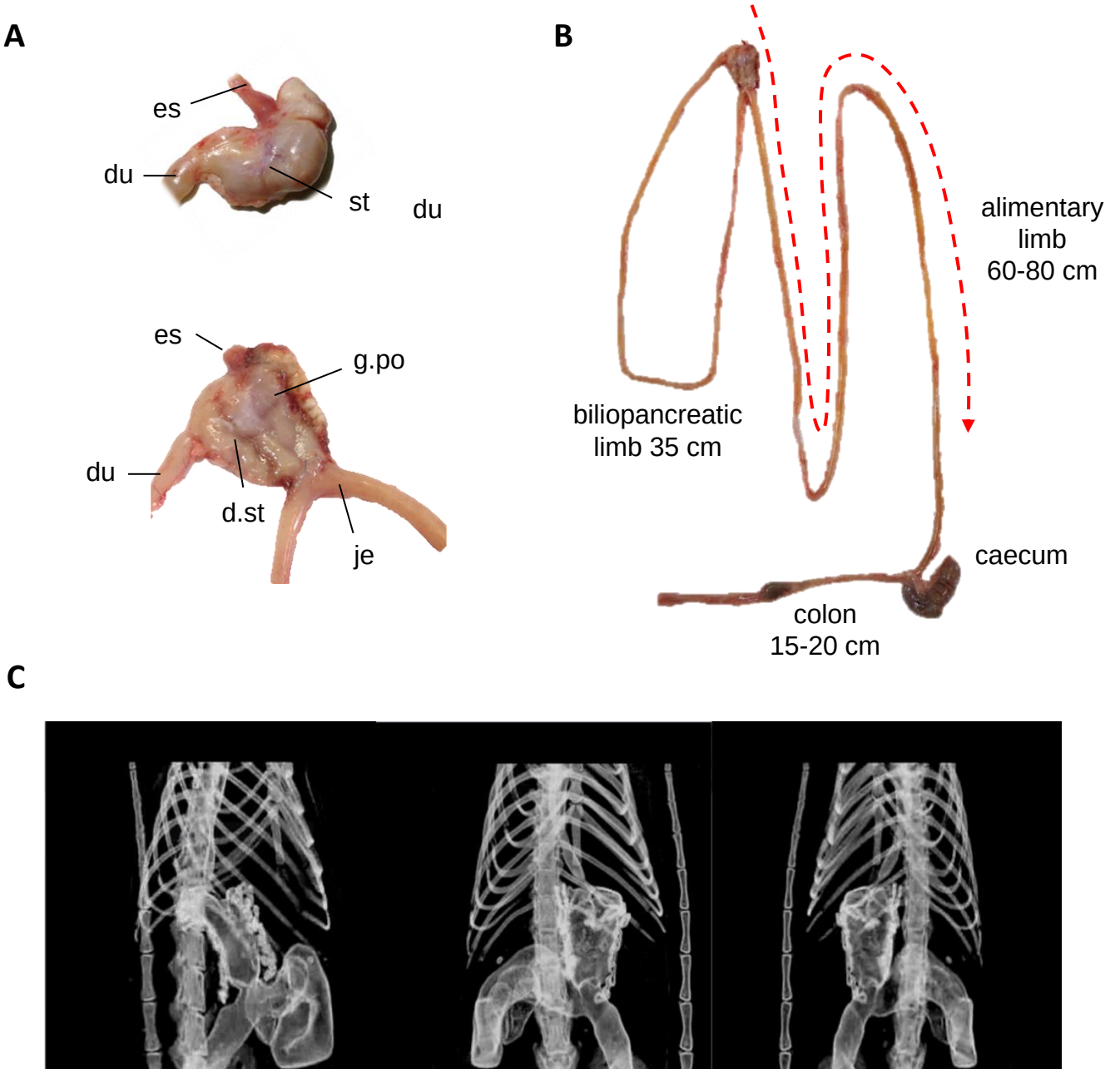
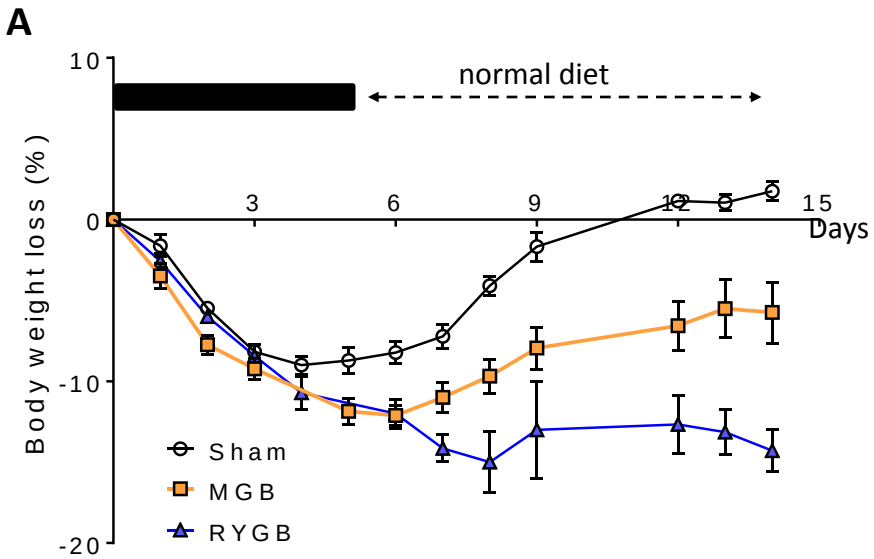
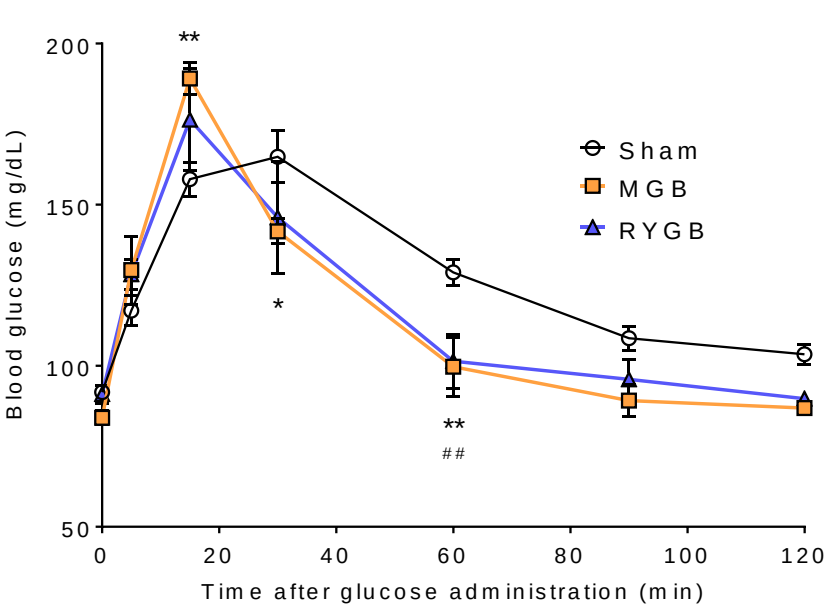
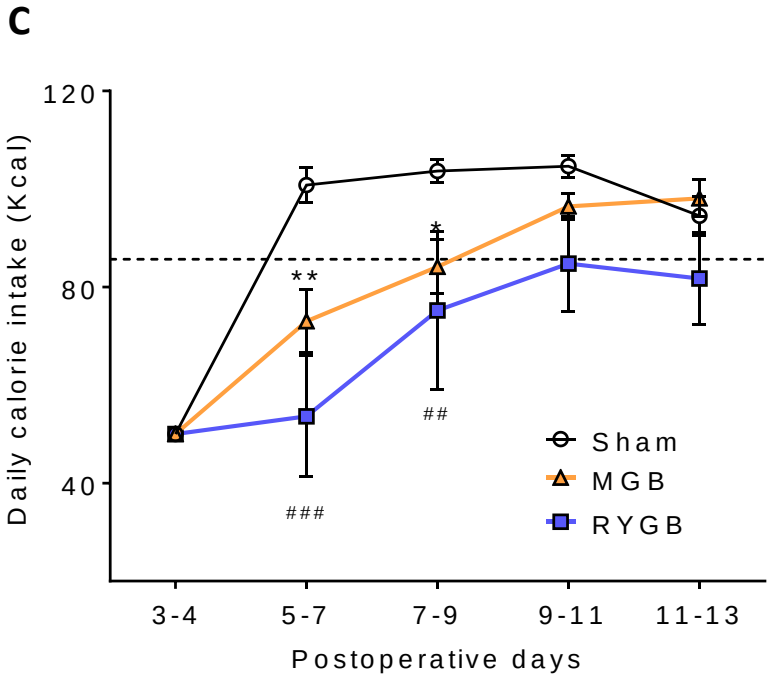

A
es
du
st
du
es
g.po
du
d.st
je
B
alimentary
limb
60-80 cm
biliopancreatic
limb 35 cm
caecum
colon
15-20 cm
C

Figure 2


A
normal diet
Body weight loss (%)
Days
Sham
MGB
RYGB


B
Blood glucose (mg/dL)
Time after glucose administration (min)
Sham
MGB
RYGB


C
Daily calorie intake (Kcal)
Postoperative days
3-4
5-7
7-9
9-11
11-13
Sham
MGB
RYGB

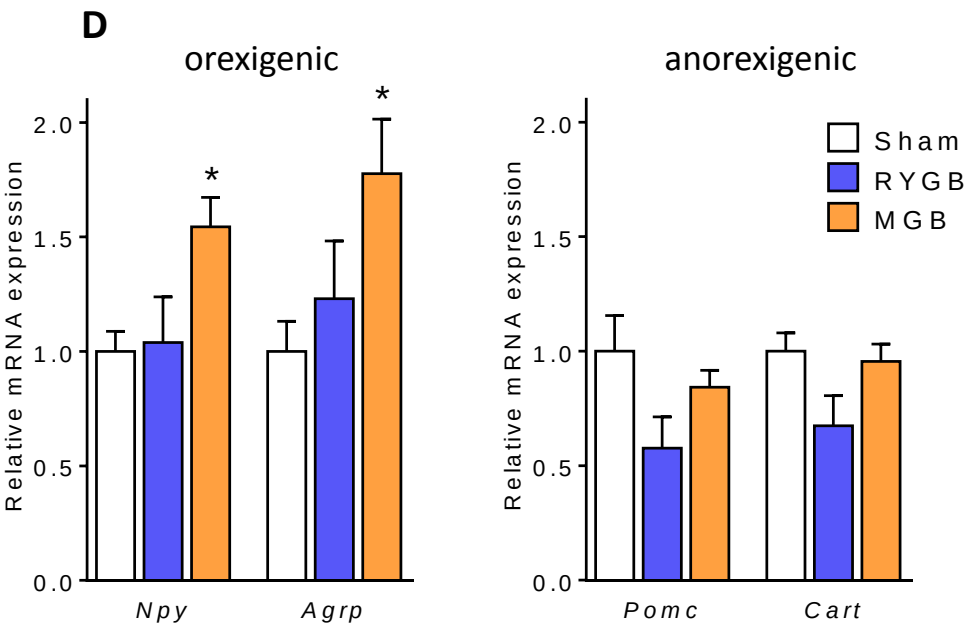

D
orexigenic
anorexigenic
Relative mRNA expression
Npy
Agrp
Pomc
Cart
Sham
RYGB
MGB

Figure 3

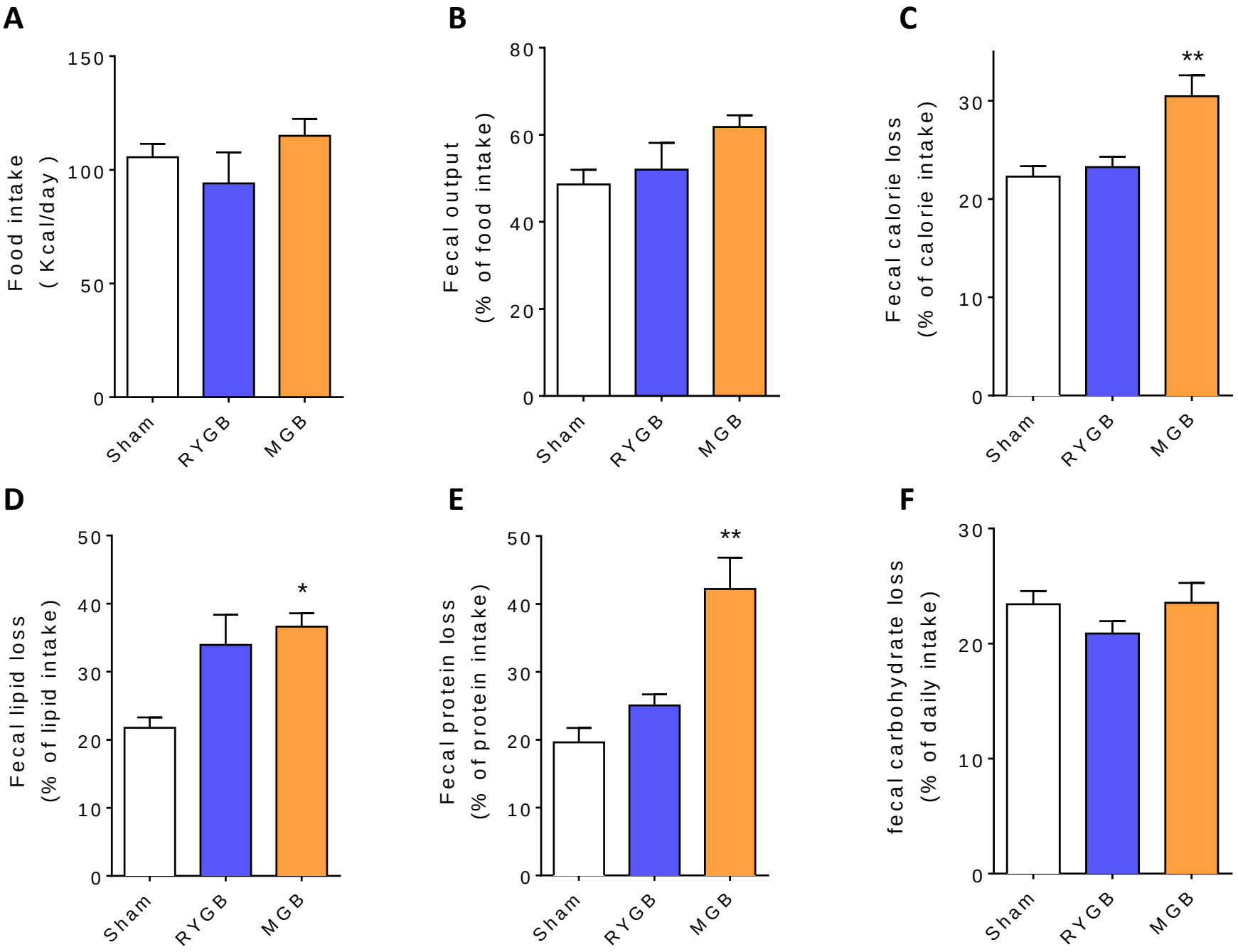
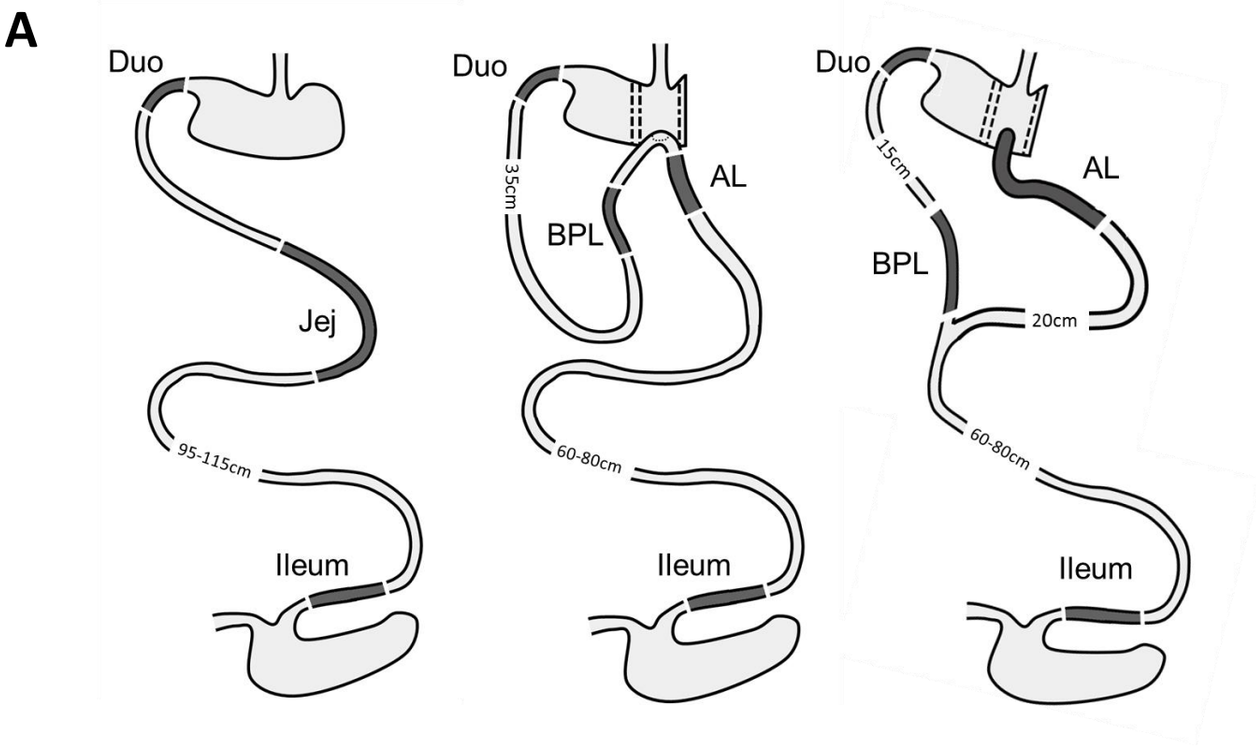
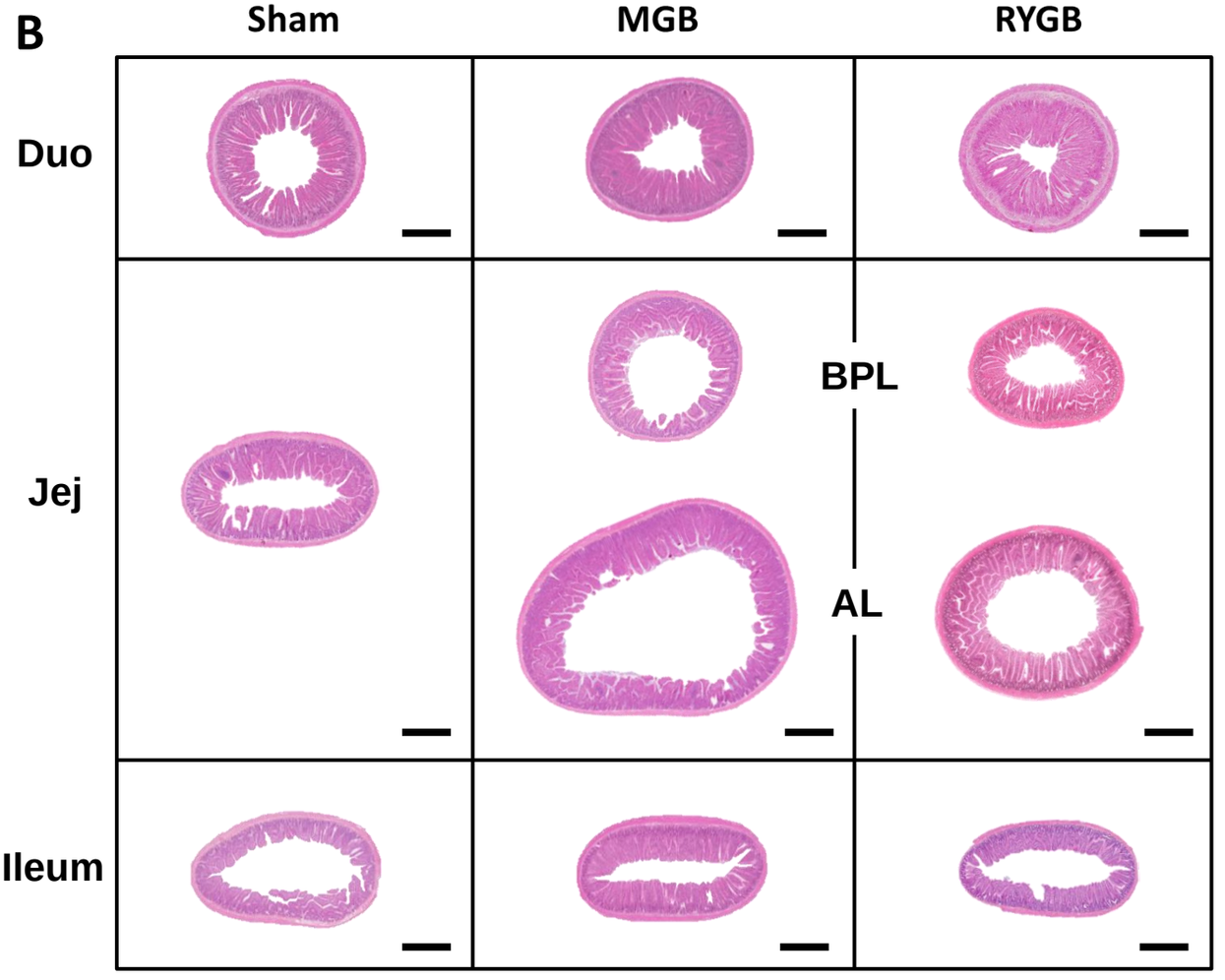
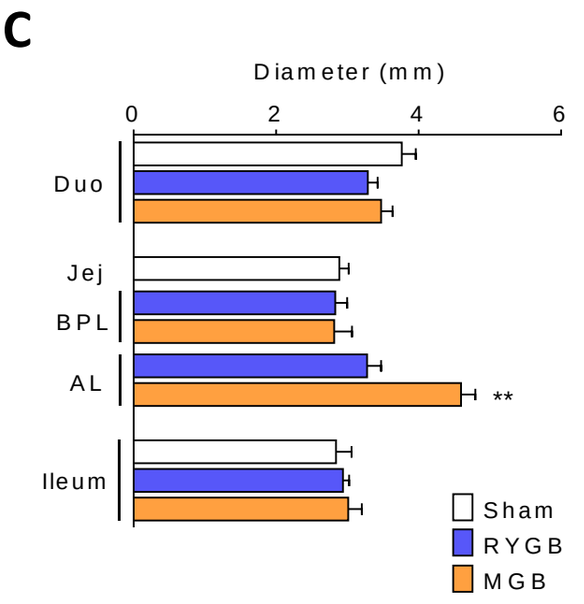
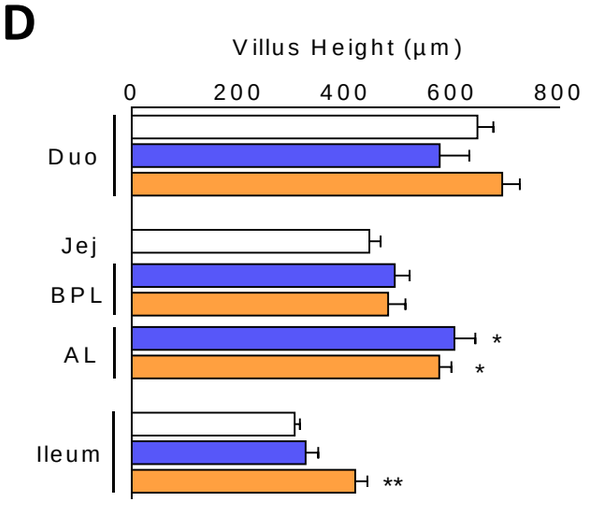

A
Food intake
(Kcal/day)
150
100
50
0
Sham
RYGB
MGB
B
Fecal output
(% of food intake)
80
60
40
20
0
Sham
RYGB
MGB
C
Fecal calorie loss
(% of calorie intake)
**
30
20
10
0
Sham
RYGB
MGB
D
Fecal lipid loss
(% of lipid intake)
*
50
40
30
20
10
0
Sham
RYGB
MGB
E
Fecal protein loss
(% of protein intake)
**
50
40
30
20
10
0
Sham
RYGB
MGB
F
fecal carbohydrate loss
(% of daily intake)
30
20
10
0
Sham
RYGB
MGB

Figure 4


A
Duo
Jej
95-115cm
Ileum
Duo
35cm
BPL
AL
60-80cm
Ileum
Duo
15cm
BPL
AL
20cm
60-80cm
Ileum


B
Sham
MGB
RYGB
Duo
Jej
Ileum
BPL
AL


C
Diameter (mm)
0
2
4
6
Duo
Jej
BPL
AL
Ileum
**
Sham
RYGB
MGB


D
Villus Height (µm)
0
200
400
600
800
Duo
Jej
BPL
AL
Ileum
*
*
**

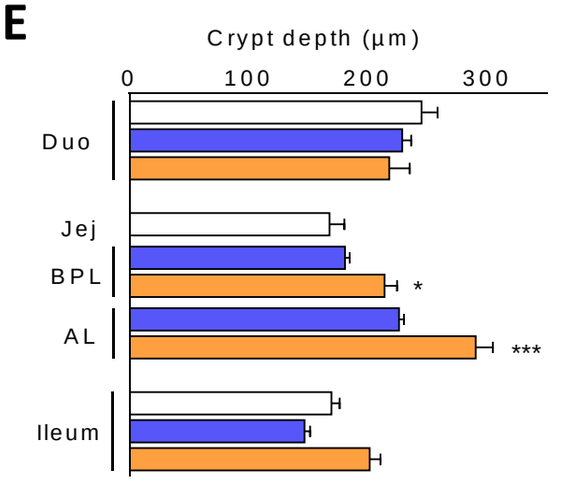
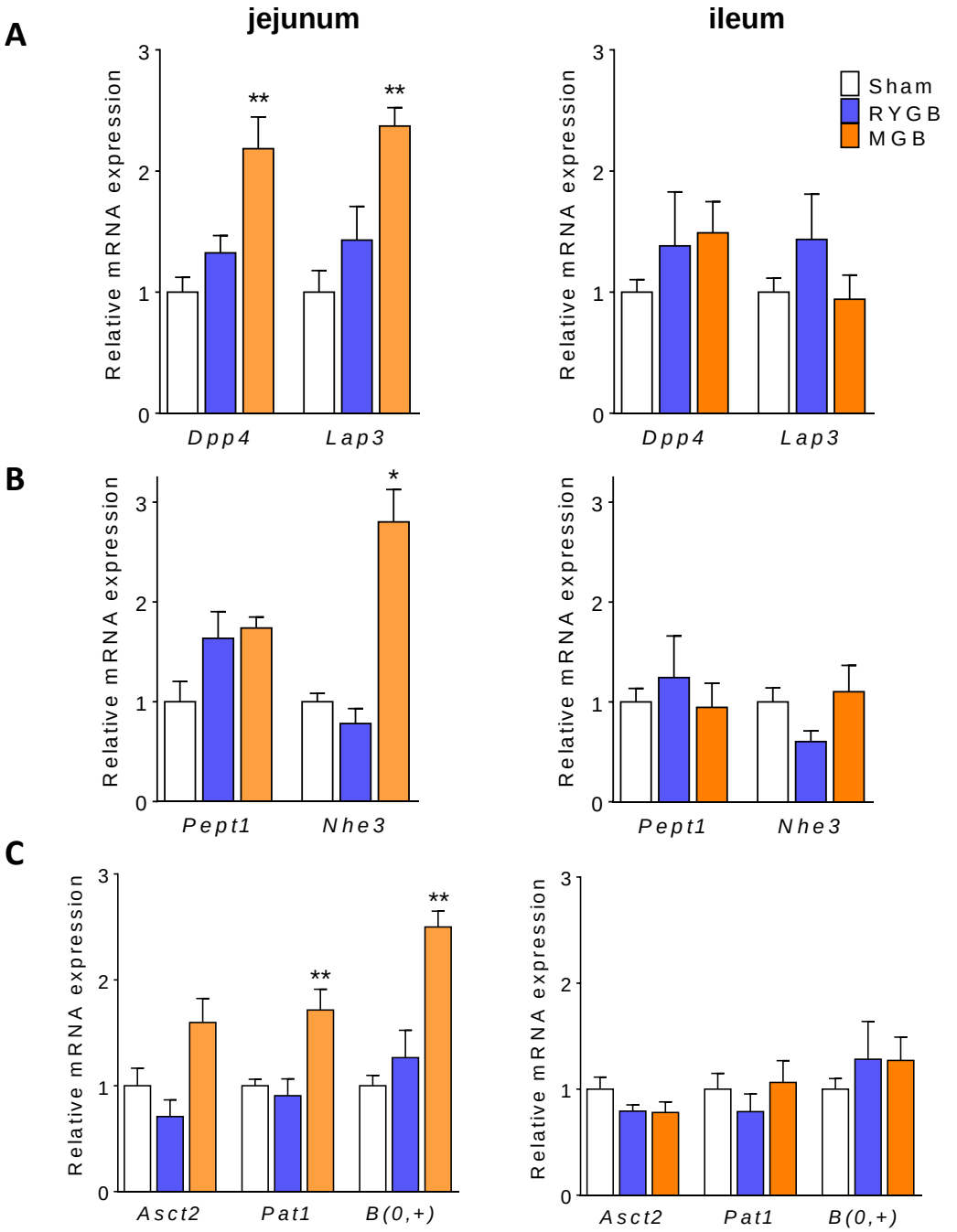

E
Crypt depth (µm)
0
100
200
300
Duo
Jej
BPL
AL
Ileum
*
***

Figure 5


A
jejunum
ileum
Relative mRNA expression
Sham
RYGB
MGB
Dpp4
Lap3
B
Pept1
Nhe3
C
Asct2
Pat1
B(0,+)