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Surgical Models of Roux-en-Y Gastric Bypass Surgery and Sleeve Gastrectomy in Rats and Mice

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Abstract

Bariatric surgery is the only definitive solution currently available for the present obesity pandemic. These operations typically involve reconfiguration of gastrointestinal tract anatomy and impose profound metabolic and physiological benefits, such as substantially reducing body weight and ameliorating type II diabetes. Therefore, animal models of these surgeries offer unique and exciting opportunities to delineate the underlying mechanisms that contribute to the resolution of obesity and diabetes. Here we describe a standardized procedure for mouse and rat models of Roux-en-Y gastric bypass (80–90 minutes operative time) and sleeve gastrectomy (30–45 minutes operative time), which, to a high degree resemble operations in human. We also provide detailed protocols for both pre- and post-operative techniques that ensure a high success rate in the operations. These protocols provide the opportunity to mechanistically investigate the systemic effects of the surgical interventions, such as regulation of body weight, glucose homeostasis, and gut microbiome.

INTRODUCTION

The worldwide pandemics of obesity and diabetes are devastating in severity, extent, and rate of growth. Over two billion adults worldwide – 30% of the population - are either overweight (body mass index (BMI) > 25) or obese (BMI > 30)¹. In the United States alone, the prevalence of obesity has increased from 15% in 1980 to nearly 34% in 2008². Obese patients have substantially increased morbidity and mortality from obesity-related complications including type 2 diabetes mellitus (T2DM), cardiovascular disease, and several types of cancer³. For instance, approximately 25 million adults in the United States suffer from obesity-associated insulin resistance and T2DM⁴. In addition to obesity in the adult population, the rate of obesity amongst children and adolescents in the USA has doubled during the past two decades and currently 17% of children aged 2–19 years are obese⁵. If this trend continues, obesity and its associated co-morbidities threaten the health

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AUTHOR CONTRIBUTIONS STATEMENTS

N.S. developed and performed the operations. B.G.B. provided figures. N.S. and B.G. wrote the manuscript. N.S. and M.L.Y. contributed to the conceptual development.

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of future generations. Given the severity of the obesity pandemic, there is an urgent need for detailed understanding of the mechanisms underlying pathophysiology of obesity to enable the development of effective therapy.

In the absence of any effective non-invasive treatments, bariatric surgery remains the only effective option that can lead to long-term sustained weight loss^{6–8}. Roux-en-Y gastric bypass (RYGB), which is the gold standard of bariatric surgery, results in up to 40% reduction in total body weight⁶ and amelioration of a wide range of obesity-related co-morbidities, including T2DM in over 80% of patients^{9–12}. In RYGB (Fig. 1a), the stomach is divided into two sections, creating a small gastric pouch (about 1–2% of total gastric volume)^{13,14}. The small intestine is also divided and rearranged to create a Y-shaped anatomy, causing the food to bypass the larger section of the stomach and upper intestine, greatly restricting stomach capacity and effective intestine length^{13–15}. Although development of laparoscopic methods has substantially reduced the mortality and complications associated with RYGB, it is still a complex operation that can lead to post-operative complications (about 0.5% mortality rates)¹⁶ after which patients require intensive post-operative care and life-long nutritional management¹⁷. Consequently, substantial efforts have focused on the development of less-invasive surgical alternatives, which can induce metabolic benefits comparable to those of RYGB. Sleeve gastrectomy (SGx) is currently the most prevalent surgical alternative to RYGB. In SGx, a substantial volume of the stomach is excised (about 80%) to create a narrow gastric sleeve, while maintaining the remainder of the gastrointestinal anatomy intact (Fig. 1b)^{13,14}. Long-term follow-up studies have demonstrated that SGx is effective in regulating body weight and ameliorating T2DM^{18,19}. Intriguingly, despite the higher technical difficulty of RYGB, there are no substantial differences in the long-term morbidity and mortality rates of RYGB and SGx²⁰.

Considering the profound effects of these operations on energy homeostasis, they represent invaluable experimental models for scientific investigations of the pathogenetic mechanisms underlying obesity and T2DM. Additionally, elucidating the molecular mechanisms underlying the therapeutic effects of these operations hold the promise of identifying new therapeutic targets. Furthermore, emerging evidence suggests that while the immediate metabolic benefits of RYGB and SGx appear to be comparable, RYGB induces more sustained long-term effects^{11,20}. Therefore, the two procedures present ideal models to study the effect of different segments of the intestine on the regulation of body weight and glucose homeostasis.

Due to practical and ethical challenges, performing studies on human subjects that underwent surgery can be difficult. Therefore, animal models of bariatric surgery are essential for complete understanding of the mechanisms that lead to regulation of body weight and blood glucose following these operations. Indeed, we, and several other groups have developed rodent models of bariatric surgery and employed them to characterize the physiological effects of the operations^{21–29}. However, the absence of a standardized technique often makes it difficult to compare the results of investigations by different research groups, and differing results have been seen, for example, contradictory results of food preference following SGx^{26,30} and the degree of weight loss following SGx^{26,31}. Furthermore, these techniques are often complex – particularly to non-physician scientists –

and lead to marginal surgical robustness. To overcome these obstacles we have developed simple techniques for rat and mouse models of both RYGB and SGx where they closely simulate the human procedures and recapitulate many of the physiological effects of human operations (please see "Experimental Design" section below for more information)^{15,26,32}. We have successfully employed these models to elucidate the role of the intestinal tissue in the regulation of energy expenditure²⁶ and diabetes resolution³². The protocols provided here are aimed to provide simple and standardized techniques for both the rodent models of RYGB and SGx that can be easily followed by scientists of different backgrounds.

Experimental Design

Experimental and Control Groups

Bariatric surgery leads to reduction in both food intake (especially in the immediate post-operative days) and body weight. Therefore, a well-designed study should control for the effects of both of these factors. Our studies usually contain the following 4 experimental groups: bariatric surgery group, sham surgery-operated group, pair-fed group, and weight-matched group. In the pair-fed group, the animals undergo the sham operation and the amount of food they receive is matched to the amount of food consumed in the surgical group on the previous day. Weight-matched animals will also be calorie restricted such that on average they reach the same body weight as the surgical animals.

Animal Randomization

There is always individual variability in the body weight and blood glucose of animals following the development of obesity. Specifically, we occasionally observe large variations in the body weight of Sprague Dawley rats. Therefore, to exclude other potential confounding factors (such as relatively high or low energy expenditure) in our lab we exclude animals with body weights greater than 15% deviation from the mean body weight of each cohort (please note that this number is selected based on our own experience and can be adjusted depending on the type of the study and the strain of the animals). Furthermore, depending on the type of study, animals can be randomized based on their body weight (e.g. for adipose tissue related studies) or their fasted blood glucose (e.g. for beta-cell related studies).

Pre-operative Acclimation

Rodents are highly sensitive animals and so the sudden introduction of new procedures can trigger stress responses which may interfere with the outcomes of the study. Therefore, it is critical to acclimate the animals to any changes that may be introduced following the surgery. In particular, it is necessary to acclimate the animals to a liquid diet and blood glucose measurements prior the surgery. If animals are suddenly provided with the liquid diet after surgery they may associate it with the pain caused by the surgery and avoid consumption. Furthermore, measurements of blood glucose, which are often used to study post-operative glucose homeostasis³², involve puncturing the tip of the sensitive tail and collecting blood from the tail vein. If not acclimated, the stress response to this process results in rapid rise in the blood glucose level. To acclimate to the blood glucose collection, the glucose tolerance test should be performed approximately 4 weeks prior to the surgery

and repeated once or twice before the operation. Liquid diet acclimation should be performed 1 or 2 weeks prior to surgery by removing the high fat diet and providing liquid diet for 48 hours. A detailed timeline of the pre-operative procedures is provided in Figure 2.

Surgery

The primary goal of this article is to provide a simple and streamlined protocol that can also be easily adopted by scientists without any medical or surgical background. This necessitates a slight modification of the RYGB operation to simplify the surgical procedure, which consequently causes modest deviations from the human operation. Most notable is the use of surgical stapler (in rats) and clip (in mice) to create the gastric pouch instead of transecting the stomach and hand-sewing the gastro-jejunal anastomosis. The key advantages of the stapler or clip technique are the ease of the method, which consequently leads to a substantially shorter operative time. On the other hand, this technique leads to generally larger pouch size compared to those in human (i.e. approximately 10% of the total stomach volume in rodents compared to about 1–2% in human). Based on our experience, the size of the gastric pouch does not significantly influence the metabolic and physiologic effects of RYGB (unpublished observation). However, the bigger pouch could lead to accumulation of solid food in the more stretchable fundus, potentially causing post-operative complications. The thorough post-operative care provided in this protocol should be followed to prevent these complications. A detailed timeline of the post-operative procedures is provided in Figure 2. An alternative method to the surgical staple (or clip) is transecting the stomach using surgical scissors and using a hand-sewn anastomosis to repair the distal stomach and connect the gastric pouch to the jejunum^{15,28,29}. This method provides significant control over the size of the gastric pouch and allows creation of pouches as small as 5% stomach volume. However, it is a more complicated technique and requires expertise with microsurgical techniques. The major complication of this technique is injury of the gastric artery leading to excessive bleeding, which could substantially increase mortality rates³³. Using the protocols we provide here we have achieved mortality rates of approximately 5–8% for RYGB and 3–5% for SGx.

MATERIALS

REAGENTS

- **Animals:** We have successfully performed RYGB and SGx operations on diet-induced obese (DIO) Sprague Dawley rats and C57BL/6 mice. However, other strains of rats and mice can be used (e.g., Zucker Diabetic Fatty (ZDF) rats, Goto-Kazaki (GK) rats, and normal lean animals). To induce obesity, the animals should be placed on a high-fat diet for a duration of 12–16 weeks, immediately after weaning. In our lab we consider DIO if the animal weighs 25% more than its age matched chow-fed counterparts (please see (Hariri et al, 2010) for a detailed discussion³⁴).

! CAUTION All experiments involving animals must conform to relevant institutional and governmental regulations. This protocol was approved by the

Institutional Animal Care and Use Committee (IACUC) of the Massachusetts General Hospital.

- Meloxicam
- Ciprofloxacin (Cipro)
- Isoflurane, 1.5–2% (wt/vol) in oxygen

! CAUTION Isoflurane is harmful if inhaled and swallowed. It may cause nausea, vomiting, nose/throat/respiratory irritation, headache, drowsiness, and skin irritation. Wear gloves and long sleeves to avoid skin contact. Carbon filters should be used to scavenge waste anesthetic gas.

- Sterile saline (0.9% NaCl solution, Baxter, cat. No. FE1323D)
- Pedialyte (Oral electrolyte maintenance solution, Abbott Nutrition, cat. No. 00336)
- Glucose (Dextrose solution, Hospira, cat. No. RL-3040)
- 100% oxygen tank (Airgas, Cat. No. OX USP200)
- Iodophor solution
- High fat diet (HFD, 60% kcal% fat, Research Diet Inc., cat. No. D12492)
- Liquid diet (Vital HN Vanilla, Abbott Nutrition, cat. No. 00766)

EQUIPMENT

- Sterile surgical gloves (Med-Vet International, cat. No. 7821 to 7828)
- Animal hair clipper (Med-Vet International, cat. No. 9757-300)
- Isoflurane anesthesia system equipped with an anesthesia induction chamber (World Precision Instruments, cat. No. EZ-B800)
- Alcohol swabs (Med-Vet International, cat. No. APREP)
- Drapes (Sterile Surgical Drapes, Med-Vet International, cat. No. DR1826)
- #11 surgical scalpel (World Precision Instruments, cat. No. 500240)
- Rat retractor (Weitlaner Retractor, 10.8 cm, World Precision Instruments, cat. No. 501314)
- Mouse retractor (Barraquer Retractor, World Precision Instruments, cat. No. 500369-G)
- Forceps (Adson-Brown Forceps, 12 cm, World Precision Instruments, cat. No. 500177-G)
- Scissors (Tenotomy Scissors, SuperCut, 10 cm, World Precision Instruments, cat. No. 14395)
- Spring scissors (14 cm curved, World Precision Instruments, cat. No. 14112)

- Needle holder (Castroviejo Needle Holder with lock, 14 cm long, World Precision Instruments, cat. No. 14137)
- Needle holder (Halsey Needle Holder, 14 cm, World Precision Instruments, cat. No. 14110-G)
- Basin (Emesis Basin, 12 oz., Ambler Surgical, cat. No. 87-102)
- Surgical Stapler (Echelon Flex™ Endocutters, 45 mm, Ethicon, cat. No. SC45A)
- Surgical clip and applier (Ethicon cat. No. LT400 and LC410)
- Gauze pads (Sterile Gauze Sponges, 2" × 2", Med-Vet International, cat. No. 224STRL)
- Cotton swabs (Sterile Cotton Tipped Applicators, 6", Med-Vet International, cat. No. MDS202010Z)
- Cautery device (World Precision Instruments, cat. No. 500392)
- Ruler/measuring tape (Med-Vet International, cat. No. P60003)
- 4-0 Silk suture (Ethicon, cat. No. K881H)
- 6-0 Silk suture (Ethicon, cat. No. K801H)
- 6-0 Prolene suture (Ethicon, cat. No. 8806H)
- 8-0 Prolene suture (Ethicon, cat. No. 8730H)
- Heating lamp and heating pad
- Rodent cages with elevated platform

REAGENT SETUP

Saline Warm up the saline solution to 37 °C before the start of the surgical operations.

EQUIPMENT SETUP

Sterile operating field Autoclave or bead sterilize instruments prior to use. Prepare an aseptic operating field by covering the operation table with a sterile drape. Place the sterile surgical instruments and a heating pad on the table. Turn on the heat pad and set the temperature to 37 °C. Cover the heating pad with sterile drape. Set up the rodent anesthesia machine.

PROCEDURE

General Pre-operative Preparation

CRITICAL Animals must have been fully acclimatized to the various changes in procedures as discussed in “Experimental Design” section.

- 1 Fast the animals overnight before the operation, removing food but leaving *ad libitum* access to water.

- 2 Induce anesthesia by transferring the animal to the anesthesia induction chamber (3–4% isoflurane and oxygen flow of 1–2 l/min).
- 3 Once the animal loses consciousness, remove the animal from the induction chamber and depilate the abdomen from sternum to groin using an electric hair clipper.
- 4 Place the animal on the heating pad in dorsal recumbency with the tail toward the surgeon (Fig. 3a).
- 5 Place the nozzle of the anesthesia system over the animal's snout and set it to 2–3% isoflurane and oxygen flow of 1–2 l/min.

CRITICAL STEP Frequently monitor the depth of anesthesia by assessing the respiration rate and tail pinch. Increase the isoflurane if rapid respiration or movement is observed. Reduce the isoflurane if breathing is too slow.

- 6 Administer ciprofloxacin (0.1 mg/kg; IP injection) 10 minutes prior to the initiation of the surgical procedure.
- 7 Disinfect the skin by scrubbing with an aqueous iodophor solution followed by 70% alcohol (Fig. 3b). Cover the entire animal with a sterile surgical drape.
- 8 Put on sterile surgical gloves.
- 9 Create an opening in the drape to expose the surgical field (Fig. 3c).

Surgical Procedures

- 10 If you wish to perform a Roux-en-Y Gastric Bypass (RYGH) follow option A for rat or option B for mouse. Alternatively, perform a Sleeve Gastrectomy following option C for rat or option D for mouse. Perform appropriate sham operations on matched control animals, following option E for RYGB and option F for sleeve gastrectomy.

Option A) Rat Roux-en-Y Gastric Bypass Surgical Procedure

- i. A video presentation of this operation is provided in Supplementary Video S1. Identify the xiphoid process (the cartilage at the lower end of the breastbone; top of the dashed line in Fig. 3a) and make a midline abdominal incision of 4 to 5 cm length using a #11 surgical scalpel from the xiphoid process down, through the skin (Fig. 3d).
- ii. Using the forceps lift the muscle and use the scissors to make a midline incision (the same size as in previous step) through the abdominal muscle (Fig. 3e).

CRITICAL STEP Carefully inspect the incision path before each cut to avoid damaging internal organs.

SUPPLEMENTARY FILE LEGENDS

Video S1. Video presentation of high-resolution procedural images for RYGB in the rat.

Video S2. Video presentation of high-resolution procedural images for SGx in the rat.

- iii. Place a self-retaining retractor to expose the abdomen (Fig. 3f).
- iv. Externalize the intestine and measure the entire length of the small intestine (Fig. 3g) to determine the length of the Roux limb (RL) and the biliopancreatic limb (BP), each limb measures about 10–15% of total length of the small intestine. The total length of rat small intestine is approximately of 85–95 cm. Please note that the length of the Roux-limb can be adjusted to mimic short and long limb RYGB surgery.

CRITICAL STEP Keep the tissues hydrated by using warm saline-soaked gauze to cover the segments that are outside the body. Regularly add saline to wet the tissue or gauze.

- v. Starting at the pylorus (i.e. the junction between stomach and small intestine), measure the 10–15% length of the intestine and place two ligations (~ 5 mm apart) on the intestine at that position using a 4-0 silk suture (Fig. 3h).

CRITICAL STEP Handle the intestine very gently and avoid using surgical instruments. Cotton swabs moistened in saline are ideal for gentle handling of the intestine.

- vi. Transect the intestine between the two ligatures (Fig. 3i). This portion of the intestine proximal to the cut (i.e. located between the stomach and the transection) will form the BP limb.

CRITICAL STEP Cut while avoiding visceral blood vessels and capillaries to prevent bleeding.

TROUBLESHOOTING

- vii. Continue measuring distally from the cut to a 10–15% length of the intestine and place a 4-0 silk suture around the intestine to mark the location (indicator suture; Fig. 3j). The intestinal portion proximal to this point (i.e. located between the transection and the indicator suture) will form the RL.
- viii. Move the free end of the RL to approximate it to the stomach.
- ix. Bluntly dissect the loose connective tissues surrounding the stomach using cotton swabs, freeing it from the spleen and the liver.
- x. Fully externalize the stomach and gently position it on a saline-soaked gauze (Fig. 3k).
- xi. Identify the visible white ridge discriminating the forestomach from the glandular stomach (dashed line in Fig. 3k).
- xii. Place and apply the laparoscopic stapler on the visible white ridge to divide the forestomach from glandular stomach (Fig. 3l–n). The dissected forestomach will form the gastric pouch (Fig. 3o).

CRITICAL STEP Ensure to avoid obstructing the esophagus.

CAUTION Please note that smaller pouch sizes can be achieved by transecting the stomach and using hand-sewn anastomosis to connect the RL. This technique is more delicate and requires a more skilled operator^{15,29} (please see the Experimental Design section for more information).

- xiii.** Place the free end of the RL next to the most lateral wall of the forestomach, just above the staple line (Fig. 3p). Using the #11 blade create an incision with the same size as the diameter of the intestine (roughly 5 mm; Fig. 3q).

CRITICAL STEP Once the incision site is created, you can ensure the patency of the esophagus by advancing a catheter through the incision into the esophagus (Fig. 3r).

? TROUBLESHOOTING

- xiv.** Connect the end of the RL to the side of the stomach using a continuous (running) stitch of 6-0 prolene suture (Fig. 3s). General technique for side-to-end anastomosis can be found in Box 1.

CRITICAL STEP It is necessary to ensure that the anastomosis is leak-proof. To do so, place two cotton swabs 1 cm from each side of the anastomosis sites and gently roll them towards each other. Closely observe for liquid leak through the anastomosis site.

? TROUBLESHOOTING

- xv.** Approximate the ligated side of the BP to the location of the indicator suture, at the distal end of the RL. Remove the ligation and indicator sutures (Fig. 3t).
- xvi.** Using the #11 blade make an incision with roughly the same size as the diameter of the intestine (roughly 5 mm) in the anti-mesenteric wall at the location of the indicator suture (Fig. 3u).

! CAUTION Choose an area away from mesenteric blood vessels and capillaries.

? TROUBLESHOOTING

- xvii.** Connect the end of the BP to the side of RL using a continuous stitch of 6-0 prolene suture as described in Box 1 (Fig. 3v).

CRITICAL STEP Ensure that the anastomosis is leak proof as described in Step xiv.

? TROUBLESHOOTING

- xviii.** Gently return the intestine into the abdominal cavity and remove the retractor.

! CAUTION Reposition the intestine as closely to normal anatomical location to avoid intestinal obstruction.

- xix.** Irrigate the muscle and the skin surrounding the laparotomy site with saline-soaked gauze.
- xx.** Close the abdominal muscle using a continuous stitch of 4-0 silk sutures (Fig. 3w).

xxi. Close the abdominal skin using an interrupted stitch of 4–0 silk sutures (Fig. 3x).

xxii. Irrigate the abdomen with saline-soaked gauze.

xxiii. Administer approximately 20 ml/kg of saline subcutaneously to prevent dehydration.

xxiv. Administer 1 mg/kg of meloxicam via subcutaneous injection (Fig. 3y).

Option B) Mouse Roux-en-Y Gastric Bypass Surgical Procedure

Option B) Mouse Roux-en-Y Gastric Bypass Surgical Procedure

i. Identify the xiphoid process (the cartilage at the lower end of the breastbone) and make a midline abdominal incision of 1.5 to 2 cm length using a #11 surgical scalpel from the xiphoid process down, through the skin.

ii. Using the forceps lift the muscle and use the scissors to make a midline incision (the same size as in previous step) through the muscle.

CRITICAL STEP Carefully inspect the incision path before each cut to avoid damaging internal organs.

iii. Place a self-retaining retractor to expose the abdomen.

iv. Externalize the intestine and measure the entire length of the small intestine (Fig. 3g) to determine the length of the Roux limb (RL) and the biliopancreatic limb (BP), each limb about 10–15% of total length of the small intestine. The total length of mouse small intestine is approximately 25 cm.

CRITICAL STEP It is essential to keep the tissues hydrated. Use a saline-soaked gauze to cover the segments that are outside the body. Regularly add saline to wet the tissue or gauze.

v. Starting from the pylorus (i.e. the junction between stomach and small intestine), measure the 10–15% length of the intestine and place two ligations (~ 5 mm apart) on the intestine at that position using a 4-0 silk suture.

CRITICAL STEP Handle the intestine very gently and avoid using surgical instruments. Cotton swabs moistened in saline are ideal for gentle handling of the intestine.

vi. Transect the intestine between the two ligatures. This portion of the intestine proximal to the cut (i.e. located between the stomach and the transection) will form the BP limb.

CRITICAL STEP Cut while avoiding visceral blood vessels and capillaries to prevent bleeding.

? TROUBLESHOOTING

vii. Continue measuring distally to the cut to a 10–15% length of the intestine and place a 4-0 silk suture around the intestine to mark the location (indicator suture). The

intestinal portion proximal to this point (i.e. located between the transection and the indicator suture) will form the RL.

viii. Move the free end of the RL to approximate it to the stomach.

ix. Bluntly dissect the loose connective tissues surrounding the stomach using cotton swabs, freeing it from the spleen and the liver.

x. Fully externalize the stomach and gently position it on a saline-soaked gauze.

CRITICAL STEP Because of the small size of the stomach in mice, it is not possible to use a surgical stapler to transect the stomach. Therefore, we use vascular surgical clips to create the two gastric sections.

xi. Place a vascular surgical clip in the white ridge to create the gastric pouch.

CRITICAL STEP Ensure to avoid obstructing the esophagus.

xii. Place the free end of the RL next to the most lateral wall of the forestomach, just above the staple line. Using the #11 blade create an incision with the same size as the diameter of the intestine (roughly 3 mm).

CRITICAL STEP Once the incision site is created, you can ensure the patency of the esophagus by advancing a catheter through the incision into the esophagus (Fig. 3r).

? TROUBLESHOOTING

xiii. Connect the end of the RL to the side of the stomach using a continuous (running) stitch of 8-0 prolene suture as described in Box 1.

CRITICAL STEP It is necessary to ensure that the anastomosis is leakproof. To do so, place two cotton swabs 1 cm from each side of the anastomosis sites and gently roll them towards each other. Closely observe for liquid leak through the anastomosis site.

? TROUBLESHOOTING

xiv. Approximate the ligated side of the BP to the location of the indicator suture, at the distal end of the RL. Remove the ligation and indicator sutures.

xv. Using the #11 blade make an incision with roughly the same size as the diameter of the intestine (roughly 3 mm) in the antimesenteric wall at the location of the indicator suture (Fig. 3u).

! CAUTION Choose an area away from mesenteric blood vessels and capillaries.

? TROUBLESHOOTING

xvi. Connect the end of the BP to the side of RL using a continuous stitch of 8-0 prolene suture as described in Box 1.

CRITICAL STEP Ensure that the anastomosis is leak proof as described in Step xiii

? TROUBLESHOOTING

xvii. Gently return the intestine into the abdominal cavity and remove the retractor.

! CAUTION Reposition the intestine as closely to normal anatomical location to avoid intestinal obstruction.

xviii. Irrigate the muscle and the skin surrounding the laparotomy site with saline-soaked gauze.

xix. Close the abdominal muscle using a continuous stitch of 6-0 silk sutures.

xx. Close the abdominal skin using an interrupted stitch of 6-0 silk sutures.

xxi. Irrigate the abdomen with saline-soaked gauze.

xxii. Administer 20 ml/kg of saline subcutaneously to prevent dehydration.

xxiii. Administer 0.1 mg/kg of buprenorphine via subcutaneous injection.

Option C) Rat Sleeve Gastrectomy Procedure

i. A video presentation of this operation is provided in Supplementary Video S2. Identify the xiphoid process (the cartilage at the lower end of the breastbone) and make a midline abdominal incision of 4 to 5 cm length using a #11 surgical scalpel from the xiphoid process down, through the skin.

ii. Using the forceps lift the muscle and use the scissors to make a midline incision (the same size as in previous step) through the muscle.

CRITICAL STEP Carefully inspect the incision path before each cut to avoid damaging internal organs.

iii. Place a self-retaining retractor to expose the abdomen.

iv. Retract the liver cranially and bluntly dissect the greater curvature of the stomach using 2 moistened cotton swabs, freeing the stomach from its lateral loose connective tissue attachments (Fig. 4a). Fully externalize the stomach (Fig. 4b).

v. Pass a 7 FR catheter through the mouth and advance to the duodenum (Fig. 4c). This tube will be used to standardize the size of the gastric sleeve.

? TROUBLESHOOTING

vi. Using a laparoscopic stapler (Fig. 4d), remove the greater curvature of the stomach (approximately 80% of the gastric volume) (Fig. 4e), while preserving the gastroesophageal junction and the pylorus. The remaining stomach tissue will form the gastric sleeve (Fig. 4f).

? TROUBLESHOOTING

vii. Gently return the stomach and the intestine into the abdominal cavity and remove the retractor.

viii. Irrigate the muscle and the skin surrounding the laparotomy site with saline-soaked gauze.

ix. Close the abdominal muscle using a continuous stitch of 4-0 silk sutures.

- x. Close the abdominal skin using an interrupted stitch of 4–0 silk sutures.
- xi. Irrigate the abdomen with saline-soaked gauze.
- xii. Administer 20 ml/kg of saline subcutaneously to prevent dehydration.
- xiii. Administer 1 mg/kg of meloxicam via subcutaneous injection.

Option D) Mouse Sleeve Gastrectomy Procedure

- i. Identify the xiphoid process (the cartilage at the lower end of the breastbone) and make a midline abdominal incision of 1.5 to 2 cm length using a #11 surgical scalpel from the xiphoid process down, through the skin.
- ii. Using the forceps lift the muscle and use the scissors to make a midline incision (the same size as in step i) through the muscle.

CRITICAL STEP Carefully inspect the incision path before each cut to avoid damaging internal organs.

- iii. Place a self-retaining retractor to expose the abdomen.
- iv. Retract the liver cranially and bluntly dissect the greater curvature of the stomach using 2 moistened cotton swabs, freeing the stomach from its lateral loose connective tissue attachments. Fully externalize the stomach.

CRITICAL STEP Due to the small size of the stomach in mice, it is not possible to use laparoscopic stapler.

- v. Attach 2 hemostats to the opposite sides of the greater curvature of the stomach and use them to fully extend the stomach.
- vi. Remove approximately 80% of the stomach by cutting in near parallel to the greater curvature, 5 mm from gastroesophageal junction to 5 mm from pylorus, leaving a narrow gastric sleeve in continuity with gastroesophageal junction and the pylorus. This opens the lumen of the stomach along the length.

? TROUBLESHOOTING

- vii. Reconstruct the gastric sleeve by suturing the incision site using 8-0 running prolene suture as described in Box 1.

CRITICAL STEP It is necessary to ensure that the stitch is leak-proof. To do so, place two cotton swabs 1 cm from each side of the anastomosis sites and gently roll them towards each other.

- viii. Closely observe for liquid leak through the anastomosis site. If any leakage is observed, apply another layer of stitch.

? TROUBLESHOOTING

- ix. Gently return the stomach and the intestine into the abdominal cavity and remove the retractor.

- x. Irrigate the muscle and the skin surrounding the laparotomy site with saline-soaked gauze.
- xi. Close the abdominal muscle using a continuous stitch of 6-0 silk sutures.
- xii. Close the abdominal skin using an interrupted stitch of 6-0 silk sutures.
- xiii. Irrigate the abdomen with saline-soaked gauze.
- xiv. Administer 20 ml/kg of saline subcutaneously to prevent dehydration.
- xv. Administer 0.1 mg/kg of buprenorphine via subcutaneous injection.

E) Sham Procedure For RYGB

- i. Identify the xiphoid process (the cartilage at the lower end of the breastbone) and make a midline abdominal incision of 1.5 to 2 cm (mouse) or 4 to 5 cm (rat) length using a #11 surgical scalpel from the xiphoid process down, through the skin.
- ii. Using the forceps lift the muscle and use the scissors to make a midline incision (the same size as in previous step) through the muscle.

CRITICAL STEP Carefully inspect the incision path before each cut to avoid damaging internal organs.

- iii. Place a self-retaining retractor to expose the abdomen.
- iv. Externalize the intestine and measure the entire length of the small intestine.

CRITICAL STEP It is essential to keep the tissues hydrated. Use a warm saline-soaked gauze to cover the segments that are outside the body. Add sterile warm saline to wet the tissue or gauze.

- v. Starting at the pylorus, measure the 10–15% length of the intestine and double ligate the intestine at that position using two 4-0 silk sutures.

CRITICAL STEP Handle the intestine very gently and avoid using surgical instruments. Cotton swabs moistened in saline are ideal for gentle handling of the intestine.

- vi. Transect the intestine between the two ligatures.

CRITICAL STEP Transect at a location with minimum number of visceral blood vessels and capillaries.

CRITICAL STEP To reduce the constriction of the anastomosis, cut the jejunum in an angle to increase circumference of the anastomosis.

vii. ? TROUBLESHOOTING

- viii. Reconnect the two end of the intestine with an interrupted 8-0 (mouse) or 6-0 (rat) prolene sutures as described in part in Box 1.
- ix. Continue measuring distally to the cut to a 10–15% length of the intestine.
- x. Create a 5 mm (for rats) or 3 mm (for mice) incision on the antimesenteric wall of the intestine.

- xi.** Close the incision with a continuous (running) 8-0 (mouse) or 6-0 (rat) prolene sutures.
- xii.** Bluntly dissect the greater curvature of the stomach using cotton swabs, freeing the greater curvature.
- xiii.** Fully externalize the stomach and gently position it on a saline-soaked gauze.
- xiv.** Make a 5 mm (for rats) or 3 mm (for mice) incision on the most lateral wall of the forestomach.
- xv.** Close the incision with a continuous (running) 8-0 (mouse) or 6-0 (rat) prolene sutures.
- xvi.** Gently return the stomach and intestine into the abdominal cavity and remove the retractor.
- xvii.** Irrigate the muscle and the skin surrounding the laparotomy site with saline-soaked gauze.
- xviii.** Close the abdominal muscle using a continuous stitch of 6-0 (mouse) or 4-0 (rat) silk sutures.
- xix.** Close the abdominal skin using an interrupted stitch of 6-0 (mouse) or 4-0 (rat) silk sutures.
- xx.** Irrigate the abdomen with saline-soaked gauze.
- xxi.** Administer 20 ml/kg of saline subcutaneously to prevent dehydration.
- xxii.** Administer 1 mg/kg of meloxicam via subcutaneous injection.

CRITICAL STEP To allow for comparison between the effects of the bariatric surgery, is critical that the sham-operated animals receive the same post-operative treatments (including nutritional regimens) as the surgically-treated animals.

Option F) Sham Procedure For SGx

- i.** Identify the xiphoid process (the cartilage at the lower end of the breastbone) and make a midline abdominal incision of 1.5 to 2 cm (mouse) or 4 to 5 cm (rat) length using a #11 surgical scalpel from the xiphoid process down, through the skin.
- ii.** Using the forceps lift the muscle and use the scissors to make a midline incision (the same size as in previous step) through the muscle.

CRITICAL STEP Carefully inspect the incision path before each cut to avoid damaging internal organs.

- iii.** Place a self-retaining retractor to expose the abdomen.
- iv.** Retract the liver cranially and bluntly dissect the greater curvature of the stomach using 2 moistened cotton swabs, freeing the stomach from its lateral attachments. Fully externalize the stomach.

- v. Gently return the stomach and intestine into the abdominal cavity and remove the retractor.
- vi. Irrigate the muscle and the skin surrounding the laparotomy site with saline-soaked gauze.
- vii. Close the abdominal muscle using a continuous stitch of 6-0 (mouse) or 4-0 (rat) silk sutures.
- viii. Close the abdominal skin using an interrupted stitch of 6-0 (mouse) or 4-0 (rat) silk sutures.
- ix. Irrigate the abdomen with saline-soaked gauze.
- x. Administer 20 ml/kg of saline subcutaneously to prevent dehydration.
- xi. Administer 1 mg/kg of meloxicam via subcutaneous injection.

CRITICAL STEP To allow for comparison between the effects of the bariatric surgery, is critical that the sham-operated animals receive the same post-operative treatments (including nutritional regimens) as the surgically-treated animals.

General Post-Operative Care Procedures

CRITICAL The rodent models of the bariatric operations are invasive procedures. Therefore, post-operative care plays a critical role in the recovery of the animals. We found that the following steps greatly increase the survival rate of the animals after these operations.

- 11 Allow the animal to recover under a heat lamp and monitor continuously until the animal regains consciousness.
- 12 Transfer the animal to a cage in isolation and keep on a raised wire platform.

CRITICAL STEP It is essential to house the animals on wire platforms to inhibit access to feces (rats and mice are coprophagic) or cage bedding (which can lead to restriction of gastrointestinal tract).

- 13 Monitor the animals continuously for another hour or until normal behavior is resumed with no signs of distress (inability to walk or stand).

? TROUBLESHOOTING

- 14 Administer meloxicam (1 mg/kg; subcutaneous injection) every 8 hours for the first 24 hours.

CRITICAL STEP Animals must be observed at least twice a day for the first 72 hours. If signs of pain or distress (such as hunched back and failure to groom) are observed, additional analgesic should be administered. Consult with the designated veterinary if the signs of stress persist.

- 15 Fully fast the animals (no food or water) for the first 24 hours after surgery.

CRITICAL STEP It is essential to fast the animals during the first post-operative night to minimize the chance of leakage and to allow healing of the incision sites.

? TROUBLESHOOTING

- 16** After 24 hours provide Pedialyte for the next two days. Fast the animals for another 24 hours.

CRITICAL STEP Pedialyte is a balanced electrolyte solution and it helps with the recovery after blood loss during the surgery. It also reduces the chance of diarrhea that would otherwise frequently occur after the surgery.

- 17** Provide liquid diet on the post-operative day 3 for 4 days. Change the liquid diet daily.

CRITICAL STEP Body weight and food intake of the animals should be closely monitored for the first 3 post-operative weeks to ensure smooth recovery from the surgery and for experimental recording of caloric intake (necessary for designing pair-fed and weight-matched control groups).

? TROUBLESHOOTING

- 18** On day 7, start transitioning to a solid diet over the course of 3 days. Provide ~2 g (rats) or >1 g (mice) of fully crushed high fat diet in a glass petri dish on the bottom of the cage. Repeat the same process on the next two days. On the fourth day provide a small pellet of high fat diet. Liquid diet must be provided during the transition period as well.

CRITICAL STEP The normal intestinal peristalsis is interrupted following the surgery. Therefore, it is necessary to slowly introduce the solid food in order to activate intestinal movement.

- 19** On the post-operative day 10 provide solid high fat diet.

CRITICAL STEP Calculate the daily food intake to ensure that animals are consuming the solid food.

? TROUBLESHOOTING

TIMING

Steps 1–9, pre-operative preparation: overnight fast + 15–20 min, see also fig 2a

Steps 10 option A, Rat RYGB: 80–90 min

Step 10 option B, Mouse RYGB: 80–90 min

Step 10 option C, Rat SGx: 30–45 min

Step 10 option D, Mouse SGx: 30–45 min

Step 10 option E, Sham surgery for RYGB: 80–90 min

Step 10 option F, Sham surgery for SGx: 30–40 min

Steps 11–19, follow up: 10+ days, see also fig 2b

TROUBLESHOOTING

See table 1 for troubleshooting guidance.

ANTICIPITATED RESULTS

Effect of bariatric surgery on body weight

All the surgical operations (RYGB, SGx, and sham) lead to a rapid reduction in body weight in the first post-operative week (Fig. 5A). In the following 2–4 weeks the bariatric surgery animals experience slow regain of their body weight until it plateaus around 80% of their initial body weight. In contrast, the sham animals continue gaining weight. By the 8th week after surgery, there is 20–25% difference between the body weight of bariatric surgery animals and sham animals.

Effect of bariatric surgery on food intake and preference

Bariatric surgery will have an effect on both the food intake and the type of food that is preferred by the animal^{26,30}. Both SGx and RYGB limit the absolute food intake to almost 50% in the first week after surgery (Fig. 5B). Food intake begins to increase 2 weeks after surgery in both surgical groups. RYGB, but not SGx, will have a marked impact on the food preference of the animals. Immediately after surgery RYGB-treated animals showed a larger intake from low-fat diet than in SGx animals. We found that SGx treated animals exhibit food preferences similar to sham-operated animal, while the RYGB-treated animals show a preference towards low fat diets (>40% of normal diet)²⁶.

Effect of bariatric surgery on glucose metabolism

Within 2–3 few days after the surgery both SGx and RYGB exhibit significantly lower fasting blood glucose levels compared to sham animals³². Several studies using pair-feeding and non-obese models type 2 diabetes have demonstrated that resolution of diabetes is mediated through a mechanism independent of body weight loss^{32,35}. These results are also in agreement with the observations in human that resolution of diabetes precedes significant weight loss following RYGB and SGx^{36,37}. Following administration of a glucose bolus in glucose tolerance test, the excursion of glucose after bariatric surgery proceeds more rapidly than in sham operated animal, with a significantly lower AUC for 120 minutes of measurement following glucose administration. RYGB and SGx animal exhibit similar response to glucose tolerance test.

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Box 1**End-to-side anastomosis between intestinal end and incision site**

1. Approximate the end of the intestine to the side incision.
2. Begin with a 'stay suture' on the lower end of the intestine from the outside inward, through the full thickness of the intestine and then from the inside outward on the opposed side incision. The sutures should be placed approximately 1 mm from the edge of the intestine.
3. Without tying the stay suture, place mosquito forceps on the two ends of the suture and apply slight traction. The weight of the forceps will position the opposing sites.
4. On the top side, place a second suture in the same way and tie the suture with 3 throws. This should align the bottom end of the intestine and the incision site. Place a mosquito forceps on the end of suture without a needle and apply slight traction to align the intestine end with the incision site.
5. Run a continuous stitch on the front side, starting at the top of the anastomosis and stitching downward. The first stitch should run outside-in on the intestine end and then inside-out on the incision side.
! CAUTION Ensure that only the front side of the intestine is included in the running stitch to prevent the intestine being closed off.
6. Once the stay suture is reached on the bottom end the continuous stitch should be locked by bringing the suture through its own loop. This locked suture does not loosen and should be positioned at the very lower end of the anastomosis.
7. Flip the intestine to show the backside. Continue suturing the back of the intestine with the same suture, again beginning from the outside- in on the intestine end to inside-out on the incision end.
8. Once the top of the anastomosis is reached lock the suture by bringing the end of the suture, which can be removed from mosquito forceps, through the final loop of the suture.
9. Secure the knot with 3 more throws, without pulling too much on the suture.
! CAUTION Excessive pull can constrict the lumen of the intestine.
10. Tie the stay suture on the bottom with 3 throws.

Figure 1.

Schematic representations of RYGB (**a**) and SGx (**b**). Normal upper gastrointestinal anatomy (**a, i**); esophagus (E), stomach (S), pylorus (P) duodenum (D), jejunum. The intestine is cut at a length of 10–15% of the total intestinal length, beginning at the pylorus. The proximal 10–15% will form the BP (between blue lines) (**a, ii**). The distal intestine is measured another 10–15% further, to form the RL (between red lines). The stomach is divided creating a smaller gastric pouch (gp) and larger gastric remnant (gr) (**a, iii**). The RL is moved up to approximate the cut end to the gastric pouch and the end of the RL to the end of the BP. The cut ends of the RL and BP are anastomosed to the gastric pouch and the jejunum, respectively (**a, iv**). Normal anatomy prior to SGx; greater curvature (gc) and lesser curvature (lc) (**b, i**). A catheter is passed through the mouth into the duodenum, running through the future gastric sleeve (**b, ii**). Division of the stomach creates a narrow gastric sleeve (gs) (**b, iii**). The greater curvature is removed (**b, iv**).

Figure 2.

Procedural and animal care timeline. Timeline for pre-operative (a) and post-operative steps (b).

Figure 3.

Roux-en-Y gastric bypass in the rat. After depilation and disinfection of the abdominal skin, the animal is draped, cutting an opening in the drape to expose the field (a–c). A 4–5 cm incision from the breastbone down is made (black interrupted line) with a scalpel and the underlying muscle is opened with scissors (d, e). A retractor is placed to expose the abdomen (f). The full length of the small intestine is measured (g). The double-ligated intestine is cut, at a distance of 10–15% intestinal length from the stomach; dividing the future RL (#) and BP (*) (h, i). An indicator suture is placed another 10–15% along the

intestine to mark the end of the roux limb (**j**). The externalized stomach shows a discreet white line, marking the border between the forestomach (fs) and glandular stomach (gs) (**k**). The laparoscopic stapler is placed between the forestomach and glandular stomach and fired, making sure not to obstruct the esophagus (**l, m**), dividing the stomach into a smaller gastric pouch (white ^) and gastric remnant (black ^) (**n**). The gastric pouch is in continuity with the esophagus (E) (**o**). The distal part of the cut intestine positioned next to the gastric pouch, forming the roux limb (**p**). The gastric pouch is incised (**q**) and the patency of the stomach to the esophagus is confirmed (**r**). The roux limb is anastomosed to gastric pouch (**s**). The proximal end of the cut intestine is positioned next to the indicator suture, forming the biliopancreatic limb (**t**). At the site of the indicator suture an incision is made (**u**) and the BP is anastomosed to the jejunum, joining with the RL and becoming the common limb (+) (**v**). The abdominal muscle and skin are closed (**w, x**) Administration of subcutaneous saline and meloxicam (**y**). White triangular indentations mark the position of the animal, indicating the side of the head (cranial direction).

Figure 4.

Sleeve Gastrectomy in the rat. Following images a-f in figure [RYGB], the stomach is externalized and the lateral attachments are bluntly dissected using cotton-tipped applicators (a), freeing the stomach (b). A 7 FR catheter is inserted into the duodenum (#), through the esophagus (*) and stomach, demarcating the gastric sleeve (c). The laparoscopic stapler is placed and fired, removing 80–90% of the stomach, creating a narrow sleeve between the esophagus and duodenum (d, e). The greater curvature of the stomach is removed (f). White triangular indentations mark the position of the animal, indicating the side of the head (cranial direction).

Figure 5. Representative body weight and food intake results^{26,32}. Percent body weight changes after RYGB (n=10), SGx (n=10) and sham (n=10) surgeries ($P<0.05$, between sham and both RYGB and SGx (*) and between RYGB and SGx (**); 2-way analysis of variance (ANOVA)) (a). Food intake relative to sham operated animals after RYGB (n=10) and SGx (n=10) ($P<0.05$, between RYGB and SGx(*); 2-way ANOVA) (b), Error bars represent

standard deviation. The experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Massachusetts General Hospital.

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Table 1

Troubleshooting table.

Step	Problem	Possible Reason	Possible Solution
10 Option A: vi and xvi 10 Option B: vi and xv 10 Option C: vi 10 Option D: vi	Bleeding	Incision site too close to the capillaries	Apply pressure using cotton swabs for approximately 1 minute. If the bleeding doesn't stop use the cautery device to stop the bleeding.
10 Option A: xiii	Esophagus obstructed	Staple was placed too close to the esophagus	Unfortunately there is no solution. Euthanize the animal.
10 Option A: xiv and xvii 10 Option B: xiii and xvi 10 Option D: vii	Leakage from anastomosis site	Anastomosis not fully closed	Add another additional stitch(es) to anastomosis
10 Option B: xii	Esophagus obstructed	Surgical clip was placed too close to the esophagus	Remove the clip and reposition it.
10 Option C: v	Catheter not advancing into the small intestine		Create a small incision on the greater curvature of the stomach and use forceps to guide the catheter into the small intestine
13	Bleeding from abdominal wall	Abdominal muscle anastomosis too tight. Kept under heat lamp for too long.	Remove the animals from the heat lamp. Bleeding should stop in less than 10 minutes.
15	Dark (or bloody) feces		Observing bloody feces in the 1 and 2 post-operative days is a good sign! It indicates that intestinal peristalsis is active.
17	Not drinking liquid diet	It is not unusual that some animals don't consume liquid diet.	If the animal doesn't drink liquid diet for 3 days, provide small amount of solid diet as described in post-operative care section.
19	Not eating solid food	Intestinal obstruction.	For reasons unknown to us, some animals stop eating solid food. Provide liquid diet for a few days. If the animal doesn't drink liquid diet the animal should be euthanized.
19	No feces	Intestinal dilation or obstruction (if the animals consumed food in the prior days).	Restrain the animals and gently massage the abdomen in a circular motion. Repeat the process twice a day.