Vertical Sleeve Gastrectomy Attenuates Hedonic Feeding Without Impacting Alcohol Drinking in Rats

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Objective: Roux-en-Y gastric bypass surgery and vertical sleeve gastrectomy (VSG) are the most commonly performed bariatric procedures. Whereas studies report new-onset alcohol misuse following Roux-en-Y gastric bypass, the impact of VSG on alcohol intake is less clear. Hedonic feeding, alcohol drinking, and hypothalamic obesity-related gene expression following VSG were evaluated.

Methods: Male Long-Evans rats underwent VSG or sham surgery. To evaluate hedonic feeding, rats received a high-fat diet following behavioral satiation on chow. Alcohol (5%-10% v/v) drinking was assessed in a two-bottle choice paradigm. Finally, polymerase chain reaction array evaluated gene expression.

Results: VSG induced moderate but significant weight loss. Sham rats significantly escalated high-fat diet intake following behavioral satiation, an effect significantly reduced in VSG rats. A moderate decrease in alcohol intake was observed in VSG rats at low (5%) alcohol concentration. However, overall, no significant between-group differences were evident. Key hypothalamic orexigenic transcripts linked to stimulation of food and alcohol intake were significantly decreased in VSG rats.

Conclusions: VSG attenuated hedonic feeding without impacting alcohol drinking, an effect potentially mediated by alterations in genetic information flow within the hypothalamus. Importantly, these data highlight VSG as an effective bariatric procedure with a potentially reduced risk of developing alcohol use disorder.

Introduction

Bariatric surgery is a well-documented treatment option for obesity. In this context, vertical sleeve gastrectomy (VSG), a procedure in which the fundus is surgically reduced to create a tubular gastric sleeve, has emerged as a prevalent surgical manipulation (1). In addition to a significant reduction in appetite and body weight, VSG patients experience improved metabolic profile. Specifically, decreased consumption of palatable or energy-dense foods, weight loss, decreased hepatic glucose production, and improvement in glucose homeostasis and dyslipidemia have been reported following VSG (2-4). One possibility explaining these observations is that VSG reduces appetite by mitigating hedonic hunger to restore metabolic homeostasis. In the central nervous system, the hypothalamus is a brain region that integrates metabolic signals with an internal need to direct behaviors that maintain homeostasis (5). The hypothalamus contains both orexigenic neuropeptides and anorectic neuropeptides, the release of which is coordinated to control energy balance and feeding behavior (6). In this regard, genetic events control the quality of a feeding event, whereas physiological mechanisms initiate or terminate a particular meal. Notably, genetic expression changes within the hypothalamus are sensitive to fluctuations in metabolic status (7) and feeding behavior (8). Currently, the behavioral and genetic mechanisms that contribute to reduced appetite and body weight loss after VSG are unresolved.

The current study tested corollaries of the central hypothesis that adaptations in feeding behavior and hypothalamic gene expression contribute to body weight loss following VSG. Separate from feeding behavior, other bariatric techniques, namely Roux-en-Y gastric bypass (RYGB) surgery, stimulate new-onset alcohol intake (9-12). We recently discovered that RYGB rats increased alcohol intake at the expense of palatable food intake, suggesting that surgery-induced changes in appetite and alcohol intake may be causal (11). Thus, a separate goal of the current work was to determine whether VSG impacts alcohol intake in rats that are otherwise nonpreferring prior to surgery. To address these issues, we utilized a rodent model of VSG in which male Long-Evans rats characterized for body weight loss underwent a battery of behavioral tests designed to assess feeding in the absence of caloric need and new-onset alcohol intake. Following behavioral characterization, polymerase chain reaction (PCR) array analysis was conducted to elucidate alterations in the obesity-related gene expression within the hypothalamus of VSG and control rats.

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Methods

Animals

Male Long-Evans rats (Harlan, Indiana) housed in an environmentally controlled vivarium on a reverse light cycle (lights off at 7 AM) were used with food and water available ad libitum, except when indicated. All procedures were approved by Institutional Animal Care and Use Committee guidelines at Washington State University. Rats (age = 14 weeks) were initially exposed to the high-fat diet (HFD; Research Diets, New Brunswick, New Jersey; 4.41 kcal/g [1.71 kcal/g from fat]) for ~8 weeks. Subsequently, rats (n = 10/group) received sham or VSG surgery. Out of this group, nine sham and eight VSG rats completed the study. Following surgery, all rats were maintained on OSMOLITE (Abbott, Lake Forest, Illinois) and water for the first 5 to 8 days and were then slowly returned to and maintained on standard rodent chow (Teklad; Envigo, South Kent, Washington; 3.41 kcal/g [0.51 kcal/g from fat]) throughout the remainder of the study.

Surgery

All rats were fasted at least 24 hours before surgery as described previously (11). On the day of surgery, rats in the VSG surgery group were anesthetized and received an incision in the abdominal wall. The stomach was gently removed from the abdomen, and the lateral stomach (70%-80% of total stomach volume) was excised using a stapler (Ethicon, Inc., New Jersey), creating a tubular gastric piece connecting esophagus and duodenum. The newly created gastric sleeve was gently placed back, and the abdominal cavity was closed. Rats in the sham surgery group were anesthetized and received an incision in the abdominal wall, and the stomach was gently removed from the cavity and placed back before closing the abdominal cavity. All rats received appropriate postoperative care and were allowed to recover until their body weight was stable. Subsequently, a subset of rats was tested in hedonic feeding or alcohol drinking paradigms.

Hedonic food intake

To assess hedonic food intake, rats were food deprived overnight as described previously (11,13). Following 21 hours, all rats received preweighed rodent chow. Food was weighed each hour for 2 hours. Following the second hour of chow access, a preweighed HFD was presented to both sham and VSG groups of rats, and food was weighed 1 hour later. The HFD intake after satisfying homeostatic caloric needs constituted the hedonic portion of this test.

Alcohol intake

Unsweetened alcohol (5%, 8%, or 10% v/v) bottles were presented on alternating days in a counterbalanced fashion in the rat home cages using a two-bottle choice paradigm (one bottle water and another alcohol). Alcohol was introduced 4 hours into the rat’s subjective dark cycle, and alcohol and water intake was evaluated 24 hours later. All animals had ad libitum access to water and food, and no water or food deprivation occurred during testing. The position of the alcohol and water bottles was switched at each testing session. Bottles were weighed, gently placed in the cages, and reweighed manually following each session to evaluate alcohol intake (grams per kilogram body weight).

PCR array

Following behavioral assessment, all rats were euthanized, and brain tissues were snap frozen and stored at ~80°C until further analysis. Hypothalamus from sham and VSG (n = 3/group) was microdissected and placed in RNA later Stabilization Solution (Ambion, Foster City, California). TissueRuptor (Qiagen, Germantown, Maryland), QIAshredder (Qiagen catalog number 79654), and RNeasy Plus Mini Kit (Qiagen catalog number 74134) were used for total RNA extraction and isolation as described in the manufacturer’s protocol. The concentration and purity of RNA samples were determined by NanoDrop spectrophotometer (Thermo Fisher, Waltham, Massachusetts). The degradation and integrity were assessed by Experion Automated Electrophoresis System (Bio-Rad, Hercules, California). All RNA samples were of high quality and passed all necessary requirements. Complementary DNA was synthesized from 350 ng of total RNA for each sample using RT² First Strand Kit (Qiagen catalog number 330401) following the manufacturer’s protocol. PCR amplification was conducted using MyiQ Real-Time PCR Detection System (Bio-Rad). Baseline threshold was manually set to 100 relative fluorescence units in primary data analysis for all arrays. The rat obesity RT² Profiler PCR Array (Qiagen catalog number PARN-017Z) was used to profile expression of a total of 84 genes (Table 1), which included orexigenic genes, anorectic genes, and genes involved in energy expenditure. All arrays passed quality control tests (PCR array

| Category of gene | Gene name                                                                 |
|------------------|---------------------------------------------------------------------------|
| Orexigenic genes | Adra2b, Agpr, Cnr1, Gal, Galr1, Mechr1, Hcrt, Hcrt1, Npy, Npy1r, Nr3c1 (Grl), Oprk1, Opm1, Sigmar1 (Opn1) |
| Neuropeptides and receptors | Ghr (Ghrelin, Obestatin), Ghhr                                                                 |
| Gut hormones and receptors | Atm, Bdnf, Bns3, Calca, Calcrl, Cartpt, Cntf, Cntr, Chh, Cntr1, Drd1, Drd2, Gh1, Ghr, Pfrhr (Gpr10), Grp, Grpr, Hrh1, Hrh2c, I1la, I1lb, I1lr1, I1lr2, I1lr3, I1lr4, I1lr5, Mc3r, Nmb, Nmbr, Nmur1 (Gpr66), Ntrk1, Nts, Ntsr1, Pnoc, Sort1, Thr, Thr1, Ucn |
| Anorectic genes | Apoa4, Cck, Cckar, Glpr1r, Pyy                                           |
| Neuropeptides and receptors | Lep (Leptin), Lepf, Tnf                                                                 |
| Gut hormones and receptors | Calcrl, Cips, Gcg, Gcgr, Glpr1r, Iapp, Ins1, Ins2, Insr, Ramp3, Sst, Sst1 |
| Adipocyte-derived peptides and receptors | Adipoq (Acrp30), Adipor1, Adipor2, Add1, C3, Ppara, Pparg, Ppargc1a (Ppargc1), Ptpr1 (Ptp), Ucp1 |
| Pancreas-derived peptides and receptors | Adcyap1, Adcyap1r1, Thrb                                                        |
| Energy expenditure |                                                                                   |
| Adipocyte-derived peptides and receptors |                                                                                   |
| CNS-derived peptides and receptors |                                                                                   |

CNS, central nervous system.
reproducibility, reverse transcription efficiency, and genomic DNA contamination. A Web-based data analysis tool (Qiagen) was used to calculate fold-change and P values. Two housekeeping genes (Hprt1 and Rplp1) were used for quantitative PCR data normalization. The cycle threshold (CT) cutoff was set to 35, and any gene with measurements > 35 was excluded from further analysis. Fold-change \((2^\Delta \text{CT})\) is the normalized gene expression \((2^\Delta \text{CT})\) in the VSG samples divided by the normalized gene expression \((2^\Delta \text{CT})\) in the sham samples. Fold-regulation represents fold-change results in a biologically meaningful way. Fold-change values greater than 1 indicate a positive or upregulation, and the fold-regulation is equal to the fold-change. Fold-change values less than 1 indicate a negative or downregulation, and the fold-regulation is the negative inverse of the fold-change.

**Statistical analysis**

Body weight, food intake, and alcohol intake data over a period of time were analyzed by a mixed-model two-way analysis of variance (ANOVA), with post hoc tests to compare within-group effects. The within-subject variable was time interval (time or conditions of measurements), and the between-group variable was surgical procedure (sham or VSG surgery). HFD intake data were analyzed using unpaired t test. PCR array data were analyzed using unpaired t test only as per the manufacturer-recommended Web-based RT² Profiler PCR Array data analysis software and others using this method to evaluate gene expression. All statistical comparisons were conducted at 0.05 α level.

**Results**

**VSG surgery: body weight**

No statistically significant between-group (P>0.05) differences existed before surgery (Figure 1A). Sham rats lost an average of 10 g (~2.0%) of their initial body weight, whereas VSG rats lost an average of 55.0 g (~10%) of their initial body weight over the first 30 days following surgery (Figure 1B). A mixed-model ANOVA identified a main effect of time \((F_{2.5,37.5}=52.255; P=0.000)\), a significant time and treatment interaction \((F_{2.5,37.5}=7.970, P=0.001)\), and a significant between-group difference \((F_{1.0,15.0}=15.420; P=0.001)\) in body weight. In addition, these significant between-group differences persisted across time during hedonic feeding \((P=0.0065)\) and alcohol drinking \((P=0.0047)\) testing (Figure 1C).

**VSG surgery: hedonic feeding**

A mixed-model ANOVA identified a significant effect of time \((F_{2.0,0.0}=10.21; P=0.004)\), but no significant \((P>0.05)\) interaction or between-group differences were evident, suggesting that chow intake in both groups decreased over the duration, but chow intake was not significantly \((P>0.05)\) different between sham and VSG rats following overnight fasting (a homeostatic-driven refeeding; Figure 2A). However, palatable (HFD) food intake following a chow preload was significantly reduced in VSG rats compared with sham controls (Figure 2B).

**VSG surgery: alcohol drinking**

Alcohol drinking at lower alcohol (5%) but not higher alcohol (8%-10%) concentrations appeared to be reduced in VSG rats compared with sham controls. However, mixed-model ANOVA did not identify any statistically significant within- or between-group differences (Figure 3). Water intake was not significantly different between sham and VSG groups.

**VSG surgery: obesity-related gene expression in hypothalamus**

A rat obesity RT² Profiler PCR Array examined the expression of 84 obesity-related genes (i.e., orexigenic, anorectic, and energy expenditure) in the hypothalamus following sham and VSG surgery. Several of these genes were significantly impacted following VSG as shown in the scatterplot (Figure 4A). A total of 71 genes were expressed in detectable amounts, whereas 13 genes (Adipoq, Agrp, Agrp2, Gcg, Gh1, Iapp, Ins1, Lep, Nmur1, Ntrk1, Pparγ, and Ucp1) were deemed undetectable (based on CT values). Table 2 provides a list of these 71 genes and their corresponding PCR array data. Of these 71 genes, 8 genes (Adipor1, Agrp, Carpt, Glp1r, Hcrt, Ilb, Lepr, and...
Mc3r) were significantly (P < 0.05) downregulated, and 1 gene (Grp) was significantly (P < 0.05) upregulated in the hypothalamus of VSG rats compared with sham controls. Furthermore, ≥ 2-fold statistical decrease were evident in Agrp, Cartpt, Gip1r, HcRt, Il1b, Lepr, and Mc3r mRNA, whereas a significant increase was apparent for Grp mRNA (Figure 4B).

Discussion

The present study was designed to test corollaries of the central hypothesis that VSG surgery stimulates body weight loss through genetic adaptations within the hypothalamus. From these efforts, we discovered that VSG in male rodents led to anticipated reductions in body weight that were sustained throughout the study period. In addition, VSG attenuated palatable food intake following a caloric preload but spared deprivation-induced refeeding behavior. This observation supports the contention that VSG is not a restrictive surgery but rather limits excess intake in the absence of a caloric need. Although alcohol drinking was moderately impacted at a lower alcohol concentration, this effect diminished as the concentration of alcohol was escalated. Behaviorally characterized VSG rats displayed decreased expression of key orexigenic hypothalamic transcripts linked to stimulation of both food and alcohol intake. Collectively, these results suggest that weight loss after VSG is accompanied by behavioral and neurobiological events that signify a reduced drive for palatable food.

RYGB surgery and VSG are the most widely performed surgical procedures to induce sustained weight loss for the treatment of obesity and related metabolic complications (1). VSG has emerged as a popular and frequently performed surgical procedure for obesity treatment given its efficiency (several metabolic benefits even at ~20% mean reduction in body weight) and allowance of revisional procedures (1). Furthermore, a significant reduction in fat and fat-free mass has been reported following VSG (14). However, a large variability was shown in body weight reduction following VSG (15), and both preclinical and clinical studies have reported body weight regain following bariatric procedures, including VSG (16-18). It is important to note that marked variability in the maximal weight loss following bariatric surgeries could be attributed to several factors, including age at which the surgical procedure is performed, preoperative BMI, percentage early weight loss following surgery, poor diet quality, incompatibility with postoperative dietary recommendations, emotional eating, increased food craving, and/or preoperative food preferences (17-22).

In this context, hedonic feeding (consumption of a palatable food in the absence of a caloric need) could be a major contributor to the individual difference in weight regain after VSG. Notably, preclinical evidence has
Figure 4 Alterations in the hypothalamic obesity-related gene expression following VSG surgery in rats. (A) Scatterplot analysis of differential expression of obesity-related genes in the hypothalamus following VSG compared with sham controls, using the rat obesity RT² Profiler PCR Array. Each dot represents one gene, and the top and bottom genes outside the dotted lines represent a twofold increase and decrease, respectively. (B) Fold-regulation (≥2-fold) is plotted for only statistically significant \( P < 0.05 \) increased or decreased gene expression following VSG compared with sham controls. **\( P < 0.01 \) and *\( P < 0.05 \), compared with sham controls.

### Table 2
Impact of vertical sleeve gastrectomy on obesity-related gene expression in hypothalamus using rat obesity RT² Profiler PCR Array

| # | Unigene | Refseq | Symbol | Description | Fold-regulation | \( P \) value |
|---|---------|--------|--------|-------------|----------------|--------------|
| 1 | Rn.202559 | NM_016989 | Adcyap1 | Adenylate cyclase-activating polypeptide 1 | -2.0 | 0.192 |
| 2 | Rn.234543 | NM_133511 | Adcyap1r1 | Adenylate cyclase-activating polypeptide 1 receptor 1 | -1.4 | 0.174 |
| 3 | Rn.104556 | NM_207587 | Adipor1 | Adiponectin receptor 1 | -1.3 | 0.021* |
| 4 | Rn.101984 | NM_001037979 | Adipor2 | Adiponectin receptor 2 | -1.2 | 0.122 |
| 5 | Rn.87064 | NM_012701 | Adrb1 | Adrenergic, beta-1-, receptor | -1.2 | 0.715 |
| 6 | Rn.137597 | NM_033650 | Agrp | Agouti-related protein homolog (mouse) | -27.1 | 0.024* |
| 7 | Rn.53846 | NM_031351 | Atn | Attractin | -1.4 | 0.226 |
| 8 | Rn.11266 | NM_012513 | Bdnf | Brain-derived neurotrophic factor | -1.9 | 0.230 |
| 9 | Rn.86415 | NM_152845 | Brs3 | Bombesin-like receptor 3 | -2.1 | 0.127 |
| 10 | Rn.11378 | NM_016994 | C3 | Complement component 3 | -4.5 | 0.300 |
| 11 | Rn.90085 | NM_017338 | Calca | Calcitonin-related polypeptide alpha | -1.6 | 0.188 |
| 12 | Rn.10062 | NM_053816 | Calcr | Calcitonin receptor | -1.0 | 0.928 |
| 13 | Rn.89164 | NM_017110 | Cartpt | CART prepropeptide | -3.0 | 0.004** |
| 14 | Rn.9781 | NM_012829 | Cck | Cholecystokinin | -1.7 | 0.547 |
| 15 | Rn.10184 | NM_012688 | Cckar | Cholecystokinin A receptor | -1.3 | 0.145 |
| 16 | Rn.89774 | NM_012784 | Cnr1 | Cannabinoid receptor 1 (brain) | 1.4 | 0.408 |
| 17 | Rn.6067 | NM_013166 | Cntf | Ciliary neurotrophic factor | -1.1 | 0.603 |
| 18 | Rn.55036 | NM_00103929 | Cntfr | Ciliary neurotrophic factor receptor | -1.1 | 0.595 |
| 19 | Rn.10349 | NM_031019 | Cth | Corticotropin-releasing hormone | 2.2 | 0.100 |
| 20 | Rn.10499 | NM_030999 | Cth1 | Corticotropin-releasing hormone receptor 1 | -1.0 | 0.905 |
### TABLE 2. (continued).  

| #   | Unigene     | Refseq    | Symbol | Description                              | Fold-regulation | P value |
|-----|-------------|-----------|--------|------------------------------------------|-----------------|---------|
| 21  | Rn.24039    | NM_012546 | Drd1   | Dopamine receptor D1A                    | 2.2             | 0.286   |
| 22  | Rn.57299    | NM_012547 | Drd2   | Dopamine receptor D2                     | 1.1             | 0.826   |
| 23  | Rn.8929     | NM_033237 | Gal    | Galanin prepropeptide                    | −1.4            | 0.234   |
| 24  | Rn.10213    | NM_012958 | Ghr1   | Galanin receptor 1                       | 1.0             | 0.955   |
| 25  | Rn.54383    | NM_012707 | Gcg    | Glucagon                                  | −1.2            | 0.490   |
| 26  | Rn.2178     | NM_017094 | Ghr    | Growth hormone receptor                   | −1.2            | 0.628   |
| 27  | Rn.42103    | NM_021669 | Ghrl   | Ghrelin/obestatin prepropeptide          | −1.2            | 0.544   |
| 28  | Rn.74241    | NM_032075 | Ghrs   | Growth hormone secretagogue receptor      | −2.3            | 0.054   |
| 29  | Rn.11408    | NM_012728 | Glp1r  | Glucagon-like peptide 1 receptor         | −2.1            | 0.023*  |
| 30  | Rn.10930    | NM_133570 | Gpr    | Gastrin-releasing peptide                | 4.6             | 0.006** |
| 31  | Rn.10316    | NM_012706 | Gpr    | Gastrin-releasing peptide receptor        | −1.0            | 0.860   |
| 32  | Rn.7628     | NM_013179 | HcrT   | Hypocretin                                | −10.5           | 0.039*  |
| 33  | Rn.88262    | NM_013064 | Hcrt1  | Hypocretin (orexin) receptor 1            | −1.5            | 0.191   |
| 34  | Rn.81032    | NM_017018 | Hhr1   | Histamine receptor H 1                    | −1.8            | 0.149   |
| 35  | Rn.9935     | NM_012765 | Htr2c  | 5-hydroxytryptamine (serotonin) receptor 2C | 1.0             | 0.800   |
| 36  | Rn.12300    | NM_017019 | Il1a   | Interleukin 1 alpha                       | 1.1             | 0.512   |
| 37  | Rn.9869     | NM_031512 | Il1b   | Interleukin 1 beta                       | −2.9            | 0.016*  |
| 38  | Rn.9758     | NM_013123 | Il1r1  | Interleukin 1 receptor, type I            | −1.2            | 0.341   |
| 39  | Rn.9673     | NM_012589 | Il6    | Interleukin 6                            | −1.1            | 0.457   |
| 40  | Rn.1716     | NM_017020 | Il6r   | Interleukin 6 receptor                    | −1.2            | 0.435   |
| 41  | Rn.989      | NM_019130 | Ins2   | Insulin 2                                | −1.7            | 0.193   |
| 42  | Rn.9876     | NM_017071 | Insr   | Insulin receptor                         | −1.1            | 0.436   |
| 43  | Rn.9891     | NM_012596 | Lepr   | Leptin receptor                          | −2.4            | 0.014*  |
| 44  | Rn.215838   | NM_001025270 | Mc3r | Melanocortin 3 receptor                   | −2.3            | 0.023*  |
| 45  | Rn.10822    | NM_031758 | Mchr1  | Melanin-concentrating hormone receptor 1  | 1.1             | 0.120   |
| 46  | Rn.18763    | NM_001109149 | Nmb | Neuromedin B                             | 1.1             | 0.715   |
| 47  | Rn.89709    | NM_012799 | Nmbr   | Neuromedin B receptor                     | 2.4             | 0.064   |
| 48  | Rn.47720    | NM_022239 | Nmu    | Neuromedin U                             | −5.0            | 0.076   |
| 49  | Rn.9714     | NM_012614 | Npy    | Neuropeptide Y                           | −1.4            | 0.088   |
| 50  | Rn.11642    | NM_00113357 | Npy1r | Neuropeptide Y receptor Y1               | −1.0            | 0.984   |
| 51  | Rn.90070    | NM_012576 | Nc3c1  | Nuclear receptor subfamily 3, group C, member 1 | −1.3            | 0.082   |
| 52  | Rn.60814    | NM_001102381 | Nts | Neurotensin                              | 3.2             | 0.089   |
| 53  | Rn.200149   | NM_001108967 | Ntsr1 | Neurotensin receptor 1                  | −1.1            | 0.708   |
| 54  | Rn.89571    | NM_017167 | Oprk1  | Opioid receptor, kappa 1                 | 1.2             | 0.206   |
| 55  | Rn.10118    | NM_013071 | Opm1   | Opioid receptor, mu 1                    | 1.3             | 0.255   |
| 56  | Rn.108195   | NM_139326 | Pomc   | Proopiomelanocortin                      | −10.3           | 0.327   |
| 57  | Rn.9753     | NM_013196 | Ppara  | Peroxisome proliferator-activated receptor alpha | −1.1            | 0.584   |
| 58  | Rn.19172    | NM_031347 | Pparc1a| Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha | 1.2             | 0.396   |
| 59  | Rn.138127   | NM_139193 | Pthr   | Prolactin-releasing hormone receptor      | 1.8             | 0.137   |
| 60  | Rn.11317    | NM_012637 | Ptpn1  | Protein tyrosine phosphatase, nonreceptor type 1 | −1.1            | 0.834   |
| 61  | Rn.13173    | NM_001034080 | Ppy | Peptide YY (mapped)                      | −1.9            | 0.143   |
| 62  | Rn.48672    | NM_020100 | Ramp3  | Receptor (G protein-coupled) activity modifying protein 3 | −1.4            | 0.147   |
indicated that preoperative food preference for palatable food is maintained after VSG (18,23), highlighting perseverance for unhealthy food as a means to stimulate body weight regain. However, it is unknown whether palatable food intake is impacted in the absence of caloric need following VSG. The data we present here indicate, for the first time, that VSG rats selectively reduced intake of palatable food when offered an HFD after consuming a caloric preload. Supporting this finding, preclinical studies indicated that VSG rats decreased preference for high-fat foods despite displaying persistence of food-motivated behavior (24). Importantly, refeeding on low-fat food after deprivation did not differ between VSG rats and sham controls, suggesting that VSG is not a restrictive surgical procedure. Instead, relative to controls, VSG rats selectively reduced intake when offered a palatable HFD after consuming a caloric preload. Consistent with this, clinical evidence has indicated that VSG reduces preference and liking for high-calorie foods (rich in fat and sugar) (3,25). Taken together, these results suggest that VSG selectively abrogates hedonic feeding in the absence of a caloric need.

Previous work from our group and others has indicated that RYGB surgery increases alcohol intake in rodents. Specifically, RYGB surgery increases sensitivity, consumption, and motivation to obtain alcohol (9-11) in rats that are nonalcohol preferring at baseline. Moreover, we recently discovered that this phenomenon occurred at the expense of reduced hedonic feeding (11), i.e., RYGB rats that displayed increased alcohol intake also selectively reduced hedonic intake of palatable food after a caloric preload. To determine whether surgical effects on alcohol extend to the VSG procedure, we examined alcohol intake over a range of alcohol concentrations. Our data indicate that VSG reduced intake of alcohol at low concentration and that this phenomenon was diminished as the concentration of alcohol was escalated. These data are in agreement with a recent preclinical study that found reduced alcohol intake after VSG (26). However, in that study, VSG rats and mice consumed significantly less alcohol at all concentrations tested. It is important to note that VSG rats were directly compared with nonsurgical controls in that study, whereas in the current study, sham rats that served as controls experienced the same degree of perioperative stress but did not undergo surgical reduction of the fundus. Thus, these differences in alcohol consumption among control groups may have contributed to the disparity in results between the two studies. In either case, we conclude that, if anything, VSG reduces alcohol intake, as opposed to RYGB, which stimulates new-onset alcohol intake in both patients (12) and rodents (9,10).

It is important to note that anatomical and resultant physiological alterations following surgeries that restructure the gastrointestinal (GI) tract can impact alcohol pharmacokinetics. For example, rapid absorption, higher blood alcohol concentration, and delayed alcohol elimination have been reported following RYGB, which may contribute to the development of alcohol use disorder (AUD) in these patients, nicely reviewed elsewhere (27). On the other hand, studies that examined alcohol pharmacokinetics and alcohol intoxication following VSG have generated conflicting results. For example, a prospective study (N=10) that compared alcohol metabolism, peak blood alcohol concentration, alcohol elimination, and intoxication before and after (3 and 12 months) VSG found no significant changes (28). Similar results were obtained by an additional study that examined the impact of VSG (n=7) or gastric banding (n=9) on alcohol pharmacokinetics and intoxication 3 and 6 months after surgery (29). These data are consistent with our preclinical finding demonstrating no change in alcohol drinking following VSG. In contrast, Maluenda et al. (30) reported increased blood alcohol levels and delayed alcohol clearance 2 months following VSG. In females. In this context, a clinical study of primarily female patients undergoing these surgeries. Because the current study examined the impact of VSG on hedonic feeding and alcohol drinking in male rats only, future studies are needed to determine whether similar effects persist in females. In this context, a clinical study of primarily female patients reported no significant changes in alcohol pharmacokinetics (examined using a Breathalyzer) or intoxication levels 3 and 12 months following VSG (28). However, a recent study argued that the Breathalyzer method may be unreliable and that more robust analytical methods (e.g., gas chromatography) are needed to assess alcohol pharmacokinetics following bariatric surgery (31). For example, Acevedo et al. found faster absorption, higher peak blood alcohol levels, and heightened intoxications in 11 women ~2.0 years following VSG using this method (31). Interestingly, a recent, large, multi-institutional study (32) based on

**TABLE 2. (continued).**

| #  | Unigene  | Refseq  | Symbol | Description | Fold-regulation | P value |
|----|---------|---------|--------|------------|----------------|---------|
| 63 | Rn.1129 | NM_030996 | Sigmar1 | Sigma nonopiod intracellular receptor 1 | 1.1 | 0.455 |
| 64 | Rn.11286 | NM_031767 | Sort1 | Sortilin 1 | −1.1 | 0.543 |
| 65 | Rn.34418 | NM_012659 | Sst | Somatostatin | 2.1 | 0.138 |
| 66 | Rn.42915 | NM_012719 | Sotr1 | Somatostatin receptor 1 | −1.0 | 0.975 |
| 67 | Rn.34019 | NM_012672 | Thrb | Thyroid hormone receptor beta | −1.6 | 0.101 |
| 68 | Rn.2275 | NM_012675 | Tnf | Tumor necrosis factor | −1.1 | 0.442 |
| 69 | Rn.22 | NM_013046 | Tph | Thyrotropin-releasing hormone | 1.7 | 0.241 |
| 70 | Rn.9962 | NM_013047 | Tthr | Thyrotropin-releasing hormone receptor | 1.2 | 0.368 |
| 71 | Rn.10190 | NM_019150 | Ucn | Urocortin | −2.1 | 0.156 |

Bold text highlights statistically significant changes. CART, cocaine- and amphetamine-regulated transcript; Refseq, reference sequence.

*P<0.05, compared with sham controls.

**P<0.01, compared with sham controls.
self-reported alcohol consumption found similar pre- and postoperative (1 and 2 years) AUD prevalence following RYGB (n=1,006; 78.4% female) and VSG (n=4,718; 78.4% female). In this study, AUD was detected 2 years following RYGB or VSG. Notably, income, education, baseline alcohol consumption, or alcohol misuse were predictive for AUD after surgery (32). Collectively, these data highlight the importance of method, time following surgery, and history of alcohol misuse as critical factors that should be considered when evaluating the impact of VSG on new onset of AUD.

The hypothalamus integrates metabolic information with the internal need to adapt behavioral responses. Specifically, GI-derived peptides target the hypothalamus to regulate energy balance through modulation of feeding behavior (5). To examine potential mechanisms for the observed reduced hedonic feeding, we measured mRNA changes in the hypothalamus of VSG rats that were behaviorally characterized by reduced hedonic feeding and low-concentration alcohol intake. Our results indicate that VSG dramatically reduced expression of key orexigenic peptides known to stimulate food intake. For example, Agouti-related protein (AgRP) is a neuropeptide with well-established effects on appetite stimulation (33). Activation of AgRP neurons was shown to drive feeding in untrained rodents (34), demonstrating that these neurons are necessary and sufficient to initiate feeding. We found that Agrp mRNA was significantly decreased after VSG relative to sham control rats. Pharmacologic AgRP selectively promotes preferences for fat and stimulates mesocortical dopamine release (35). Thus, reduced hedonic feeding in VSG may derive from reduced Agrp expression. We also detected decreased Hcrt (orexin) mRNA in the hypothalamus of VSG rats. Notably, orexin signaling was shown to be required for hedonic feeding behavior in rodents (36). In addition, orexin stimulates alcohol intake in rodents (37). Thus, decreased orexin expression may also contribute to the reduction in hedonic feeding and/or low-concentration alcohol intake observed after VSG. We also detected decreased expression (a strong trend [P = 0.05]) of ghrelin receptor-1a (GHSR), the central target of the appetite hormone ghrelin (38). In addition to promoting appetite, ghrelin promotes alcohol intake (39). VSG reduces circulating ghrelin in humans (40) and rodents (41), indicating that hypothalamic reduction in GHSR mRNA expression may derive from reduced circulating ghrelin. In support of this contention, we recently discovered that RYGB rats with reduced circulating ghrelin had diminished GHSR control of dopamine neuronal firing (11), suggesting that reduced plasma ghrelin may lead to reductions in central GHSR function. A significant decrease in leptin receptor mRNA expression was also observed in the hypothalamus of VSG rats, an observation in agreement with decreased circulating leptin hormone following VSG (42). It is important to note that leptin concentration and sensitivity closely correlate with body weight as obesity increases, whereas weight loss decreases plasma leptin levels (43). Therefore, decreased leptin receptor expression in the present study could be a consequence of reduced body weight following VSG. Interestingly, leptin was shown to positively regulate hypothalamic cocaine- and amphetamine-regulated transcript protein mRNA levels (44). We observed a significant decrease in the cocaine- and amphetamine-regulated transcript prepropeptide mRNA expression in the hypothalamus of VSG rats, which is in agreement with the decreased leptin functional activity following VSG. We also discovered that VSG led to upregulation of gastrin-releasing peptide, a peptide produced in the GI tract and hypothalamus that inhibits food intake (45). Collectively, these results indicate that VSG surgery exerts dramatic genetic programming changes in the brain’s endogenous appetite center, which potentially contribute to a reduced drive to feed.

It is important to note that it is unclear whether similar changes could be attributed to the weight loss itself, independent of surgery, which requires further investigation.

To summarize, the current data indicate that VSG surgery may exert positive benefits on body weight loss through reductions in hedonic intake of palatable food, an effect that occurs without risk of new-onset alcohol misuse and is potentially explained by alterations in genetic information flow within the hypothalamus. These data can inform the development of new therapies designed to reduce body weight with a reduced risk of developing AUD.

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