Brief Communication

MOLECULAR METABOLISM

Vertical sleeve gastrectomy corrects metabolic perturbations in a low-exercise capacity rat model

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ABSTRACT

Objective: Bariatric surgery is currently our most effective strategy at weight loss, yet the mechanisms for its success remain unknown. Low exercise capacity, in humans and rodents, predicts poor metabolic outcome. The objective of this manuscript was to determine if bariatric surgery could restore metabolic perturbations in rats with low intrinsic exercise capacity.

Methods: We performed vertical sleeve gastrectomy (VSG) or sham surgery in high fat-fed rats selectively bred for low running capacity.

Results: We found that VSG reduced body mass through a reduction in fat mass, caused early reductions in food intake, and shifted macronutrient preference away from fat and toward carbohydrates. VSG had no impact on basal glucose but did improve the return to baseline after an oral glucose load. As has been shown previously, VSG increased postprandial insulin, GLP-1, and bile acids. There was no significant impact of VSG on plasma triglycerides, hepatic triglycerides, or cholesterol. Interestingly, the brown adipose tissue to white adipose tissue ratio tended to be greater in VSG compared to sham surgery animals. While VSG positively impacted several aspects of metabolism, it did not enhance maximal oxygen capacity and seemed to lower metabolic efficiency as indicated by lower resting oxygen consumption and fat and carbohydrate oxidation.

Conclusion: VSG can improve the metabolic status of animals with a low exercise capacity independently of exercise capacity.

Keywords Bariatric surgery; Metabolism; Exercise

1. INTRODUCTION

Increasing rates of obesity and type 2 diabetes mellitus (T2DM) threaten our health and overburden our health care system. A large part of this threat comes from the relative dearth of effective therapies for both conditions. Although a variety of lifestyle interventions and medications can produce some weight loss and improvements in glucose regulation [1], none of them provides the long-term benefits observed with bariatric surgery. The fastest growing bariatric surgery procedure performed worldwide is vertical sleeve gastrectomy (VSG), a procedure in which ~80% of the stomach along the greater curvature is removed. This procedure produces sustained weight loss and rapid improvements in glucose and lipid metabolism [2]. While the VSG procedure is quite effective, the mechanism(s) underlying this success are unclear. Understanding the mechanism(s) for this success could lead to less invasive treatments as well as expand our current knowledge of the pathophysiology of obesity.

Low exercise capacity and cardiovascular fitness are highly predictive of poor metabolic health, including higher fat mass, reduced insulin sensitivity, increased blood pressure, and, importantly, increased age-adjusted mortality [3]. Evidence for the impact of inherent exercise capacity has been extensively studied in a rat model derived from a founder population of N:NIH stock rats and artificially selectively bred for intrinsic (untrained) treadmill running capacity [4]. In this model, as in humans, exercise capacity is a heritable trait [5,6], and, like humans who differ in running capacity, rats with low vs. high capacity running ability (LCR vs. HCR) diverge in susceptibility to metabolic disease [7–10]. Specifically, compared to HCR, LCR weigh significantly more and despite similar food consumption have decreased capacity for substrate oxidation throughout their lifespan [11]. The phenotype of LCR is coincident with a host of metabolic problems [12] along with a 28–40% decreased lifespan [7]. Given the widespread metabolic benefits of VSG, we hypothesized that VSG would induce weight loss and correct the metabolic perturbations associated with LCR.

2. MATERIAL AND METHODS

2.1. Animals

LCR rats were developed as previously described [4]. In the running capacity selection process, rats were exercise tested across 5 consecutive days so that estrous cycle dropped out as a random variable [4]. Twenty-two female LCR rats aged 4–5mos old were individually housed and maintained on a 12:12-h light–dark cycle (lights off at 1400) at 25 °C and 50–60% humidity with ad libitum access to water and a high-fat butter diet (HFD, 4.54 kcal/g; 41% fat; Research Diets, New Brunswick, NJ) for 12 weeks. Only female rats

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were used in this study as 80% of bariatric surgery patients are women. After 12-weeks of high fat feeding, body fat and lean mass were assessed using NMR (Echo MRI, Houston, TX), and surgical groups (VSG, n = 11 vs. sham, n = 9) were assigned in a counter-balance fashion based on fat mass. All procedures for animal use were approved by the University of Michigan Institutional Animal Care and Use Committee. Two VSG animals died within 2-weeks of surgery. All of their data have been eliminated from the study. Besides the exercise selection process, estrous cycle was not assessed on days of testing. The rationale for this is that we wanted to determine the overall impact of surgery on metabolic responses and were not interested in the potential variation across the estrous cycle.

2.2. Surgeries

VSG was performed as described previously [13–17]. Briefly, a laparotomy incision was made in abdominal wall, allowing the stomach to be isolated outside the abdominal cavity and placed on saline-moistened gauze pads. Loose, gastric connections to the spleen and liver were released along the greater curvature, and the suspensory ligament supporting the upper fundus was severed, widening the angle between lower esophagus and the fundus. The lateral 80% of the stomach was excised using an ETS 35-mm staple gun, leaving a tubular gastric remnant in continuity with the esophagus and the pylorus and duodenum. This gastric sleeve was then reintegrated into the abdominal cavity. Finally, the abdominal wall was closed in layers.

Sham surgery was performed as described previously [13–17] and involved abdominal laparotomy incision and removal of the stomach from the abdominal cavity followed by manually applying pressure with blunt forceps along a vertical line between the esophageal sphincter and the pylorus of the stomach.

For 3 days preoperatively, the high-fat diet was replaced with Ensure Plus liquid diet (Abbott Nutrition, Columbus, OH). After recovery from surgery, animals were studied using a battery of *in vivo* physiological studies described below. Body mass was measured weekly throughout the study, and food intake was measured weekly for the first 4-weeks postoperatively. Body composition was assessed again at 4, 8, and 24 weeks after surgery.

2.3. Macronutrient preference

We previously demonstrated that bariatric surgery alters macronutrient preference. To determine whether the LCR rats responded similarly, approximately 18 weeks after surgery, we provided three pure macronutrient diets casein, high fat lard, and cornstarch; (Harlan Teklad, Madison, WI), which were presented in separate containers simultaneously and assessed food intake for each macronutrient for 4d.

2.4. Glucose tolerance tests

7-weeks after surgery, an oral glucose tolerance test was performed. Prior to each test, rats were fasted for 5h and blood glucose was measured via a hand-held glucose analyzer (Accuchek; Roche Diagnostics, Indianapolis, IN) from tail vein samples at 0, 15, 30, 45, 60, and 120 min after administration of 25% dextrose (2 g/kg). Fifteen minutes after the nutrient load, blood was collected in tubes containing 1 ml of a cocktail made of EDTA (4.65 g), aprotinin (92 mg), heparin (40,000 U), and a DPP4-Inhibitor (1 μL; Millipore # DPP4-010) for assessment of plasma glucagon levels.

2.5. Mixed meal tolerance test

Approximately 12 weeks after surgery, 4–5-hour fasted rats were gavaged with 3 mL (4.46 kcal) Ensure Plus Liquid diet. This load was based on the volume of liquid diets rats will voluntarily consume [17]. Fifteen minutes after the nutrient load, blood was collected in tubes containing 1 ml of a cocktail made of EDTA (4.65 g), aprotinin (92 mg), heparin (40,000 U), and a DPP4-Inhibitor (1 μL; Millipore # DPP4-010). Blood glucose was assessed using the hand held glucometer as in the OGTT. Plasma levels of total bile acids (Genway Biotech, San Diego, CA) and insulin (Crystal Chem, Downers Grove, IL) were assessed using commercially available ELISA kits while GLP-1 (7–36) was measured by an electrochemiluminescence assay (Meso Scale Discovery, Gaithersburg, MD).

2.6. Lipid metabolism

Ad lib-fed and 24h-fasted blood was taken from the tail vein for subsequent analysis of plasma triglycerides and cholesterol. At the end of the study liver from ad lib-fed rats was harvested and immediately flash frozen in liquid N_2 for subsequent analysis of hepatic triglyceride levels.

Briefly, hepatic lipids were folch extracted, and tissue and plasma triglycerides and cholesterol were assayed by the University of Cincinnati MMPC using commercially available kits (Cholesterol: Infinity® Cholesterol, Fischer Scientific, Waltham, MA; Triglycerides, Randox Trigs, Randox Laboratories, Crumlin, UK).

2.7. Estimation of endurance running capacity

Maximum oxygen consumption (VO_{2max}) was measured by the UM Animal Phenotyping Core using an integrated open-circuit calorimeter (CLAMS, Columbus Instruments). All exercise tests were performed between 0900 and 1500h. Rats were weighed and placed into the treadmill chambers ($305 \times 51 \times 44$ mm^3) for approximately 10 min to acclimate them to the treadmill environment. The slope of the treadmill was set at 10° to the horizontal for each rat within the initial speed set at 10 m/min and increased every 2 min until the rat sat on the electric shocker for 5 consecutive seconds indicating exhaustion. VO_2 and VCO_2 were sampled every 5s.

2.8. Statistical analysis

Normally distributed data were analyzed utilizing standard parametric statistics including One- and Two-way ANOVAs with repeated measures and t-tests where applicable. Statistical analyses were performed using either GraphPad Prism v.6.02 or Statistica v.13 for Windows. Data are expressed as mean ± SEM, and statistical significance was accepted when $p < 0.05$.

3. RESULTS

3.1. Body mass & composition changes

VSG caused a significant decrease in body weight at week 1, compared to presurgery values, and at week 24, compared to their sham surgery counterparts, whether shown as absolute or as a % delta over time (Figure 1A,B; surgeryXtime interaction; $p < 0.05$). Further, sham but not VSG animals had greater body mass over presurgical values at weeks 11–24 (Figure 1A,B; surgeryXtime interaction; $p < 0.05$). Fat mass was significantly lower at 24 weeks after the surgery in VSG vs. Sham animals (surgeryXtime interaction; $p < 0.05$), and there were no significant differences between surgeries in lean mass at any post-operative time point (Figure 1C,D).

3.2. Food intake & preference

Similar to what we have previously reported [13,17], food intake was significantly lower in VSG vs. sham animals the first two weeks after surgery but was similar thereafter (Figure 1E; surgeryXtime interaction; $p < 0.05$). Twenty-weeks after surgery when body mass changes

