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Development of Murine Bariatric Surgery Models: Lessons Learned

Heather A. Frohman, MD^{1,2}, Piotr G. Rychahou, MD^{1,2}, Jing Li, PhD², Tong Gan, MD^{1,2}, and B. Mark Evers, MD^{1,2}

¹Department of Surgery, University of Kentucky, 800 Rose St. Lexington, Kentucky, 40536, USA

²Markey Cancer Center, University of Kentucky, 800 Rose St. Lexington, Kentucky, 40536, USA

Abstract

Roux-en-Y gastric bypass (RYGB) improves comorbidities such as diabetes and hypertension and lowers the risk of obesity-related cancers. To better understand the physiologic and genetic influences of bariatric surgery, a reliable murine model is needed that can be extended to genetically engineered mice. Given the complexity of these procedures, few researchers have successfully implemented these techniques beyond larger rodent models. The purpose of our study was to develop a technically feasible and reproducible murine model for RYGB and sleeve gastrectomy (SG). Mice were converted to liquid diet perioperatively without fasting and housed in groups on raised wire platforms. SG involved significant reduction of stomach volume followed by multi-layer repair of the gastrotomy. RYGB procedure consisted of side-to-side, functional end-to-side bowel anastomoses and exclusion of the stomach medial to gastroesophageal junction. Sham surgeries consisted of enterotomies and gastrotomy followed by primary repair without resection or rerouting. Survival after incorporation of the aforementioned techniques was 100% in the SG group and 41% in the RYGB group at one month after surgery. Only 26% of RYGB mortality was attributed to leak, obstruction, or stricture; the majority of postoperative mortality was due to stress, dumping, or malnutrition. Much of the survival challenge for this surgical model was related to perioperative husbandry, which is to be expected given their small stature and poor response to stress. Utilization of the perioperative and surgical techniques described will increase survival and feasibility of these technically-challenging procedures, allowing for a better understanding of mechanisms to explain the beneficial effects of bariatric surgery.

Corresponding Author: B. Mark Evers, M.D., Markey Cancer Center, University of Kentucky, 800 Rose Street, CC140, Lexington, Kentucky 40536, Phone: 859-323-6556, Fax: 859-323-2074, mark.evers@uky.edu.

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Keywords

Bariatric Surgery; murine model; Roux-en-Y Gastric Bypass

INTRODUCTION

Over the past decade, the prevalence of obesity has increased by 70% in the United States, with approximately 35% of men and 40% of women being classified as obese (BMI >30) in 2014.^{1,2} A major public health concern is that morbid obesity is associated with a number of comorbidities.³ Obesity results in a pro-inflammatory state, which promotes insulin resistance, hypertension, dyslipidemia, atherogenesis, coronary heart disease, stroke, and congestive heart failure, as well as increased risk for development of obesity-related cancers and type 2 diabetes mellitus.⁴⁻⁶

Bariatric surgery is the most efficient way to lose excess body weight with long-term success in the obese population. Several studies have demonstrated that, along with weight loss, bariatric surgery is more effective than nonsurgical treatments in better controlling type 2 diabetes mellitus, hypertension, obstructive sleep apnea, hyperlipidemia, and decreasing the risk of specific obesity-related cancers.^{7,8} Extensive literature describes the short-term outcomes from bariatric surgery; however, few studies have followed patients for greater than five years after surgery, thus the long-term outcomes and consequences of bariatric surgery are not yet well described.⁸⁻¹⁰ Furthermore, the mechanisms by which bariatric surgery affects specific co-morbidities, such as diabetes and cancer risk, are largely unknown. Literature exists demonstrating that the beneficial effects of diabetes control occurs much sooner than weight loss, suggesting a hormonal or genetic etiology.^{11,12} In order to better define these genetic and hormonal impacts in a controlled environment, bariatric surgery should be studied in a genetically engineered transgenic species – which is currently most commonly utilized in a murine model. There is robust literature on large animal models of bariatric surgery, specifically pigs, and dogs, as well as larger rodent species.¹³⁻¹⁷ However, few groups have studied bariatric surgery in a small rodent model, specifically mice.¹⁸⁻²¹

Within currently published literature, there is considerable variation in the technique described for successful bariatric surgery in mice, specifically Roux-en-Y gastric bypass (RYGB).¹⁸⁻²² Aside from technical differences in performing the surgery, there is also much variation in the pre-operative and post-operative care of the animals. Our group sought to develop a technically feasible murine model for two of the most commonly performed bariatric surgery techniques, RYGB and vertical sleeve gastrectomy (SG), which provided long-term survival and can be extended to transgenic species. Herein, we describe the lessons learned in the development of murine models of RYGB and SG. Key steps to successful procedures include: closure of gastrotomy in two layers (for SG); side-to-side, functional end-to-side anastomoses (for RYGB); adequate compensation for intra-operative insensate heat loss; and conversion to a liquid diet for the perioperative period.

MATERIALS & METHODS

All experiments involving mice were approved by the Institutional Animal Care and Use Committee (IACUC) and our approved protocol included provision of animals for establishment of a feasible surgical technique, where animals were subsequently euthanized immediately after the operation. All animal experiments were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Animals were under the direct care of the surgeon, with additional daily monitoring provided by the Division of Laboratory Animal Resources (DLAR) staff. Mouse stress level was subjectively assessed by examination of changes in the following metrics: mouse activity, self and peer grooming, wound licking, orbital tightening, and visits to food trough.

Mice

Female Swiss-Webster mice, aged seven weeks, were purchased from Taconic Biosciences and housed in groups under a 12h light/dark cycle at room temperature. Only female mice were utilized in the development of our surgical techniques in order to limit confounding variables, specifically, female mice are less likely to fight than male mice, and thus less likely to have death due to injuries unrelated to the surgery. Mice were fed a high fat diet (60% kcal% fat, Research Diets, New Brunswick, New Jersey) for 20w until they reached a body weight greater than 40g, ideally >50g. Three days prior to surgery, high fat diet was removed from the cage, and mice were changed to a liquid diet (Two Cal HN, Vanilla, Abbott Nutrition, Columbus, Ohio). Mice were maintained on a liquid diet until 10d after surgery. Solid high fat diet was reintroduced to mice starting on post-operative day 7. Mice were provided water *ad libitum* prior to surgery and maintained on a 50% Pedialyte water solution (Pedialyte®, Unflavored, Abbott Nutrition) for 10d post-operatively. We did not fast the mice. In order to limit ingestion of feces or bedding, bedding was removed from cages 3d prior to surgery and replaced with a raised wire platform floor until post-operative day 10, when standard bedding was re-introduced and wire bottom platforms were removed. At time of surgery, abdominal skin was shaved and disinfected with betadine and alcohol swabs.

Surgical Materials & Reagents

Prior to initiation of surgery, Ciprofloxacin 0.1mg/kg (Claris Lifesciences, Inc., North Brunswick, New Jersey) was injected intra-peritoneal (ip). Inhaled Isoflurane 2–3% was utilized for general anesthesia, and Buprenorphine SR 1.2 mg/kg SQ (Zoofarm, Fort Collins, Colorado) for analgesia. Insensate fluid loss was compensated with subcutaneous (sc) injection of sterile normal saline at 20ml/kg/hour of operation (0.9% wt/vol NaCl Solution; Med-Vet International, Mettawa, Illinois). Additional subcutaneous normal saline fluid boluses were provided daily until post-operative day 3 to limit dehydration. Intraoperative heat source was provided by a Far Infrared Surgical Warming Pad (Kent Scientific Corporation, Torrington, Connecticut). Microscopic surgery instruments were utilized for all surgeries (Fisher Scientific Pittsburgh, Pennsylvania; Harvard Apparatus, Holliston, Massachusetts) and hemostasis was assured with use of a Bovie cautery device (Bovie Deluxe High Temperature Cautery Kit, World Precision Instruments, Sarasota, Florida). Prolene suture (8-0) on a tapered needle was used for bowel anastomoses, and 6-0 vicryl suture on a

reverse-cutting needle was utilized for fascia and skin closure (Ethicon, Somerville, New Jersey).

Surgical Technique

All procedures were completed within 75min (including anesthesia induction time). Sham procedures were extended to 75min to mimic true procedure time.

Opening & Closure of Abdomen for All Procedures—Abdominal skin was incised sharply with a #15-scalpel blade, extending from xiphoid process to mid-pelvis. Fascia was divided sharply with scissors and retracted with self-retaining retractor (Fig. 1A). At regular intervals during the procedure, internal organs were saturated with sterile 0.9% normal saline and covered with soaked gauze. At the end of the procedure, intestines were covered with the bilateral intra-abdominal fat pads to create a barrier between anastomoses and the abdominal wall (Fig. 1J). Fascia and skin were approximated with continuous running vicryl suture in two layers (Fig. 1, K and L). Established hemostasis throughout the procedure with use of a cautery device.

Key Components of Roux-en-Y Gastric Bypass—After evisceration and measurement of total length of small bowel, the length of the Roux limb and biliary limb was calculated as 10–15% of total intestinal length. Starting at the ligament of Treitz, we measured the previously determined length of intestine for the biliary limb, and sharply transected the small bowel at this location (Fig. 1, B–D). Short segments of pediatric scalp vein IV tubing was threaded into both proximal and distal bowel lumens to stent the lumen open for the creation of anastomoses (1.5cm segment of tubing from Winged Scalp Vein Set, ID 0.1mm, OD 0.2mm, Amsino International, Pomona, California) (Fig. 1E). Next, we measured distally from the transected small intestine the previously determined length of intestine for the Roux limb and advanced the intraluminal IV tubing to this point (Fig. 1F). An anti-mesenteric enterotomy was performed 5mm proximal to the transected end of the proximal small intestine, and a similar enterotomy was created in the distal small bowel at the point of the future biliary-to-Roux limb anastomosis (Fig. 1G). The small intestine enterotomies were aligned for creation of the side-to-side, functional end-to-side jejuno-jejunosomy (J-J) anastomosis. The posterior wall of the J-J anastomosis was approximated by running the suture in a continuous fashion. The anterior wall of the J-J anastomosis was approximated with a series of interrupted simple stitches. After creation of the anastomosis, the IV tubing was advanced distally and removed from the blind opening of the biliary limb. The blind end of the biliary limb was ligated with a suture tied in a purse-string fashion (Fig. 1H). The remaining IV tubing (within the Roux limb) was slid retrograde within the Roux limb toward the open end of the transected jejunum and an anti-mesenteric enterotomy was performed approximately 5mm from the proximal end of this intestinal limb. After externalizing the stomach, an obliquely angled gastrotomy was performed along the white ridge of the stomach. The gastro-jejunosomy (G-J) was created in a similar manner as for the J-J anastomosis (continuous running suture along the posterior wall followed by simple interrupted stitches along the anterior wall of the anastomosis). The IV tubing was then advanced through the blind opening of the jejunum adjacent to the G-J and removed. The blind end of the jejunum was then ligated with a suture tied in a purse-string fashion. Finally,

the small gastric pouch was created by applying a vascular clip just medial to the gastro-esophageal (G-E) junction to exclude 60% of the stomach volume (Titanium Vascular LigaClip, size M/L, Ethicon, Somerville, New Jersey) (Fig. 1I and Fig. 2).

The sham RYGB procedure involved transection of the small intestine as if to create the biliary and Roux limbs, similar to the above description. However, after transection of the jejunum, the jejunum was anastomosed in a side-to-side, functional end-to-end fashion. For proper simulation of the RYGB procedure, a gastrotomy was also made, then primarily repaired. Finally, a small enterotomy was created in the distal jejunum at the location of the J-J anastomosis and repaired with simple interrupted stitches, taking care not to narrow the bowel lumen.

Key Components of Vertical Sleeve Gastrectomy—As a first step, the stomach was bluntly dissected from surrounding structures in order to allow externalization. A gastrotomy was then made along the greater curve of the stomach and any residual gastric contents were evacuated. A short segment of pediatric scalp vein IV tubing was placed within the stomach along the lesser curvature (1.5cm segment of tubing from Winged Scalp Vein Set, ID 0.1mm, OD 0.2mm, Amsino International, Pomona, California). The stomach is then sharply excised along the greater curvature, extending from 8–10mm distal to the G-E junction to 5mm proximal to the pylorus, such that the remaining stomach could be brought together without tension and the resulting stomach lumen approximating the diameter of the IV tubing. Hemostasis along the borders of the gastrectomy site was achieved with the cautery device. After removal of the IV tubing, the gastric sleeve was constructed by closure of the gastrotomy in two layers with the first layer as a running continuous stitch, incorporating full thickness bites of stomach, and the second layer utilizing a running Lembert technique, incorporating only gastric serosa (Fig. 3).

The sham SG was performed by the creation of a gastrotomy the same length as in the true SG procedure. Then, the gastrotomy was simply re-approximated in two layers, as described above, without excision of any portion of the stomach (*See supplemental material for detailed surgical protocols*).

Statistical Methods

Percent weight change from pre-op to one month after surgery and excess body weight loss were calculated and compared between groups using analysis of variance with Holm's p-value adjustment for multiple pairwise testing. Significance was determined as $p < 0.05$.

RESULTS

Intra-Operative techniques resulting in improved survival

Applying a different suture technique to approximate the posterior and anterior walls of the bowel anastomoses, specifically a continuous running suture for the posterior wall and interrupted sutures for the anterior wall, results in a lower frequency of anastomotic leak and obstruction (Table 1). Further, creation of a side-to-side anastomosis, as compared to end-to-side, decreased the incidence of leaks related to anastomotic necrosis.

Utilization of a far-infrared heating pad during the operation led to improved intraoperative temperature maintenance, as assessed by immediate arousal after surgery. Prior to implementation of the intraoperative heating pad, all mice died within 7d of surgery. In contrast, less than 18% of mice died prior to day 7 when the heating pad was utilized.

Perioperative techniques resulting in improved survival

Housing mice in groups with at least two other mice, one of which did not undergo a surgical procedure appeared to lower animal stress, as noted subjectively by increased grooming, decreased wound licking, increased visits to the food trough, and increased mobility in the cage (Table 1). When housed alone, mice tended to hover in a corner of the cage and infrequently visit the food trough. Another notable technique was to forego fasting and instead place mice on liquid diet 3d prior to operation to evacuate solid food content from the gut without a period of starvation. Furthermore, removal of the cage bedding and addition of a wire-bottom platform limited ingestion of fecal matter and obstruction.

Survival

Prior to implementation of the final protocol (as described in the Methods above), survival at 1-month after surgery was 0% for all RYGB and sham RYGB mice (n=15 and 7, respectively), 86% for SG and 100% for sham SG (n=7 and n=5, respectively). The longest survival following the RYGB surgery was 3d. The longest survival following the sham RYGB surgery was 16d.

After implementation of the protocol described above, survival at one week after surgery was 60% for RYGB and 68% for sham RYGB. At one month after surgery, survival was 41% for RYGB and 50% for sham RYGB (n=22 and n=12, respectively), and 100% for both SG and sham SG (n=8 and n=8, respectively). Of the mice that died, many had no obvious cause of death noted at time of necropsy (58%, n=11); only 26% of mortality was directly attributed to leak, obstruction, or stricture (n=6) (Table 2).

Weight loss

After implementation of the protocol described above, average percent weight loss at one month after surgery compared to pre-op was 21.2% for RYGB (standard deviation, SD $\pm 13\%$, $p < 0.0001$), 4.5% for sham RYGB (SD $\pm 10\%$, $p = 0.2$), 1.4% for SG (SD $\pm 5\%$, $p = 0.7$), and a gain of 0.9% of weight in the sham SG group (SD $\pm 4\%$, $p = 0.8$) (Fig. 4A). Comparison of percent weight loss was significantly different between RYGB vs. sham RYGB ($p = 0.005$); however, there was no difference in weight loss between SG vs. sham SG ($p = 0.8$). In reference to excess body weight lost (ideal body weight: 28g), the RYGB group lost 42% of excess weight, which was significantly greater weight loss in comparison to sham RYGB, SG, or sham-SG ($p = 0.01$, $p = 0.001$, $p = 0.0003$, respectively) (Fig. 4B). Following RYGB, mice had solid feces until approximately 24h after surgery when stools were noted to be loose and remained loose for 5–7d following surgery. We do not believe this is related to the liquid diet, as mice had solid formed stools when consuming the liquid diet in the 3d prior to surgery. No other surgery group (sham RYGB, or SG groups) exhibited loose stools post-operatively. At the time of planned sacrifice (one month after surgery), the stomach volume in the SG group was surprisingly similar to the size of the

stomach in mice with sham SG, despite resection of nearly 1cm width of stomach tissue during the SG surgery one month prior. Gastric dilatation and failed weight loss (or weight re-gain) is commonly noted in humans who continue to make poor diet choices following SG, thus our murine model of SG mimics this finding.^{23–25}

DISCUSSION

Bariatric surgery in a mouse model is technically challenging but feasible. Unfortunately, we were not consistently successful when mimicking previously described models of bariatric surgery in mice, especially for the RYGB model. As such, we developed our unique protocols for both RYGB and SG, as well as the sham versions of these surgeries.

Pre-Operative Essentials

As with any murine study, it is essential to acclimate the mice prior to the procedure in order to reduce undue stress. This includes acclimation to the liquid diet as well as removing cage bedding and adding the raised wire platform multiple days before the surgery. Additionally, foregoing a pre-operative fasting period is important in minimizing stressors leading up to the surgery, especially for procedures scheduled in the morning, as mice tend to be nocturnal.

Intra-Operative Essentials

The one factor that affected survival most significantly was application of a heating source that maintained the mouse's internal temperature despite an open abdomen for the length of the procedure. Much has been published regarding the risk of hypothermia resulting from anesthetic-induced inhibition of thermoregulatory control and exposure of internal organs to ambient temperature.²⁶ This risk is amplified in small rodents, given their large surface area relative to body mass.^{27,28} Far-infrared rays, which are electromagnetic waves with properties of both light and wave, are effective for both radiation and thermal treatment. The far-infrared rays cause local vasodilation, increased circulation, and have even been shown to improve wound healing.^{29,30} While traditional external heat pads can similarly provide surface warming properties, the advantage of using a heat pad with far-infrared rays is the transfer of heat to deep tissues, which allows the animal to better maintain its physiologic temperature during a long operation.³¹ We advocate the use of a similar product in order to minimize early post-operative mortality related to hypothermia.

Additionally, we advocate for the creation of bowel anastomoses using a side-to-side, functional end-to-side technique. We found that end-to-side anastomosis of the small bowel was limited by the small size of the transected proximal jejunal lumen, which predisposed the animal to anastomotic stricture and obstruction. By creating a side-to-side anastomosis, we were able to dictate the diameter of the anastomosis and decrease risk of mechanical obstruction. Another advantage of a lateral rather than end anastomosis is the increase in blood flow to the anastomosis, which has been demonstrated in several studies.^{32–34}

Post-Operative Essentials

The use of sustained-release buprenorphine, as compared to buprenorphine-HCl, for analgesia prevented the need for intermittent dosing of analgesia post-operatively, thus limiting postoperative handling. Studies also support that this medication choice provides more adequate pain coverage, as noted by increased general activity, decreased wound licking, and decreased orbital tightening post-operatively.³⁵ Unfortunately, one notable side effect of buprenorphine in mice is pica-like behaviors, which we noted by increased ingestion of cage bedding after surgery rather than consuming the available food source. In order to circumvent this problem, mice were placed on raised wire platforms and bedding was removed from the cage until post-operative day 10. Removal of cage bedding also decreased incidence of early mechanical obstruction at anastomoses due to ingestion of the woodchips or paper products.

Conclusions

These surgical procedures do not require an expert surgeon, merely someone with a steady hand who is comfortable utilizing microscopic instruments. Utilization of the perioperative and operative techniques described should increase postoperative survival and feasibility for these technically-challenging small animal procedures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Flegal KM, Kruszon-Moran D, Carroll MD, Fryar CD, Ogden CL. Trends in Obesity Among Adults in the United States, 2005 to 2014. *JAMA*. 2016; 315(21):2284–2291. [PubMed: 27272580]
2. Sturm R, Hattori A. Morbid obesity rates continue to rise rapidly in the United States. *International journal of obesity*. 2013; 37(6):889–891. [PubMed: 22986681]
3. Bae J, Shade J, Abraham A, et al. Effect of Mandatory Centers of Excellence Designation on Demographic Characteristics of Patients Who Undergo Bariatric Surgery. *JAMA Surg*. 2015; 150(7):644–648. [PubMed: 25993654]
4. Skolnik NS, Ryan DH. Pathophysiology, epidemiology, and assessment of obesity in adults. *J Fam Pract*. 2014; 63(7 Suppl):S3–S10.
5. Lavie CJ, McAuley PA, Church TS, Milani RV, Blair SN. Obesity and cardiovascular diseases: implications regarding fitness, fatness, and severity in the obesity paradox. *J Am Coll Cardiol*. 2014; 63(14):1345–1354. [PubMed: 24530666]
6. Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M. Health and economic burden of the projected obesity trends in the USA and the UK. *Lancet*. 2011; 378(9793):815–825. [PubMed: 21872750]

7. Adams TD, Stroup AM, Gress RE, et al. Cancer incidence and mortality after gastric bypass surgery. *Obesity*. 2009; 17(4):796–802. [PubMed: 19148123]
8. Courcoulas AP, Yanovski SZ, Bonds D, et al. Long-term outcomes of bariatric surgery: a National Institutes of Health symposium. *JAMA surgery*. 2014; 149(12):1323–1329. [PubMed: 25271405]
9. Hutter MM, Schirmer BD, Jones DB, et al. First report from the American College of Surgeons Bariatric Surgery Center Network: laparoscopic sleeve gastrectomy has morbidity and effectiveness positioned between the band and the bypass. *Ann Surg*. 2011; 254(3):410–420. [PubMed: 21865942]
10. Puzziferri N, Roshek TB 3rd, Mayo HG, Gallagher R, Belle SH, Livingston EH. Long-term follow-up after bariatric surgery: a systematic review. *JAMA*. 2014; 312(9):934–942. [PubMed: 25182102]
11. Bojsen-Moller KN. Mechanisms of improved glycaemic control after Roux-en-Y gastric bypass. *Dan Med J*. 2015; 62(4):B5057. [PubMed: 25872541]
12. Pories WJ, Swanson MS, MacDonald KG, et al. Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. *Annals of surgery*. 1995; 222(3):339–350. [PubMed: 7677463]
13. Rao RS, Rao V, Kini S. Animal models in bariatric surgery--a review of the surgical techniques and postsurgical physiology. *Obes Surg*. 2010; 20(9):1293–1305. [PubMed: 20383602]
14. Hankir MK, Seyfried F, Hintschich CA, et al. Gastric Bypass Surgery Recruits a Gut PPAR-alpha-Striatal D1R Pathway to Reduce Fat Appetite in Obese Rats. *Cell Metab*. 2017; 25(2):335–344. [PubMed: 28065827]
15. Arapis K, Cavin JB, Gillard L, et al. Remodeling of the residual gastric mucosa after roux-en-y gastric bypass or vertical sleeve gastrectomy in diet-induced obese rats. *PLoS One*. 2015; 10(3):e0121414. [PubMed: 25822172]
16. Glenn IC, Bruns NE, Ponsky TA. Use of 5-mm Laparoscopic Stapler to Perform Open Small Bowel Anastomosis in a Neonatal Animal Model. *J Laparoendosc Adv Surg Tech A*. 2016; 26(10):840–844. [PubMed: 27462959]
17. Gandarillas M, Hodgkinson SM, Riveros JL, Bas F. Effect of three different bariatric obesity surgery procedures on nutrient and energy digestibility using a swine experimental model. *Exp Biol Med* (Maywood). 2015; 240(9):1158–1164. [PubMed: 25711878]
18. Bruinsma BG, Uygun K, Yarmush ML, Saeidi N. Surgical models of Roux-en-Y gastric bypass surgery and sleeve gastrectomy in rats and mice. *Nat Protoc*. 2015; 10(3):495–507. [PubMed: 25719268]
19. Yin DP, Boyd KL, Williams PE, Abumrad NN, Wasserman DH. Mouse Models of Bariatric Surgery. *Curr Protoc Mouse Biol*. 2012; 2012:2012.
20. Hao Z, Zhao Z, Berthoud HR, Ye J. Development and verification of a mouse model for Roux-en-Y gastric bypass surgery with a small gastric pouch. *PLoS One*. 2013; 8(1):e52922. [PubMed: 23326365]
21. Seyfried F, Lannoo M, Gsell W, et al. Roux-en-Y gastric bypass in mice--surgical technique and characterisation. *Obes Surg*. 2012; 22(7):1117–1125. [PubMed: 22527601]
22. Lan Z, Zassoko R, Liu W, et al. Development of techniques for gastrojejunal bypass surgery in obese mice. *Microsurgery*. 2010; 30(4):289–295. [PubMed: 20049916]
23. Langer FB, Bohdjalian A, Felberbauer FX, et al. Does gastric dilatation limit the success of sleeve gastrectomy as a sole operation for morbid obesity? *Obes Surg*. 2006; 16(2):166–171. [PubMed: 16469218]
24. Baumann T, Grueneberger J, Pache G, et al. Three-dimensional stomach analysis with computed tomography after laparoscopic sleeve gastrectomy: sleeve dilation and thoracic migration. *Surg Endosc*. 2011; 25(7):2323–2329. [PubMed: 21298527]
25. Disse E, Pasquer A, Pelascini E, et al. Dilatation of Sleeve Gastrectomy: Myth or Reality? *Obes Surg*. 2017; 27(1):30–37. [PubMed: 27334645]
26. Lenhardt R. The effect of anesthesia on body temperature control. *Front Biosci (Schol Ed)*. 2010; 2:1145–1154. [PubMed: 20515846]
27. Clark JD, Gebhart GF, Gonder JC, Keeling ME, Kohn DF. Special Report: The 1996 Guide for the Care and Use of Laboratory Animals. *ILAR J*. 1997; 38(1):41–48. [PubMed: 11528046]

28. Rembert MS, Smith JA, Hosgood G. A comparison of a forced-air warming system to traditional thermal support for rodent microenvironments. *Lab Anim.* 2004; 38(1):55–63. [PubMed: 14979989]
29. Wong CH, Lin LC, Lee HH, Liu CF. The analgesic effect of thermal therapy after total knee arthroplasty. *J Altern Complement Med.* 2012; 18(2):175–179. [PubMed: 22339105]
30. Toyokawa H, Matsui Y, Uhara J, et al. Promotive effects of far-infrared ray on full-thickness skin wound healing in rats. *Exp Biol Med (Maywood).* 2003; 228(6):724–729. [PubMed: 12773705]
31. Zarndt BS, Buchta JN, Garver LS, Davidson SA, Rowton ED, Despain KE. Use of a Far-Infrared Active Warming Device in Guinea Pigs (*Cavia porcellus*). *J Am Assoc Lab Anim Sci.* 2015; 54(6): 779–782. [PubMed: 26632788]
32. Hallbook O, Johansson K, Sjodahl R. Laser Doppler blood flow measurement in rectal resection for carcinoma--comparison between the straight and colonic J pouch reconstruction. *Br J Surg.* 1996; 83(3):389–392. [PubMed: 8665202]
33. Trencheva K, Morrissey KP, Wells M, et al. Identifying important predictors for anastomotic leak after colon and rectal resection: prospective study on 616 patients. *Annals of surgery.* 2013; 257(1):108–113. [PubMed: 22968068]
34. Jones DG, KA. Anastomotic technique-Does it make a difference? *Seminars in Colon and Rectal Surgery.* 2014; 25:79–84.
35. Kendall LV, Wegenast DJ, Smith BJ, et al. Efficacy of Sustained-Release Buprenorphine in an Experimental Laparotomy Model in Female Mice. *J Am Assoc Lab Anim Sci.* 2016; 55(1):66–73. [PubMed: 26817982]

Figure 1.

Roux-en-Y Gastric Bypass (RYGB) in the Mouse. (A) After a midline incision, the fascia was retracted to expose small intestine. (B) Identification of the ligament of Treitz. (C) Measurement of the biliary limb. (D) Transection of the small intestine. (E) Short segments of pediatric scalp vein IV tubing fed into both the proximal and distal small bowel lumens to stent the lumen open. (F) One segment of IV tubing was gently advanced distally in the small bowel to the location of the biliary and Roux limb anastomosis. (G) Anti-mesenteric enterotomies were made in the small bowel at the point of the future jejuno-jejunostomy. (H) After construction of the side-to-side, functional end-to-side jejuno-jejunostomy, the IV tubing was slid out of the blind opening in the biliary limb and the blind end of the biliary limb was suture ligated. (I) Completed RYGB anatomy. (J) Intra-abdominal fat pads placed between bowel and fascia. (K) Closure of the fascia. (L) Skin closure.

Figure 2. Roux-en-Y Gastric Bypass (RYGB). (A) Schematic of RYGB procedure. Both the jejuno-jejunostomy (J-J) and gastro-jejunostomy (G-J) are side-to-side, functional end-to-side anastomoses. Approximately 60% of the stomach volume is excluded by application of a vascular clip across the body of the stomach, just medial to the esophagus. (B) Completed RYGB.

Figure 3. Sleeve Gastrectomy. (A) Schematic of sleeve gastrectomy procedure. Approximately 80% of the stomach volume is removed and the gastrotomy is repaired in two layers. (B) Completed sleeve gastrectomy.

Figure 4. Weight Loss Following Bariatric Surgery. (A) Average weight loss following bariatric surgery. (B) Percentage of excess body weight lost following bariatric surgery. Abbreviations: (R) Roux-en-Y gastric bypass; (RS) Sham Roux-en-Y gastric bypass; (S) Sleeve gastrectomy; (SS) Sham sleeve gastrectomy; (*) denotes statistical significance of $p < 0.01$.

Table 1

Summary of Lessons Learned During Development of Murine Bariatric Surgery Protocol.

Attempted Technique	Reason for Failure	Adaptation
End-to-side anastomosis	Necrosis, leak, or obstruction at the anastomosis	Side-to-side, functional end-to-side anastomosis
Continuous suture entire circumference	Obstruction at the anastomosis	Continuous suture on posterior wall, simple interrupted suture pattern on the anterior wall of the anastomosis
Interrupted suture on entire circumference	Leak at anastomosis	Continuous suture on posterior wall, simple interrupted suture pattern on the anterior wall of the anastomosis
Gastric pouch size 20% of stomach volume (transect entire stomach, suture partial stomach closed)	Stomach retracts into thorax; major size mismatch at anastomosis; necrosis of gastric remnant, due to ligation of left gastric artery (supplies 75% of mouse stomach)	G-J along lateral fundus of stomach, apply vascular clip across body of stomach to create gastric pouch ~40% of stomach volume
Staple stomach to create gastric remnant/pouch	Stapler too large to apply to small rodent	Hand sewn anastomosis with clip across stomach to create pouch
House mice individually after surgery	Increased mouse stress, they are very social animals	House mice in groups of at least 3
House mice with only other mice who underwent surgery same day	Mice are stressed from surgery, do not groom each-other or interact	House mice with at least one mouse that does not undergo surgery – provides care/comfort to post-operative mice
Fast mice pre-operatively	Increased mouse stress	Provide liquid diet pre-operatively without fasting
Fast mice post-operatively 24h	Increased mouse stress	Provide liquid diet immediately after surgery
Continue solid diet until within 24h of surgery	Stomach continues to be full of solid food, stomach fully distended, difficult to control gastric contamination during surgery	Stop solid food 3d prior to surgery, but provide <i>ad libitum</i> liquid diet until surgery – less contents in stomach
Providing 20cc/kg/h of operation NS sq only immediately after operation	Only compensated for insensate losses from operation, mice do not consume enough volume for days after surgery and become dehydrated	Administer 20cc/kg NS sq prior to operation, 20cc/kg NS ip at end of operation, 20cc/kg NS sq immediately after operation, 20cc/kg NS sq Q24h after operation for 72h
Utilize a heating lamp for surgery	Much of the heat was absorbed by surgeons hands rather than providing heat to the mouse, mouse was cold by end of surgery	Switch to Far Infrared heating pad – lead to mouse maintaining homeostatic temperature during surgery and significantly improved survival
Returning mouse to wire- bottom cage immediately after surgery	Stresses mouse to have no bedding in cage, causes barrier between mouse and heating pad, so mouse becomes cold	Did not add the wire-bottom to cage until 48h after surgery, when mouse was recovered from surgery and attempting coprophagy again rather than consumption of liquid diet. Maintained on heating pad for 7d post-operative to provide additional temperature support
Return mouse to Aspen chip bedding immediately post-operatively	Mice eat the bedding, possibly related to pica from buprenorphine, causes early obstruction at anastomoses	Provide only the crinkle paper bedding in cage for first 10d, as mice do not attempt to consume this

Table 2

Etiology of Death After Bariatric Surgery. Only 26% of mortality was directly attributed to the surgery (e.g., leak, necrosis, obstruction, blood loss).

Etiology of Death	No. of Mice	Post-Operative Day
Normal Necropsy	11	1, 2, 2, 4, 5, 6, 7, 8, 9, 13, 20
Anastomotic Leak	3	1, 1, 2
Hemorrhage	2	1, 3
Obstruction	1	2
Ischemia at Anastomosis	1	5
Cannibalized	1	17