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A Protocol for Roux-en-Y Gastric Bypass in Rats Using Linear Staplers
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1 **TITLE:**
2 A Protocol for Roux-en-Y Gastric Bypass in Rats Using Linear Staplers
3
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23 **KEYWORDS:**
24 Roux-en-Y gastric bypass, gastric bypass, metabolic surgery, bariatric surgery, rat
25

26 **SUMMARY:**
27 Roux-en-Y gastric bypass (RYGB) is performed to treat obesity and diabetes. However, the
28 mechanisms underlying RYGB's efficacy are not fully understood, and studies are limited by
29 technical difficulty leading to high mortality in animal models. This article provides instructions
30 on how to perform RYGB in rats with high success rates.
31

32 **ABSTRACT:**
33 Roux-en-Y gastric bypass (RYGB) is commonly performed for the treatment of severe obesity and
34 type 2 diabetes. However, the mechanism of weight loss and metabolic changes are not well
35 understood. Multiple factors are thought to play a role, including reduced caloric intake,
36 decreased nutrient absorption, increased satiety, the release of satiety-promoting hormones,
37 shifts in bile acid metabolism, and alterations in the gut microbiota.
38
39 The rat RYGB model presents an ideal framework to study these mechanisms. Prior work on
40 mouse models have had high mortality rates, ranging from 17 to 52%, limiting their adoption. Rat
41 models demonstrate more physiologic reserve to surgical stimulus and are technically easier to
42 adopt as they allow for the use of surgical staplers. One challenge with surgical staplers, however,
43 is that they often leave a large gastric pouch which is not representative of RYGB in humans.
44

45 In this protocol, we present a RYGB protocol in rats that result in a small gastric pouch using
46 surgical staplers. Utilizing two stapler fires which remove the forestomach of the rat, we obtain
47 a smaller gastric pouch similar to that following a typical human RYGB. Surgical stapling also
48 results in better hemostasis than sharp division. Additionally, the forestomach of the rat does not
49 contain any glands and its removal should not alter the physiology of RYGB.
50
51 Weight loss and metabolic changes in the RYGB cohort were significant compared to the sham
52 cohort, with significantly lower glucose tolerance at 14 weeks. Furthermore, this protocol has an
53 excellent survival of 88.9% after RYGB. The skills described in this protocol can be acquired
54 without previous microsurgical experience. Once mastered, this procedure will provide a
55 reproducible tool for studying the mechanisms and effects of RYGB.
56
57 **INTRODUCTION:**
58 Obesity and type 2 diabetes have become worldwide epidemics[1]. Although medical weight loss
59 can improve diabetes in patients, those with severe diabetes benefit most from bariatric surgery.
60 Bariatric surgery has proven to be safe and effective at weight loss and improving or curing type
61 2 diabetes[2,3], even in those with long-standing disease[4]. Metabolic bariatric procedures, such as
62 the current gold-standard Roux-en-Y gastric bypass (RYGB) surgery, induce rapid and sustained
63 improvements in glucose homeostasis while also reducing the need for diabetic medications[5–7].
64
65 After RYGB, glucose homeostasis improvement occurs rapidly and is independent of the weight
66 loss[8]. Two major theories have been proposed to explain the metabolic changes associated with
67 diabetes remission that occur following metabolic surgery. First, the hindgut hypothesis
68 postulates that, after bypass, higher concentrations of undigested nutrients reach the distal
69 intestine enhancing the release of hormones such as GLP-1. On the other hand, the foregut
70 hypothesis suggests that bypassing the proximal intestine reduces the secretion of anti-incretin
71 hormones. Both of these effects could lead to early improvement of glucose metabolism[9].
72
73 Animal models have the potential to be a powerful tool to study these mechanisms. However, a
74 major barrier in utilizing mouse or rat models is the technical difficulty in performing these
75 procedures. Most studies have relied on mouse or rat models[10–12]. Mouse models have been
76 difficult as the mouse stomach is too small to use stapler devices[11], and mortality rates are
77 unacceptably high, ranging from 17 to 52%[13]. In rats, some protocols remain technically difficult
78 to perform due to complex ligation of gastric vessels prior to dividing the stomach[12,14]. Other
79 models divide the stomach using a stapler but leave a large pouch not consistent with the post
80 RYGB human anatomy[11]. In this model, we provide detailed instructions on how to perform RYGB
81 using linear staplers in a rat model resulting in a gastric pouch more in keeping with that of human
82 anatomy. Overall, this procedure was associated with excellent survival rates and metabolic
83 outcomes.
84
85 **PROTOCOL:**
86 Animal use protocols were approved by the Health Science Animal Care and Use Committee at
87 the University of Alberta (AUP00003000). See **Figure 1** for a diagram demonstrating the RYGB
88 anatomy.

1.   Roux-en-Y gastric bypass

1.1.   Preparation of animals and operative setup

1.1.1.   One week prior to the surgery, provide the rats with oral rehydration therapy and liquid diet in addition to their solid diet and water to acclimatize them to this new diet.

1.1.2.   Fast rats with only access to water for 12-18 h prior to the surgery.

1.1.2.1.   Ensure rats are fasted on a raised wire platform so that they cannot consume bedding material.

1.1.3.   Inject rats with subcutaneous long buprenorphine sustained release (SR) at a dose of 1 mg/kg immediately before surgery.

1.1.4.   Autoclave all surgical instruments, towels, and drapes.

1.1.5.   Clean the operating surface, heating pad, and anesthetic nose cone with 70% ethanol.

1.1.6.   Set up the operating surface with an operating microscope, anesthetic machine, and supplies in a manner that is ergonomic for the operating surgeon.

1.1.7.   Use a temperature-regulated heating pad and set to 37 °C.

1.1.8.   Place a sterile drape or towel over the heating pad.

1.1.9.   Fill a 50 mL sterile conical tube with 0.9% saline.

1.2.   Anesthetic induction and preparation

1.2.1.   Induce anesthesia using 4% isoflurane as per previously established protocols[15].

1.2.2.   Apply pressure to the hindfoot of all four limbs to ensure there is no pain response.

1.2.3.   Check for adequate anesthesia and respiratory rate after every 5 min.

1.2.4.   Apply lubricant to both eyes to prevent drying.

1.2.5.   Shave hair from the abdomen.

1.2.6.   Clean the abdomen with a povidone-iodine solution. Allow the solution to dry, and change into sterile gloves.

133   1.2.7. Drape the rat with an opening in the drape to expose the abdomen.

134

135   1.2.8. Place instruments, sutures, cotton swabs, and a 10 mL syringe in a location that permits
136   easy access during the procedure.

137

138   1.3.   Median laparotomy

139

140   1.3.1. Make a 3 cm incision in the upper midline of the abdomen using a scalpel, just below the
141   xyphoid process as a landmark.

142

143   1.3.2. Using scissors, divide the fascia and peritoneum, with care to stay midline on the linea
144   alba to reduce bleeding from the rectus abdominus. If there is bleeding, control it with thermal
145   or electrocautery.

146

147   1.4.   Mobilizing the stomach

148

149   1.4.1. Using two wet cotton swabs, bluntly dissect gastric attachments.

150

151   1.4.2. When encountering dense adhesions, use thermal cautery to divide gastric attachments
152   with care to avoid cauterizing the stomach. Sharply divide the ligament between the stomach
153   and the accessory liver lobe to reduce the risk of liver tearing with stomach mobilization.

154

155   1.4.3. For larger blood vessels, especially at the short gastric arteries, ligate using 6-0
156   polypropylene suture.

157

158   1.4.4. Create a window on the right distal side of the esophagus but proximal to the left gastric
159   artery. Ensure that a cotton swab can reach into this area posteriorly. The stomach is adequately
160   mobilized when it can be exteriorized outside of the abdomen.

161

162   1.5.   Identify and divide the jejunum

163

164   1.5.1. Identify the ligament of Treitz by following the jejunum proximally until observing it is
165   attachment to the transverse mesocolon.

166

167   1.5.2. Measure 7 cm distally, identify a location between mesenteric vessels, and divide the
168   bowel with micro scissors. Avoid Peyer's patches when dividing the bowel. Take care to only
169   divide the bowel and not the mesentery.

170

171   1.5.3. Place a clean, saline soaked sponge prior to dividing the bowel to minimize
172   contamination.

173

174   1.5.4. Check for the presence of a small crossing vessel in the mesentery at the border of the
175   small bowel and divide this with cautery to avoid bleeding.

176

177   1.5.5.   Continue to divide the mesentery 1 cm towards the mesenteric base.

178

179   1.5.6.   Identify the proximal and distal jejunum. Place the proximal jejunum under a wet gauze
180   on the rat's right and the distal jejunum on the rat's left.

181

182   1.6.   Stapling the stomach

183

184   1.6.1.   Insert a 45 mm linear cutting stapler with 3.5 mm staple height across the white line of
185   the forestomach to create a smaller pouch. Wait for 10 s before firing the stapler.

186

187   1.6.2.   Place pressure using gauze on the staple lines for 1 min to ensure hemostasis. If
188   hemostasis is not achieved with pressure alone, bleeding along the staple line is oversewn using
189   6-0 polypropylene figure of eight sutures.

190

191   1.6.3.   Perform a second staple fire across the stomach into the window created previously. Wait
192   for 10 s before firing the stapler. Pressure is held along the staple line to ensure hemostasis, and
193   oversewing may be needed.

194

195   1.7.   Gastrojejunostomy

196

197   1.7.1.   Make a gastrotomy immediately after stapling the stomach. Delays in this can cause
198   gastric distension and aspiration as the stomach is discontinuous after the second gastric staple.

199

200   1.7.2.   Using an 11-blade scalpel, create a gastrotomy at the distal pouch. Express gastric
201   contents through the gastrotomy. This is important to prevent gastric distension and aspiration.
202   Lengthen this gastrotomy using micro scissors to approximately 5 mm. The gastrotomy is made
203   large enough for the cotton swab tip just to fit through.

204

205   1.7.3.   Mobilize the distal end of the jejunum adjacent to the gastrotomy and place such that the
206   mesentery is not twisted.

207

208   1.7.4.   While suturing the anastomosis, ensure that the bowel is kept moist by covering it with
209   saline-soaked gauze and reapplying saline regularly.

210

211   1.7.5.   Using 6-0 polydioxanone or polypropylene suture, place a stay suture at the inferior
212   margin of the anastomosis and gently retract using a snap. Tie with three knots.

213

214   1.7.6.   Place a stay suture at the superior margin of the anastomosis and gently retract using a
215   snap. Tie with six knots.

216

217   1.7.7.   Suture the anterior side of the anastomosis in a continuous fashion, taking bites 1 mm
218   wide and 1 mm apart with care to avoid taking the backside.

219

220   1.7.8.   Once the suture has reached the inferior stay suture, tie these together with an additional

221   six knots.

223   1.7.9.   Once the anterior side is complete, flip the bowel and stomach over and pass the inferior
224   stay suture through the mesenteric defect. Reapply the snap and retract inferiorly.

226   1.7.10. For the posterior side of the anastomosis, place full thickness interrupted 6-0 sutures, 1
227   mm wide, and spaced 1 mm apart, with care to avoid taking the backside. These are tied with six
228   knots each.

230   NOTE: The anterior side of the anastomosis is sutured in a continuous fashion while the posterior
231   side is done in an interrupted fashion. This prevents potential stricture or stenosis associated
232   with a continuous circumferential closure.

234   1.7.11. Check for the leakage by gently pushing luminal contents across the anastomosis. If there
235   are areas with leakage, carefully reinforce them with interrupted sutures. Take care to avoid
236   taking the backwall when reinforcing with extra sutures.

238   1.8.   Jejunojejunostomy

240   1.8.1.   From the gastrojejunostomy, measure 20 cm distally.

242   1.8.2.   Create a jejunotomy on the antimesenteric side using the 11-blade scalpel. Avoid making
243   the jejunotomy over Peyer's patches.

245   1.8.3.   Extend this jejunotomy using micro scissors, such that it is the same size as the
246   biliopancreatic limb. Ensure that a cotton swab just fits inside.

248   1.8.4.   Place the biliopancreatic limb such that there is no twisting of the mesentery.

250   1.8.5.   Perform the anastomosis similarly to the gastrojejunostomy with 6-0 stay sutures on the
251   superior and inferior sides. The anterior side is performed with continuous sutures while the
252   posterior side is performed with interrupted sutures.

254   1.8.6.   Ensure that the bowel is kept moist with saline during this anastomosis.

256   1.8.7.   Check for leakage by gently pushing luminal contents through the anastomosis. If there
257   are areas with leakage, reinforce them with interrupted sutures.

259   1.9.   Reposition the bowel and stomach

261   1.9.1.   Ensure that there is no twisting of the pouch, remnant stomach, or liver. Ensure that the
262   left lobe of the liver is anterior to the stomach and not trapped behind the pouch as this can
263   cause compressive liver ischemia.

264

265   1.9.2. Position the bowel in the abdomen in its natural position such that there is no twisting.

266

267   1.10.   Abdominal closure

268

269   1.10.1. Close the fascia with 3-0 polyglactin in a continuous fashion. 3-0 polydioxanone may also
270   be used.

271

272   1.10.2. Close the skin with 2-0 silk in a continuous fashion.

273

274   1.11.   Anesthetic emergence

275

276   1.11.1. Decrease isoflurane to zero but continue supplemental oxygen.

277

278   1.11.2. Administer a local anesthetic as a splash block to the incision.

279

280   1.11.3. Administer 10 mL of subcutaneous 5% dextrose in normal saline (D5NS) in the
281   subcutaneous tissue behind the neck.

282

283   1.11.4. Place an Elizabethan rat collar before the rat is fully awake. Take care to fit it snugly but
284   not too tight to cause discomfort.

285

286   NOTE: The collar is kept on until day 5 to prevent wound dehiscence.

287

288   **2.   Sham surgery**

289

290   NOTE: Sham surgery is performed similar to RYGB, however, no anastomoses are performed.

291

292   2.1.   A gastrotomy is created and then closed with 6-0 polydioxanone or polypropylene
293   sutures.

294

295   2.2.   A jejunotomy is created 7 cm distal to the ligament of Treitz and then closed with 6-0
296   polydioxanone or polypropylene sutures.

297

298   **3.   Postoperative care**

299

300   3.1.   Postoperative care

301

302   3.1.1. House rats individually and keep them on raised wire platforms until solid food is
303   reintroduced to prevent consumption of bedding and luminal obstruction.

304

305   NOTE: Postoperative diet is resumed gradually as edema at the gastrojejunostomy can cause
306   obstruction with the early resumption of solid diet.

307

308   3.1.2. Inspect the feet daily while rats are on raised wire platforms for any skin changes.

309
310   3.1.3. Keep rats on water and oral rehydration therapy diet for the first 72 h.

311
312   3.1.4. Administer 10 mL of D5NS every 12 h for the first 72 h.

313
314   3.1.5. Administer subcutaneous short-acting buprenorphine at 0.01 mg/kg if rats appear to be
315   in pain. The Rat Grimace Scale is used to assess for pain[16].

316
317   3.1.6. On postoperative day 3, add rodent liquid diet. Continue to provide water and oral
318   rehydration therapy.

319
320   3.1.7. On postoperative day 5, restart high-fat diet. Continue to provide water and liquid diet.
321   Remove the Elizabethan collar.

322
323   3.1.8. On postoperative day 7, discontinue liquid diet.

324
325   3.1.9. Remove skin sutures on postoperative day 10-14.

326
327   **REPRESENTATIVE RESULTS:**

328
329   **Animals and housing**
330   36 male Wistar rats were housed in pairs and were fed 60% sterile rodent high-fat diet starting
331   from six weeks of age (**Figure 2**). At 16 weeks of age, they underwent RYGB or sham surgery.
332   After the first postoperative week, rats were resumed on a high fat diet. Half of the rats were
333   euthanized at 2 weeks post-operative and the other half were euthanized at 14 weeks
334   postoperative.

335
336   **Mortality**
337   Overall, 33 (91.7%) rats survived to the planned study endpoint. All rats undergoing early
338   euthanasia underwent necropsy by a veterinarian. Two rats were euthanized within 24 hours.
339   One RYGB had aspiration pneumonitis and one sham rat had fascial dehiscence with
340   unsalvageable bowel. Another RYGB rat was euthanized at two weeks due to anastomotic leak
341   from the gastrojejunostomy. Overall, 88.9% of RYGB rats survived to study endpoint.

342
343   **Body Weight**
344   Rats undergoing RYGB had a lower postoperative weight than sham rats. **Figure 3** demonstrates
345   absolute weights for rats postoperatively while **Figure 4** demonstrates postoperative percentage
346   weight change which was statistically significant at all timepoints postoperatively. At 14 weeks,
347   rats who had RYGB had a mean percentage weight change of 6.4% while rats with sham surgery
348   had 23.7% (p = 0.0001).

349
350   **Intraperitoneal glucose tolerance testing**
351   Fasting blood glucose was not significantly different between any of the cohorts. However, the
352   area under the curve was significantly lower in RYGB compared with sham at 13 weeks (18.1 vs

353 23.8 mmol-h/L, p=0.046, **Figure 5**) but was the same for RYGB vs sham at 1 week (20.8 vs 23.3
354 mmol-h/L, p=0.68).

356 **FIGURE AND TABLE LEGENDS:**

358 **Figure 1: Roux-en-Y gastric bypass anatomy**

360 **Figure 2: Preoperative absolute weight on high fat diet; RYGB, Roux-en-Y gastric bypass**

362 **Figure 3: Postoperative absolute weight on high-fat diet; RYGB, Roux-en-Y gastric bypass**

364 **Figure 4: Postoperative percentage weight change on high-fat diet; RYGB, Roux-en-Y gastric**
365 **bypass**

367 **Figure 5: Intraperitoneal glucose tolerance testing in gastric bypass vs sham at 13 weeks. RYGB,**
368 **Roux-en-Y gastric bypass**

370 **DISCUSSION:**
371 RYGB involves the creation of a small gastric pouch (less than 30 mL), and the creation of a
372 biliopancreatic limb and a Roux limb (Error! Reference source not found.). In humans, the
373 biliopancreatic limb is typically 30 to 50 cm and transports secretions from the gastric remnant,
374 liver, and pancreas. The Roux limb is typically 75 to 150 cm in length and is the primary channel
375 for ingested food. The common channel is the remaining small bowel distal to where the two
376 limbs join and is where the majority of digestion and absorption occur, as pancreatic enzymes
377 and bile mix with ingested food[17].

379 The mechanism of weight loss in RYGB is multimodal. The small gastric pouch reduces food intake
380 through mechanical restriction. The bypass results in a malabsorptive component as a significant
381 portion of the small intestine is not absorbing calories and nutrients. More recently, studies have
382 demonstrated that gut hormones play a significant role in weight loss after RYGB as well. These
383 are primarily through ghrelin, peptide-YY, cholecystokinin (CCK), and GLP-1 hormone pathways[18].

385 Rat models provide a powerful method to study the mechanisms behind both the weight and
386 metabolic effects of RYGB. In this paper, we present a RYGB protocol that has low mortality with
387 significant weight loss and metabolic effects. Once the operator became familiar with the
388 technique, the procedure took approximately 90 minutes to perform. The protocol can also be
389 modified with longer biliopancreatic and Roux limb lengths to potentially increase weight loss
390 and metabolic effect. Furthermore, it is technically more feasible than other models as it allows
391 the use of surgical staplers to achieve hemostasis and minimize operative time. Models that rely
392 on sharp division of the stomach without stapling often result in higher mortality due to
393 significant blood loss. The technical skills required to perform the procedure were relatively easy
394 to acquire, and learners were able to comfortably perform the procedure after approximately
395 five to ten non-recovery procedures.

397 One of the critical steps of this protocol is to limit blood loss during mobilization of the stomach.
398 Careful use of thermal cautery combined with suture ligation of vessels is important. It is also
399 important to perform at least half the circumference of the anastomoses in an interrupted
400 manner. This prevents excessive stricturing at the anastomoses. Furthermore, checking for leaks
401 is crucial as these can lead to sepsis and death. Prior to closing the abdomen, it is essential that
402 the left lobe of the liver is placed in its natural, anterior position and that there is no rotation in
403 the bowel or the stomach as this can lead to visceral ischemia.

405 Postoperative care is vital to this protocol. Raised wire platforms are required during both fasting
406 and postoperative periods as the consumption of solid material leads to anastomotic
407 obstructions. It is vitally important to provide subcutaneous fluid as the rats may not tolerate
408 oral fluids in the immediate postoperative period. The rats should be acclimatized to oral
409 rehydration therapy and liquid diet as rats may avoid new diets due to associations with
410 postoperative pain. This dietary protocol contributes to significant weight loss in the immediate
411 postoperative period in both the RYGB and sham cohorts, and weight recovery in the sham group
412 took about five weeks. However, strict adherence to this postoperative protocol is vital to reduce
413 morbidity and mortality after RYGB. Additionally, frequent examination of the rats using the Rat
414 Grimace Scale is important to detect for morbidity. In our study, one rat developed a late
415 anastomotic leak which was rapidly detected using this scale and allowed for early euthanasia to
416 reduce suffering.

418 One of the advantages of this method is that it results in a smaller pouch through the use of
419 surgical staplers to reduce gastric bleeding. When we attempted to sharply divide the stomach
420 without staplers, it leads to excessive bleeding and a much higher mortality rate. However, this
421 also leads to the removal of the forestomach, and this may lead to physiologic changes that are
422 different from that of human RYGB. However, the forestomach is unique to rodents and contains
423 no glands, and should not cause any changes to gut hormones.

425 The most important limitation of this method is that it requires two surgical stapler reloads per
426 rat, which can be costly. However, excellent survival outcomes potentially reduce cost by
427 requiring less rats for a study, resulting in better utilization of husbandry facilities, surgical
428 equipment and research personnel.

437 **DISCLOSURES:**
438 Ethicon supplied two 45 mm linear cutting staplers, multiple 3.5 mm stapler reloads, and 6-0
439 polypropylene sutures. Authors have no other conflicts of interest to declare.
440

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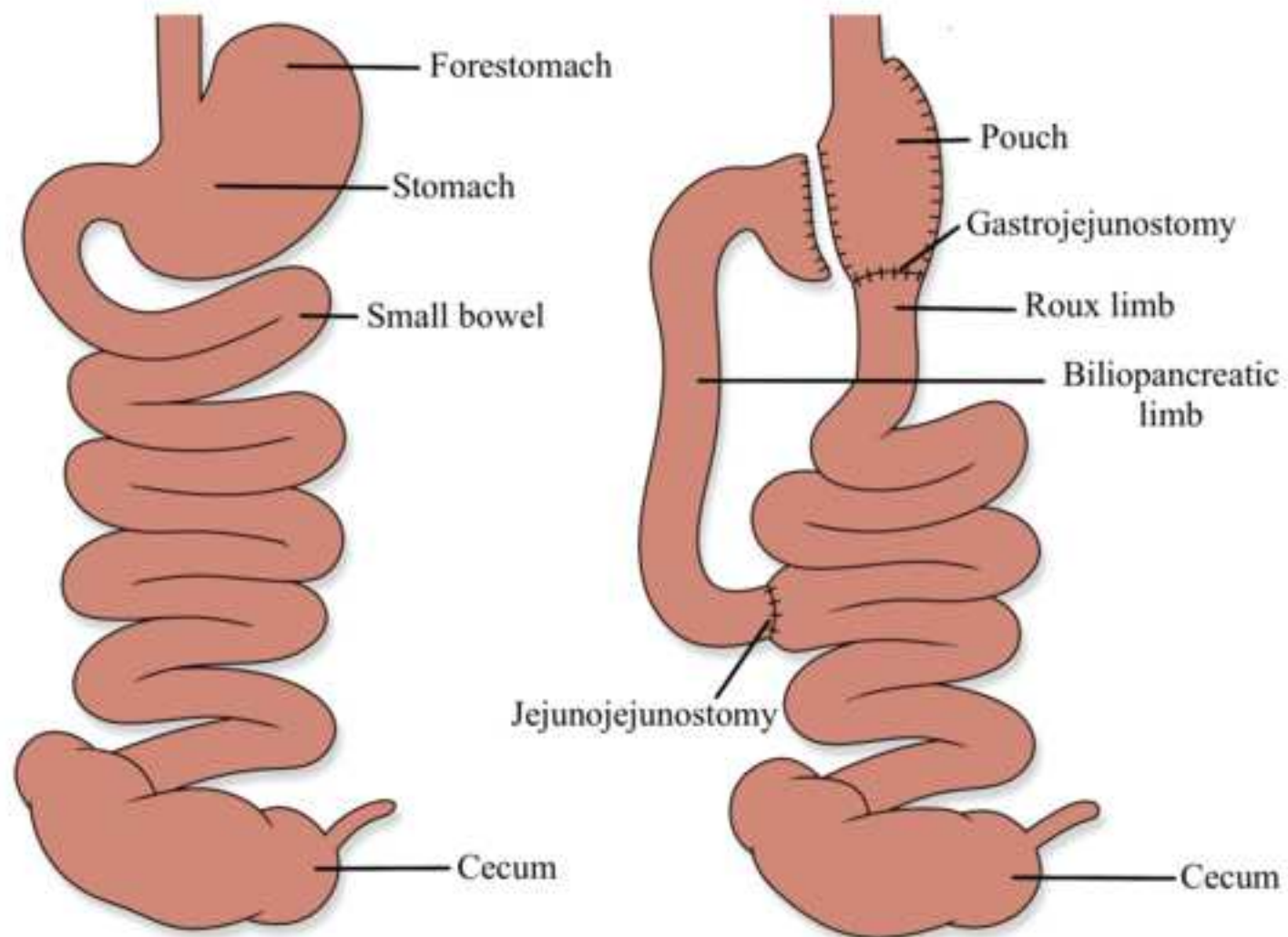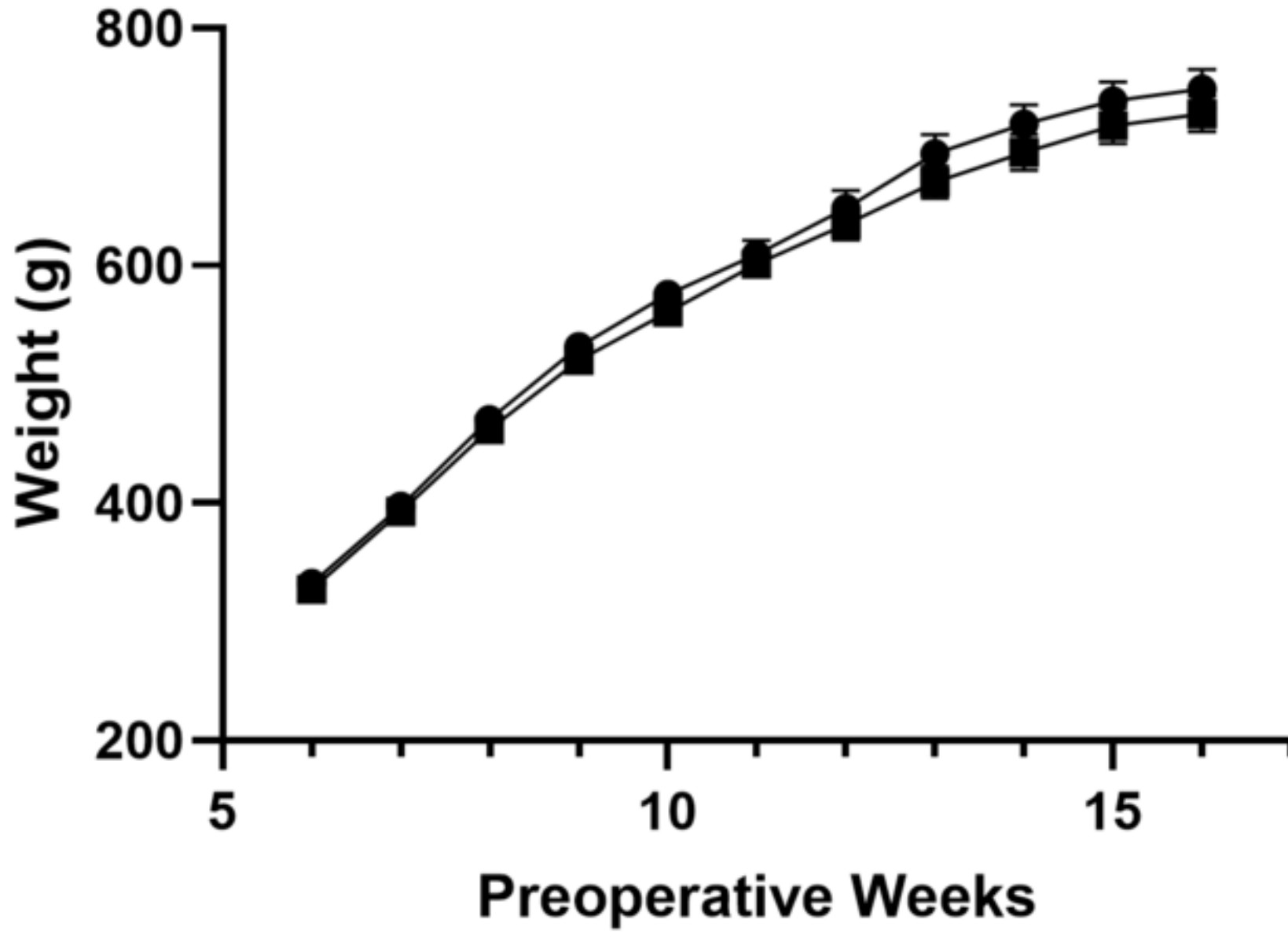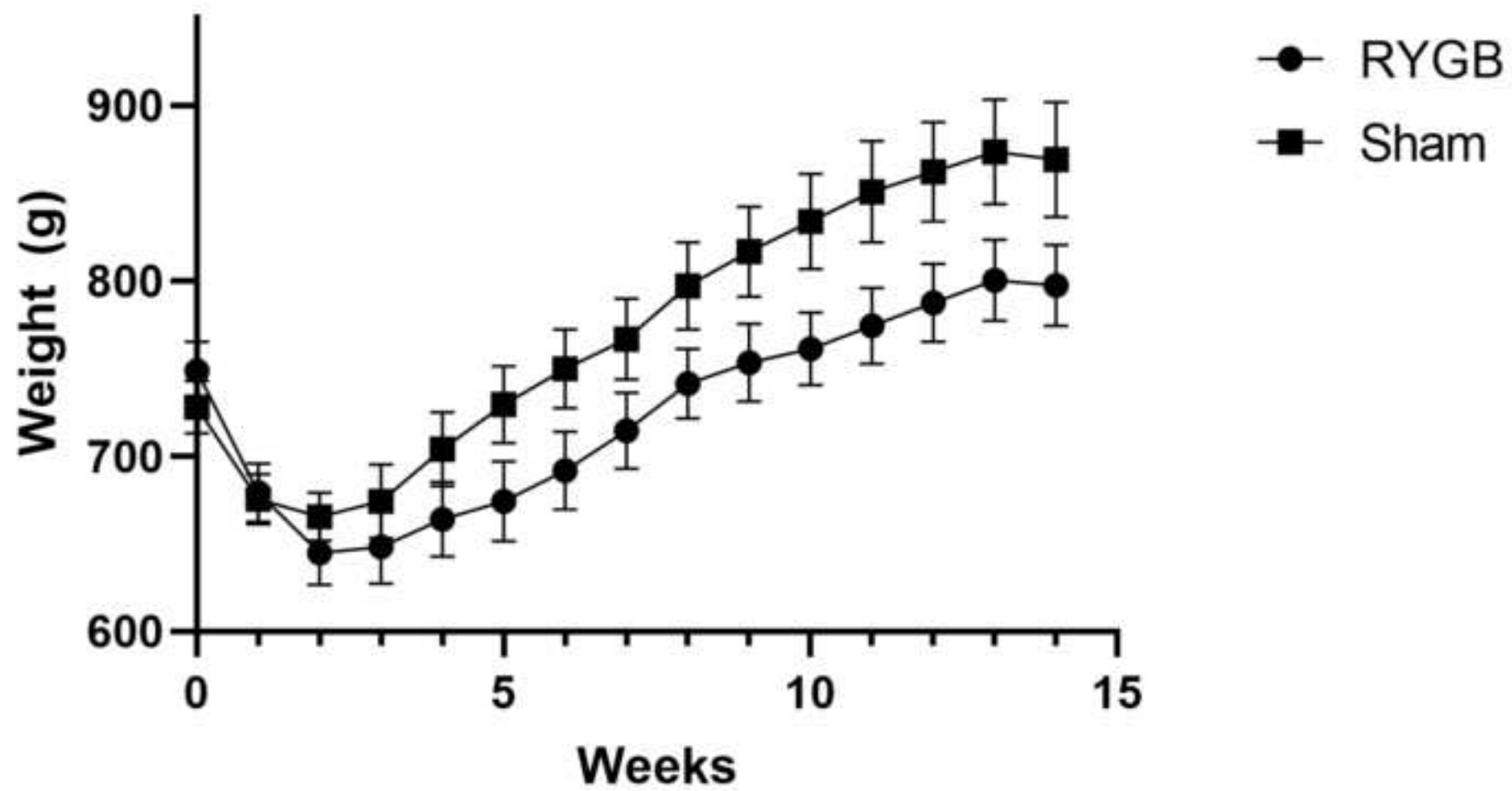
Figure 1

Figure 1

Figure 2

Figure 3

Figure 4

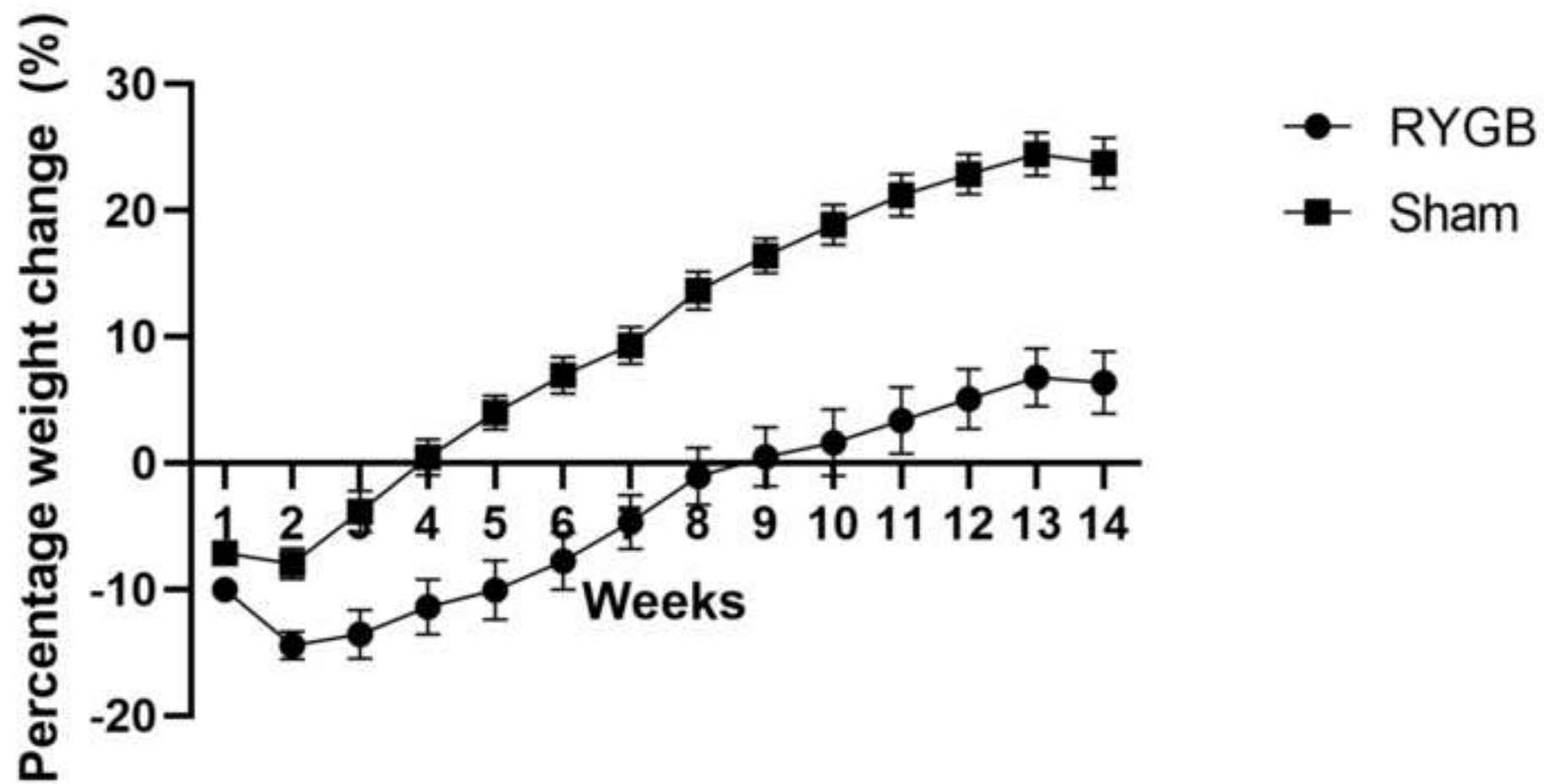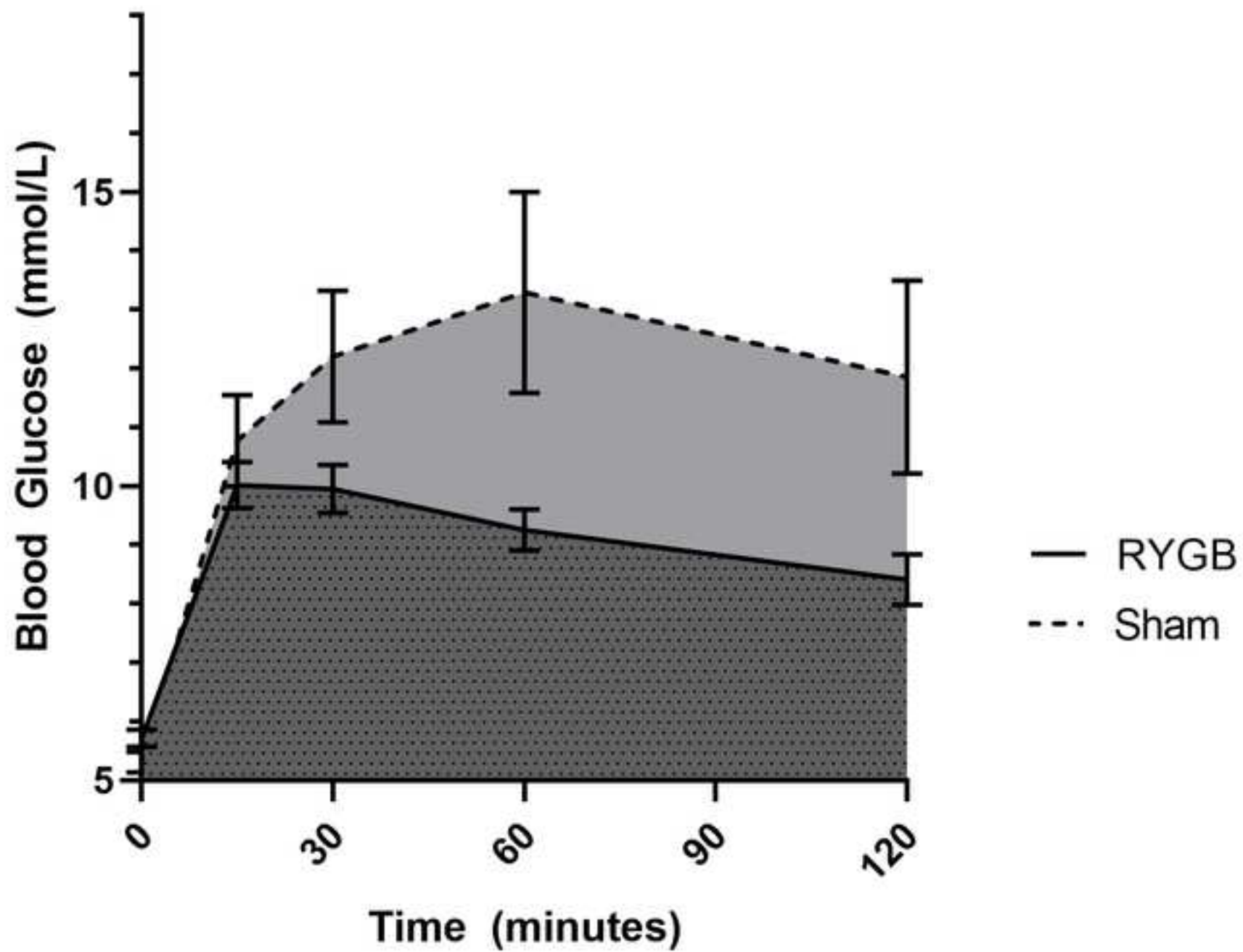Click here to access/download;Figure;Figure 4.tif ⬇

Figure 5

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Table of Materials
JoVE_Materials.xls

Thank you for the valuable feedback from the Editor. We have made all changes as requested.

Editorial and production comments:
Manuscript:
1. The editor has formatted the manuscript to match the journal's style. Please retain and used the attached file for revision.

We have revised this version of the manuscript.

2. Please address specific comments marked in the manuscript.

All comments in the manuscript have been addressed in the manuscript.
3. Please proofread the manuscript well.

The manuscript has been proofread by all authors.
4. Please sort the materials table in alphabetical order and remove copyright or trademark symbol if any.

We have removed copyright and trademark symbols and the list is sorted in alphabetical order.

Video:
1. Please include figure 1 somewhere in the video.

We have added Figure 1 to the end of the video.
2. Not all steps need to be shown in the video as long as the video is a cohesive story.

We have removed steps unnecessary steps.
3. Please remove the substep numbering from the video. (1.2, 1.3, etc.)

All substep numbering has been removed.
4. Title Cards:
• Please capitalize the first letter of every important word in your chapter title cards.

We have capitalized first letters.
5. Video Editing Content:
• Please reduce the duration of the video by removing some content that is not necessary for the video.
• Rather using text or title cards all over the video to explain the content. Consider using video or images which will be easier to explain the content to the audience.

We have removed all unnecessary parts of the video
6. Audio Editing and Pacing:
• Audio levels are very high. Please ensure audio level peaks average around -9 dB.

We have adjusted our audio levels.
• Please start the narration after the Main title i.e 00:10

The narration now starts after the main title.