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Development of murine bariatric surgery models: lessons learned

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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 multilayer repair of the gastrotomy. RYGB procedure consisted of side-to-side, functional end-to-side bowel anastomoses and exclusion of the stomach medial to the 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 1 mo 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.

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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 (body mass index > 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 proinflammatory 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, in few studies, patients are followed for more than 5 y 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 comorbidities, 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] 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 used 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 preoperative and postoperative 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 intraoperative insensate heat loss; and conversion to a liquid diet for the perioperative period.

Materials and methods

All experiments involving mice were approved by the Institutional Animal Care and Use Committee, 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 staff. Mouse stress level was subjectively assessed by examination of changes in the following metrics: mouse activity, self-grooming and peer grooming, wound licking, orbital tightening, and visits to food trough.

Mice

Female Swiss-Webster mice, aged 7 wk, were purchased from Taconic Biosciences and housed in groups under a 12-h light/dark cycle at room temperature. Only female mice were used in the development of our surgical techniques 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, NJ) for 20 wk until they reached a body weight more than 40 g, ideally >50 g. Three days before surgery, mice were changed from the high-fat diet to a liquid diet (Two Cal HN, vanilla; Abbott Nutrition, Columbus, OH). Mice were maintained on a liquid diet until 10 d after surgery. Solid high-fat diet was reintroduced to mice starting on postoperative day 7. Mice were provided water *ad libitum* before surgery and maintained on a 50% Pedialyte water solution (Pedialyte, unflavored; Abbott Nutrition) for 10 d postoperatively. We did not fast the mice. To limit ingestion of feces or bedding, bedding was removed from cages 3 d before surgery and replaced with a raised wire platform floor until postoperative day 10, when standard bedding was reintroduced and wire-bottom platforms were removed. At time of surgery, abdominal skin was shaved and disinfected with Betadine and alcohol swabs.

Surgical materials and reagents

Before initiation of surgery, ciprofloxacin 0.1 mg/kg (Claris Lifesciences, Inc, North Brunswick, NJ) was injected intraperitoneally. Inhaled isoflurane 2%-3% was used for general anesthesia, and subcutaneous buprenorphine SR 1.2 mg/kg (Zoo Farm, Fort Collins, CO) for analgesia. Insensate fluid loss was compensated with a subcutaneous injection of sterile normal saline at 20 mL/kg/h of operation (0.9% wt/vol NaCl solution; Med-Vet International, Mettawa, IL). Additional subcutaneous normal saline fluid boluses were provided daily until postoperative day 3 to limit dehydration. Intraoperative heat source was provided by a far-infrared surgical warming pad (Kent Scientific Corporation, Torrington, CT). Microscopic surgery instruments were used for all surgeries (Fisher Scientific, Pittsburgh, PA; Harvard Apparatus, Holliston, MA), and hemostasis was assured with the use of a Bovie cautery device (Bovie Deluxe High Temperature Cautery Kit; World Precision Instruments, Sarasota, FL). Prolene suture (8-0) on a tapered needle was used for bowel anastomoses, and 6-0 vicryl suture on a reverse-cutting needle was used for fascia and skin closure (Ethicon, Somerville, NJ).