Video Article

A Murine Model of Vertical Sleeve Gastrectomy

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Abstract

Bariatric surgery, such as vertical sleeve gastrectomy (VSG), is a surgery of the gastrointestinal tract that is performed for the purpose of weight loss. Bariatric surgery is currently the most effective long-term treatment for obesity. In addition to weight loss, bariatric surgery produces additional health benefits such as remission of type 2 diabetes, remission of hypertension, and decreased risk of developing certain types of cancer. The mechanisms beyond weight loss for these benefits remain incompletely defined. Therefore, animal models of bariatric surgery are being developed and validated to identify the mechanisms leading to these benefits, with the goal of improving understanding of gastrointestinal physiology and identifying new therapeutic targets. VSG has become the most commonly performed bariatric procedure in the clinic in the United States because it is highly effective at producing weight loss and metabolic improvement, and is simpler to perform than other bariatric procedures. Therefore, we have developed and validated a murine model of VSG. This murine VSG model recapitulates many of the effects of VSG seen in humans, including improved glucose and blood pressure regulation. The method is based on isolation of the stomach, ligation of gastric vessels, and removal of 70% of the stomach by transecting along the greater curvature of the stomach. We have successfully applied this surgical protocol to various genetically modified mouse lines to define the mechanistic contributors to the benefits of VSG. Furthermore, this murine VSG model has been combined with other surgical techniques, to achieve deeper mechanistic insight. Therefore, this is a simple and versatile model for studying gastrointestinal physiology and the health benefits of bariatric surgery.

Video Link

The video component of this article can be found at https://www.jove.com/video/56534/

Introduction

As the obesity epidemic continues to grow worldwide bariatric surgery has gained popularity as it is the most effective long-term treatment for obesity[1]. Unfortunately, weight loss by diet and exercise is difficult to achieve and relatively ineffective over the long-term[2,3]. Bariatric surgery, such as vertical sleeve gastrectomy (VSG), is defined as the manipulation of the gastrointestinal tract for the purpose of weight loss[1,4]. Although weight loss is a prominent outcome of bariatric surgery, bariatric surgery provides other health benefits such as improving obesity comorbidities and extending lifespan[5]. For example, bariatric surgery results in high rates of remission of type 2 diabetes and hypertension, and reductions in the lifetime risk of the development of certain types of cancer[1,6,7]. Of note, the effect of bariatric surgery causing remission of type 2 diabetes and hypertension is often observed soon after surgery and prior to weight loss[8,9]. This highlights the concept that there are mechanisms independent of body weight contributing to the health benefits observed after surgery. Animal models of bariatric surgery have been developed and are utilized to study the mechanisms by which these health benefits occur[10,11,12].

We have validated a mouse model of VSG, which we have applied to various genetically modified mouse models to study the mechanisms by which bariatric surgery improves obesity comorbidities such as type 2 diabetes, hypertension, and colorectal cancer[10,11,12]. Rodent models allow more experimental control and the ability to perform genetic or pharmaceutical manipulation to define the role of specific genes or signaling pathways of interest. We are focusing primarily on VSG because VSG is the most commonly performed bariatric procedure in the clinic in the United States[13]. Additionally, VSG is a simple surgical model with fewer anatomic modifications compared to other procedures such as Roux-en-Y gastric bypass or biliopancreatic diversion.

Our mouse model of VSG recapitulates the following effects of bariatric surgery observed in humans: weight loss, reduced food intake, improved glucose regulation, improved islet function, increased post-prandial glucagon-like peptide-1 (GLP-1) secretion, reduced arterial blood pressure, and increased circulating bile acid concentrations[10,11,12,13,14,15]. Therefore, this is an ideal model to study the body weight dependent and independent mechanisms by which VSG improves or resolves obesity comorbidities. In addition, it is a reliable model that can be combined with other surgical procedures, allowing for the investigation of the impact of VSG under various disease conditions with greater mechanistic insight[12].

Protocol

All experimental protocols have been approved by the Cornell University Institutional Animal Care and Use Committee.

1. Pre-surgical Preparation

NOTE: Study mice are typically on a C57BL diet-induced obese background to make studies translationally relevant to human obesity and insulin resistance. Male and female mice may be studied as described in the subsequent steps.

1. At an age of 2 months, place mice on a 45% or 60% high fat diet for 2 months (see **Table of Materials**).
   NOTE: Mice are fed a high fat diet to create an obese and insulin resistant phenotype. Shorter or longer periods of high fat diet feeding may be used depending on the goals of the study.
2. Continue to feed mice *ad libitum* until surgery. If baseline measurements are needed, after 7 weeks of high fat diet feeding, fast mice for 6 h or overnight and take a baseline blood sample for measurement of metabolites of interest (*e.g.,* glucose).
3. Continue mice on high fat diet and allow mice one week to recover after blood collection prior to sham surgery or VSG. Place mice on a liquid diet (see **Table of Materials**) for 4 days before surgery.
   NOTE: Mice are maintained on a liquid diet before and after surgery to clear the digestive tract of particulate matter that may impair the healing response.
4. Sterilize the following items prior to surgery: foil, gauze, cotton tipped applicators (CTAs) and surgical instruments (hemostat, operating scissor, iris scissor, dumont forcep, serrated Brown-Adson forcep, gavage needle, microneedle driver, spring scissor).

2. Vertical Sleeve Gastrectomy and Sham Procedures

1. On the day of surgery weigh mice to obtain a baseline weight measurement.
2. Equip the surgical room with an anesthesia system, heated water bath, and heating pad. Set the water bath to 37 °C and use it to warm 0.9% saline solution for irrigation. Clean the surgical field with 70% ethanol and use sterile technique to open autoclaved surgery pack and tools.
   NOTE: The heating pad is used to keep the animal warm during surgery and recovery from anesthesia.
3. Using an induction chamber, anesthetize the mouse with 5% isoflurane and O_2 flow rate of 1 L/min. Confirm that the mouse is in the appropriate plane of anesthesia by performing a toe pinch. Maintain the mouse with 1-3% isoflurane and an O_2 flow rate of 0.6 L/min once a consistent plane of anesthesia has been achieved.
4. **Once the mouse is anesthetized, place eye ointment (see Table of Materials) on its eyes to keep them moist during surgery. At this time also administer an analgesic, such as meloxicam (2 mg/kg subcutaneously). Clip the hair from the umbilicus to the axilla holding the skin down gently to provide tension.**
   1. As the mouse's skin is thin and delicate, take care to not cause skin lesions when clipping the hair. Clean the skin with povidone-iodine and alcohol.
   2. Don sterile gloves and prepare the sterile surgical field and instruments. Perform the rest of the procedure using sterile technique. Prepare the sterile surgical field by placing sterile surgical drapes on either side of the mouse. Use autoclaved tin foil to create a surgical drape for the mouse.
   3. Cut a small hole in the tin foil to allow access to the mouse's abdomen.
5. Make an incision of the skin from the mid-abdomen (umbilicus) to the level of the xiphoid cartilage using an iris scissor. Identify the linea alba and use iris scissors to cut through the body wall along the linea alba. Use CTAs to gently elevate the stomach out of the abdomen and then bluntly dissect the greater omentum off of the greater curvature of the stomach.
6. Ligate the short gastric artery that runs between the fundus of the stomach and the spleen by placing two ligatures using 7-0 monofilament absorbable suture. Use spring scissors to cut between the two ligatures. Once the artery is transected, fully exteriorize the stomach from the abdominal cavity. Place gauze under the stomach and wet with saline to keep the tissue moist.
7. **Sham Procedure**
   1. For the sham procedure, place a loose simple continuous pattern of suture using 6-0 monofilament absorbable suture with a taper needle. Begin 2 mm to the right (surgeon's right) of the esophagus. Place the suture line along the ventral gastric wall and then continue along the dorsal gastric wall.
      NOTE: For all gastric manipulations, use only monofilament absorbable suture to decrease the risk of infection. Furthermore, use only suture with a taper needle for all gastric manipulations, as use of a cutting needle increases the risk of suture "pull-through" leading to gastric tissue damage and wound dehiscence.
   2. Pass the needle completely through one gastric wall while placing the suture. Make sure the suture is lying flat, but not restricting the stomach. Gently tie the suture off using 3-4 throws and skip to step 2.9.
   3. For the VSG procedure skip step 2.7 and proceed with step 2.8.
8. **VSG procedure**
   1. For the VSG procedure, ligate the prominent branches of the gastric artery and vein with 7-0 monofilament absorbable suture with a taper needle using 3 throws for each knot. Place the ligatures just below (*i.e.*, towards the lesser curvature of the stomach) the intended line of transection.
      NOTE: The intended line of transection starts at ~2 mm above (meaning towards the greater curvature of the stomach) the cardiac notch of the stomach and at least 2 mm below the margo pilcatus and extends to the proximal end of the right lobe of the pancreas. Typically, there are 4 vessels per gastric wall that need to be ligated; however, this may vary between mice.
   2. To prevent spillage of gastric contents during the gastrectomy, place a simple continuous line of suture passing through both gastric walls just below the intended line of transection using 6-0 monofilament absorbable suture with a taper needle. Begin the suture line to the surgeon's right of the esophagus and below the margo pilcatus and end just above the pancreas.
   3. Place thin-tipped hemostats above the suture line and use spring scissors to cut between the suture line and hemostats. Remove the transected gastric tissue from the sterile surgical field. Use CTAs to clean blood and digesta off of the stomach.
   4. Reinforce apposition of the gastric walls using 6-0 monofilament absorbable suture with a taper needle using a simple discontinuous pattern.
      NOTE: A simple discontinuous suture pattern is recommended since this provides more secure closure than the simple continuous suture pattern.

5. Flush the gastric remnant with saline throughout the procedure in order to keep the tissue moist and clean. Use a 20G gavage needle attached to a 20-mL syringe to perform gastric lavage with saline. Use this size to ensure adequate pressure is provided without risking gastric tissue damage from excessive pressure or accidental gastric tissue damage from use of a regular pointed needle.
6. Use a minimum of 20 knots to securely close the stomach; pay attention to closure along the esophageal side, as this side is more difficult to access and therefore is often a site of dehiscence.
7. Ensure that there are no leaks by gently pressing on the stomach with CTAs. If leaks are identified, place additional simple discontinuous knots on the areas of leakage and then leak test again. Leak test until no leakage is detected.
8. Do a final thorough lavage of the stomach using at least 60 mL of saline to ensure that no infectious particulate matter has been left behind.

9. Place the stomach back into the abdominal cavity under the liver using a CTA. Place CTAs along the dorsal aspect of the abdominal cavity to absorb all excess fluid. Using a blunt 18G needle, inject lactated Ringer's solution (LRS) with or without antibiotics (0.5 mL LRS +/- 20 mg/kg Enrofloxacin) directly into the abdominal cavity, just prior to closure.
   NOTE: This replaces fluid loss experienced during surgery and provides a method for direct application of antibiotics to the surgical site to assist with recovery.
10. Close the abdominal muscle layer using 6-0 monofilament absorbable suture with a taper needle in a simple discontinuous pattern. Then close the skin layer with 6-0 monofilament absorbable suture in a simple continuous pattern.
11. Place tissue adhesive on the skin and fold the skin over the suture line to bury the suture so that the mouse cannot disrupt the wound closure post-operatively.
12. Turn the isoflurane off and let the mouse recover on the heating pad for 10-15 minbefore returning it to its home cage. Do not leave the animal unattended until it has regained consciousness and is able to move.
13. Keep animals housed singly until fully recovered from surgery.
    NOTE: If accurate food intake measurements are to be obtained throughout study, mice should continue to be singly housed throughout study. There is a risk of foreign body obstruction from eating home cage bedding. Animals should be housed in cages free of bedding except for nesting material to provide enrichment (see **Table of Materials**).

3. Post-operative Mouse Care and Measurements

1. Return the mouse to its home cage and place the cage on a heating pad. Maintain mice on a liquid diet for at least 7 days after surgery. Administer an analgesic, such as meloxicam (2 mg/kg subcutaneously), for 2 days after surgery.
   NOTE: An antibiotic, such as enrofloxacin (20 mg/kg), may be given for up to 7 days after surgery. Antibiotics are given to minimize risk of infection from the surgical procedure and may or may not be required depending on the level of sterility maintained throughout the procedure. However, if antibiotics are used, they must be administered to all mice in the same study to maintain consistency.
2. During the post-operative period assess body weight, food intake, defecation, activity level, and disposition at least daily to ensure proper healing and recovery. Conduct post-operative monitoring for 14 days after surgery.
   NOTE: Surgical failure is typically seen either immediately after surgery or within three days post- operatively. Animals that are not eating or drinking on the second day after surgery must be closely monitored for surgical complications.
3. After successful completion of the post-operative period, measure body weight and food intake regularly throughout study to ensure maintenance of the appropriate phenotype.
4. **Perform an oral glucose tolerance test (OGTT) to assess glucose tolerance, glucose-stimulated insulin secretion, and gut hormone secretion.**
   1. Perform the OGTT in mice after fasting them for 6 h and then gavage mice with 50% dextrose solution at a 1 g/kg dose.
   2. Collect tail blood samples at 0, 2.5, 5, 15, 30, 60, and 120 minutes after the gavage. Measure blood glucose with a glucometer at these time points. Collect serum samples at each time point for measurement of various hormones, such as insulin and GLP-1.
   3. Fast mice for 6 h prior to euthanasia. Take a final blood sample and glucose measurement. Compare the final glucose measurement to the baseline measurement. Euthanize mice with an intraperitoneal injection of pentobarbital at a 200 mg/kg dose.
   4. Use the baseline and final serum samples to measure additional analytes and compare pre- and post-operative surgical values.

Representative Results

The sham and VSG procedures are depicted in **Figure 1**. **Figure 1A** shows where the suture line is placed along the gastric walls during the sham procedure. This same area is where the stomach is cut during VSG surgery. **Figure 1B** shows the tubular remnant of stomach left after performance of VSG.

Statistics and Data Analysis
Data are presented as mean ± SEM. Data were analyzed by ANOVA with Tukey's post-test or by Student's t-test as indicated. Differences were considered significant at $P<0.05$.

VSG decreases energy intake and body weight and improves glucose tolerance

High fat diet fed male C57BL mice were operated on and studied as described above. VSG-operated mice exhibited reduced energy intake and body weight compared to *ad libitum* fed sham-operated mice (Cumulative energy intake: Sham = 474 ± 19, VSG = 385 ± 14 kcal; Final body weight: Sham = 34.5 ± 2.1, VSG = 29.9 ± 1 g; **Figure 2A**-B, **P*<0.05). Three weeks after surgery an oral glucose tolerance test was performed. Glucose measurements were made using a glucometer (see **Table of Materials**). Serum insulin and GLP-1 concentrations were measured by multiplex sandwich electrochemiluminescence immunoassay (see **Table of Materials**). VSG improved glucose tolerance (Glucose AUC_{0-120}: Sham =1,467 ± 76, VSG = 1,061 ± 72 mmol/L x 120 minutes; **Figure 3A**-B, *P* <0.01), increased glucose-stimulated insulin secretion (Percent increase in insulin from baseline to 15 minutes after the glucose gavage: Sham = 92 ± 54, VSG = 2,720 ± 1,241%; **Figure 3C**, *P* <0.05), and increased post-prandial GLP-1 secretion compared with sham-operated *ad libitum* fed control mice (GLP-1 AUC_{0-120}: Sham = -124 ± 45, VSG = 111 ± 48 pmol/L x 120 minutes; **Figure 3D**, **P* <0.01). These results are consistent with what is seen in humans and other rodent models after bariatric surgery[9,17,18,19].

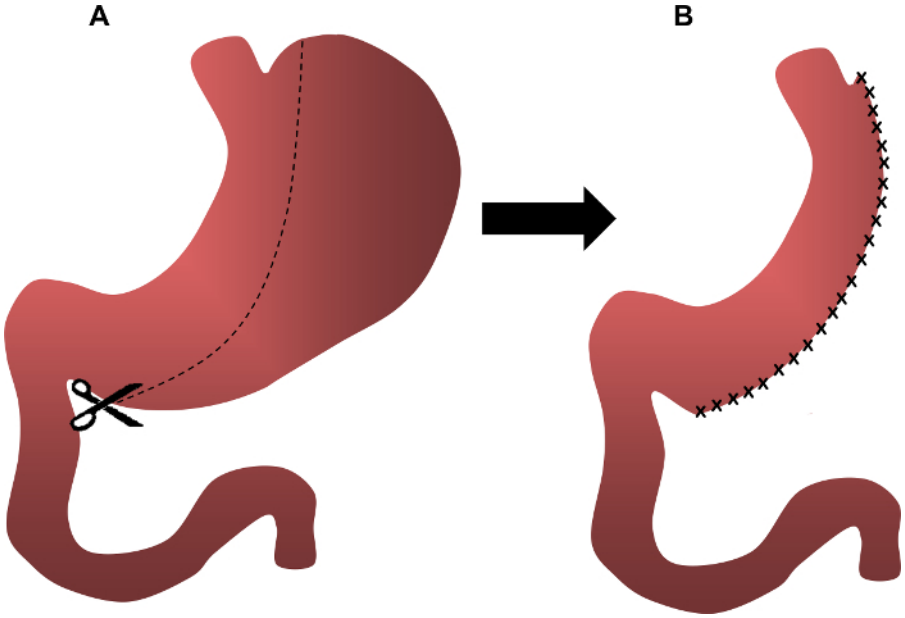

Figure 1: Diagram of VSG. Depiction of line of transection during VSG. **(A)** This same area is where a simple continuous line of suture is placed along the gastric walls during the sham procedure. **(B)** Depiction of the tubular remnant left after completion of the VSG procedure. Please click here to view a larger version of this figure.

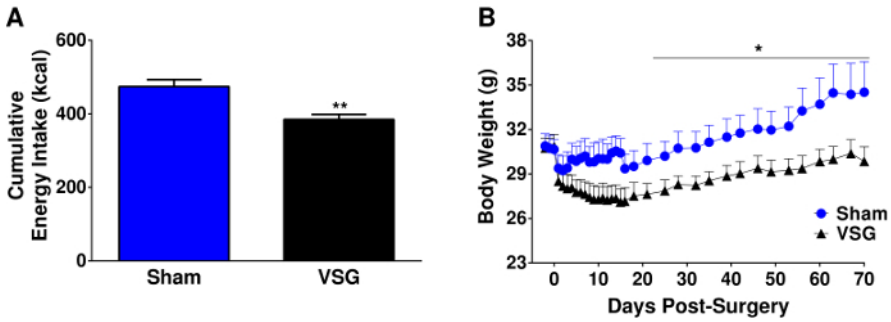

Figure 2: VSG lowers energy intake and body weight. Cumulative energy intake **(A)** and body weight **(B)**. ***P* <0.05 compared with Sham by Student's *t*-test and **P* <0.05 Sham compared with VSG by two-factor ANOVA. Results shown as mean ±SEM. *n* = 6-7 per group. Please click here to view a larger version of this figure.

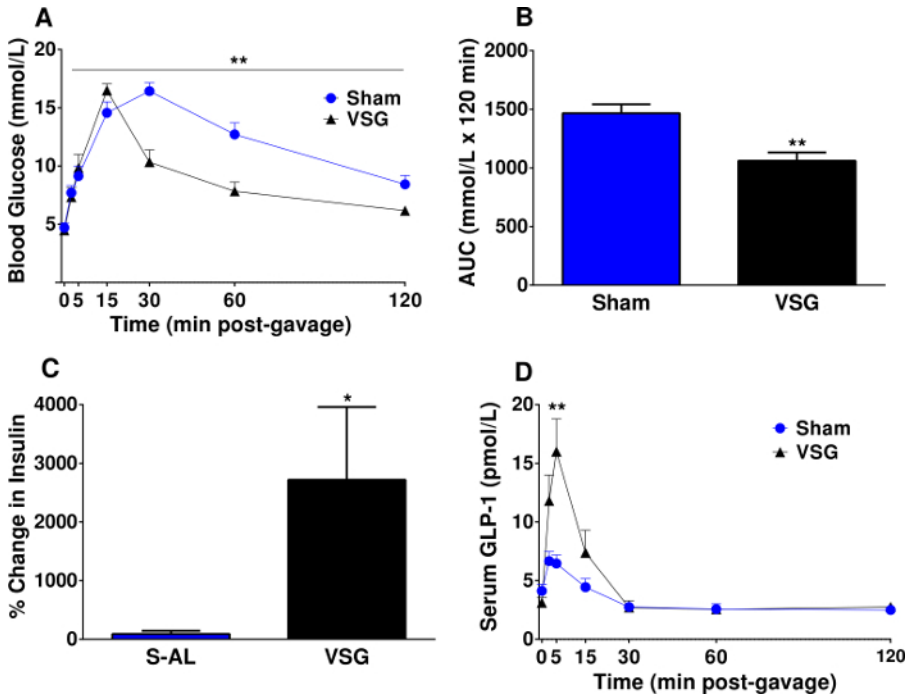

Figure 3: VSG improves glucose tolerance, increases glucose-stimulated insulin secretion and increases post-prandial GLP-1 secretion during an OGTT. (A) Blood glucose concentration, **(B)** glucose area under the curve (AUC), **(C)** percent increase in serum insulin concentrations from baseline to 15 minutes after glucose gavage, and **(D)** serum total GLP-1 concentrations during an OGTT. **P* <0.05, ***P* <0.01, ****P* <0.001 VSG compared with Sham by Student's *t*-test of the AUC or percent change in insulin. Results shown as mean ± SEM. *n* = 6-7 per group. Please click here to view a larger version of this figure.

Discussion

Bariatric surgery is the most effective long-term treatment for obesity and results in other health benefits such as high rates of type 2 diabetes and hypertension remission[1,9,15]. Murine models of bariatric surgery provide a powerful tool with which to identify the mechanisms by which bariatric surgery causes rapid and pronounced improvements in obesity comorbidities. Furthermore, murine models of bariatric surgery provide a novel paradigm for studying the basic biology by which the gut regulates various processes, such as metabolism, cardiovascular function, and carcinogenesis[10,11,12].

We have developed a mouse model of vertical sleeve gastrectomy to study the mechanisms by which bariatric surgery improves obesity comorbidities such as type 2 diabetes and hypertension[10,11,12]. This model of VSG consists of ligation of stomach vessels followed by removal of 70% of the stomach. The control condition is a sham surgery in which suture is placed along the gastric wall in the same location where the stomach would be transected in a VSG procedure. Herein, we present data where sham-operated mice were fed *ad libitum* after surgery. In order to investigate body weight-independent effects, a sham-operated control group that is food restricted in order to match their body weight to VSG-operated mice should be studied, as we have previously described[11,12]. Our data demonstrate that this VSG model achieves both reductions in body weight and food intake compared to *ad libitum* fed sham-operated controls. Oral glucose tolerance tests are done at least two weeks after surgery to allow animals enough time to fully recover from surgery, so that surgical recovery is not a confounding factor. Consistent with the decrease in body weight, the presented model exhibits improved glucose tolerance. Similar to what is observed in human patients after bariatric surgery, VSG-operated mice exhibit remarkable increases in glucose-stimulated insulin secretion and postprandial GLP-1 secretion[14,17].

Lab members are thoroughly trained on sham surgery and VSG and practice for several months to master this technique and achieve a survival rate of greater than 95%; however, this will vary based on prior experience. Prior to initiating a full study, it is recommended that a new surgeon validate his/her VSG and sham surgeries by measuring body weight, food intake, and glucose tolerance in practice sham and VSG-operated mice to ensure that the appropriate phenotype is being achieved. Furthermore, it is important to closely monitor mice after surgery for signs of surgical failure. The most common post-operative complication is leakage of stomach contents. Post-operative signs of this complication include lack of food consumption, lack of fecal production, and a palpable upper abdominal mass. Animals that exhibit signs of surgical complication should be promptly euthanized. If post-operative complications are a reoccurring problem, a reassessment of surgical technique must be performed. The most common problem is inadequate closure of the gastric wall leading to leakage of gastric contents. Closure of the gastric wall is the most important step in the protocol. Once suture placement is complete, there should be no visible gaps between knots. It is advised that CTAs are used to verify that there is no leakage of stomach contents prior to closing the abdomen. Another less common issue is induction of gastrointestinal stasis by inadvertent manipulation of the small intestine when initially removing the stomach from the abdominal cavity. Care must be taken to not manipulate the small intestines because the mouse small intestine is very fragile and excessive manipulation of the small intestine can lead to stasis.

Our mouse model of VSG has similar surgical outcomes to that of other groups and survival rates greater than 95%. Similar to human patients and other mouse models of VSG, the presented mouse VSG models exhibited weight loss, reduced food intake, improved glucose tolerance, and increased post-prandial GLP-1 secretion[4,18,19]. This sham procedure differs from other models as it involves placement of suture while

other models only apply pressure to the stomach with blunt forceps[19,20]. Placing sutures allows control of the effect of placing foreign material in the stomach while also providing surgical manipulation of the gastric tissue. Like any mouse model, the limitations of this technique are that the translation of data generated from this mouse model to humans is limited by species differences. However, mice allow for greater control and experimental manipulation, providing deeper mechanistic insight than can be achieved in human patients. Although the majority of murine bariatric studies have primarily focused on the glucoregulatory benefits of these procedures, we use the presented murine VSG model to define the mechanisms driving the impact of bariatric surgery on hypertension and cancer[12]. This highlights important future applications of this model.

Murine models of bariatric surgery provide an important tool to study the mechanisms by which bariatric surgery produces weight loss and confers health benefits. Furthermore, bariatric models provide a novel paradigm with which to study how the gut interfaces with other physiologic processes in the body. Described here is a model of VSG which has been previously validated and recapitulates many of the effects seen in humans after VSG[10,11,12]. Furthermore, this is a versatile model that can be successfully combined with other surgical procedures for assessment of different disease processes and/or more detailed mechanistic assessment[12].

Disclosures

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References

1. Buchwald, H. *et al.* Bariatric surgery: a systematic review and meta-analysis. *JAMA.* **292** (14), 1724-1737 (2004).
2. Wadden, T. A., Butryn, M. L., & Byrne, K. J. Efficacy of lifestyle modification for long-term weight control. *Obes Res.* **12 Suppl** 151s-162s (2004).
3. Yanovski, S. Z., & Yanovski, J. A. Obesity. *N Engl J Med.* **346** (8), 591-602 (2002).
4. Abbatini, F. *et al.* Long-term effects of laparoscopic sleeve gastrectomy, gastric bypass, and adjustable gastric banding on type 2 diabetes. *Surg Endosc.* **24** (5), 1005-1010 (2010).
5. Sjostrom, L. *et al.* Effects of bariatric surgery on mortality in Swedish obese subjects. *N Engl J Med.* **357** (8), 741-752 (2007).
6. Adams, T. D. *et al.* Cancer incidence and mortality after gastric bypass surgery. *Obesity (Silver Spring).* **17** (4), 796-802 (2009).
7. Vest, A. R., Heneghan, H. M., Schauer, P. R., & Young, J. B. Surgical management of obesity and the relationship to cardiovascular disease. *Circulation.* **127** (8), 945-959 (2013).
8. Ahmed, A. R. *et al.* Laparoscopic Roux-en-Y gastric bypass and its early effect on blood pressure. *Obes Surg.* **19** (7), 845-849 (2009).
9. Pories, W. J. *et al.* Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. *Ann Surg.* **222** (3), 339-350; discussion 350-332 (1995).
10. Garibay, D. *et al.* beta-Cell Glucagon-Like Peptide-1 Receptor Contributes to Improved Glucose Tolerance After Vertical Sleeve Gastrectomy. *Endocrinology.* **157** (9), 3405-3409 (2016).
11. McGavigan, A. K. *et al.* TGR5 contributes to glucoregulatory improvements after vertical sleeve gastrectomy in mice. *Gut.* **66** (2), 226-234 (2017).
12. McGavigan, A. K. *et al.* Vertical sleeve gastrectomy reduces blood pressure and hypothalamic endoplasmic reticulum stress in mice. *Dis Model Mech.* **10** (3), 235-243 (2017).
13. Esteban Varela, J., & Nguyen, N. T. Laparoscopic sleeve gastrectomy leads the U.S. utilization of bariatric surgery at academic medical centers. *Surg Obes Relat Dis.* **11** (5), 987-990 (2015).
14. Laferrere, B. *et al.* Effect of weight loss by gastric bypass surgery versus hypocaloric diet on glucose and incretin levels in patients with type 2 diabetes. *J Clin Endocrinol Metab.* **93** (7), 2479-2485 (2008).
15. Adams, T. D. *et al.* Health benefits of gastric bypass surgery after 6 years. *Jama.* **308** (11), 1122-1131 (2012).
16. McGavigan, A. K. *et al.* TGR5 contributes to glucoregulatory improvements after vertical sleeve gastrectomy in mice. *Gut.* (2015).
17. Peterli, R. *et al.* Improvement in glucose metabolism after bariatric surgery: comparison of laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy: a prospective randomized trial. *Ann Surg.* **250** (2), 234-241 (2009).
18. Ryan, K. K. *et al.* FXR is a molecular target for the effects of vertical sleeve gastrectomy. *Nature.* **509** (7499), 183-188 (2014).
19. Wilson-Perez, H. E. *et al.* Vertical sleeve gastrectomy is effective in two genetic mouse models of glucagon-like Peptide 1 receptor deficiency. *Diabetes.* **62** (7), 2380-2385 (2013).
20. Pressler, J. W. *et al.* Vertical sleeve gastrectomy restores glucose homeostasis in apolipoprotein A-IV KO mice. *Diabetes.* **64** (2), 498-507 (2015).

