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

3
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9
10 **Author's contributions**

11 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.,
12 M.L.G., and A.B. performed experiments; E.V. performed animal surgeries; N.K. supervised
13 stool analyses; J.-B.C., E.V., M.L.G., and A.B. analyzed and interpreted data; J.-B.C.,
14 M.L.G., and A.B. wrote the manuscript with comments from N.K., J.-M.C., and S.M.

15
16 **Running head:**

17 **Protein malabsorption after Mini Gastric Bypass**

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36 **Abstract**

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

56

57 **New and Noteworthy**

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

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

66

67 **Introduction**

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

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

93 **Materials and Methods**

94 **Animal surgeries and post-surgery procedures**

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

103 **MGB:** The forestomach was resected using an Echelon 45-mm stapler with blue cartridge
104 (Ethicon). The lesser curvature was then dissected and the vascular supply isolated in this
105 region. A silastic tube was passed behind the esophagus to delimit the position of the stapler
106 TA-DST 30 mm-3.5mm (Covidien). The retaining pin of the stapler was locked through the
107 dissected lesser curvature, the stapler positioned in a parallel line with the transection line of
108 the forestomach, and the gastric pouch created. The jejunum was then anastomosed to the
109 gastric pouch 35 cm from the pylorus with 6-0 Polydioxanone (PDS) running sutures (Fig.
110 1A-B). The survival rate was 100% (6/6).

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

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

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

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

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

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

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

136 **Tomodensitometry (TDM) with oral opacification of the gastrointestinal tract**

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

142 **Oral glucose tolerance test**

143 Rats were fasted for 16 h before being subjected to an oral glucose tolerance test (OGTT) 16
144 days after the surgery. Blood was sampled from the tail vein before ($t = 0$) and 5, 15, 30, 60,
145 90 and 120 min after oral gavage of glucose (1g/kg body weight). Blood glucose levels were
146 measured using the AccuChek System (Roche Diagnostics) and expressed in mg/dL.

147 **Stool analyses**

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

161 **Histology and morphometric analyses**

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

168 **Reverse transcription and Quantitative Real-time PCR**

169 Total RNA was extracted from frozen hypothalamus and intestinal mucosa scrapings with
170 TRIzol reagent (Invitrogen). One microgram from each sample was converted to cDNA using
171 the Verso cDNA Synthesis Kit (Thermo Scientific). Primers were designed using Roche assay
172 design center or were based on previous studies; they were all synthesized by Eurofins. Real-
173 time PCR was performed using the LightCycler 480 system (Roche Diagnostics) according to
174 the manufacturer's instructions. Ct values of the genes of interest were normalized against
175 three different reference genes (*LI9*, *Hprt*, and *Rpl22*), which were chosen after multiple
176 comparisons with numerous reference genes. The primers used in this study are presented in
177 Table1.

178 **Results**

179 **A rat model of MGB**

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

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

190 **MGB induces weight loss and better glucose tolerance similar to RYGB but increases** 191 **orexigenic neuropeptides**

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

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

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

210 Hypothalamic levels of mRNA encoding the orexigenic peptides, neuropeptide Y (*Npy*) and
211 agouti-related polypeptide (*Agrp*), were 40 and 75% higher in MGB- than in sham-operated
212 rats, respectively, whereas levels of mRNA encoding the anorexigenic peptides, Pro-
213 opiomelanocortin (*Pomc*) and Cocaine- and amphetamine-regulated transcript (*Cart*) were
214 similar between the two groups (Fig. 2D). In contrast, hypothalamic mRNA levels for the
215 orexigenic peptides, *Npy* and *Agrp*, of RYGB rats were similar to those for sham rats (Fig.
216 2D). The levels of mRNA for the anorexigenic peptides, *Pomc* and *Cart*, were slightly lower
217 in the hypothalamus of RYGB-operated rats than in sham or MGB-operated rats, but the
218 difference was not statistically significant (Fig. 2D).

219 **Fecal protein loss is higher after MGB than RYGB**

220 MGB, RYGB, or sham surgery rats were kept in metabolic cages from post-operative day 12
221 to 15 to evaluate the intestinal absorptive capacity after the surgery. The experiment was set
222 up so that daily food intake was not significantly different between the three groups during
223 this analysis (Fig. 3A). Overall stool excretion (expressed as the percentage of food intake)
224 was slightly, but not significantly, higher by MGB rats than by sham or RYGB rats (Fig. 3B).
225 However, fecal caloric loss was 25% higher in MGB rats than in sham or RYGB rats (Fig.
226 3C). This higher overall caloric loss was due to greater fecal lipid loss (+ 40% in MGB-
227 operated rats vs sham), and a doubling of fecal protein loss (+ 100% in MGB-operated rats vs
228 sham) (Fig. 3D-E). We also noted a greater fecal lipid loss in RYGB-operated rats, although it
229 was not significantly different from that of sham-operated rats. Finally, there was no
230 difference in fecal carbohydrate loss (evaluated mathematically) between the three groups
231 (Fig. 3F).

232 **Intestinal morphological adaptation is comparable after MGB or RYGB**

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

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

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

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

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

257 **Discussion**

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

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

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

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

291 An additional explanation for the reduced weight loss of MGB rats may involve energy
292 expenditure and thermogenesis. An increase in energy expenditure has been demonstrated in
293 RYGB rats (9) but it has never been studied after MGB. A specific effect of RYGB in rats is a
294 resistance to decrease in energy expenditure and thermogenesis after body weight loss relative
295 to food restricted animals (1). This resistance was not observed after vertical sleeve
296 gastrectomy and it is possible that it did not appear after MGB either. In agreement, increased
297 expression of orexigenic neuropeptides NPY and AgRP has been associated with decreased
298 energy expenditure (19) and decreased NPY expression has been associated with increased
299 thermogenesis and browning of white adipose tissue (31). Our observation that NPY and
300 AgRP increase only after MGB, but not after RYGB, suggests that MGB rats may reduce
301 their energy expenditure and thermogenesis and that these reductions contribute to the limited
302 weight 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 hyperplastic 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

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

338 It is still unclear why protein malabsorption occurred solely in the MGB-operated rats.
339 Previous studies in rats suggested that gastric acid secretion and gastric pepsin may not be
340 essential for protein digestion since complete gastrectomy does not cause severe protein
341 malabsorption (6). In contrast, the absence of pancreatic secretion was shown to be
342 responsible for severe protein malabsorption (12). After MGB and RYGB surgeries, protein
343 digestion is more likely to occur in the common limb, where pancreatic secretions and food
344 are mixed together. In this study, we made certain to exclude a similar length of intestine in
345 both models (35cm); leaving the same intestinal fragment exposed to food and pancreatic
346 secretions. Rerouting a part of the bile flux through the stomach pouch could affect digestive
347 capacities by modifying the pH of the different digestive compartments. pH plays a crucial
348 role in a normally functioning digestive tract and most digestive enzymes are sensitive to it.
349 (13). Stomach proteolytic enzymes, such as pepsin, operate in an acidic environment (20),
350 whereas the activity of pancreatic enzymes, such as trypsin, chymotrypsin, and
351 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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380 **Disclosure**

381 None of the authors have anything to disclose.

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478

479 **Figure legends**

480 **Figure 1. MGB procedure**

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

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

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

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

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

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

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

504 are the means \pm SEM. *P < 0.05, **P < 0.01 in MGB versus sham; ###P < 0.01, ####P < 0.001 in
505 RYGB versus sham, in a two-way ANOVA for repeated measures followed by Bonferroni
506 correction for multiple comparisons.

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

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

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

514 **Figure 3 Protein malabsorption after MGB or RYGB**

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

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

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

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

525 **Figure 4. Intestinal remodeling after MGB or RYGB**

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

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

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

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

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

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

546

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

Gene	NCBI Accession #	Sequence
<i>Agrp</i>	NM_033650	CAGAGTTCTCAGGTCTAAGTC TTGAAGAAGCGGCAGTAGCAC
<i>Asct2</i>	NM_175758	TTCCCTCCAATCTGGTGT CTCTGTGGACAGGCACCAC
<i>B0+At</i>	NM_053929	CAACGGAGCTCTTGCAGTC GATGCCGATAGAGAACACG
<i>Cart</i>	NM_017110	TACGGCCAAGTCCCCATGTG GGGGAACGCAAACCTTTATTGTTG
<i>Dpp4</i>	NM_012789	AGGCTGGTGCAGGAAGATT CCATCTTGTCACTGACGATTT
<i>Hprt</i>	NM_012583	GACCGTTCTGTCATGTCG ACCTGGTTCATCATCACTAATCAC
<i>L19</i>	NM_031103	TGCCGGAAGAACACCTTG GCAGGATCCTCATCCTTCG
<i>Lap3</i>	NM_001011910	GCAGGAGAGAATTTTAATAAGTTGGT TGAGAGGAGGTCCCGATATG
<i>Nhe3</i>	NM_012654	CAGCTTGCCAAAATCGT GCACTCTCCGGGACAACA
<i>Npy</i>	NM_012614	CCGCTCTGCGACACTACAT TGCTCAGGGCTGGATCTCT
<i>Pat1</i>	NM_130415.1	CCTGGATTCCGAACCACTC TGAGTGACGACGAGGAAGAA
<i>Pept1</i>	NM_057121	AGGCATTTCCCAAGAGGAAC CATTATCTTAATCTGCGAGATGAGC
<i>Pomc</i>	NM_139326	AGGACCTCACCACGAAAG CCGAGAGGTCGAGTCTGC
<i>Rpl22</i>	NM_031104	GCCGCCATGGCTCCTGTGAAAA ACAGGGTGAGTGCAGTCAAGGGT

548

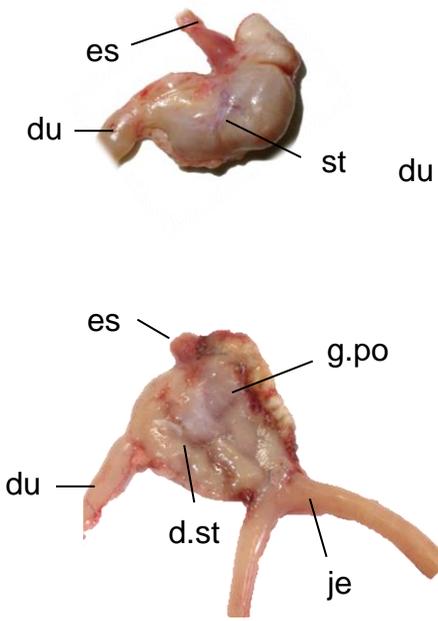
549 **Table 2 Plasma parameters of operated rats**

	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

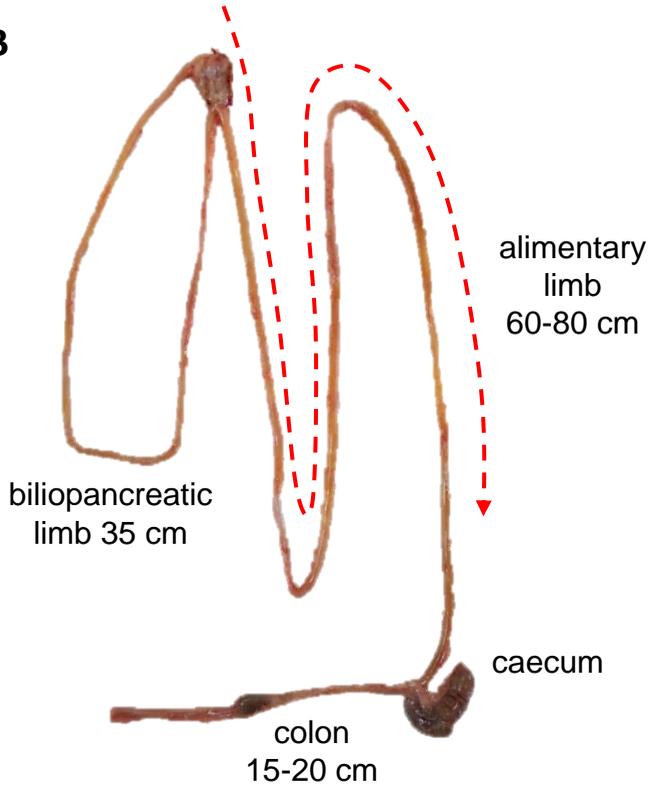
550 Plasma levels of albumin, triglycerides, cholesterol, and non-esterified fatty acids (NEFA) 20
551 days after surgery RYGB (n = 5), MGB (n = 6), sham (n = 7).
552 Results are expressed as the means ± SD. *P < .05, vs sham-operated rats in a Kruskal-Wallis
553 with Dunn's multiple comparison test.

Figure 1

A



B



C



Figure 2

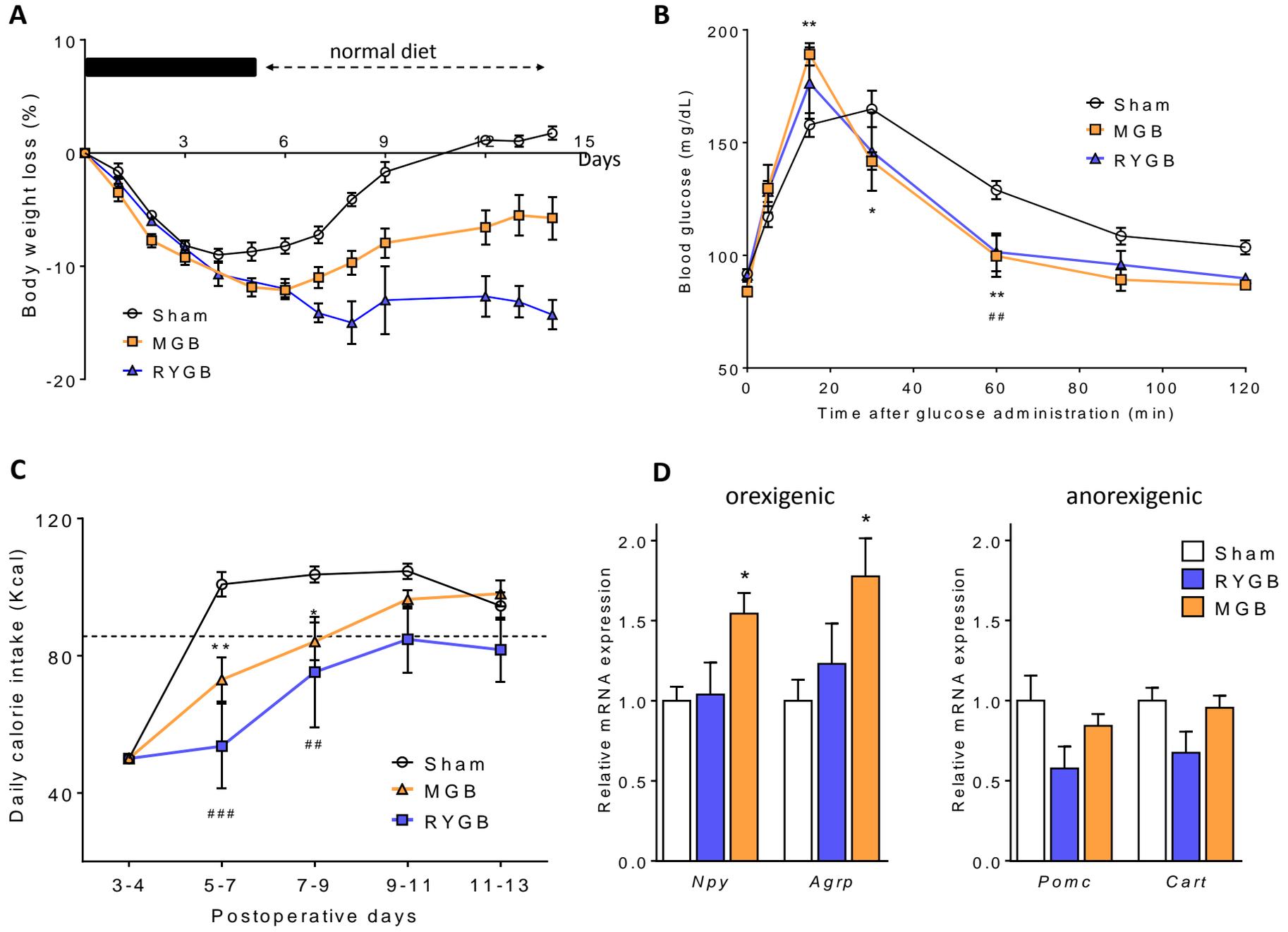


Figure 3

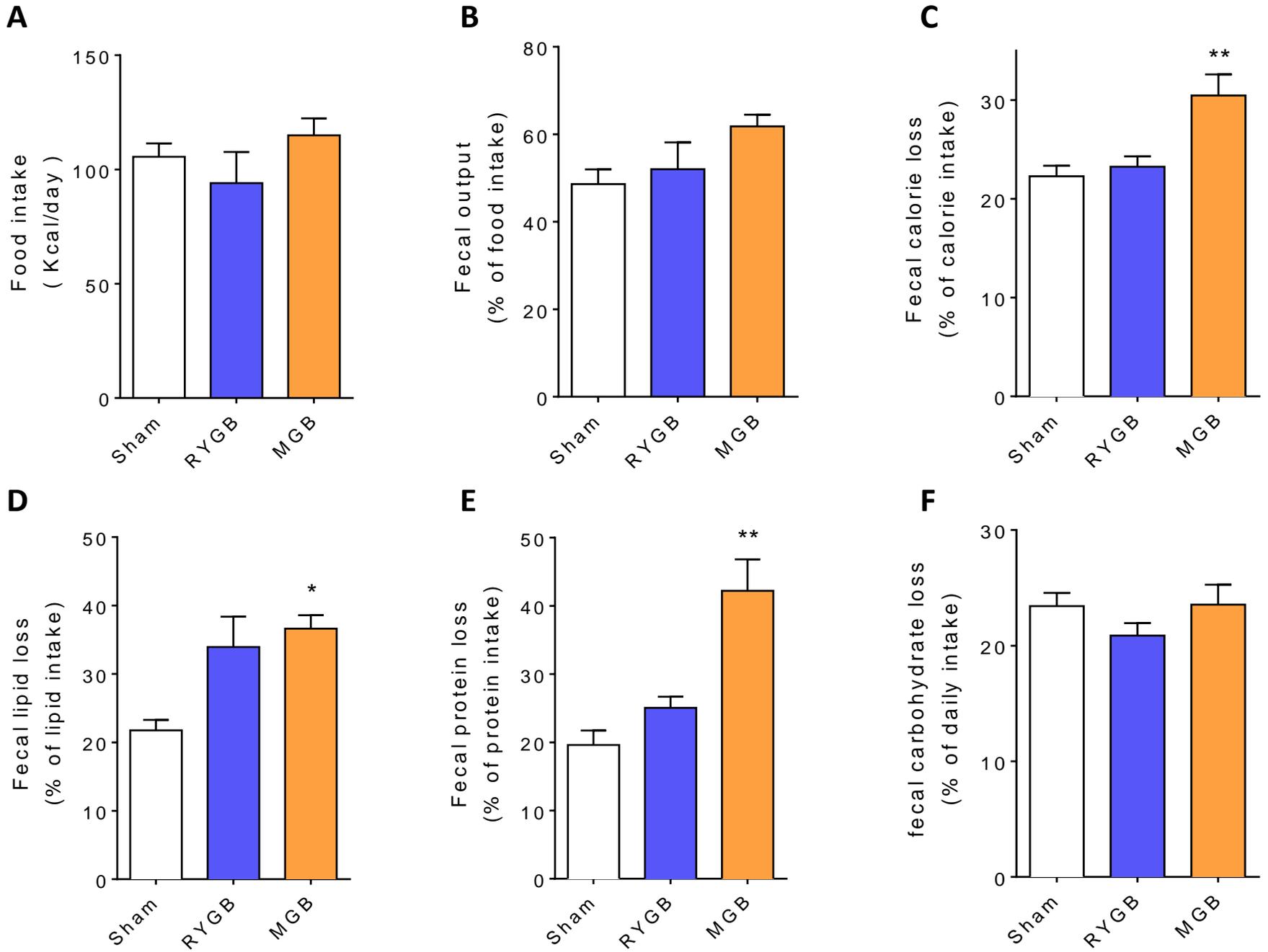


Figure 4

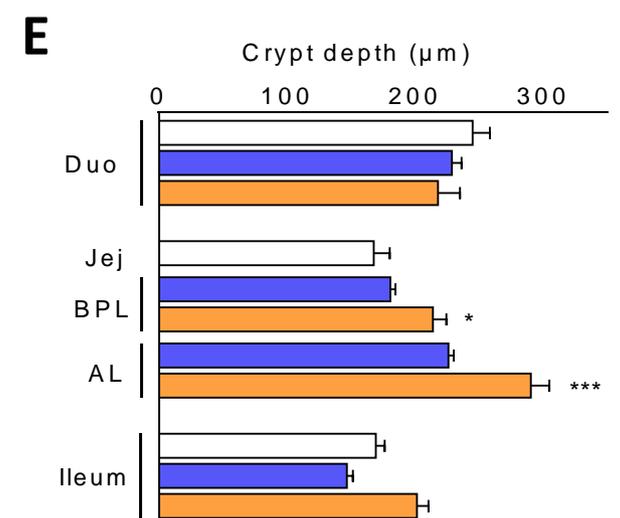
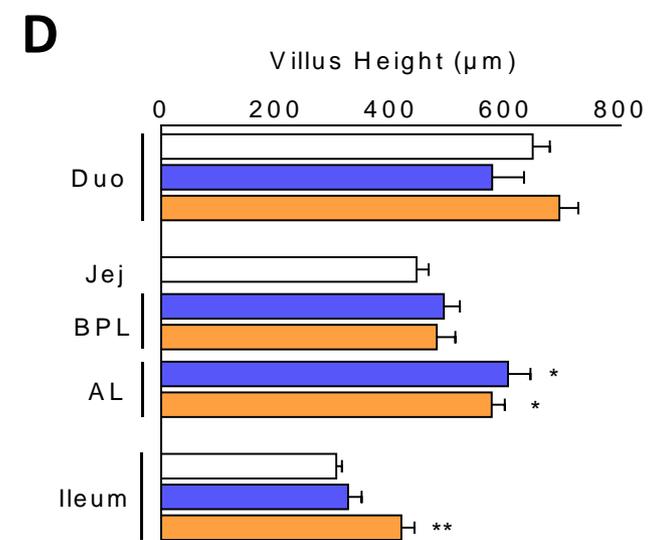
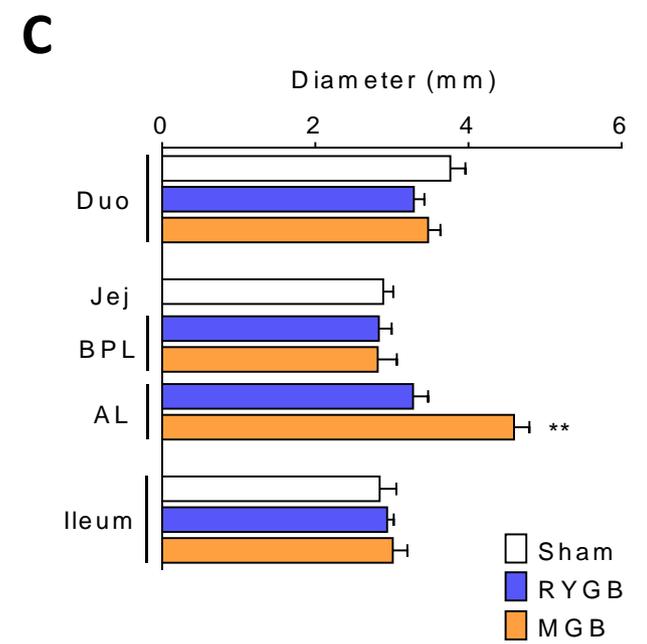
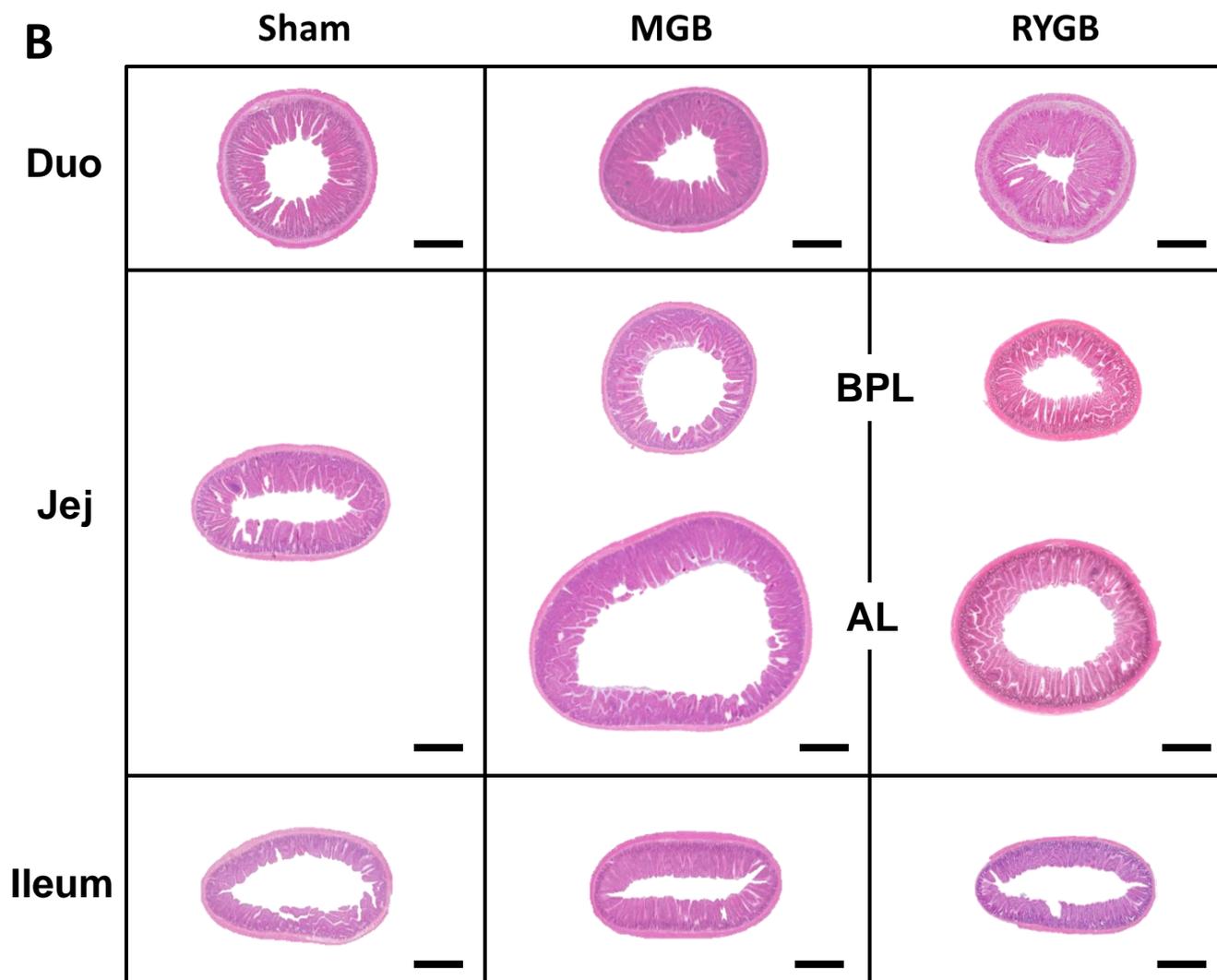
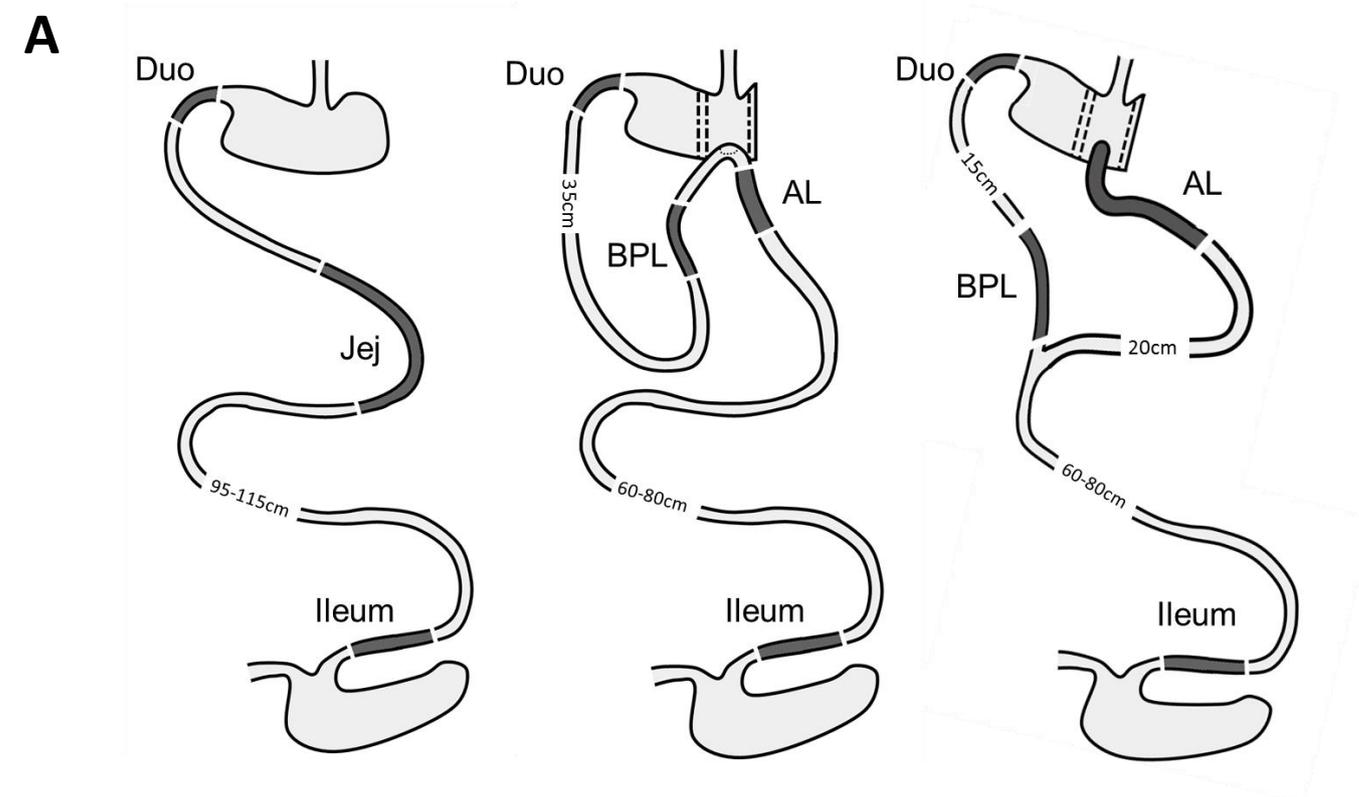
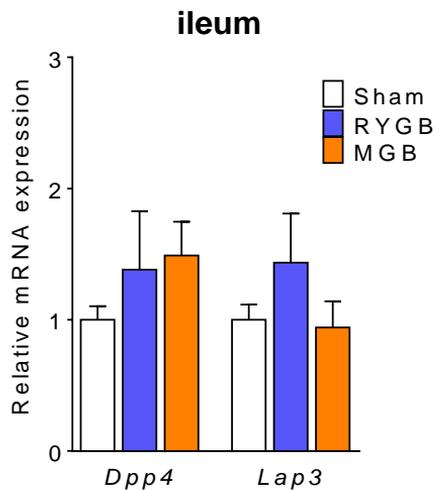
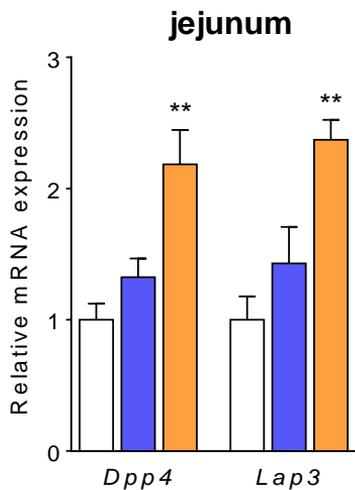
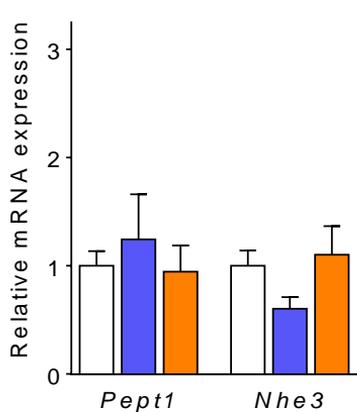
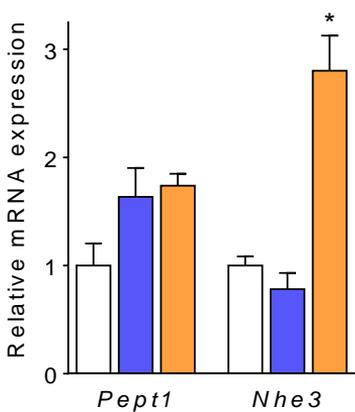


Figure 5

A



B



C

