Effect of vertical sleeve gastrectomy on food selection and satiation in rats

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Chambers AP, Wilson-Perez HE, McGrath S, Grayson BE, Ryan KK, D’Alessio DA, Woods SC, Sandoval DA, Seeley RJ. Effect of vertical sleeve gastrectomy on food selection and satiation in rats. Am J Physiol Endocrinol Metab 303: E1076–E1084, 2012. First published August 28, 2012; doi:10.1152/ajpendo.00211.2012.—Vertical sleeve gastrectomy (VSG) is a restrictive procedure that reduces food intake to produce weight loss. Here we assess volume and nutrient effects on the ingestive behavior of VSG and sham surgery animals. Rats given access to Ensure or pelleted chow were used to determine if liquid foods would adversely affect weight loss after surgery. Volume effects were studied by altering the caloric density of Ensure, and dietary preferences for fat and carbohydrate (sucrose) were assessed using a two-bottle test. c-Fos was used to measure neuronal activation in the nucleus of the solitary tract and area postrema in response to intragastric infusions of water, sucrose, or Intralipid. The degree of colocalization with catecholaminergic neurons was also assessed. VSG rats did not show the expected preference for a liquid diet over chow and lacked dietary preferences for fat seen in shams. Preferences for carbohydrate/sucrose solutions were unaffected by surgery. Meal size was reduced by VSG; however, VSG rats were able to alter their volume of intake to compensate for changes in caloric density, and intragastric infusions of water produced similar levels of neuronal activation among VSG, sham, and pair-fed rats. In comparison, nutrient-induced c-Fos activation was substantially increased by VSG. Colocalization between c-Fos and catecholaminergic-expressing neurons was similar among rats treated with water, sucrose, or Intralipid. VSG alters nutrient sensing in a manner that lowers the threshold for satiety and reduces fat preference to induce and maintain weight loss.

BARITRIC SURGERY IS CURRENTLY the most effective treatment for obesity and many obesity-related comorbidities (9). In purely restrictive procedures, such as vertical-band gastroplasty and the adjustable gastric band, a restrictive pouch is created in the forestomach that limits meal size to induce weight loss. In other procedures, such as Roux-en-y gastric bypass surgery (RYGB), restriction is combined with malabsorption and changes in nutrient delivery. Compared with banding, RYGB results in superior weight loss and significant changes in dietary preferences, including a reduced preference for foods that are high in fat (22) and the avoidance of high-calorie liquids, milk, ice cream, and other sweets (7, 8, 13, 18, 22, 33, 34). In other restrictive procedures, postsurgical increases in the consumption of high-energy soft foods and liquids is associated with poorer-than-expected weight loss (7, 8, 38). Because both banding procedures and RYGB cause considerable gastric restriction, the superior effect of RYGB surgery on food choice (7, 8, 13, 15, 38) has been hypothesized to result from surgical alterations of the gastrointestinal tract.

More recently, a novel bariatric procedure, the vertical sleeve gastrectomy (VSG), has emerged as a third alternative. In VSG, the majority of the stomach is resected along the greater curvature leaving a tubular gastric remnant of reduced size, but intestinal anatomy is left intact. In several small clinical studies, VSG was found to improve glucose tolerance and produce similar weight loss as RYGB surgery (12, 15, 17, 21, 26–28, 32). However, the mechanism by which VSG exerts these metabolic benefits is not clear, and there has been some debate as to whether it is simply another restrictive procedure.

The aim of the present study was to determine if VSG alters dietary preferences in a way we would expect based on other purely restrictive procedures. Preferences for a liquid diet (Ensure) over pelleted chow, as well as preferences for carbohydrate and fat, were used to determine how VSG alters food choice. Volume effects were studied in rats given access to two different caloric densities of Ensure, and c-Fos was used to measure the degree of neuronal activation produced by an intragastric infusion of water, sucrose, or Intralipid. Because catecholaminergic neurons in the brain stem respond markedly to distention, the degree of colocalization of c-Fos and dopamine β-hydroxylase (DBH) was assessed in each treatment.

METHODS

Animals. Adult male Long-Evans rats (n = 96, 250–300 g) (Harlan Laboratories, Indianapolis, IN) were individually housed and maintained in a room on a 12:12-h light-dark cycle (lights off at 1400) at 25°C and 50–60% humidity. All procedures for animal use were approved by the University of Cincinnati Institutional Animal Care and Use Committee. Before surgery, rats were given ad libitum access to water and a high-fat butter diet (HFD, 4.54 kcal/g; 41% fat; Research Diets, New Brunswick, NJ) previously documented to produce metabolic impairments (42). After 8 wk on the HFD, the rats were assigned to surgical groups (RYGB, VSG, ad libitum-fed sham, or sham-operated pair fed) that were counterbalanced for lean and fat tissue mass.

Surgery. VSG was performed as previously described (12, 36). A laparotomy was made, and ~80% of the stomach, including all of the fundus and a portion of the antrum, was excised along the greater curvature using an ETS 35-mm staple gun. The remaining gastric sleeve was reintegrated into the abdominal cavity, and the laparotomy was closed in layers. In the VSG sham procedure, the stomach was in the same way, but no cut was made. Subcutaneous injections of Buprenex (0.3 ml), Metacam (0.25 mg/100 g body wt), saline (10 ml), and gentamicin were given on the day of surgery. Buprenex and saline (5–10 ml) were given twice daily over the next 2–3 days as needed.

Food intake, body weight, and body composition. Body composition was assessed using an EchoMRI analyzer (Houston, TX). Beginning 3 days preoperatively, the high-fat diet was replaced with Ensure Plus liquid diet (Abbott Nutrition, Columbus, OH). Two cohorts of
rats were used. In the first, the HFD was returned on postoperative day 3. This cohort was used to assess the early effect of the surgery on food intake and body weight, as well as on nutrient-induced c-Fos activation in the brain stem. Sham-operated rats in the pair-fed group were given access to the amount of food eaten by the VSG animals on the previous day. In a second cohort, rats were maintained on Ensure except where indicated. These animals had previously been used in another study that examined the effect of VSG on blood glucose parameters (12); part of the food intake data in that manuscript (postoperative days 100–120) overlap with data reported in the present manuscript. The experimental progression of each cohort is outlined in Fig. 1.

Liquid vs. solid diet. To determine if liquid diets negatively affect weight loss after VSG, we assessed preferences for Ensure (1.41 kcal/g; fat 29%, carbohydrate 56%, protein 15%) relative to solid chow (3.1 kcal/g; fat 17%, carbohydrate 58%, protein 25%). On postoperative day 123, sham and VSG rats were given simultaneous access to Ensure Plus liquid diet and a standard pelleted rat chow. Rats were allowed to acclimate to the presence of both diets for 4 days. The ratio of Ensure/chow consumed over the last 3 days was averaged as an indication of dietary preference. The long-term effect of consuming each diet separately was studied on postoperative days 100–129 and 183–210 for the Ensure diet and on postoperative days 130–182 for the chow diet.

Volume effects and meal size after VSG. Six-hour-fasted rats were given access to Ensure Plus or Ensure Plus diluted with water (1:2) for 60 min via a repeated-measures design that was counterbalanced across days. Thus, rats were already familiar with this diet, and the only adjustment made by the animals was in response to the different caloric densities of the Ensure. The volume of intake and kilocalories were used to indicate the effect of restriction on meal size. Sham rats, and sham-operated pair-fed rats, were used for comparison.

Carbohydrate and fat preference. Beginning at 1000, food was replaced with a bottle containing plain water (control), as well as a test bottle containing various concentrations of sucrose (0.1–30%). The intake of each bottle was monitored over the next 4 h. Increasing concentrations of sucrose were presented over consecutive days. Preference is indicated by the relative consumption of sucrose over the control bottle, i.e., % of the control value. To determine if VSG alters dietary preferences for fat, the experiment was repeated 1 wk later using 0.2% methyl cellulose and various concentrations of corn oil (1–100%).

Water- and nutrient-induced c-Fos activation. Six-hour-fasted VSG, sham, or sham-operated pair-fed rats were gavaged with isoca-
stored at 4°C in PBS until representative sections from the rostral (-13.3 mm), mid (-13.6 mm), and caudal (-14.0 mm) nucleus of the solitary tract (NTS) were selected and stained for c-Fos. Free-floating sections were incubated for 20 min in 1% H2O2 in methanol solution before being washed 5 × 5 min in PBS + Triton (0.1%) and placed into 10% normal donkey serum for 1 h. Sections were then incubated in c-Fos primary antibody (1:5,000, SC-52 rabbit polyclonal antibody) (Santa Cruz Biotechnology) diluted in blocking solution overnight at 4°C before being placed into a secondary antibody (Biotinylated donkey a-rabbit; 1:200) for 1 h at room temperature. Sections were then placed into aviden-biotylated complex (Vector ABC) 1 h before being exposed to a nickel-diaminobenzidine (DAB) solution for 8 min. Sections were rinsed 3 × 10 min in between each stage with PBS and washed 5 × 10 min in PBS sections after undergoing DAB. Sections were mounted onto Superfrost slides and covered with a cover slip, and areas of interest were photographed at ×5 using a Zeiss 510 Meta microscope under white light. Bilateral counts were made using Axiovision4.4 software under identical conditions by persons blind to each treatment. Representative unilateral sections of the NTS and area postrema (AP) of rats treated with Intralipid were photographed at ×10 magnification and presented in Fig. 7. Cumulative totals were used to indicate c-Fos activation in the NTS, and c-Fos expression in the AP (bregma -13.6 mm) was counted separately. In a second experiment, dual labeling was used to determine if c-Fos (1:1,000) colocalized with DBH (1:1,000) (MAB308; Chemicon)-expressing neurons. Sections were blocked for 1 h in 5% normal donkey serum before being incubated overnight at 4°C in both antibodies. The following day, sections were rinsed and incubated at room temperature in donkey anti-rabbit CY3 (1:100) and donkey anti-mouse fluorescein isothiocyanate (1:100) (Jackson ImmunoResearch) antibodies on an orbital shaker for 1 h at room temperature. Unilateral sections were visualized as before and photographed under 550 and 488 nm light under ×10 magnification. c-Fos- and DBH-expressing neurons and neurons expressing both were counted by hand.

Statistics. Data were analyzed using one-way and two-way independent and repeated-measures ANOVAs where appropriate and expressed as means ± SE. Differences between treatments were followed up using Bonferroni’s Multiple Comparisons test, with the α-level set at P < 0.05.
RESULTS

Food intake, body weight, and body composition. Daily food intake was significantly reduced in VSG rats relative to sham-operated controls for the first several weeks after surgery ($P < 0.05$; Fig. 2A), and pair feeding produced comparable weight loss (Fig. 2B). As food intake gradually approached that of sham-operated animals, differences in body weight among treatments stabilized. At this time, sham-operated rats were in an anabolic rather than a stable metabolic state. By 5 wk after surgery, lean tissue mass had increased to a similar extent in each group ($P < 0.05$, Bonferroni repeated-measures posttest; Fig. 3C), whereas postsurgical fat mass was reduced by approximately half in VSG and pair-fed animals relative to ad libitum-fed sham rats ($P < 0.05$).

Liquid diet vs. solid chow diet. At the time of the diet preference study (postoperative day 100), sham rats weighed more than VSG rats ($699 \pm 23 \text{ vs. } 525 \pm 17 \text{ g}, P < 0.05$). VSG rats lacked the baseline preference for the palatable liquid diet that was apparent in sham rats ($P < 0.001$; Fig. 3, A and B) and consumed a similar number of calories from each diet during the final 3 days of the study ($P > 0.05$; Fig. 3A). Results from the second half of the study, in which the effect of each diet on long-term food intake and body weight was assessed (Fig. 4, A–D), show that exposure to a liquid diet does not adversely affect weight loss after VSG. Sham-operated rats ate significantly more liquid diet than VSG rats ($P < 0.05$), whereas daily food intake on chow was similar among groups ($P > 0.05$).

Volume effects and meal size. To test the hypothesis that meal size was directly altered by mechanical restriction, 6-h-fasted rats were given access to Ensure or Ensure diluted with water (Fig. 5A). Each group compensated for reductions in caloric density by increasing their volume of intake ($P < 0.05$; Fig. 5A). VSG rats were able to maintain the same caloric intake in each condition ($P > 0.05$; Fig. 5B), whereas ad libitum and pair-fed sham rats were unable to fully compensate when exposed to the diluted diet ($P < 0.05$; Fig. 5B), implying that they could not accommodate the volume necessary to make up for reductions in caloric density. The results demonstrate that rats are able to increase the volume of nutrients ingested when necessary for weight maintenance after VSG and imply that caloric density, rather than gastric restriction per se, may be the primary determinant of meal size following VSG.

Carbohydrate and fat preference. The effect of VSG on dietary preferences for sucrose and Intralipid are shown in Fig. 6, A and B. Differences in sucrose preference among groups were nonsignificant ($P > 0.05$). At concentrations of

Fig. 5. A: rats compensated for a decrease in calories by volume by increasing their volume of intake during the Ensure + H$_2$O condition (hatched bars) compared with Ensure alone (open bars), $P < 0.05$. B: VSG rats ($n = 7$) compensated almost perfectly by eating a similar amount of calories in each condition, $P > 0.05$. Ad libitum ($n = 10$) and pair-fed ($n = 10$) rats increased their volume of intake in the Ensure + H$_2$O condition but ate significantly fewer calories compared with Ensure alone, $P < 0.05$. *$P < 0.05$ compared with Ensure.

Fig. 6. Preferences for sucrose were unaltered by VSG (filled triangles, $n = 7$, $P < 0.05$) (A); however, preferences for corn oil were significantly reduced compared with ad libitum sham rats (open circles, $n = 10$, $P < 0.05$) (B). At the highest concentrations tested, the total intake (kcal) of both sucrose (C) and corn oil (D) was also reduced by VSG (filled bars) compared with sham rats (open bars), $P < 0.05$. *$P < 0.05$ compared with sham.
1% or higher, VSG and sham-operated rats consumed significantly more sucrose relative to the control bottle ($P < 0.05$). Figure 6B shows that, in sham-operated rats, preferences for corn oil also increased in a concentration-dependent manner ($P < 0.05$). Remarkably, however, VSG rats ate similar amounts from each bottle at each concentration tested ($P > 0.05$), indicating that dietary preferences for fat were abolished by VSG.

**Nutrient-induced c-Fos activation.** To assess neuronal activation in areas of the brain that have been implicated in satiety, c-Fos activation in response to distention induced by water vs. carbohydrate (sucrose) or fat (Intralipid) was studied in sham, VSG, and sham-operated pair-fed rats (Fig. 7). The volume and number of calories gavaged were consistent across the lipid and carbohydrate conditions and were based upon the mean voluntary intake of Ensure in VSG rats following 6 h of fasting. c-Fos expression was similar among groups in rats administered water intragastrically ($P > 0.05$). However, sucrose and Intralipid induced significantly more c-Fos-like immunoreactivity in the NTS and AP of VSG rats ($P < 0.05$), implying that VSG enhanced the sensitivity to nutrients, but not to simply volume effects. Dual labeling with an antibody raised against DBH showed that c-Fos-expressing neurons were infrequently colocalized with catecholaminergic neurons ($P < 0.05$; Figs. 8 and 9). The number of DBH-containing neurons was similar across groups and conditions ($P > 0.05$), and increases in c-Fos expression in VSG rats given intragastric infusions of sucrose and Intralipid did not result in a higher percentage of c-Fos colocalization with DBH-expressing neurons ($P > 0.05$; Fig. 10). In ad libitum and pair-fed sham rats, the caloric content of the sucrose and Intralipid was insufficient to induce significantly more c-Fos than water alone ($P > 0.05$; Fig. 10). Table 1 summarizes levels of c-Fos activation in all regions studied.

**DISCUSSION**

We used a rat model of VSG to study how meal-related stimuli are processed after surgery relative to animals that remain obese, or in rats that lost a similar amount of weight through caloric restriction. VSG’s effects were inconsistent with the profile produced by purely restrictive procedures in that preferences for calorically dense liquids were not observed, and weight loss was not adversely affected by access to a high-calorie liquid diet. Surgically induced absences in fat preference and changes in c-Fos expression in response to intragastric infusions of sucrose and Intralipid imply that the effect of VSG on meal size results from increases in sensitivity to the caloric content of the food, rather than being a product of a small stomach resulting in increased distension-related signals.
We examined the responsiveness of neurons in the NTS and AP after VSG because they receive direct input from vagal afferent fibers that innervate the gut and code for key aspects of meal-related stimuli, including mechanical distention, caloric content, and changes in postprandial glucose and hormone levels. Distention of the stomach wall activates mechanoreceptors located in the gastric mucosa that generate impulses carried by vagal afferent neurons and interneurons of the myenteric plexus (1). Most of the vagal afferent fibers that innervate the stomach project to the NTS, rostral to the level of obex. Many of these neurons are adrenergic (31). These neurons are unique in that their activity correlates with gastric distention but not with caloric or osmotic intake (31). We found that the percentage of adrenergic positive cells in the NTS expressing c-Fos in our study was <15% and similar among groups.

Activity in this region is a good correlate for meal termination (30). However, preference and reward pathways are likely

Fig. 8. Micrograph (x10) showing c-Fos (red)- and dopamine β-hydroxylase (DBH) (green)-expressing neurons in the NTS just rostral to obex in a VSG rat given an intragastric infusion of water (scale bar 100 μm).

Fig. 9. Micrograph (x10) showing c-Fos (red)- and DBH (green)-expressing neurons in the NTS just rostral to obex in a VSG rat given an intragastric infusion of sucrose (scale bar 100 μm).
the product of a more complex pathway that includes higher forebrain circuits. We postulate that reductions in fat preference are one reason that VSG rats eat less, and gain less weight, than sham-operated animals when exposed to diets that are high in fat. In contrast, the higher levels of c-Fos activation in the brain stem of VSG rats in our study reflect increased sensitivity to nutrients in areas of the brain that convey sensations of fullness and satiety. It seems likely that these effects work in concert to lower body weight and fat mass in VSG animals relative to sham-operated rats.

It should be noted that many of the mechanoreceptors in the gastric mucosa are removed during VSG, which could potentially limit the representation of activity by mechanosensitive neurons in VSG rats in our study. However, the hepatic and gastric branches that innervate the stomach and duodenum are spared, as are the celiac branches that innervate the rest of the alimentary tract. In addition to receiving input from the gut, the NTS has reciprocal connections with the hypothalamus and ventral tegmental area that make it responsive to both homeostatic and nonhomeostatic inputs on meal size (5). Apart from a reduction in activity in catecholaminergic neurons in the NTS, the pattern of c-Fos expression in VSG rats given sucrose or Intralipid in our study is similar to that reported in others (14, 31).

In those studies, the activation of c-Fos in the NTS and AP was contingent upon the animals having access to the same number of calories they would eat voluntarily. c-Fos expression in rats given one-third to one-half of what they normally eat was not significantly different from levels seen in unfed rats (14, 31). The all-or-nothing nature of this response is consistent with the results of our current study in which sucrose and Intralipid had no effect on c-Fos expression in ad libitum and pair-fed animals. This is likely related to the fact that the stimulus was only 20% of what ad libitum and pair-fed sham animals eat voluntarily under similar conditions (Fig. 5), indicating that the size of the caloric stimulus was insufficient to elicit a response in these animals. The lower threshold for activation in our VSG rats, combined with reductions in fat preference after surgery, provide a neural correlate for clinical data showing that VSG results in greater weight loss, significant reduction in hunger ratings, and alterations in food choice when compared with purely restrictive procedures such as the adjustable gastric band (15). These effects are consistent with observations made in rat models of RYGB surgery that have previously been shown to produce a similar effect on meal-induced c-Fos (34) expression and fat preference (10, 33).

Although the physiology that underlies the effects on satiation and food preference following these procedures has not been identified, it is tempting to speculate that it is related to changes in postprandial hormone profiles. Glucagon-like peptide (GLP)-1, peptide YY (PYY), and insulin are all greatly increased after VSG and RYGB (3, 12, 17, 28, 39), and these three hormones modulate intake of certain kinds of foods or the choice among foods in a manner consistent with the observed food choices evident after surgery. The GLP-1 receptor agonist exendin-4 caused a relative increase in carbohydrate intake in rats (29) and was more effective at reducing food intake following an intragastric preload of fat than of carbohydrate (2); PYY injected into the paraventricular nucleus of the hypothalamus stimulates carbohydrate intake to a greater degree than fat intake (35); and insulin injected directly into the arcuate nucleus reduces preferences for fat in rats (20, 40).

Losses in preferences for dietary fat may explain why VSG rats gained less weight than sham animals maintained on Ensure

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Table 1. The expression of c-Fos in different regions of the NTS and AP following exposure to water, sucrose, or Intralipid

<table>
<thead>
<tr>
<th>Water</th>
<th>Sucrose</th>
<th>Intralipid</th>
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</thead>
<tbody>
<tr>
<td><strong>Sham</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R NTS</td>
<td>52 ± 11</td>
<td>42 ± 7</td>
</tr>
<tr>
<td>C NTS</td>
<td>46 ± 11</td>
<td>47 ± 8</td>
</tr>
<tr>
<td>M NTS</td>
<td>25 ± 7</td>
<td>29 ± 4</td>
</tr>
<tr>
<td>AP</td>
<td>22 ± 5</td>
<td>19 ± 4</td>
</tr>
<tr>
<td><strong>VSG</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R NTS</td>
<td>44 ± 16</td>
<td>100 ± 18*#</td>
</tr>
<tr>
<td>C NTS</td>
<td>45 ± 13</td>
<td>105 ± 9*#</td>
</tr>
<tr>
<td>M NTS</td>
<td>47 ± 11</td>
<td>124 ± 8*#</td>
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<tr>
<td>AP</td>
<td>10 ± 3</td>
<td>58 ± 6*#</td>
</tr>
<tr>
<td><strong>Pair fed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R NTS</td>
<td>55 ± 15</td>
<td>41 ± 5</td>
</tr>
<tr>
<td>C NTS</td>
<td>26 ± 6</td>
<td>27 ± 4</td>
</tr>
<tr>
<td>M NTS</td>
<td>20 ± 4</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>AP</td>
<td>13 ± 4</td>
<td>10 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. NTS, nucleus of the solitary tract; AP, area postrema; R, rostral; C, caudal; M, medial; VSG, vertical sleeve gastrectomy.

*P < 0.05 compared with sham. #P < 0.05 compared with pair fed.
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AUTHOR CONTRIBUTIONS


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GRANTS

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