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Sleeve Gastrectomy Induces Loss of Weight and Fat Mass in Obese Rats, but Does Not Affect Leptin Sensitivity

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BACKGROUND & AIMS: Surgical intervention produces sustainable weight loss and metabolic improvement in obese individuals. Vertical sleeve gastrectomy (VSG) produces dramatic, sustained weight loss; we investigated whether these changes result from improved sensitivity to leptin. **METHODS:** VSG was performed in Long–Evans rats with diet-induced obesity. Naïve or sham-operated rats, fed either ad libitum or pair-fed with the VSG group, were used as controls. Following surgery, body weights and food intake were monitored. We investigated energy expenditure, meal patterns, leptin sensitivity, and expression of pro-opiomelanocortin/agouti-related peptide/neuropeptide Y in the hypothalamus of the rats. **RESULTS:** We observed sustained losses in weight and body fat in male and female rats after VSG. Weight loss persisted after the disappearance of a transient, post-surgical food intake reduction. Resting energy expenditure was similar between control and VSG rats. VSG rats maintained their reduced body weights. However, they responded to a chronic food restriction challenge by overeating, which resulted in prerestriction, rather than pre-VSG, body weights. Consistent with lower adiposity, VSG decreased plasma leptin levels. Although VSG slightly improved leptin's anorectic action, the response was comparable to that observed in controls matched for adiposity by caloric restriction. Changes in hypothalamic neuropeptide expression were consistent with the lower body weight and lower leptin levels but cannot account for the sustained weight loss. **CONCLUSIONS: VSG causes sustained reduction in body weight, which results from loss of fat mass. The maintenance of weight loss observed did not result from changes in sensitivity to leptin.**

Keywords: Bariatric Surgery; Hypothalamus; Arcuate; Stomach.

BASIC–ALIMENTARY TRACT

Body fat is regulated by a complex neuroendocrine system, making it difficult to maintain weight loss achieved through caloric restriction. A key component of this regulatory system is the adipocyte hormone leptin. Leptin is secreted from white adipose tissue and the stomach, and it reduces food intake and body weight through its actions at the long leptin receptor (ObRb) in the central nervous system (CNS). In the arcuate nucleus of the hypothalamus, a major CNS energy balance control area, leptin exerts its catabolic action by stimulating pro-opiomelanocortin (POMC) neurons, while inhibiting expression of the endogenous MC3/4R antagonist agouti-related peptide (AgRP) and the potent orexigen neuropeptide Y (NPY).[1] As a result, when injected directly into the 3rd-cerebral ventricle adjacent to the arcuate nucleus of the hypothalamus, leptin reduces food intake and body weight.[2]

However, in most obese individuals, leptin levels are elevated[3,4] in direct proportion to body fat, and exogenous leptin treatment produces little or no weight loss.[5,6] This failure of leptin to produce the same effects in obese individuals as it can in lean individuals is termed *leptin resistance*. Thus, a key question for any weight-loss regimen is whether it acts to reverse leptin resistance as a part of how weight loss is maintained.

Bariatric surgery produces greater weight loss and weight loss that is more durable than caloric restriction and, therefore, is currently the most effective therapy for obesity.[7] Vertical sleeve gastrectomy (VSG) is one such bariatric surgical procedure that involves the creation of a reduced stomach lumen along the lesser curvature of the stomach through the removal of gastric tissues along the greater curvature from the fundus to the antrum. Stomach capacity is typically reduced ≥80%, and the intestine remains intact. This procedure produces dramatic weight loss in humans[8–10] and in rodents.[11,12] In fact, recent reports indicate that its efficacy is close to that of the more common Roux-en-Y gastric bypass.[13,14]

Although VSG is typically referred to as a "restrictive" procedure, evidence suggests that stomach volume reductions alone are unlikely to account for the profound efficacy of the procedure.[15] We hypothesized that VSG-

Abbreviations used in this paper: AgRP, agouti-related peptide; CNS, central nervous system; MBH, mediobasal hypothalamus; NPY, neuropeptide Y; PF, pair-fed; POMC, pro-opiomelanocortin; RQ, respiratory quotient; VSG, vertical sleeve gastrectomy.

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treated rats would actively defend their new lower body weights and would do so through changes in the leptin-hypothalamic axis. Such changes could occur either with increased leptin secretion, increased leptin action, or direct changes on the key targets of leptin action in the hypothalamus. These mechanistic issues are difficult if not impossible to address solely in human subjects. Consequently, we developed a rat model of VSG used for the present studies.

Materials and Methods

Animals

Male and female Long–Evans rats (Harlan Laboratories, Indianapolis, IN; 250–300 g) were fed either a high-fat butter oil-based diet (HFD; Research Diets, New Brunswick, NJ, D12451; 45% fat; 4.73 kcal/g) or standard chow (Harlan-Teklad, Indianapolis, IN) for 8 weeks prior to surgery. Rats were housed at the University of Cincinnati at the Metabolic Diseases Institute under controlled conditions (12:12-hour light–dark cycle, 50%–60% humidity, 25°C with free access to water and food except where noted). All procedures for animal use were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Experimental groups are outlined in Supplementary Table 1. Four cohorts, labeled A, B, C, and D, contained 24 to 67 rats each. Cohort A contained naïve (n = 8 male, n = 8 female), sham (n = 10 male, n = 10 female), and VSG (n = 9 male, n = 9 female) rats. Cohorts B and D consisted of chow (n = 10 in cohort D, n = 20 in cohort B), sham (n = 10 in cohort D, n = 14 in cohort B), pair-fed (PF) (n = 8 in cohort D, n = 13–15 in cohort B), and VSG (n = 7 in cohort D, n = 18 in cohort B). Cohort C included sham (n = 8), PF (n = 7), and VSG (n = 9) rats. Rats fed HFD or chow diet prior to surgery were maintained on their respective diets after surgery. Where indicated, a sham-operated subgroup was PF to match the intake of the VSG group. To do this, the amount of food eaten by the VSG rats during the previous 24 hours was provided at random times during the light:dark cycle to the PF rats. Fat and lean tissue mass was measured using nuclear magnetic resonance (Echo MRI: Echo Medical Systems, Houston, TX).

Blood was taken from the tip of the tail just before the onset of dark after 4 hours of fasting for quantification of plasma leptin (postsurgical day 50) and after 2 hours of fasting for quantification of plasma insulin and glucose (postsurgical day 125). At the end of each study, animals were placed briefly in a CO_2 chamber and then sacrificed by decapitation during the light phase.

Surgical Procedures

For VSG, a laparotomy incision was made in the 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, thus 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 superiorly and the pylorus and duodenum inferiorly. This gastric sleeve was then reintegrated into the abdominal cavity. Finally, the abdominal wall was closed in layers. Sham surgery involved abdominal laparotomy incision and placement of the stomach out of 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. Following surgery, rats received intensive postoperative care for 3 days, consisting of twice-daily subcutaneous injections of 10 mL saline and 0.3 mL Buprenex. Rats were fasted 24 hours prior to surgery and had postsurgical access to Osmolite OneCal liquid diet only until food was returned 3 days after surgery.

Energy Expenditure and Meal Patterns

A continuous monitoring system (TSE Systems, Inc, Chesterfield, MO) was used to determine energy expenditure and meal patterns 28 to 30 days after surgery. Rats from each group (n = 14–18/surgical condition) were placed in the system for 96 hours. The first 24 hours were considered adaptation and the data from the next 72 hours were analyzed. Data for indirect calorimetry analysis were sampled every 45 minutes. Data for food intake and meal pattern analysis were sampled every 15 minutes and accumulated in 6-hour blocks.

Fecal Lipid Content

Dietary lipid absorption was assessed using the Behenate method (n = 7–8/group), as described previously.[16] Briefly, rats were temporarily placed on a diet containing 5% sucrose polybehenate (behenic acid). After 24 hours of acclimation to the diet, cages were changed and fecal pellets were collected after another 24 hours. Fecal samples of about 10 mg were collected and fecal lipid content was assayed by gas chromatography of fatty acid methyl esters. Fat absorption was calculated from the ratio of behenic acid to other fatty acids in the diet and feces. During this time, PF rats were also fed the Behenate diet.

Food Restriction Study

On postoperative day 50, rats within each dietary group (n = 14–20/group) were divided into 2 groups (n = 7–10) balanced on the basis of body weight and fat mass. One group received ad libitum access to food, while the other was food restricted by 73% for a period of 22 days to induce weight loss. This was followed by a recovery period in which all rats had ad libitum feeding.