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**Vertical Sleeve Gastrectomy: Mechanisms for Weight Loss and Lessons for Obesity Therapy**

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Vertical Sleeve Gastrectomy: Mechanisms for Weight Loss and Lessons for Obesity Therapy

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Abstract

Bariatric surgery has emerged as a superior treatment for obesity because of its ability to produce potent, durable weight loss. Vertical sleeve gastrectomy (VSG) is one bariatric procedure that is gaining popularity as an obesity therapy. It is less invasive and as effective as other bariatric procedures including the Roux-en-Y gastric bypass (RYGB). Here, we perform VSG in rats and demonstrate dramatic, long-term weight loss in rats following surgery. This weight loss was a specific loss of fat mass. Initial weight loss was due to early postsurgical reductions in food intake and was maintained a lack of compensatory hyperphagia in response to the postoperative weight loss. Ingestive behavior after the surgery was characterized by smaller, more frequent meals than in sham-operated controls. Importantly, we demonstrated that VSG does not impair ability to overeat in response to additional weight loss but instead appears to reduce motivation to overeat. Energy expenditure was unaffected by VSG, substantiating the idea that changes to food intake are primary determinants of a newly defended, postsurgical body fat level.

Because leptin resistance is a feature of obesity that often precludes the maintenance of diet-induced weight loss, we initially hypothesized that enhanced leptin sensitivity contributes to defended body weight after VSG. Food intake reduction following an exogenous dose of leptin was greater in VSG- than sham-operated rats. However, because the response after VSG was comparable to the anorexia elicited in pair-fed rats, we concluded that behavioral sensitization to leptin after surgery is secondary to weight loss and is unlikely to drive reduced motivation of VSG-operated rats to overeat. This conclusion is supported by the absence of changes in the expression of genes in the mediobasal hypothalamus that regulate activity of the melanocortin axis.
Highlighting the power of VSG as a treatment not only for obesity but also for obesity-related comorbidities, we show that the procedure produces significant, weight-independent improvements to lipid homeostasis. This benefit is primarily a postprandial phenomenon. Our data indicate that lower postprandial plasma lipid levels are due to reduced intestinal triglyceride secretion in the absence of any changes to hepatic triglyceride production. We did not detect any changes to the expression of genes regulating lipid transport and/or triglyceride production in either the proximal intestine or the liver. We hypothesize that changes to intestinal biology following VSG are not due to permanent, transcriptional changes but, rather, to altered patterns of intestinal nutrient delivery after surgery. Meal patterns and/or gastric emptying might elicit these changes. We also report weight-related enhancement of plasma bile acid levels, leading us to propose that bile acids may mediate some weight-dependent metabolic benefits to weight loss following VSG and potentially other bariatric procedures.

Together, these data provide exciting promise for the use of VSG as a metabolic surgery for obese populations. Although it is unclear what mechanism(s) may suppress hyperphagic behavior and thus lower defended body fat level after VSG, our data contribute significantly to the understanding of the cascade of metabolic changes elicited by VSG. Additionally, we provide the first evidence that VSG induces weight-independent changes to lipid homeostasis. Therefore, VSG is a procedure that holds the potential to treat not only obesity, but obesity-related comorbidities which may include atherosclerosis and hyperlipidemia. Further understanding the mechanisms for these improvements, including the role of intestinal nutrient sensing, is an important area for future research.
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CHAPTER 1

General Introduction and Background:

Putative Mechanisms for Weight Loss After Vertical Sleeve Gastrectomy and Insights from Other Bariatric Procedures
Introduction

Obesity is a growing epidemic in the United States as well as other westernized nations. More than two-thirds of the U.S. population is overweight and over one-third obese\(^1\). There is an urgent need for more effective and widely available treatments for these individuals, who comprise such a large part of our population. Traditionally, diet and exercise have been used as primary modes of treatment for obesity. We now know, however, that body weight is very tightly regulated\(^2\) and so dieting can be nearly impossible for obese individuals. Although dieting almost always produces weight loss, strong homeostatic feedback mechanisms can lead patients to regain much of this weight. Currently available pharmaceutical treatments produce similarly modest weight loss\(^3\).

Bariatric surgeries produce dramatic, long-term weight loss that is superior to traditional weight loss treatments in both magnitude and durability. Importantly, these procedures also dramatically reduce obesity-related comorbidities, thereby making a profound impact on mortality rates. Bariatric surgery has been shown to reduce long-term mortality due to disease, especially diabetes, heart disease, and cancer\(^4\). Among the benefits of bariatric surgery are improved plasma lipids, including total cholesterol, LDL-C, HDL-C, and triglycerides, decreased systolic and diastolic blood pressure, and decreased incidence of diabetes\(^4,5\). As a result, pharmacologic treatment for diabetes and other elements of the metabolic syndrome such as hyperlipidemia and hypertension can often be discontinued\(^5\). For these reasons, bariatric procedures might be treatments for weight loss but for other metabolic disorders as well. More and more, “weight loss surgeries” are considered also to be “metabolic surgeries.” Current guidelines dictate that bariatric surgery is appropriate for adults with BMI $\geq 40$ or for adults with BMI between 35 and 40 in addition to obesity-related comorbidities including dyslipidemia and
Type 2 diabetes\textsuperscript{6}. However, based on the success of these surgeries to improve not only body weight but other metabolic parameters as well, it is likely that these criteria will be expanded in the near future.

Non-surgical treatments for obesity are highly likely to fail in the long-term. In order to produce more effective and widely available treatments for obesity and diabetes, it is important to understand the mechanisms by which these surgeries produce sustained weight loss and metabolic improvements. However, these mechanisms remain largely undefined. The increasing popularity of bariatric surgery and the recent development of rodent models for these procedures, however, have given impetus to the field of research around mechanisms for surgically-induced weight loss. This introduction will focus on VSG and mechanisms by which the procedure may reduce body weight and produce dramatic metabolic improvements. A key element of this discussion will be the postulated and known mechanisms by which other procedures produce similar improvements.

**Bariatric surgery: historical perspectives and the emergence of the vertical sleeve gastrectomy**

The first weight loss surgeries were performed in the 1950s at the University of Minnesota\textsuperscript{7}. The earliest procedure, the jejuno-ileal bypass (JIB) surgery, redirected nutrient flow to bypass most of the small intestine and was intended to produce weight loss by malabsorption. Because of a severe syndrome of complications including arthritis, skin problems, and liver failure which occurred after this procedure, JIB is now rarely performed. Later, in 1967, Mason and Ito\textsuperscript{8} introduced a gastric bypass procedure which produced weight loss without these side effects. This procedure was designed based on the weight loss observed
after partial gastric resection for the treatment of gastric ulcers. This procedure involved the creation of a very small stomach pouch connected to limb of distal intestine, bypassing the proximal intestine. The currently-performed Roux-en-Y gastric bypass (RYGB, Figure 1A) procedure is a modification of this early procedure. The RYGB has a much smaller gastric pouch size than Mason and Ito’s original procedure, and the intestinal component of the surgery has been modified to avoid bile reflux, but the basic principle remains the same. Another currently used bariatric procedure is adjustable gastric banding (AGB, Figure 1B), a restrictive procedure in which a saline-filled silicon band is fitted around the stomach. The level of gastric restriction imposed by the band may be adjusted by infusing saline via a subcutaneous port. Together, AGB and RYGB are the two most commonly performed bariatric procedures.

Procedures also currently performed include biliopancreatic diversion (BPD, Figure 1C), duodenal-jejunal bypass (DJB, Figure 1E), ileal interposition (IT, Figure 1F), vertical banded gastroplasty (VBG, not pictured), and vertical sleeve gastrectomy (VSG, Figure 1G). BPD is a malabsorptive procedure in which the size of the stomach is reduced and the proximal small intestine is anastamosed to the distal intestine. Bile and pancreatic juices enter the distal intestine through a common channel but because of the proximal small intestinal bypass, do not contact nutrients in the duodenum. A variation on the BPD is coupled with a VSG and is called the BPD-duodenal switch (BPD-DS; Figure 1D). DJB is bypass of the duodenum and proximal jejunum without any gastric component. IT is the transplantation of a segment of ileum to a more proximal region of small intestine. VSG, another procedure which has been gaining attention recently, is the removal of 80% or more of the stomach, including the fundus and greater curvature. VSG produces weight loss and diabetes remission which are comparable to
the effects of RYGB$^{15}$, and one study suggests that VSG may achieve even greater appetite suppression than RYGB$^{16}$.


Vertical Sleeve Gastrectomy (VSG)

VSG was initially designed as a procedure to be used in conjunction with other surgeries, either preliminarily to reduce weight-related risk prior to another bariatric procedure or in conjunction with another procedure in order to maximize excess weight loss. The VSG was first described in 1998 as a part of the BPD-DS procedure$^{18}$. It has since been used alone as a staging
procedure in super-obese patients (BMI >50 kg/m²) due to its lack of invasiveness and to its ability to produce significant weight loss. Increasingly, however, VSG is gaining popularity as an independent weight-loss procedure. The procedure is attractive as a single-stage weight loss intervention for several reasons related to reduced surgical risk and to reduced postsurgical complication. These include the maintenance of an open pathway for future endoscopic studies, the lack of any implanted foreign material, and the lack of malabsorption that could hinder drug absorption from the GI tract. Complication rates range from 0%-24% for VSG, and the procedure has an overall mortality rate of 0.39%. Increasing evidence highlights VSG as a relatively low-risk procedure that can produce substantial weight loss comparable to weight loss produced by more invasive procedures like RYGB. One study reported excess weight loss (EWL) of 71% 6 months after VSG and, 12 months postoperatively, EWL of 83%. Weight loss in this study was associated with resolution of diabetes in 100% of patients, dyslipidemia in 75%, and hypertension in 93%.

The central hypothesis for this thesis is that VSG’s mechanism of action to produce sustained weight loss and to ameliorate hyperlipidemia involves physiologic changes which counteract the effects of high-fat diets to produce leptin resistance and dysregulated lipid metabolism. A key element of this hypothesis is the prediction that VSG will induce changes unique to the surgery, which are distinct from or more dramatic than those which occur during weight loss due to caloric restriction. This may include changes to the level and/or function of several circulating hormones, including gut-derived and adipose-derived hormones.
Central Nervous System Control of Energy Homeostasis

Body weight defense is dependent upon the brain’s ability to respond to internal cues relaying information about both long-term and short-term energy availability. The arcuate nucleus (ARC) of the hypothalamus is an important energy balance mission-control center (Figure 2). The ARC is composed of two neuronal populations thought to be important effectors of hormonal and local fuel signaling. The first population contains catabolic pro-opiomelanocortin (POMC)-producing neurons. POMC mRNA expression is increased in the ARC by leptin and insulin\(^{26-28}\). POMC is cleaved to produce alpha-melanocyte stimulating hormone (\(\alpha\)-MSH), a hormone whose role in peripheral cells is to regulate skin and hair pigmentation but which decreases food intake and induces weight loss when administered exogenously\(^{29,30}\). This effect is thought to be mediated by the MC4 melanocortin receptor subtype, found concentrated in the hypothalamus. Increased food intake and body weight in MC4 knockout animals\(^{31}\) suggest a role for the endogenous stimulation of MC4 receptors by \(\alpha\)-MSH to affect energy balance.

A second population produces the anabolic transmitters neuropeptide Y (NPY) and agouti-related peptide (AGRP). AgRP is found exclusively in the ARC and acts as a competitive antagonist/ inverse agonist at MC4 receptors\(^{32}\). During times of energy deficiency, AgRP blocks the catabolic effects of \(\alpha\)-MSH, resulting in increased food intake and in weight gain. Indeed, exogenous AgRP administration or genetic AgRP overexpression has been shown to produce weight gain and to stimulate food intake\(^{33,34}\). However, genetic disruption of AgRP has no effect on either food intake or weight gain\(^{35}\). Perhaps contributing to the anabolic effects of AgRP, NPY stimulates food intake and weight gain\(^{36,37}\).
The MC4 receptor is found in several brain regions, including hypothalamus, forebrain, and hindbrain.\textsuperscript{38, 39} One such area is the paraventricular nucleus (PVN), which appears to be a center for integration of signals from multiple brain regions involved in the regulation of food intake and body weight. Site-specific injection of an MC4 receptor agonist such as MTII or α-MSH into the PVN elicits an anorectic response; conversely, local administration of an MC4 receptor antagonist such as AgRP, SHU9199, or HS014 stimulates feeding. Either response is observed only after feeding has been initiated, for example by the onset of the dark cycle, and therefore it is hypothesized that PVN MC4 signaling is involved in the regulation of meal duration rather than of meal initiation.\textsuperscript{44}

MC4 receptors are also found in the dorsomedial hypothalamus (DMH), an area known to be important in the mediation of responses to stress. Intra-DMH infusion of α-MSH decreases food intake and local injection of the MC4 antagonist AgRP elicits hyperphagia.\textsuperscript{42, 45} The hyperphagic response to intra-DMH AgRP involves a preference for sucrose but not for corn starch, suggesting that the DMH is involved in the regulation of food intake in response to palatable foods.

In the brainstem, both the dorsal motor nucleus of the vagus nerve (DMX) and the nucleus of the tractus solitarius (NTS) express MC4 receptors.\textsuperscript{44} Both areas are involved in autonomic control and appear to contribute to the regulation of food intake behavior. Administration of the MC3 and MC4 receptor agonist MTII and the melanocortin receptor antagonist SHU9119 into the fourth ventricle reduces and stimulates, respectively, food intake with a magnitude similar to the response to lateral ventricle MTII or SHU9119.\textsuperscript{46} These results are supported by the hypophagic or hyperphagic response to the injection of MTII or SHU9119, respectively, into the dorsal vagal complex (DVC).\textsuperscript{47}
Melanocortin signaling also converges upon the brain’s reward circuitry. Like drugs of abuse, food can have reward value, eliciting the release of dopamine into the nucleus accumbens. Animals deficient in brain dopamine will not feed and die of starvation unless fed via gavage\textsuperscript{44}. The lateral hypothalamic area (LHA) is hypothesized to connect ARC neurons to reward pathways. Blockade of alpha-amino-3-hydroxy-5-methylisoxazole (AMPA) receptors in the nucleus accumbens elicits hyperphagia; this response is abolished when the LHA is inhibited via the administration of a GABA-A agonist\textsuperscript{48}. Electrical stimulation of the LHA elicits food intake in satiated rats\textsuperscript{44}. The LHA contains two types of neurons, those containing melanin concentrating hormone (MCH) and those containing orexin. Intracerebroventricular administration of AgRP induces c-fos exclusively in orexin-containing neurons of the LHA\textsuperscript{49} but selectively increases MCH gene expression\textsuperscript{50}. Whether either type of neuron is implicated as a downstream target of the melanocortin system has yet to be determined, as drugs targeting the MC4 receptor have little effect on ingestive behavior when administered into the LHA\textsuperscript{43, 45}.

Despite the precision by which energy balance is regulated, obesity is a problem. Physiologic mechanisms exist to protect against starvation in the face of famine, with orexigenic feedback loops which increase hunger in response to fasting. Although anorexigenic circuits exist and do decrease food intake during times of plenty, these mechanisms often break down over time in obese individuals. Perhaps the most widely-studied example of this is leptin resistance. Recently, bariatric surgery has emerged as a therapeutic option aimed to circumvent those “thrifty” aspects of energy homeostasis that are not adaptive to a modern, overnutritive environment.
Figure 2. CNS control of energy balance. The arcuate nucleus of the hypothalamus (ARC) sits in the mediobasal hypothalamus and houses AgRP and POMC neurons for the integration of peripheral energy balance cues such as leptin and insulin. POMC and AgRP agonize and antagonize, respectively, melanocortin type 4 receptors (MC4R) in downstream brain regions. Figure taken from a review by Seeley and Woods\textsuperscript{51}.

Putative Mechanisms for Weight Loss and Metabolic Improvement after VSG Restriction

It has been conventionally thought that weight loss after VSG results from reduced food intake as a direct consequence of gastric volume restriction\textsuperscript{52-55}. Because the volume of the stomach is reduced by VSG, a small increase in volume results in a much greater increase in pressure in the gastric sleeve as compared with the intact stomach\textsuperscript{56}. Furthermore, removal of the highly distensible gastric fundus, which normally allows for gastric pressure to remain
somewhat stable when volume increases, is likely to result in very high gastric pressure after a meal. This “restrictive” dogma, however, has been challenged by recent data describing both humans and rodents after VSG. Melissas et. al.⁵⁷ argue that VSG should not be viewed as a restrictive procedure. To support their argument, the authors point out that the volume of the stomach remaining after VSG in humans (about 150-200cc⁵⁸) is much larger than the volume remaining after gastric banding (typically 15-20cc⁹), while weight loss observed after VSG is similar or even greater than that which is produced by gastric banding. Gan et. al.⁵⁹ point out that weight loss is less dramatic and the diabetes resolution slower after “restrictive” procedures than after “bypass” procedures. Because VSG produces weight loss and glycemic improvement which is comparable to that which is achieved by RYGB¹⁵, it might be hypothesized that restriction alone cannot account for these effects of VSG.

If the primary mechanism for weight loss following VSG was caloric restriction due to volume reduction, one would expect that gastric dilatation after surgery would abrogate weight loss. Gastric dilatation is not common, occurring in only 1 of 14 patients one year after surgery⁵⁵, but is unlikely to limit surgical success, as gastric tube size does not predict excess weight loss in humans⁶⁰. Furthermore, weight regain has been reported in association with gastric dilatation following surgery⁵⁸, but gastric dilatation without weight regain has also been reported⁵⁵. These are only associative reports, however. Animal studies will be necessary to test any potential causal relationship between stomach volume and weight loss after VSG.

**Gastric emptying**

Delayed gastric emptying has been proposed to reduce hunger by increasing gastric volume and pressure. Afferent vagal fibers lining the stomach⁶¹,⁶² and small intestine⁶³ express
stretch receptors, and so it has been proposed that gastric stretch might elicit satiety\textsuperscript{64}. Based in part on reports of early satiety after VSG, delayed gastric emptying has been proposed to play an important role to produce weight loss after VSG. This hypothesis, however, does not appear to be supported by the literature. Reports suggest that VSG either increases\textsuperscript{57, 65, 66} or does not change gastric emptying rate\textsuperscript{67}, as measured via scintigraphic measurement of emptying for a labeled, mixed meal. GI transit time, the time required for an ingested meal to reach the ileum, has also been reported to be enhanced after VSG\textsuperscript{66}. Inconsistencies in the data regarding gastric emptying after VSG might be explained by differences in surgical technique or bougie size, as very small pouch size might actually impair gastric emptying\textsuperscript{68}, or by failure to distinguish between diabetics and nondiabetics in an obese study population, as diabetes can affect GI motility\textsuperscript{65}.

Other procedures which reduce gastric volume have been shown to alter gastric emptying rates. VBG does not alter total gastric emptying, but enhances emptying of the proximal gastric pouch created by the band\textsuperscript{69}. Emptying of this proximal pouch is most likely to affect satiety, as the pressure within this pouch is highest and most altered by a meal, but no correlation seems to link satiety or weight loss to gastric emptying rate after VBG\textsuperscript{70}.

Removal of gastric mucosa during VSG requires the transection of vagal nerve fibers which may normally regulate gastric emptying. After total vagotomy, gastric emptying is accelerated\textsuperscript{71}, probably due to the removal of vagally-removed negative feedback mechanisms. For example, gastrin secreted by a vasovagal reflex\textsuperscript{72} in response to antral distension slows gastric emptying\textsuperscript{73}. Due to vagotomy but also because stomach distensibility may be compromised after transaction of the muscular stomach wall during VSG, this feedback loop might be lost. Additionally, manipulation of the stomach during VSG may elicit vagal
remodeling. Chronic ligation of the fundus, a procedure anatomically similar to AGB, alters vagus nerve fiber receptive field\textsuperscript{74}. Such vagal plasticity might alter not only gastric emptying, but feelings of satiety or hormone release as well. Of course, which vagal fibers are severed or remodeled may determine the direction of change of gastric emptying, as vagal efferents are important for promoting gastric emptying and intestinal transit. Future characterization of vagal nerve fiber function and distribution after VSG may clarify whether vagal disruption affects gastric emptying after VSG.

Pyloric preservation is a feature of VSG as well as RYGB and AGB. The pylorus is important for the effect of several enteroendocrine hormones, including PYY, CCK, and GLP-1, to slow gastric emptying. Paradoxically, resection of the pylorus alone impairs gastric emptying for liquids\textsuperscript{75}. Although the pylorus is preserved by RYGB, this region is excluded from the flow of nutrients, and so slower gastric emptying and longer intestinal transit time after RYGB\textsuperscript{76} may be due to dysregulation of the “pyloric brake.” Nutrients do contact the pylorus after AGB, but accelerated gastric emptying might reflect an ability for the pressure imposed by the band to override this “brake.” It is unclear how pyloric preservation may affect gastric emptying after VSG, but more specific hypotheses may be formed as an understanding of VSG-induced endocrine effects develops.

**Hormonal Mechanisms and the Hypothalamic Melanocortin Axis**

Recently, several groups have hypothesized that a hormonal mechanism underlies weight loss after VSG. Compared with dieting, VSG is unique in that it is not associated with rebound hyperphagia. Data described in this thesis suggest that this is not due to restriction. Increasingly, attention has been turned to potential hormonal mechanisms for this lack of
hyperphagia and for the defense of a new, lower body weight. Although the intestines are not cut during VSG, it is clear that several hormonal parameters are altered after surgery. A key difference between weight loss induced by VSG and by dietary interventions is a lack of rebound weight gain after VSG. The following is a discussion of potential candidate hormones and their central nervous system (CNS) targets by which VSG may promote a leaner defended body mass.

**Ghrelin**

Ghrelin is produced in both the stomach and duodenum, but about 2/3 to 3/4 of circulating ghrelin is produced by X/A-like cells of the gastric mucosa, particularly by the fundus. Ghrelin is a 28-amino acid peptide cleaved from preproghrelin and activated via posttranslational modification by octanoic acid. This modification is required for binding to the ghrelin receptor, GHS-R1a. GHS-R1a is widely expressed, but of particular importance to ghrelin’s role in energy balance is its CNS expression. Via its action in the CNS, ghrelin is orexigenic, a unique feature of centrally-acting hormones. Ghrelin stimulates food intake when administered either peripherally or centrally. The main effect of ghrelin on food intake is to initiate meals. Consistent with its postulated role as a hunger hormone, plasma ghrelin levels rise during periods of fasting and fall in response to feeding.

Because VSG involves the removal of ghrelin-producing mucosa, considerable attention has been given to the hypothesized role of reduced ghrelin to mediate weight loss and metabolic improvement following the surgery. Plasma ghrelin is decreased after VSG in humans and in rodents. This reduction is immediate (present on postsurgical day 1 in both humans and rats) and persistent. By contrast, ghrelin levels are increased in response to weight loss by caloric restriction. Given this difference in plasma ghrelin levels between VSG-operated
animals and naïve, calorically restricted animals, ghrelin has emerged as an attractive candidate to explain the absence of hyperphagia in response to VSG-induced weight loss.

The decrease observed after VSG is presumably due to resection of ghrelin-producing stomach tissue. It should be noted, however, that plasma ghrelin levels are reduced but not completely diminished following VSG and, therefore, ghrelin is probably also secreted from other areas of the stomach and intestine. Extra-fundal ghrelin-producing regions appear to be nutrient-responsive, as ghrelin secretion 12 months after VSG is depressed by a meal\(^\text{16}\).

Postprandial fluctuation in plasma ghrelin levels implies some basal ghrelin secretion in VSG patients. Supporting the presence of intestinally-derived ghrelin, alterations in plasma ghrelin levels have been reported after surgeries not involving the stomach\(^\text{90}\). Postprandial changes to plasma ghrelin are lost after RYGB\(^\text{16}\), indicating that factors from the distal stomach or proximal small intestine may influence plasma ghrelin levels.

Fasting plasma ghrelin levels are increased after gastric banding\(^\text{91, 92}\), highlighting an important difference between VSG and “restrictive”-type gastric surgeries. Unlike gastric banding, VSG includes removal of gastric mucosa. Because this mucosa remains intact after gastric banding, ghrelin secretion is preserved. Postsurgical increases in circulating ghrelin most likely reflect a normal response to negative energy balance. RYGB also involves gastric restriction without removal of gastric mucosa, but is associated with reduced circulating ghrelin levels\(^\text{76, 89, 93-98}\). Because unlike after banding, ghrelin-producing mucosa is excluded from access to nutrients, gastric ghrelin secretion might be reduced due to the deprivation of nutrient stimulation to ghrelin-producing cells. When plasma ghrelin levels were measured intraoperatively during RYGB, the most dramatic reduction occurred immediately after gastric transection, resulting in plasma ghrelin levels which approximated postoperative levels\(^\text{99}\); how
these findings relate to RYGB’s effects on ghrelin secretion in an alert, free-fed animal, however, is unclear. Furthermore, changes to ghrelin sensitivity after VSG may negate any potential effect of reduced ghrelin on defended body weight. One of the most important sites of ghrelin action is the CNS, where ghrelin acts to potently reduce food intake. A recent report suggests that CNS ghrelin sensitivity may be increased following VSG, compensating for the potential effects of reduced plasma ghrelin. Wang et. al.\textsuperscript{87} reported circulating ghrelin which is decreased by as much as 60\% after VSG but found a 50\% increase in hypothalamic ghrelin receptor (GHS-R 1a) protein expression. Because the hypothalamus houses important feeding centers, increased local sensitivity to ghrelin would be expected to dampen weight loss if ghrelin were required. However, reduced ghrelin action at other receptors or sites cannot be ignored.

If reduced ghrelin levels contribute to a lower level of defended body fat after VSG, then the reduction should be required for weight loss and maintenance. Indeed, Langer et. al.\textsuperscript{84} report, in a recent study, complete weight regain in the sole patient whose ghrelin did not decrease postoperatively. However, obesity is also associated with low plasma ghrelin levels\textsuperscript{100, 101}, suggesting that low ghrelin may not be protective against obesity. In fact, ghrelin levels after AGB are elevated and correlate inversely with BMI\textsuperscript{91, 102}. Plasma ghrelin levels after AGB do not correlate with satiety\textsuperscript{102}, suggesting other mechanisms for food intake reduction which override a normal elevation in plasma ghrelin in response to weight loss. This response is not observed following either RYGB or VSG, probably due to the exclusion of most ghrelin-producing cells from nutrient flow.

Recent reports suggest that the currently available data regarding ghrelin after bariatric surgery paint an incomplete picture. Until recently, the scientific community has focused on ghrelin as a “hunger hormone,” produced by the GI tract during nutrient deprivation. New data,
however, have inspired a re-evaluation of ghrelin’s functional role. It is now known that ghrelin produced in the stomach during fasting is primarily inactive, desacyl-ghrelin. Desacyl-ghrelin must be acylated and activated by the enzyme ghrelin O-acetyltransferase (GOAT) in order to activate ghrelin receptors. Additionally, octanoylation may be required for ghrelin to cross the blood-brain barrier\textsuperscript{103}. Surprisingly, highest expression of GOAT is found after feeding\textsuperscript{104}. These data seem to position active ghrelin not as a hunger hormone but rather as an indicator of feeding status.

A problem with the existing data describing ghrelin levels after bariatric surgery, including VSG, is that most studies do not distinguish active ghrelin from total ghrelin and do not measure meal-stimulated GOAT levels. To illustrate the importance of this distinction between active and total ghrelin, in one rodent study\textsuperscript{53} unfasted total ghrelin levels were unchanged but active ghrelin levels were very low after VSG, as compared with lean controls. VSG and RYGB in humans and rats reduce fasting plasma ghrelin levels\textsuperscript{87, 88, 105} without altering the ratio of octanoyl to total ghrelin\textsuperscript{105}. It is unknown how active ghrelin levels may change postprandially in patients after VSG, but it is expected that the response might be similar to the response seen post-RYGB. In RYGB patients, both total and octanoylated ghrelin levels exhibit dramatic suppression following a liquid test meal\textsuperscript{105}. In rats, this active ghrelin reduction is weight-independent\textsuperscript{94}. Similar studies are needed for VSG, in order to parse the postprandial total versus active ghrelin response. A key element to these studies will be to characterize expression of ghrelin and GOAT in extra-fundal gastric tissues and in the small intestine under fasting and fed conditions. VSG clearly reduces total plasma ghrelin levels, but whether this change provides any mechanistic basis for weight loss and maintanence is uncertain. Insights from other surgical procedures such as AGB and RYGB may form the basis of several
hypotheses regarding the role of the ghrelin axis after VSG, but these hypotheses must be tested directly.

*Cholecystokinin (CCK)*

Cholecystokinin (CCK) is a classic satiety hormone responsible for modulating hunger in response to meal onset. CCK is secreted as two forms, CCK-33 and CCK-8, from I cells of the duodenum and jejunum. In response to a meal, CCK is released rapidly from the duodenum and jejunum into the circulation. Fat- and protein-rich meals are particularly potent stimuli for CCK release. CCK is also released in response to gastric distension. CCK acts on CCK-1 (CCK-A) receptors in the periphery and CCK-2 (CCK-B) receptors in the CNS. Via its actions at CCK-1 receptors, CCK stimulates pancreatic enzyme secretion and gallbladder contraction and delays gastric emptying. Together, these actions serve not only to attenuate food intake but to promote effective digestion of fats and protein in a meal.

CCK suppresses food intake by reducing meal size. CCK is produced not only by the intestine but in the CNS as well. Exogenous delivery of CCK into either the peritoneal cavity or the CNS reduces food intake, but ip CCK will only reduce food intake if administered within 15 minutes of the meal’s onset. This is due to CCK’s short half-life of only 1-2 minutes. Chronic administration of CCK alone does not result in weight loss, but coadministration of leptin can uncover an anorectic response.

It is clear that the stomach plays an important role to modulate CCK release. Total gastrectomy increases CCK release in humans and in rats. Normally, total gastrectomy produces weight loss and reduced food intake, but these effects were normalized by chronic CCK-A or –B receptor blockade in rats. Additionally, some evidence indicates increased
central sensitivity to CCK after total gastrectomy as shown by enhanced CCK-A receptor-dependent NTS activation after a meal\textsuperscript{122}. Removal of stomach tissue is a common feature of VSG and total gastrectomy and so it is possible that CCK is also implicated in VSG-induced body weight reduction.

CCK measurements in the plasma after VSG have not yet been reported. However, several outcomes of VSG might affect CCK release. CCK release might be altered by enhanced gastric distension after volume restriction. VBG, as the prototypical restrictive gastric procedure, has been reported not to change plasma CCK levels after a meal\textsuperscript{123}, but it is difficult to compare the changes to gastric distension which may occur after banding versus VSG. Additionally, altered meal patterns and/or gastric emptying after VSG may alter the kinetics by which nutrients are delivered to the small intestine and perhaps affect CCK release. Further studies are needed to describe changes to fasting and meal-stimulated CCK levels after VSG and to test whether altered CCK release and/or action might contribute to reduced adiposity and lack of hyperphagia after VSG.

\textit{Glucagon-Like Peptide-1 (GLP-1)}

A major effect of bariatric procedures, including VSG, is improved glucose tolerance and, often, complete and rapid reversal of Type 2 Diabetes. In many cases, this improvement appears to be weight independent, an effect hypothesized to result from altered secretion of gut-derived, or enteroendocrine, hormones. GLP-1 is one important enteroendocrine hormone intimately involved in the control of glucose homeostasis, and several currently-used pharmacologic therapies for obesity target the GLP-1 axis\textsuperscript{124}. GLP-1 is a 30 amino acid product of the preproglucagon (PPG) gene which is produced by L-cells of the intestine, mainly the
ileum. Nutrient delivery is required for the release of GLP-1, which stimulates insulin secretion from pancreatic beta cells. This mechanism is called the “enteroinsular axis.” Stimulation of GLP-1 release by intestinal nutrients may not require nutrients to contact the ileum, but may result from signals arising in the proximal intestine. GLP-1 has an additional effect, mediated by the CNS, to inhibit appetite. GLP-1 may therefore improve glucose homeostasis not only via a direct effect on insulin secretion but indirectly via reductions in food intake.

Glycemic improvement after RYGB in humans is rapid and weight-independent, an effect which could be due to immediate changes in the levels of circulating GLP-1 after surgery. Not only does improved glucose tolerance occur prior to weight loss after RYGB, but RYGB-induced weight loss produces glycemic improvement which is superior to weight loss due to dieting. In a glucose tolerance test comparing BMI- and weight loss-matched patients after dietary intervention or RYGB, improved glucose tolerance after surgical intervention was associated with enhanced insulin secretion and dramatically increased postprandial active GLP-1, total GLP-1, and GIP levels. Enhanced meal-stimulated GLP-1 secretion has been observed in humans and in rats after RYGB and is also associated with secretion of all products of the preproglucagon gene. In obese individuals, postprandial GLP-1 release is blunted and the effect is restored after weight loss. Lean control groups, therefore, are important to fully understand the role of any bariatric surgery to produce weight-independent changes to GLP-1 release.

Because GLP-1 is synthesized primarily in the distal small intestine, it would be predicted that procedures altering the flow of nutrients to the ileum would have the most profound impact on GLP-1 secretion. The “hindgut hypothesis” states that the more rapid
delivery of nutrients to the distal small intestine following RYGB enhances local hormone release. Consistent with this hypothesis, fasting GLP-1 levels are unaltered after RYGB as compared with control subjects\textsuperscript{95, 138}. BPD\textsuperscript{142}, IT\textsuperscript{13, 143, 144}, and JIB\textsuperscript{145}, procedures including proximal small intestinal bypass, exaggerate postprandial GLP-1 secretion. IT increased GLP-1 secretion after a meal in diabetic rats as well as chow-fed, euglycemic rats, demonstrating an independent effect rather than the reversal of a HFD-induced or obesity-induced impairment in GLP-1 secretion\textsuperscript{146}. Ileal PPG expression is enhanced in fasted rats after IT\textsuperscript{13}, suggesting that chronically increased PPG expression may prepare the L-cell for enhanced postprandial GLP-1 release. Other bariatric procedures that do not alter nutrient flow through the intestine, however, do not appear to produce changes to the enteroinsular axis. AGB, for example, does not enhance meal-stimulated GLP-1 secretion as compared with weight-matched controls\textsuperscript{138, 147}, lean controls\textsuperscript{147}, or preoperative controls\textsuperscript{95}.

Surprisingly, although VSG does not involve intestinal re-routing, VSG in humans appears to produce improvements to glucose homeostasis which are very comparable to those observed after RYGB\textsuperscript{15}. Enhanced release of GLP-1 might underlie this effect. As early as 10 days after either VSG or RYGB, postprandial GLP-1 and insulin release are enhanced\textsuperscript{15}. This effect is expected to underlie the rapid improvement to glucose tolerance that is observed after either procedure. Enhanced GLP-1 release has also been observed after VSG in diabetic GK rats\textsuperscript{88}. While several lines of evidence indicate changes to the enteroinsular axis following VSG, it is still unclear whether VSG’s beneficial effects on glucose tolerance are weight-independent and/or directly related to GLP-1. Future studies need to explore a direct link between GLP-1 and improvements to glucose tolerance in order to parse changes elicited by GLP-1 versus other enteroendocrine hormones.
Peptide YY (PYY)

Like GLP-1, peptide YY (PYY) is secreted from intestinal L-cells. PYY circulates as PYY<sub>1-36</sub> and PYY<sub>3-36</sub>. PYY<sub>3-36</sub> is the predominant circulating form of the hormone<sup>148,149</sup> and is formed via cleavage of PYY<sub>1-36</sub> by the enzyme di-peptidyl peptidase IV (DPP-IV)<sup>150</sup>. PYY is released from the gut into the circulation in response to nutrients, reaching peak plasma concentration 1-2 hours after a meal and remaining elevated in the blood for several hours<sup>151</sup>. PYY may be implicated in obesity, as decreased circulating PYY levels have been reported in obese rodents<sup>152</sup> and humans<sup>153-156</sup>. PYY functions in the GI tract to increase ileal fluid and electrolyte absorption and to inhibit pancreatic and gastric secretions, gallbladder contraction, and gastric emptying<sup>157</sup>, but PYY is also known to play a role in the control of food intake.

Intraperitoneal administration of PYY<sub>3-36</sub> dose-dependently reduces food intake in rodents and humans<sup>158</sup>. PYY<sub>1-36</sub> administration has the same effect but is much less potent<sup>159</sup>. In the CNS, however, PYY appears to have opposing actions in various regions. Ventricular administration of either PYY<sub>1-36</sub><sup>160</sup> or PYY<sub>3-36</sub><sup>161</sup> increases food intake by a mechanism which is dependent on Y1 and Y5 PYY receptor subtypes<sup>162</sup>. An orexigenic effect is also observed after direct infusion of PYY<sub>1-36</sub> into the PVN<sup>163</sup> or hippocampus<sup>164</sup>, but intra-ARC administration of PYY<sub>3-36</sub> inhibits food intake<sup>158</sup>. This phenomenon is thought to be due to the presence of PYY receptor subtype Y2 in the ARC. Intra-ARC infusion of the Y2 receptor antagonist BIIE0246 abolishes the anorectic response to peripherally-administered PYY<sub>3-36</sub><sup>165</sup>. In addition to a direct role for PYY to interact with receptors in the ARC, peripheral PYY may also act centrally by activating vagal afferents; Y2 receptors are present within the nodose ganglion and on vagal afferents.
PYY may also influence body weight by altering energy expenditure; chronic peripheral administration of PYY$_{3-36}$ to rodents promotes fat oxidation$^{166-168}$. In humans, infusion of PYY$_{3-36}$ increases energy expenditure and fat oxidation rates$^{169}$. These effects appear to be physiologic: fasting PYY levels are negatively correlated with 24-hour resting energy expenditure rate$^{170}$ and postprandial levels correlate positively with energy expenditure$^{171}$.

Recently, it has been proposed that PYY may mediate weight loss after bariatric surgery, including VSG. After both RYGB and VSG, fasting$^{16}$ as well as postprandial$^{15, 16, 139, 172}$ PYY has been found to be elevated in humans. This response corrects obesity-induced resistance to meal-stimulated PYY secretion and is present as early as one week after surgery$^{15}$. In rats, RYGB$^{139}$ as well as IT$^{144}$ enhances postprandial PYY secretion. For RYGB, this effect increases over time after surgery$^{139}$. It is unknown whether this is also true for VSG, but when combined with an ileal interposition, either with or without duodenal diversion, VSG exaggerated meal-induced PYY secretion as compared to preoperative values$^{173}$.

Because decreased PYY levels have been reported in obese individuals, weight loss after bariatric surgery might influence plasma PYY levels without any direct effect of surgery on L-cell response. Whether or not VSG may increase PYY in a weight-independent manner is unknown, but studies describing changes in PYY levels after other types of weight loss surgeries might provide some clues. After RYGB, fasting PYY levels are unchanged relative to either lean or overweight controls in humans$^{105, 148}$ and rats$^{94}$. However, postprandial PYY response is enhanced$^{94, 105, 147, 148, 174}$ and more rapid$^{105}$ after RYGB as compared to lean and either overweight or weight-matched controls. These data demonstrate that RYGB’s effect on PYY secretion is weight-independent, an effect which might be due to enhanced ileal nutrient delivery. This hypothesis is consistent with increased meal-stimulated PYY responses after ileal
interposition and increased ileal PYY expression after ileal transposition. Both surgeries result in premature delivery of nutrients to the ileum, perhaps enhancing L-cell stimulation. To support this idea, enteroglucagon (also produced by L-cells) is increased in the circulation following RYGB.

Since AGB, like VSG, does not involve any intestinal re-routing, an obvious hypothesis might be that VSG and AGB similarly affect PYY secretion. AGB does not enhance meal-stimulated PYY secretion as compared to lean controls. However, Valderas et. al. have demonstrated increased postprandial plasma PYY after VSG when compared with BMI-matched patients who had undergone medical treatment for weight loss. While the enhancement was lower after VSG than RYGB, these data suggest that the effect might be weight-independent, as it appears to be after RYGB. These findings have not yet been recapitulated, as other reported changes to PYY secretion after VSG have only compared subjects to the preoperative condition. Further studies are needed to clarify whether PYY is causally linked to weight loss after VSG, but it is hypothesized that observed changes are weight-dependent and do not drive development of the lean phenotype.

Gastric Inhibitory Peptide (GIP)

Gastric inhibitory peptide (GIP) is another key component of the enteroendocrine system that can affect glucose tolerance. Secreted from duodenal K cells, GIP inhibits intestinal motility and modulates insulin secretion after glucose load. Minimal changes to fasting GIP secretion have been observed after RYGB. However, meal-induced GIP secretion appears to be blunted after RYGB, an effect which presumably results from bypassing the proximal small intestine, the primary site for GIP secretion. VBG, on the other hand, enhances the GIP
response to a meal, perhaps contributing to improved glucose tolerance\(^{145}\). GIP levels have not yet been reported following VSG, but these measurements will reveal important information regarding the mechanism for improvements in glucose tolerance after the procedure.

**Adipokines: Leptin and Adiponectin**

Much attention has been given to the role of intestinally-derived hormones to mediate the effects of VSG. The role of adipose-derived hormones, on the other hand, cannot be ignored. Leptin is a key component of energy homeostasis but leptin function is often disrupted in obese individuals. Secreted from white adipose tissue and the stomach, leptin circulates in proportion to body fat and acts in the CNS to reduce body weight primarily by suppressing ingestive behavior. Most obese individuals have very high levels of circulating leptin\(^{177,178}\), but exogenous leptin treatment produces little or no weight loss\(^{179,180}\). Impaired leptin action in obese individuals is termed “leptin resistance” and is assumed to contribute to the difficulty of most traditional obesity therapies to produce weight loss without hyperphagia. Bariatric surgeries, including VSG, are effective to reduce body weight in obese individuals who are likely to be leptin-resistant. It has been hypothesized that altered leptin resistance might provide a mechanism for the weight loss and lack of hyperphagia observed after VSG\(^{181}\). Plasma leptin levels in humans\(^{182}\) are decreased following VSG, consistent with decreased adiposity. Similar findings have been reported after RYGB in humans\(^{91,95,105,139,148,183}\) and in rats\(^{94}\) and after AGB in humans\(^{91,92,95}\). Interestingly, the reduction in plasma leptin levels after RYGB exceeds the reduction observed in weight-matched control subjects\(^{148}\). Because leptin is produced in the gastric fundus\(^{184}\), nutrient-exclusion of fundic mucosa might produce more exaggerated reductions in plasma leptin levels than expected for the level of observed weight loss.
Although these studies report changes to plasma leptin levels which may reflect a
reversal to obesity-related leptin dysfunction, none have directly addressed whether VSG or
other bariatric surgeries alter leptin sensitivity or whether restored leptin function may drive the
maintenance of weight loss after surgery.

Adiponectin is another adipose-derived hormone whose levels in the plasma reflect total
body adiposity. Circulating adiponectin increases in response to weight loss\textsuperscript{185, 186}, including
weight loss induced by RYGB\textsuperscript{175, 187, 188} and VBG\textsuperscript{90}. Low circulating levels of adiponectin have
been linked to insulin resistance via actions in peripheral tissues\textsuperscript{189}. However, whether
adiponectin plays a role centrally to modulate food intake and energy expenditure is
controversial, and it has been argued that adiponectin may not cross the blood-brain barrier\textsuperscript{190, 191}. Perhaps for this reason, adiponectin has not yet been studied as a mechanistic player in the
maintenance of surgically-induced weight loss, but it is possible that elevated adiponectin levels
with postsurgical weight loss may promote improved glucose homeostasis. As with leptin, it will
be important to determine whether VSG produces weight loss-independent changes to the levels
of and tissue sensitivity to adiponectin.

**Taste and Reward**

Although energy homeostasis is strongly defended, homeostatic mechanisms appear to be
biased to protect against famine. It has been argued\textsuperscript{192} that obesity may be due to the ability of
an overnutritive environment to take advantage of reward-driven neural circuits that may
override the hypothalamic control of energy homeostasis. Additionally, obese individuals have
been found to experience decreased satiation, as indicated by a slower decline in salivation after
a taste of lemon juice or yogurt\textsuperscript{193, 194} or by self-reported reward values of high-sugar and high-
fat. Based on these ideas, therapies targeted to alter or enhance reward processing might augment diet-induced weight loss. Because bariatric surgery is so successful at producing long-term weight loss, several groups have become interested in whether these procedures alter the rewarding properties of food. Several qualitative studies support this hypothesis. Surveys indicate that reduced food intake after RYGB stems from a “lack of desire” for high-fat and high-carbohydrate foods and patients report that they are “not interested in sweets and desserts after surgery”.

Changes to food preference may be mediated by ingestive or postingestive cues. Ingestive cues include properties such as taste and texture, whereas postingestive cues include feelings of satiety and hedonics. Taste signals from the mouth and small intestine are integrated in the primary taste cortex, which includes the frontal operculum and primary taste cortex. Signals from these regions may be relayed to secondary brain regions, including the amygdala and the orbitofrontal cortex, in order to influence reward signaling. Changes to both taste and reward have both been identified following RYGB and other bariatric procedures. Taste intensity was attenuated in 92% of AGB patients and 59% of RYGB patients in one study. In the same study, 67-68% of patients from both groups reported new taste aversions, a phenomenon which could arise from changes to taste and/or reward. Another study demonstrated enhanced taste acuity for bitter and sour and attenuated taste acuity for sweet and salty after RYGB. The rewarding properties of foods may also be altered after bariatric surgery. RYGB increases dopamine receptor availability, perhaps altering neuronal activity in reward centers like the nucleus accumbens. Some data have indicated that a history of mood disorder may attenuate weight loss after RYGB or VSG, a phenomenon perhaps explained by altered reward signaling.
Changes to taste and/or reward after surgery may affect the types of foods which are selected. Specifically, a decreased preference for fatty foods has been demonstrated in humans after several bypass procedures, including RYGB\textsuperscript{204, 205} and JIB\textsuperscript{206}, and in rats after RYGB\textsuperscript{207}. While altered macronutrient selection cannot explain weight loss in rodents fed a fixed diet, alterations to food reward might contribute to total caloric intake and thus to weight loss after surgery. “Dumping syndrome” in RYGB patients is an unpleasant syndrome induced by overconsumption of calorically dense foods and may induce aversion to such foods. Permanent changes to food perception and tolerance have been documented after RYGB\textsuperscript{204, 208, 209}. One possibility is that new food preferences after surgery might result from learned taste aversions which are conditioned by the unpleasant symptoms of “dumping-syndrome” associated with consumption of specific foods or macronutrients. According to this hypothesis, RYGB patients might avoid overeating, especially high-fat, high-carbohydrate foods, in order to avoid these symptoms.

Very little information exists about changes in taste or hedonics that may occur after VSG. Recently, decreased D2 receptor availability has been reported in brain reward areas after VSG and RYGB\textsuperscript{182}. Although this change was hypothesized to be due to increased dopamine levels, it is unknown how this change might alter food reward in VSG patients. Additionally, the decreased receptor availability observed after RYGB does not seem to agree with the earlier finding that D2 and D3 receptor availability are increased following RYGB, so it is unclear what to take from either of these studies.

Dumping syndrome has not been reported after VSG and so conditioned taste aversions are unlikely to develop. However, changes to the taste or reward value of foods after VSG are very plausible. These changes might be entrained by meal patterns, altered lipid metabolism,
and/or alterations to the endocrine system. PYY release after a meal is enhanced after VSG172, and PYY has been shown to activate reward centers in the brain, including the ventral striatum, OFC, and insular cortex210. Meal-stimulated GLP-1 release is also enhanced after VSG88; GLP-1 receptors have been identified in reward centers211 and in taste buds212. Although a link between hormonal and hedonic alterations has not yet been tested, these data might provide indirect evidence for reward- and/or taste-driven changes to food preference after VSG.

**Summary and Aims**

Collectively, these data indicate that VSG produces weight loss and metabolic improvements which are similar to the response to RYGB both in timecourse and magnitude, although VSG is a much less anatomically-aggressive surgery. Proposed mechanisms for the effects of VSG initially included restriction and gastric emptying, but more recent studies suggest that the surgery may induce hormonal and/or behavioral changes which produce these effects. The studies described in this dissertation use a rat model of VSG. An advantage to this model is the ability to use certain controls not available for human studies. One such control is a sham-operated group, whose presurgical body weight and fat mass are matched to VSG-operated rats. Additionally, by pair-feeding a sham-operated group to the VSG-operated group, a weight-matched control can distinguish which effects of VSG are weight-independent.

The central hypothesis for the studies included in this dissertation was that VSG acts to improve body adiposity and plasma lipids via weight-independent physiologic changes. The data included in chapter two aimed to address the hypothesis that body weight reductions after VSG are due to alterations to central leptin sensitivity. The studies described in chapter three address the hypothesis that VSG improves postprandial lipid clearance. Collectively, the data
presented here suggest that VSG induces sustained body weight loss which is defended and which is independent of changes to lipid clearance. Body weight reduction is due to a selective loss of fat mass and is produced by early reductions in food intake followed by absence of a hyperphagic response to weight loss, but these changes are not produced by weight-independent changes to leptin sensitivity. These studies are important, because they aim to dissect mechanisms for weight loss which, after VSG, are more dramatic and more durable than weight loss produced by dieting alone. The hope is that an understanding of mechanisms for the effects of bariatric surgeries, including VSG, will elucidate targets for more effective, less invasive, and more universally accessible treatments for obesity as well as obesity-related comorbidities.
CHAPTER 2

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

The data presented in this chapter appear in the following publication:
Abstract

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 (POMC)/agouti-related peptide (AgRP)/neuropeptide Y (NPY) 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 pre-restriction, 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
Background

Body fat is regulated by a complex neuroendocrine system, making it difficult to maintain weight loss achieved via 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 (ARC), a major CNS energy balance control area, leptin exerts its catabolic action by stimulating pro-opiomelanocortin (POMC) neurons while inhibiting the expression of the endogenous MC3/4R antagonist Agouti-related peptide (AgRP) and the potent orexigen Neuropeptide Y (NPY). As a result, when injected directly into the 3rd-cerebral ventricle adjacent to the ARC, leptin reduces food intake and body weight.

However, in most obese individuals, leptin levels are elevated in direct proportion to body fat, and exogenous leptin treatment produces little or no weight loss. 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. 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% or more, and the intestine remains intact. This procedure produces dramatic weight
loss in humans\textsuperscript{19, 214, 215} and in rodents\textsuperscript{216, 217}. In fact, recent reports indicate that its efficacy is close to that of the more common Roux-en Y gastric bypass\textsuperscript{15, 16}.

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\textsuperscript{57}. We hypothesized that VSG-treated rats would actively defend their new lower body weights and would do so via 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.

**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-h 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 Supplemental Table 1. Four cohorts, labeled A, B, C and D, contained 24-67 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),
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 pair-fed to match the intake of the VSG group. To do this, the amount of food eaten by the VSG rats during the previous 24 h 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 (NMR, Echo MRI: Echo Medical Systems, Houston, TX).

Blood was taken from the tip of the tail just before the onset of dark after 4 h of fasting for quantification of plasma leptin (postsurgical day 50) and after 2 h of fasting for quantification of plasma insulin and glucose (postsurgical day 125). At the end of each study, animals were placed briefly in a CO2 chamber and then sacrificed by decapitation during the light phase.

Surgical Procedures. For VSG, 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, 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 post-operative care for 3 days, consisting of twice-daily subcutaneous injections of 10 mL saline and 0.3 mL Buprenex. Rats were fasted 24 h prior to surgery and had post-surgical access only to Osmolite OneCal liquid diet 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 28-30 days after surgery. Rats from each group (N = 14-18/surgical condition) were placed in the system for 96 h. The first 24 h were considered adaptation and the data from the next 72 h were analyzed. Data for indirect calorimetry analysis were sampled every 45 min. Data for food intake and meal pattern analysis were sampled every 15 min, and accumulated in 6-h blocks.

Fecal lipid content. Dietary lipid absorption was assessed using the Behenate method (N = 7-8/group), as described previously. Briefly, rats were temporarily placed on a diet containing 5% sucrose polybehenate (behenic acid). After 24 h of acclimation to the diet, cages were changed and fecal pellets were collected after another 24 h. 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 post-operative Day 50, rats within each dietary group (N = 14-20/group) were divided into two 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.

**Leptin sensitivity test.** Sensitivity to exogenous leptin was assessed at 20-22 days following surgery, using subgroups of N = 7-10 animals per surgical group. Rats received 3 consecutive intraperitoneal (i.p.) injections of either saline (vehicle) or leptin (PeproTech, Inc., Rocky Hill, NJ) at 24-h intervals. Leptin was administered at a dose of 1.46 mg/kg lean mass, as determined by NMR, in a volume of 0.5 mL water per 100 g total body mass. This dose is based on average lean body mass for a sham-operated, HFD-fed control rat and on a dose of 1 mg/kg leptin. Food was removed on the evening prior to the study, 2 h after the onset of the dark, and the injection was made 4 h prior to the onset of the dark cycle on each study day. Food was returned at the onset of the dark on each day, and food intake and body weight were monitored at 24-h intervals.

**Gene Expression Studies.** Neuropeptide expression was measured in the mediobasal hypothalamus (MBH) by dissecting a wedge bounded rostrally by the optic chiasm, caudally by the mammillary bodies, and laterally by a cut connecting the optic tract to the 3rd ventricle. Tissue was homogenized in RLT buffer using a tissue lyser (QIAGEN, Inc., Valencia, CA) and RNA was extracted using a QIAGEN miniprep RNA extraction kit. An iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA) was used, and quantitative PCR was performed using SYBR Green detection. 6-9 rats per group were studied in each experiment. Primers used in these studies are listed in Supplemental Table 2.
Plasma leptin, insulin, and glucose quantification. Plasma leptin levels after 4 hours of fasting were quantified 50 days after surgery, using a rat leptin ELISA (Crystal Chem, Downers Grove, IL). Groups of 14-19 rats were analyzed. Plasma insulin levels were quantified 125 days after surgery, using a rat insulin ELISA (Crystal Chem, Downers Grove, IL). Plasma glucose levels on postsurgical day 125 were quantified using a plasma glucose analyzer (Analox Instruments, USA, Lunenberg, MA). Groups of 13-18 rats were analyzed for plasma insulin and glucose measurements. Plasma insulin and glucose were measured during the light phase of the light-dark cycle, after 2 hours of fasting.

Statistical Analysis. All data are expressed as mean ± SEM. Body weight, food intake, NMR, and TSE data were analyzed via 2-way ANOVA (Variables: treatment & time) with a Bonferroni post hoc test where appropriate. Pair-fed controls were excluded from meal pattern analysis because meal patterns were imposed by feeding schedule. Plasma leptin levels and hypothalamic gene expression profiles were analyzed using 1-way ANOVA followed by a Tukey post-hoc test where appropriate.

Results

Weight loss and fat mass loss following VSG persists despite transient reductions in food intake. Following surgery, VSG rats lost about 20% of initial weight (Figures 1A-B). After the initial period of weight loss (10 days), VSG animals gained an average of 1.03 ± 0.11 g/day for males and 1.63 ± 0.14 g/day for females. This is comparable to weight gain in both NAÏVE (females: 1.12 ± 0.05 g/day, males: 1.54 ± 0.14 g/day) and SHAM animals (females: 1.43 ± 0.10 g/day,
males: 1.57 ± 0.11 g/day). Body weight remained reduced in VSG rats compared to SHAM and NAÏVE throughout the duration of the study. VSG induced a significant loss of fat mass, as assessed on Day 122 (Figure 1C). Lean mass did not change in VSG rats relative to SHAM or NAIVE (Figure 1D). VSG induced a significant, initial reduction in daily food intake (Figures 1E-F). This anorexia was transient and daily food intake for VSG rats and SHAM rats was no longer significantly different after Day 16 for males (Figure 1E). Females with VSG had similar changes with early significant reductions in body mass but a similar rate of regain (Figure 1B), reductions in fat mass (Figure 1C), maintenance of lean mass (Figure 1D), and transient anorexia (Figure 1F) compared to SHAM and NAÏVE animals.

Weight loss following VSG is not due to altered energy expenditure or malabsorption. Anorexia following VSG is transient whereas the weight loss is persistent. Two possible explanations for this are altered energy expenditure or intestinal malabsorption following VSG. Using the Behenate method for analysis of lipid absorption, VSG rats have normal lipid absorption and actually had significantly less lipid in the feces than rats in the SHAM group (Figure 2A). This strongly implies that fat mass loss after VSG is not due to intestinal malabsorption. We used indirect calorimetry to determine oxygen consumption on days ranging from 29 to 42 after surgery. At this time, VSG rats weighed less than SHAM (Figure 2B, P<0.01). VSG and SHAM animals also had equivalent daily caloric intake at this time, and so the PF group serves as a weight-matched control. There were no differences in energy expenditure (Figure 2C) or locomotor activity (Figure 2D) among the three tested groups. In fact, there was a trend toward decreased energy expenditure in both VSG and sham-operated pair-fed (PF) rats. Respiratory quotient (RQ) was significantly reduced (P<0.01) during the light phase in VSG and PF
compared with SHAM (overall interaction, P<0.001, Figure 2E), indicating increased fat utilization. Interestingly, during the dark phase, the RQ reduction was unique to PF rats (Figure 2E). Because both weight reduced groups exhibited similar reductions in RQ during the light phase, we attribute this increase to reduced body mass.

*The time course of altered hypothalamic neuropeptide expression is not consistent with a causal role in VSG-induced weight loss.* VSG induced a specific loss of fat mass which endured after the anorexia abated. Whereas weight loss due to caloric restriction is often followed by hyperphagia and weight regain, VSG rats were not hyperphagic and did not regain lost weight gain following the initial anorectic period. We asked whether improved central leptin sensitivity might prevent overeating in VSG rats. If central leptin sensitivity were improved, there should be changes in the expression of hypothalamic genes regulated by leptin (i.e. increased expression of the hypothalamic neuropeptide POMC and reduced expression of AgRP and NPY). However, there were no significant changes in the expression of NPY (Figure 3A, P = 0.7155), AgRP (Figure 3B, P = 0.8222), or POMC (Figure 3C, P = 0.5501) in MBH lysates among VSG, SHAM, and NAÏVE animals at 122 days post-surgery when body weights were relatively stable.

One shortcoming of this experiment is that we could not determine whether the gene expression pattern is comparable to that of an animal in a weight-reduced state produced by food restriction. To address this, we examined the expression of the same genes in a separate cohort of VSG rats and compared them to PF rats at earlier time post-surgical points. Both VSG and PF resulted in similar loss of fat mass by postsurgical Days 9 and 34 (Figure 4A, P < 0.001 vs. presurgical fat mass for each time point). Body weight and food intake data for this cohort are shown in Supplemental Figure 1. POMC (Figure 4C), NPY (Figure 4D), and AgRP (Figure 4E)
expression in MBH lysates were unchanged (P > 0.05 for all group comparisons at each time point except P<0.05 for sham vs PF on day 35) at 10 and 35 days post-surgery. Although we found increased AgRP and NPY expression in PF rats, this may have been secondary to the longer period of fasting in these animals as a result of the pair-feeding regimen. Taken together, these data do not support the hypothesis that these key targets of leptin action are crucial to the food intake reduction observed after VSG. Rather, these data point to the observed changes in hypothalamic expression as resulting from reduced intake, weight loss and lower leptin levels.

**VSG produces altered meal patterns.** Using continuous monitoring to record meal patterns, we found that VSG rats display a unique pattern of meal ingestion. At the time of the assessment, daily food intake was equivalent for SHAM and VSG animals. While rats typically eat the majority of their calories during the dark, VSG rats ate small meals of consistent size throughout the light-dark cycle (Figure 5A). These meals were smaller (Figure 5A) and more frequent (Figure 5B) compared to SHAM. Meal duration (Figure 5C) and ingestive speed (Figure 6D) were consistent among groups across the light-dark cycle. VSG rats spent more time eating than SHAM (Figure 5E). Total 24-h food intake for VSG rats was different than that of other groups (Figure 5F).

**VSG does not impair regulatory responses to food restriction.** Because VSG is considered a restrictive procedure, sustained weight loss has been hypothesized to be the result of an inability to consume sufficient calories to regain presurgical body mass. To test this hypothesis, we asked whether VSG rats are able to consume additional calories in response to a period of food restriction and additional weight loss. Twenty-two days of chronic food restriction produced
significant weight loss in all groups (P < 0.0001, Figure 6A). After 11 days, rats from all groups had regained the majority of the lost weight and did so by consuming more calories than the rats that were not restricted, and there were no differences among groups in this regard (Figure 6B). Thus, the VSG rats have the capacity to ingest increased numbers of calories when challenged to do so but choose not to do so to regain the weight lost after the VSG procedure.

*VSG does not improve leptin sensitivity beyond the expected consequence of weight loss.* As indicated above, although VSG rats are capable of overeating, they do not do so to return their reduced weight to normal following surgery. We hypothesized that improved leptin sensitivity might suppress hyperphagic behavior after surgery and limit weight regain at later time points. This was assessed directly by administering exogenous leptin. After 3 daily i.p. injections of leptin, VSG and PF rats exhibited significant and comparable reductions in food intake (P < 0.01 for VSG leptin vs. vehicle; p < 0.05 for PF leptin vs. vehicle, Figure 7A). SHAM rats, as expected, were leptin resistant (p > 0.05 leptin vs. vehicle, Figure 7A).

Plasma leptin levels on Day 50 were lower in VSG rats than in PF (p < 0.001) or CHOW (P <0.001) controls (Figure 7B). Similar trends persisted on day 125 (Supplemental Figure 2A). Plasma insulin (Supplemental Figure 2B) and glucose (Supplemental Figure 2C) were unaffected by VSG on day 125. When regression lines for fat mass vs. plasma leptin concentration on day 50 were compared, y-intercepts (P < 0.0001) but not slopes (P = 0.72) differed significantly between groups (Supplemental Figures 3A-E). We interpret this outcome to mean that basal leptin output by adipose tissue is lower for VSG and Sham-chow rats. Because PF and VSG rats had improved leptin sensitivity compared with both obese and chow-fed controls, we looked at hypothalamic leptin receptor (ObRb) and melanocortin 4 receptor (MC4R) expression.
Expression of MC4R (P = 0.28, Figure 7C) and ObRb (P = 0.64, Figure 7D) in MBH lysates obtained on Day 125 after the surgery was similar between groups.

**Discussion**

The current rat model of VSG produces significant and durable weight loss in obese male and female rats. Importantly, this rodent model of VSG leads the rat to defend a new, lower body weight. Unlike what is seen following weight loss by caloric restriction, rats following VSG do not overeat to compensate for this reduced fat mass. Additionally, we provide strong evidence that the weight loss cannot be attributed to an improvement in leptin sensitivity.

Weight loss secondary to VSG was due to the selective loss of fat tissue, with lean tissue expanding along the rat’s normal growth curve. The loss of weight was almost entirely due to food restriction since there was no effect of VSG on overall energy expenditure or caloric absorption from the GI tract. Interestingly, food intake was reduced for only 3 weeks immediately following VSG and then the animals went back to eating the comparable calories as SHAM-treated rats. The VSG rats did this by eating smaller but more frequent meals. These data suggest that the VSG rats adapt their meal patterns to the reduced stomach volume caused by the surgery. Nevertheless, VSG rats are clearly capable of consuming even more calories when necessary as indicated by the hyperphagic response to the chronic caloric restriction (Figure 7). The VSG rats are capable of defending their body weight after chronic food restriction and do so by increasing their food intake (Figure 7). In fact, the VSG rats had comparable efficiency to regain lost weight after chronic food restriction as controls. After chronic caloric restriction, VSG rats overate relative to their own pre-restriction baseline intake and, perhaps more strikingly, ate more relative to SHAM and CHOW baseline intake. This
experiment has two important implications. First, like the control groups, VSG rats appear to defend their body weight, albeit it at a lower level. Thus, instructing patients who have undergone this procedure to lose additional weight by dieting is likely to be no more successful than comparable advice to patients who have not had the procedure. Second, whatever the effect of the physical restriction in the VSG rats, they are willing to overcome such restriction to overeat when given the appropriate metabolic stimulus. Thus, it is unlikely that physical restriction per se is what prevents VSG rats from responding to the weight loss and anorexia that occur in the weeks following the procedure.

If restriction is not the primary reason why VSG rats consume less food, there remains a key question about the underlying mechanism. We hypothesized that the surgery may reverse the leptin resistance that is normally associated with exposure to HF diets. First, we determined that leptin levels are low in VSG rats. In fact, leptin levels at Day 50 after the surgery may be inappropriately low in VSG rats as compared to either SHAM or even PF rats (Supplemental Figure 3). This would be consistent with the hypothesis that it is not additional leptin but rather increased leptin sensitivity that drives the reduced body weight. However, if VSG rats are more sensitive to leptin, leptin-regulated genes should be expressed at a level consistent with those of an animal with greater leptin levels; i.e., hypothalamic POMC should be elevated and AgRP and NPY should be reduced. However, over the course of a number of experiments, the results indicated no change or increased AgRP and NPY gene expression while POMC was either not changed or decreased.

Such results are not consistent with increased leptin sensitivity. However there is also evidence that leptin actions are not mediated solely via MBH systems \textsuperscript{219-221}, so we performed a more direct assessment of leptin’s ability to reduce food intake in VSG, SHAM and PF rats. As
expected, leptin produced only a small, non-significant reduction of food intake over 3 days in SHAM rats. VSG rats significantly reduced their food intake in response to leptin, but PF rats had a similar reduction, implying that hypophagia as opposed to surgery per se is important. Collectively, the evidence does not support a conclusion of increased activity of the leptin-melanocortin axis following VSG. On the other hand, our findings are consistent with data from Lopez and colleagues\textsuperscript{53} demonstrating weight loss after VSG in obese, leptin-resistant Zucker rats. Thus, the ability of VSG surgery to result in weight loss is based on some other effect of the surgery, one that bypasses leptin resistance (and leptin deficiency) rather than reversing it. Nonetheless, it may be the case that increased leptin sensitivity in VSG rats contributes to the maintenance of the weight loss as they are losing weight.

VSG is often considered to be a restrictive procedure which produces weight loss by reducing caloric intake during meals\textsuperscript{52}. The current results support part of that contention in that a temporary reduction in food intake appears responsible for the profound initial body fat loss observed and VSG does result in smaller meal size. However, the results are not consistent with the possibility that this is driven primarily by the restriction caused by the surgery. VSG rats defend their new, albeit lower, body weight and can increase their food intake to do so. Further, smaller meal sizes are eventually compensated by increased numbers of meals. Thus, VSG does not impair an animal’s \textit{ability} to overeat, but suppresses the animal’s \textit{drive} to overeat. Further, this change in the rat’s motivation to eat is not the result of important alterations in leptin production or the actions of the leptin-melanocortin axis. These insights into one bariatric surgery highlight both the need and the difficulty of understanding how these procedures produce such powerful effects. Answers to these key questions will provide important insights into how future treatments, both surgical and non-surgical, can be designed.
Figure Legends

Figure 1. Changes in body weight, body composition, and food intake following VSG in male and female rats. VSG resulted in loss of body weight in males (A, $P < 0.01$ vs. SHAM and NAÏVE) and females (B, $P < 0.05$ vs. SHAM and NAÏVE) at all time points measured. Body weight change was equivalent for SHAM and NAÏVE rats at all time points ($P > 0.05$). (Overall interaction of time vs. surgical treatment: $P = 0.0042$ for males, $P < 0.0001$ for females.) Weight loss after VSG was due to loss of fat tissue (C, $P < 0.001$ vs. SHAM or NAÏVE for both male and female rats; overall effect of surgical treatment, $P < 0.0001$). Lean tissue was not affected by surgical treatment (D, $P > 0.05$ for overall effect of treatment and for all male-male or female-female comparisons). Daily food intake was suppressed immediately following VSG in both males (E, $P < 0.001$ vs. SHAM and NAÏVE until Day 16) and females (F, $P < 0.001$ vs. SHAM and NAÏVE until Day 16, $P < 0.05$ vs. SHAM on Day 24). After Day 16 for males and Day 24 for females, daily caloric intake did not differ between groups ($P > 0.05$ for all group comparisons).

Figure 2. Intestinal absorption and energy expenditure. VSG is not a malabsorptive procedure as indicated by fecal lipid analysis (A, $P = 0.0193$). Energy expenditure was measured when VSG rats weighed significantly less than SHAM (B, $P < 0.01$). Energy expenditure was unchanged after VSG (C, interaction, $P = 0.9699$). Locomotor activity was increased during the dark phase in VSG vs. SHAM (D, $P < 0.05$) but did not differ for VSG vs. PF ($P > 0.05$). Light-phase RQ was depressed for VSG rats compared to SHAM (E, $P < 0.01$), but was unchanged relative to PF ($P > 0.05$). During the dark phase, RQ resembled SHAM ($P > 0.05$ for VSG vs. SHAM or PF, $P < 0.01$ for SHAM vs. PF).
Figure 3. Hypothalamic melanocortin axis following VSG. Expression of melanocortin system neuropeptides in MBH lysates was unaffected by VSG. Transcripts examined include NPY (A, P = 0.7155), AgRP (B, P = 0.8222), and POMC (C, P = 0.5501).

Figure 4. Effects of food intake after VSG on body composition and the hypothalamic melanocortin axis. VSG-induced fat mass loss is due primarily to caloric restriction, a phenomenon not mediated by hypothalamic melanocortin signaling. Fat mass was significantly reduced in all groups at 9 days (A, P < 0.01 for PF and P < 0.001 for VSG vs. presurgical fat mass) and at 34 days (A, P < 0.01 for PF and P < 0.001 for VSG vs. presurgical fat mass). At 34 days, fat mass was reduced compared to SHAM for PF (P < 0.001), CHOW (P < 0.001), and VSG (P < 0.001) animals (one-way ANOVA for fat mass at 34 days, P < 0.0001). A small reduction in fat mass was observed at 9 days in SHAM rats, but this fat mass was regained by 34 days (A, P < 0.05, 9 day vs. 34 day fat mass). Lean mass is unchanged by 34 days for all animals (B, P > 0.05 presurgical vs. 34 day fat mass for all groups). Small reductions in lean mass were observed at 9 days for VSG (P < 0.01) and PF (P > 0.05) animals, but lean mass recovery was evident by Day 34 (VSG, P > 0.05 for all comparisons at each time point). Lean mass was not different (P > 0.05) among groups at each of the three measured time points. Expression of POMC did not differ among groups (C, P > 0.05 for all comparisons at each time point) and did not change differentially among the groups over time (C, interaction P = 0.5934, effect of treatment P = 0.0340, effect of time P = 0.5809). The same is true for NPY (D, P > 0.05 for all comparisons at each time point) and AgRP (E, P > 0.05 for all comparisons at each time point except P < 0.05 for SHAM vs. PF on Day 35).
Figure 5. Meal patterns after VSG. VSG alters meal patterns. After VSG, rats consume smaller meals than SHAM (A, effect of treatment $P = 0.0004$). Dark phase meal number was increased following VSG (B, effect of treatment $P < 0.0001$, interaction between treatment and time, $P < 0.0001$). These meals were shorter in duration than those consumed by SHAM rats (C, effect of treatment, $P = 0.0003$). Ingestive speed for meals in VSG rats was unchanged over time (D, interaction, $P=0.7297$), but overall time spent eating was greater for VSG rats (E, effect of treatment, $P < 0.0001$, interaction between treatment and time, $P = 0.0064$). Total food intake did not differ for VSG and SHAM rats (F, $P = 0.2758$).

Figure 6. Regulatory responses to food restriction after VSG. VSG does not impair the ability to overeat or to regain body weight after caloric restriction. After 22 days of caloric restriction, all rats regained body weight to exceed pre-restriction weight and did so along a similar trajectory (A, interaction $P = 0.9937$). During the first 24 h of ad libitum feeding after the period of caloric restriction, all rats consumed significantly more calories than pre-restriction baseline (B, $P < 0.001$ for SHAM, PF, CHOW, $P < 0.01$ for VSG).

Figure 7. Leptin sensitivity after VSG. VSG improves leptin sensitivity secondary to weight loss. After VSG, rats exhibited an enhanced anorectic response to exogenous leptin as compared to SHAM (A, $P < 0.01$ for VSG, $P > 0.05$ for SHAM or CHOW). However, a similar response was observed after leptin injection in PF rats (A, $P < 0.05$ for PF). Plasma leptin levels (B) were reduced after VSG as compared to PF ($P < 0.0001$), SHAM ($P<0.001$), or CHOW ($P < 0.001$).
Effect of treatment, $P = 0.0001$. VSG affected the expression of neither MC4R (C, $P = 0.28$) nor ObRb (D, $P = 0.64$).

**Supplemental Table 1. Overview of experimental groups.** Experimental groups used for each of the reported experiments.

**Supplemental Table 2. Primer sequences.**

**Supplemental Figure 1. Body weight and food intake.** VSG resulted in body weight loss beginning on the second postsurgical day (A, $P < 0.05$ on Days 2-3 vs SHAM, $P < 0.01$ on Day 4, $P < 0.001$ on Days 5 and after). Body weight change was equivalent for VSG and PF rats at all time points. (Overall interaction of time vs. surgical treatment: $P < 0.0001$). Daily food intake was suppressed immediately following VSG (B, $P < 0.05$ vs. SHAM on Days 3-12 and on Day 14).

**Supplemental Figure 2. Plasma leptin, insulin, and glucose after VSG.** Plasma leptin levels on postsurgical day 125 (A, overall effect of treatment $P<0.0001$; SHAM vs. VSG, $P<0.01$; SHAM vs. CHOW, $P<0.001$). Plasma insulin levels were decreased in CHOW animals as compared to both SHAM (B, $P<0.01$) and VSG ($P<0.01$) animals, but were not decreased in VSG animals as compared to SHAM ($P>0.05$). Overall effect of treatment on plasma insulin levels, $P=0.0011$. Plasma glucose levels were unaffected by diet or surgery (C, overall effect of treatment, $P=0.64$).
Supplemental Figure 3. Relationship between body fat mass and plasma leptin concentration. Plasma leptin levels generally increased as a function of expanded fat tissue mass (A, $R^2 = 0.3380$, $P = 0.0292$; B, $R^2 = 0.5510$, $P = 0.004$; C, $R^2 = 0.3773$, $P = 0.0148$; D, $R^2 = 0.7074$, $P < 0.0001$). Slopes of body weight vs. plasma leptin did not differ significantly among the four groups studied (E, $P = 0.7223$). However, the elevations (y-intercepts) of the four regression lines differed ($P < 0.0001$).
Figures

Figure 1. Changes in body weight, body composition, and food intake following VSG in male and female rats.
Figure 2. Intestinal absorption and energy expenditure.
Figure 3. Hypothalamic melanocortin axis following VSG.
Figure 4. Effects of food intake after VSG on body composition and the hypothalamic melanocortin axis.
Figure 5. Meal patterns after VSG.
Figure 6. Regulatory responses to food restriction after VSG.

Figure 7. Leptin sensitivity after VSG.
<table>
<thead>
<tr>
<th>Cohort</th>
<th>Total N</th>
<th>Treatment groups</th>
<th>Experiments</th>
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| A      | 54 (male and female) | • NAIVE (N=8 male, 8 female)  
    • SHAM (N=1 male, 10 female)  
    • VSG (N=9 male, 9 female) | • Food intake, body weight  
    • MBH POMC, NPY, AgRP expression |
| B      | 67 (male only) | • CHOW (N=20)  
    • SHAM (N=14)  
    • PF (N=13-15)  
    • VSG (N=18) | • Food intake, body weight  
    • Energy expenditure  
    • Meal patterns  
    • Refeeding response to restriction  
    • Plasma leptin, insulin, glucose levels (no PF for measurements on day 125)  
    • MBH ObRb and MC4R expression (no PF group)  
    • Leptin sensitivity |
| C      | 24 (male only) | • SHAM (n=8)  
    • PF (n=7)  
    • VSG (N=9) | • MBH POMC, NPY, AgRP expression (10 days post-surgery) |
| D      | 33 (male only) | • CHOW (N=10)  
    • SHAM (N=10)  
    • PF (N=8)  
    • VSG (N=7) | • MBH POMC, NPY, AgRP expression (35 days post-surgery)  
    • Fecal lipid content |

Supplemental Table 1. Overview of experimental groups.
Supplemental Table 2. Primer sequences.

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<th>Primer</th>
<th>Sequence</th>
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<td>Rat POMC- Reverse</td>
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<td>5’-CTCTGCGACACTACATCAA-3’</td>
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Supplemental Figure 1. Body weight and food intake.
Supplemental Figure 2. Plasma leptin, insulin, and glucose after VSG.
Supplemental Figure 3. Relationship between body fat mass and plasma leptin concentration.
CHAPTER 3

Sleeve Gastrectomy Improves Postprandial Lipid Clearance by Reducing Intestinal Triglyceride Secretion

The following authors contributed to this chapter by contributing either to data collection or to study design:

Abstract

Postprandial hyperlipidemia is a potent risk factor for atherosclerotic heart disease and is associated with the consumption of high-fat diets and with obesity. Bariatric surgeries result in more robust, durable weight loss than dieting. These surgeries are also associated with multiple metabolic improvements, including reduced plasma lipid levels. We sought to determine whether the beneficial effects of vertical sleeve gastrectomy (VSG) on plasma lipid levels are weight-independent. Compared with either obese or lean (pair-fed) sham-operated controls fed a high-fat diet (HFD), HFD-fed VSG animals had much lower levels of plasma triglycerides, cholesterol, and phospholipids after a 4-hour fast. Plasma triglyceride, cholesterol, phospholipid, and non-esterified fatty acid levels were comparable for VSG and chow-fed animals. These differences were less dramatic after long periods of fasting, leading us to the hypothesis that VSG’s main effect on lipid homeostasis is postprandial. We used injections of the lipase inhibitor poloxamer 407 alone or prior to an oral lipid gavage to approximate hepatic and intestinal triglyceride secretion, respectively. We report that while hepatic triglyceride secretion in fasted animals is unaffected by VSG, animals who have had this surgery display a marked, weight-independent reduction in intestinal triglyceride secretion. VSG also did not affect hepatic lipid storage, weight, or triglyceride secretion. Hepatic gene expression studies revealed no significant changes. Use of a $^{13}$C-riolein-enriched olive oil gavage in the presence of poloxamer revealed no significant changes to postprandial intestinal lipid uptake. We did not observe any changes to total intestinal lipid storage or to the expression of intestinal target genes known to control triglyceride metabolism and synthesis. These data reveal an important effect of VSG to produce strong, weight-independent reductions to postprandial plasma lipids. Here, we show
that this effect is due to reduced intestinal triglyceride secretion following ingestion of a lipid meal. We speculate that this effect may be due to smaller meal size in VSG-operated animals.

Keywords: Hyperlipidemia, triglyceride, chylomicron, VLDL, bile acid, meal pattern, vertical sleeve gastrectomy, bariatric surgery, obesity.
Background

Cardiovascular disease in obese patients is a leading cause of mortality, which can arise from hyperlipidemia associated with consumption of high-fat diets. In fact, even mild obesity has been reported to increase risk of coronary heart disease by 50%, with more severe obesity increasing risk as much as 3-fold\textsuperscript{222}. Cardiovascular disease is currently the leading cause of death in the United States\textsuperscript{223}, not surprising for a nation with an increasingly obese population. Obesity-related dyslipidemia commonly includes elevated total cholesterol, elevated LDL, elevated triglycerides and low HDL\textsuperscript{224}. Hyperlipidemia in addition to excess fat is therefore a target of any ideal weight loss regimen.

Bariatric surgery produces outcomes associated with improved cardiovascular health, including both symptoms of and risk factors for cardiovascular disease\textsuperscript{4}. Hyperlipidemia is a potent cardiovascular risk factor shown to be reduced in at least 70% of patients after bariatric surgery\textsuperscript{5}. Improvements have been documented following RYGB\textsuperscript{6-8}, gastric banding\textsuperscript{9,10}, biliary-intestinal bypass\textsuperscript{10}, duodenal-jejunal bypass\textsuperscript{11}, biliopancreatic diversion\textsuperscript{12}, ileal interposition\textsuperscript{13}, and vertical sleeve gastrectomy (VSG)\textsuperscript{14-16}. The degree to which lipids are improved appears to depend on the procedure performed, but it is yet unknown which gastrointestinal manipulations are most effective at reversing hyperlipidemia. Importantly, for each of these procedures, it is also unclear whether improvements are due to weight loss or to an independent effect of the surgery. In this study we focused on the VSG, where a reduction in gastric volume is created by the removal of gastric mucosa along the greater curvature and including the fundus. We have previously shown that this surgical model produces profound and persistent weight loss in rats\textsuperscript{181}. We hypothesized that this surgical model is also associated with improved hyperlipidemia and
that this improvement is due to altered intestinal biology after surgery. In order to determine if changes in plasma lipids were a direct result of the surgery, a critical comparison for this study was made between the VSG-operated rats and a group of sham-operated animals that were pair-fed (PF) to the VSG rats and who lost an equivalent amount of body fat during the study. Here, we demonstrate that VSG improves plasma lipids in a weight-dependent manner.

Methods

Animals. Male 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 and were maintained on their respective diet post surgery. Rats were housed at the University of Cincinnati at the Metabolic Diseases Institute under controlled conditions (12:12-h light-dark cycle, 50-60% humidity, 25 °C with free access to water and food except where noted). Where indicated, a subgroup of sham-operated rats was pair-fed to match the intake of the VSG group. To do this, the amount of food eaten by the VSG rats during the previous 24 h was divided and then provided at random times during the light/dark cycle to the PF rats. Fat and lean tissue mass were measured using nuclear magnetic resonance (NMR, Echo MRI: Echo Medical Systems, Houston, TX). At the end of each study, animals were placed briefly in a CO2 chamber and then sacrificed by decapitation during the light phase. All procedures for animal use were approved by the University of Cincinnati Institutional Animal Care and Use Committee. A summary of animals and of group sizes used for these studies is provided in Supplemental Table 1.
Surgical Procedures. For VSG, 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, 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 (Ethicon Endo-Surgery, Cincinnati, Ohio), 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 post-operative care for 3 days, consisting of twice-daily subcutaneous injections of 10 mL warm saline and 0.3 mL Buprenex. Once-daily, subcutaneous injections of the nonsteroidal anti-inflammatory drug meloxicam were also administered during the first 2 postoperative days. Solid food was withdrawn 24 hours prior to surgery and rats were given access only to Osmolite OneCal liquid diet until solid food was returned 3 days after surgery.

Measurement of plasma lipids. All blood was sampled from the tip of the tail except when taken as trunk blood during sacrifice. Plasma cholesterol, NEFA, phospholipid, and triglycerides were measured in 4-hour-fasted blood via colorimetric assay using Infinity Reagents for each analyte (Thermo Fisher Scientific, Inc., Waltham, MA). For measurement of plasma lipids in fractions also used for FPLC analysis, blood was collected at sacrifice after 24h of fasting. For
the fasting timecourse study, blood was sampled after 0, 4, 8, and 24 h of fasting. For the study of triglyceride production rate, rats were fasted for 24 h and baseline blood samples were taken prior to i.p. injection of 1 g/kg poloxamer 407 (P-407; Sigma-Aldrich, St. Louis, MO). Blood was taken at 0, 2, 4, 6, and 24 hours either following P-407 injection only for the fasted study and at 0, 1, 2, 4, and 6 hours following an intragastric gavage of 0.5 g/kg olive oil administered 1 hr after P-407. For the GLP-1 antagonist study, animals were fasted for 24 hours prior to baseline blood sampling and i.p. delivery of 50 µg/kg Exendin (9-39) (EX-9) or saline vehicle. An intragastric gavage containing 0.5 g lipid per kg body weight was administered 30 minutes following EX-9 and blood was sampled 1, 2, 3, and 6 hours after gavage. Plasma triglycerides were measured via colorimetric assay using Infinity Triglyceride Reagent and Standard (Thermo Fisher Scientific, Inc., Waltham, MA). Plasma cholesterol was measured, via a colorimetric assay (Infinity Cholesterol Reagent and Standard, Thermo Fisher Scientific, Inc., Waltham, MA). For determination of plasma lipoprotein composition, trunk blood was collected into EDTA after 24 h of fasting and plasma was stored at 4°C for fractionation via fast protein liquid chromatography (FPLC) within 7 days. Cholesterol in FPLC fractions was determined as described above. At the initiation of fasting in all studies, cages were changed to minimize coprophagia.

**Lipid absorption.** Dietary lipid absorption of a 5 mL/kg intragastric load of 20% soybean oil emulsification (20% soybean oil, 1.2% egg phospholipid, 2.5% glycerin, 2.5% sucrose polybehenate) was measured as previously described\(^2\). Briefly, 24 h-fasted rats received an intragastric gavage of the emulsion. Cages were changed at the initiation of fasting and at the time of gavage. Fecal samples of about 10 mg were collected 24 h after gavage and fecal lipid
content was assayed by gas chromatography of fatty acid methyl esters. The ratio of total fatty acids to sucrose polybehenate in stool samples was used to estimate dietary lipid absorption.

*Stable isotope study.* On the day prior to the study, all rats were placed in clean cages and given 10 g HFD at lights out and 5 g HFD 6 hours into the dark phase of the light-dark cycle. On the day of the study, 1 g/kg P-407 was administered followed 1 hour later by a 0.28 g intragastric lipid gavage containing 0.18 g olive oil and 0.10 g \(\text{1,1,1-}^{13}\text{C3-Triolein}\). All rats were sacrificed 4 hours after the gavage. Intestines were removed immediately after sacrifice and dissected into 4 sections of equal length (M1, M2, M3, and M4 from proximal to distal, respectively). After removal of 1.5 cm from each segment for PCR and histology, each gut segment was cleaned thoroughly by removing outer mesenteric fat and rinsing inner surface in 1% SDS followed by PBS. Each segment was placed immediately in 10 mL chilled PBS and homogenized immediately on ice. Lipids were extracted from aliquots of homogenate using the method described by Folch et. al.\textsuperscript{232} Ratio of \(^{13}\text{C}\) to \(^{12}\text{C}\) in the lipid fraction was measured using gas-chromatography combustion isotope ratio mass spectroscopy (GCC-IRMS) and reported as excess enrichment above baseline, as measured using samples from pluronic-treated, ungavaged animals.

*Intestinal and liver gene expression.* Tissue was homogenized in RLT buffer using a tissue lyser (QIAGEN, Inc., Valencia, CA). RNA was extracted using a QIAGEN miniprep RNA extraction kit and cDNA was made using an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). Quantitative PCR was performed using TaqMan gene expression assays (Supplemental
Table 2) or, for intestinal melanocortin axis genes, SYBR green detection (Supplemental Table 3).

**Histology.** To visualize fat deposition in the intestine, tissue samples were collected into 4% paraformaldehyde and, following fixation, stored in 30% sucrose. 7 μm cryosections were stained with Oil Red O and Cresyl Violet and digital images of sections were acquired using a digital camera attached to a Zeiss microscope (Zeiss, Thornwood, NY).

**Biochemical Assays.** Western blots were used to measure plasma ApoB48 and ApoB100 content after a lipid gavage in the presence of i.p. P-407. Briefly, plasma samples were denatured in one volume of RIPA buffer (Santa Cruz Biotechnologies, Santa Cruz, CA) plus three volumes Laemmli buffer (Bio-Rad Laboratories, Hercules, CA) and diluted with distilled water. Following 10 minutes at 95°C, samples were applied to a 4-15% Tris-HCl polyacrylamide gel (Bio-Rad Laboratories, Hercules, CA) and run at 50 V for 4.5 hours. Proteins were transferred to nitrocellulose overnight at 30 V. Blots were blocked at room temperature in 5% BSA. Goat anti-human apolipoprotein B antibody (Millipore, Temecula, CA) was diluted 1:5000 in 5% BSA and incubated overnight at 4°C. Blots were incubated in secondary antibody, 1:5000 HRP-conjugated rabbit anti-goat IgG (Millipore, Temecula, CA) in 5% milk for 1 h at room temperature. Immunoreactivity was quantified using Alpha Ease software (Alpha Innotech Corporation, San Leandro, CA).

For measurement of triglycerides and cholesterol in liver and intestine, tissues from rats fasted for 24 h were flash-frozen in isopentane and the lipid from 50 mg of tissue was extracted in 2:1 chloroform:methanol. Triglyceride and cholesterol content were measured using
colorimetric assays (Infinity Triglyceride Reagent and Standard and Infinity Cholesterol Reagent and Standard, Thermo Fisher Scientific, Inc., Waltham, MA). Hepatic cholesterol esters were measured using a Cholesterol/Cholesteryl Ester Quantitation Kit (EMD Chemicals, Gibbstown, NJ) from homogenates containing 10 mg of tissue.

Plasma sample bile acid concentrations were determined using the total bile acids assay kit from BioQuant (San Diego, CA).

Statistical Analysis. All data are expressed as mean ± SEM. Body weight, food intake, NMR, and changes in plasma triglyceride or cholesterol levels over time were analyzed via 2-way ANOVA (Variables: treatment & time) with a Bonferroni post hoc test where appropriate. Area-under-the-curve comparisons, triglyceride production rates, plasma lipid analytes at single time points, plasma bilirubin and bile acid measurements, single-gene expression comparisons, comparison of gavage sizes, single-day body weight and body composition measurements, stable isotope enrichment in intestinal lipid samples, and total tissue cholesterol and triglyceride content were analyzed using 1-way ANOVAs followed by Tukey’s post-hoc test where appropriate.

Results

VSG reduces plasma lipids. Plasma lipids were measured after 4 h of fasting 50 d post surgery, when rats were weight-stable. At this time, obese, sham-operated (SHAM) animals were heavier than VSG, sham-operated, chow-fed (CHOW), and sham-operated, pair-fed (PF) groups. Weight loss after VSG is a selective loss of fat mass\textsuperscript{181}. All animals in PF, SHAM, and VSG groups were maintained on HFD for the duration of the study. Plasma cholesterol (Figure 1A,
P<0.001), triglycerides (Figure 1B, P<0.01), and phospholipids (Figure 1C, P<0.001), but not non-esterified fatty acids (NEFA) (Figure 1D) were decreased after VSG as compared to SHAM controls. Importantly, this observed reduction after VSG is weight-independent, as cholesterol (P<0.05), triglycerides (P<0.01), and phospholipids (P<0.001) were also reduced after VSG as compared to the PF animals. Further, levels of all four lipids measured (cholesterol, triglycerides, phospholipids and NEFA) were similar in chow and VSG groups despite lower body weight and fat mass in the CHOW group (Supplemental Figure 1), again supporting a weight-independent effect of VSG on lipid regulation.

Plasma triglycerides increase dramatically with feeding and rate of triglyceride clearance during fasting can be affected by several factors. In order to explore whether reductions in plasma triglycerides were dependent on the duration of fasting, we measured triglycerides in plasma sampled after 0, 4, 8, and 24 hours of fasting. All animals consumed 15 g of HFD on the day prior to the study. Consistent with the data from Figure 1C, triglycerides were reduced in unfasted plasma from VSG animals as compared with SHAM (P<0.001) and PF (P<0.01). This difference was gradually diminished along the course of the fast (Figure 2A). At 4 and 8 hours of fasting, plasma triglycerides were lower in VSG animals than SHAM (P<0.001 at both time points) but no longer significantly different from PF. By 24 hours of fasting, no significant differences existed between any of these groups, but the area under the curve for triglycerides during the 24-hour experiment was lower for VSG than for PF (P<0.05) or SHAM (P<0.001; Figure 2B). Plasma cholesterol was reduced at a similar rate among the three groups (Figure 2C) and the area under the curve for 24-hour cholesterol was unaffected by treatment (Figure 2D). Body weight was significantly reduced in VSG and PF animals on the day of the study. Body weight, food intake, and body composition are presented in Supplemental Figure 2.
**Lipoprotein content of plasma is unaltered after VSG.** Selective reductions in VLDL or HDL, the two major lipoproteins in rats, might suggest a mechanism for the observed reductions in plasma lipid levels after VSG. For this reason, we used fast protein liquid chromatography (FPLC) to characterize cholesterol content in these lipoprotein fractions from 24-hour-fasted plasma. This experiment was performed 35 days after surgery, when body weight was stably reduced by either VSG or PF. No shifts in peak cholesterol content were observed among these fractions, indicating no change in particle size, but VSG animals exhibited reduced cholesterol content in both HDL and VLDL peaks (Figure 3A). The pattern of cholesterol content in both peaks appears to correlate with total plasma cholesterol content for these samples. Although not significant, VSG appeared to elicit some reduction in total plasma cholesterol content as compared with SHAM and PF controls (Figure 3B). Perhaps indicating reversal of HFD-induced change to plasma cholesterol, VSG displayed decreased plasma cholesterol as compared to CHOW rats (P<0.05). Total plasma triglycerides were reduced after VSG as compared with SHAM rats (P<0.05, Figure 2C), an effect consistent with reduced VLDLs in VSG animals. Trends for decreased triglycerides in PF and CHOW vs. SHAM did not reach statistical significance.

**Triglyceride production rate and postprandial lipid clearance.** To determine whether reduced plasma triglyceride levels after VSG are due to attenuated hepatic VLDL production and/or secretion, we administered an intraperitoneal injection of the lipase inhibitor poloxamer 407 (P-407) (1 g/kg) to 24 h-fasted animals. P-407 blocks triglyceride breakdown, so the appearance of triglycerides in the plasma after P-407 injection is due only to secretion by the liver and intestine.
We chose to use P-407 for this study because, unlike Triton-WR1339, it does not affect hepatic VLDL secretion rate\(^{234}\). In the fasting state, plasma triglyceride appearance rate after P-407 should approximate hepatic triglyceride secretion rate. We found no changes in rate of triglyceride appearance in the plasma across groups following administration of P-407 to 24 h-fasted (Figures 4A and 4B). This result suggests that neither accelerated endothelial and/or hepatic lipid metabolism nor impaired VLDL secretion can explain the reduced plasma triglyceride levels observed after VSG.

Based on the observation that plasma triglycerides were reduced most dramatically in VSG animals after short periods of fasting, we hypothesized that VSG might enhance postprandial lipid clearance. We next investigated whether intestinal chylomicron assembly and secretion are affected by VSG. In order to answer this question, we administered P-407 to 24-hour fasted rats 1 hour prior to a 0.5 g/kg olive oil gavage and measured plasma triglycerides for 6 hours. This dose was chosen based on evidence that larger doses of lipids are malabsorbed when administered via gavage (Supplemental Figure 3) and based on the fact that blood volume, which will affect plasma lipid concentrations, should correlate with body weight. Plasma triglyceride excursions following this gavaged dose are shown in Supplemental Figure 4. We found a reduced rate of triglyceride appearance in the blood for VSG animals which was significant by 4 hours after the gavage and which remained low throughout the 6-hour duration of the study (treatment X time interaction, P<0.0001; Figure 4C). Plasma triglycerides were reduced in VSG rats at the 6-hour time point as compared with SHAM (P<0.01 and P<0.001 at 4- and 6-hour time points, respectively) and PF animals (P<0.001 at 4- and 6-hour time points). Rate of triglyceride appearance during the 6-hour experiment was lower in VSG animals than in either SHAM (P<0.01) or PF (P<0.001) animals (Figure 4D). No significant changes to ApoB48
or to ApoB100 content in the plasma were detected in samples collected at the 6-hour time point (Figure 4E).

*GLP-1 does not mediate impaired intestinal triglyceride synthesis in VSG rats after a lipid meal.* GLP-1 release has been shown to regulate postprandial lipid metabolism by reducing intestinal chylomicron secretion\(^2^{35}\). To address the hypothesis that VSG attenuates chylomicron synthesis through enhanced meal-stimulated GLP-1 release, we delivered a lipid gavage at a dose previously shown to stimulate GLP-1 release (0.5 g/kg)\(^2^{36}\) 30 minutes after i.p. administration of the selective GLP-1 receptor antagonist, Exendin (9-39) (EX-9; 50 µg/kg), or vehicle (VEH). This dose of EX-9 has been reported to impair glucose tolerance in VSG animals (Adam Chambers, unpublished data). In VSG (Figure 5A), SHAM (Figure 5B), and PF (Figure 5C) rats, EX-9 did not alter triglyceride excursion after a lipid gavage. Area under the curve during the 6-hour experiment was also unaffected by EX-9 in all groups (Figure 5D).

*Hepatic cholesterol content, but not triglyceride content, is enhanced by VSG.* Although hepatic triglyceride secretion appears to be unchanged after VSG, other metabolic changes might occur in the liver to affect lipid homeostasis. For example bile acid metabolism has been found to be related to lipid metabolism, glucose homeostasis and even gut peptide secretion\(^2^{37}\). Serum bile acids have been reported to be increased following bariatric surgery\(^2^{38},^{239}\) but have not previously been measured following VSG. We found that plasma bile acids were decreased in SHAM animals compared to CHOW, VSG, and PF animals (Figure 6A). Therefore, VSG seems to have no weight-independent effects on plasma bile acid levels but obesity in this experiment was associated with reduced plasma bile acid concentration. Plasma bilirubin levels were
unaffected by weight or diet (Figure 6B). We did not detect any significant differences in expression of hepatic CYP7A1, CYP27A1, HMG-CoA reductase (HMGCR), or scavenger receptor class B, member 1 (SCARB1; Figure 6C). A trend toward reduced CYP7A1 expression in VSG-operated rats is perhaps related to increased plasma bile acid levels. Neither hepatic triglyceride (Figure 6D) nor cholesterol (Figure 6E) content was affected by treatment in this study. Ratio of cholesterol ester content to total cholesterol content did not differ among groups (Figure 6F), indicating that long-term hepatic cholesterol storage was also unaffected by either VSG or pair-feeding. Consistent with this result, we did not detect any differences in whole liver weight among groups (Figure 6G). We next assayed the expression of several genes related to hepatic lipid uptake (CD36, FATP4, L-FABP, ApoB48R; Figure 6H) and esterification (ACAT2; Figure 6H), intracellular triglyceride synthesis and packaging (MGAT2, DGAT1, MTP; Figure 6I), lipoprotein composition (ApoA2 and ApoB; Figure 6J), and lipoprotein lipase (LPL) activity (ApoC2 and ApoC3; Figure 6K). Few changes were detected, with the exception of ApoB (ANOVA, P=0.0181; PF vs. SHAM, P<0.05) and ApoB48R (ANOVA, P=0.0104; PF vs. VSG, P<0.01; Figure 6G).

**VSG does not affect the size of intestinal lipid storage pools.** Enterocytes of the upper small intestine are known to contain lipid storage pools\(^{240}\). We hypothesized that reduced postprandial intestinal triglyceride secretion after VSG might be due to enhanced intestinal lipid storage postprandially. Following P-407 administration and a 1,1,1,\(^{13}\)C3-Triolein-enriched olive oil gavage containing a total of 0.28 g of lipid, uptake of lipids into the proximal intestine was comparable among groups (Figure 7A). Animals were fed on the day prior to the study; this was done in a manner aimed to minimize differences in meal patterns among groups. We did not find
any differences in intestinal weight (Figure 7B) or in triglyceride (Figure 7C) or cholesterol content (Figure 7D) from whole intestinal segment homogenates. Oil Red O-stained sections from M2 were examined, revealing no obvious differences in lipid content between groups (Figure 7E). However, we detected very little lipid in these sections, despite the fact that lipid content was highest in M2 among the 4 gut regions. We did not observe any apparent changes to villus length or morphology, which could affect lipid absorption. Triglyceride and cholesterol content in proximal duodenal or terminal ileal samples from 24 h-fasted fasted animals were also consistent across groups (Supplemental Figure 5). No differences in plasma triglycerides or cholesterol were detected at this time point (Supplemental Figure 6). Analysis of the expression of genes known to control intestinal triglyceride metabolism and chylomicron synthesis did not reveal any significant changes in VSG-operated animals. Samples from M1 and M2 represent duodenum and jejunum, respectively, and so any changes to chylomicron formation or secretion are expected to be affected in these regions. Gene expression studies within these regions did not reveal any significant changes to transcripts known to affect intestinal lipid uptake (CD36, FATP4, L-FABP, I-FABP, and ACAT2; Figure 8A), triglyceride synthesis and packaging (DGAT1, MGAT2, and MTP; Figure 8B), chylomicron composition and size (ApoAIV and ApoB; Figure 8C), and LPL activity (ApoC2 and ApoC3; Figure 8D). Because ileal DGAT1 expression has recently been linked to local GLP-1 release\(^{241}\), we also assayed M4 DGAT1 expression, finding no significant alteration (Supplemental Figure 8).

Recently, local intestinal leptin sensitivity has been proposed to regulate intestinal lipid metabolism\(^{242}\). In order to test the hypothesis that VSG-induced reduction intestinal triglyceride synthesis is secondary to improved intestinal leptin sensitivity, we assayed the expression of a few key melanocortin axis components in M2 (jejunum). In SHAM animals, we detected
expression of leptin receptors (ObRb), melanocortin receptor type 4 (MC4R), Agouti-related peptide (AgRP), and Neuropeptide Y (NPY) in all 4 sampled regions of intestine (M1, M2, M3, M4; Figure 8E). We did not, however, detect the expression of pro-opiomelanocortin (POMC) in any region of the intestine. Expression of neither AgRP nor ObRb was altered by VSG in M2 (Figure 8F).

**Discussion**

It has been speculated that plasma lipids are reduced in humans after bariatric surgery in part due to conscious dietary changes. Here, we show that the improvement to lipid homeostasis after VSG is instead a physiological consequence of the surgery. We report lower plasma lipid levels in VSG-operated rats relative to weight-matched (PF) rats despite the fact that both groups were maintained on the same amount of HFD. In fact, after VSG and after sham surgery, rats eat an equivalent number of daily calories, arguing that lipid processing and not lipid ingestion is the primary mechanism for reduced plasma triglycerides and cholesterol.

These data show that plasma triglycerides are markedly reduced in VSG-operated rats, especially during short periods of fasting. This observation led us to our initial hypothesis that VSG’s primary effect on lipid homeostasis is to improve postprandial lipid levels. The data presented here argue for an intestinal mechanism for reduced postprandial lipid excursions in VSG-operated rats. Figure 4 demonstrates that the secretion of intestinally-derived triglyceride-rich particles is reduced in VSG animals as compared either with SHAM or PF controls. We did not find any changes to plasma ApoB48 content in plasma at the 6-hour time point, suggesting that reduced intestinal triglyceride output might reflect the secretion of smaller, rather than
fewer, chylomicrons. Lymphatic sampling of postprandial lipid is necessary in order to conclusively determine whether VSG alters chylomicron size and/or composition.

Intestinal enterocytes contain lipid storage pools which may be acutely mobilized by the consumption of a meal. Thus, triglycerides in the plasma following a meal might actually have been consumed at an earlier meal. Until recently, it was thought that 95%-98% of the lipid contained in a meal is absorbed during the immediate postprandial period, but it is now thought that enterocytes abide by a “last in- last out” phenomenon whereby triacylglycerols consumed at breakfast may not appear in the plasma until after lunch. Although the lipids are not derived from the meal itself, the degree of lipemia observed postprandially is determined by the fat content of the meal, a phenomenon possibly mediated cephalically by oral fat taste receptors. Although for our experiments we delivered, via intragastric gavage, meals that were comparable in fat content for all groups, this mode of delivery is not physiological, as it bypasses oral taste receptors and lingual lipase in the rat and it does not elicit a cephalic phase of eating, two elements of a meal thought to be critical to the regulation of postprandial lipid homeostasis. Additionally, studies linking postprandial plasma lipid levels to meal size have used fasted subjects and have not considered the effects of a standardized meal on plasma lipids in subjects with differing meal patterns.

In humans, it has been speculated that small, frequent meal intake may have benefits for metabolic health. Our data raise the possibility that smaller meals consumed by VSG animals may reduce plasma lipid excursions by reducing the size of the lipid pool which is released from the enterocyte postprandially. Thus, we hypothesize that eating smaller, more frequent meals may alter the rate by which stored intestinal lipids are mobilized, in effect “metering out” triglycerides more gradually as they are released in smaller, more frequent boluses. According to
this hypothesis, one would expect to find reduced preprandial intestinal lipid stores in VSG-operated animals, as these animals consume smaller meals than SHAM or PF animals. These lipid stores contain secretable lipid and may represent either all or a subset of intestinal lipid content. Consistent with equal daily caloric intake in VSG and control rats, we do not observe any alterations to the total storage pool in any of the four anatomical sections of the intestine (Figure 7 and Supplemental Figure 5).

One potential limitation to this study is that, 6 hours after the $^{13}$C-enriched lipid gavage, we did not observe differences in plasma triglycerides between groups (Supplemental Figure 6) as we did in the experiment shown in Figure 4. A key difference between this experiment (Figures 6-8) and the original experiment (Figure 4) was the feeding status of the animals at the time of the gavage. Figure 4 shows markedly reduced intestinal triglyceride secretion following the lipid gavage given to 24 h-fasted animals. In designing the stable isotope experiment, however, we were concerned that the fasted intestine might exhibit exaggerated absorption of the lipid gavage and thus might wash out any inter-group differences in the absorption of the isotope. The feeding paradigm we chose was intended to minimize differences in meal patterns between groups. We propose that, by normalizing preprandial intestinal lipid stores, we may have eliminated a possible difference in $^{13}$C-Triolein uptake. This proposal follows from our hypothesis that smaller meals in VSG-operated animals might limit the size of intestinal lipid storage pools in a manner proportional to meal size, thereby attenuating intestinal triglyceride secretion after a subsequent bout of feeding. Although we did not observe any changes to fasted intestinal triglyceride storage (Supplemental Figure 5), this assay may not be sensitive enough to detect very small changes to intestinal lipid storage. Furthermore, these data represent only
small samples of the proximal and distal intestine. In order to conclusively address these issues, the experiment should be repeated in 24 h-fasted animals.

Consistent with the hypothesis that smaller meals reduce intestinal triglyceride secretion without inducing long-term changes to intestinal lipid metabolism, we did not detect any significant changes to the expression of any of a large panel of genes known to regulate intestinal triglyceride metabolism and/or chylomicron synthesis (Figure 8). High-fat diets have been reported to increase intestinal absorptive capacity by enhancing the expression of several genes including FATP4, I-FABP, L-FABP, CD36, ApoC2, ApoAIV, and MTP\textsuperscript{246}. In light of this report, we interpret our data to mean that neither VSG nor pair-feeding can reverse the hyperabsorptive effect of HFD on the intestine. In other words, dietary composition appears to be more important than body weight to affect intestinal lipid absorption on a transcriptional level. We cannot, however, eliminate the possibility that the activity of enzymes such as DGAT1, MGAT2, and MTP may be differentially regulated across groups.

Gastric mucosa produces leptin which avoids proteolytic degradation in the stomach\textsuperscript{184, 247-249} and which may play some role to regulate intestinal function\textsuperscript{242}. Furthermore, leptin receptors are present in the small intestine, mainly in the jejunum but also weakly in the ileum\textsuperscript{250}. Recently, the intestine has been demonstrated also to contain other components of the leptin-sensitive melanocortin axis, and this system has been reported to affect lipid homeostasis through control of MTP expression\textsuperscript{242}. Proximal intestinal samples from leptin-deficient, \textit{ob/ob} mice show diminished MTP expression and activity which is thought to underlie phenotypical features including low postprandial triglyceride levels\textsuperscript{242}. VSG animals have low postprandial intestinal triglyceride secretion despite low plasma leptin levels\textsuperscript{181}, but we tested the hypothesis that intestinal leptin sensitivity might be enhanced following the surgery. As shown recently by
Iqbal et al.\textsuperscript{242}, we report the intestinal expression of AgRP, ObRb, and MC4R (Figure 8). We did not, however, detect any significant changes to expression of any of these genes in proximal small intestinal samples. This finding is consistent with the lack of intestinal MTP regulation seen with either weight loss or VSG (Figure 8) and is not surprising given that CNS leptin sensitivity does not appear to mediate weight loss after VSG\textsuperscript{181}.

Importantly, although we have previously reported a lack of intestinal malabsorption during a 24-hour period of \textit{ad libitum} HFD consumption\textsuperscript{181}, our most recent data suggest that VSG-operated animals have an impaired ability to absorb a large, gavaged bolus of lipid. This effect is likely due to the nonphysiologic nature of the gavaged meal. First, intragastric gavage eliminates any cephalic response which may be important for absorption in VSG animals. Second, restricted stomach volume in VSG animals may enhance the rate by which gavaged lipids reach the duodenum, flooding the absorptive capacity of the small intestine. The gavaged bolus did not exceed the size of meals consumed \textit{ad libitum}: VSG animals freely consume small meals of about 5.77 kcal (1.27 ± 0.10 grams of HFD per meal, with a caloric density of 4.54 kcal/g\textsuperscript{181}), whereas the malabsorbed gavage dose contained an average of 4.5 kcal (0.5 fat g). However, delivery of nutrients to the stomach and perhaps to the duodenum is more rapid following a gavage than during \textit{ad lib} meal consumption, perhaps overriding mechanisms necessary to properly absorb fats.

Supported by an increased rate of appearance of triglycerides in the plasma during the first hour after the gavage (Supplemental Figures 2 and 3), reports demonstrate accelerated gastric emptying after VSG\textsuperscript{57, 65, 66}. This acceleration could perhaps flood the intestine’s absorptive capacity. Incomplete absorption of normally-sized meals could drive the consumption of smaller meals in order to achieve maximal caloric extraction from consumed
nutrients. Further study is needed in order to understand gastric and intestinal kinetics after VSG, as they may relate to absorption and meal patterns. These data raise a potentially important clinical point, however, suggesting that binge eating after VSG may elicit malabsorption and may not provide an explanation for failure to maintain weight loss in the case of unsuccessful outcomes.

VSG does not include any surgical manipulation to the intestine, yet the surgery appears to alter intestinal physiology. Changes to meal patterns and/or intestinal transit time might effect these intestinal consequences of VSG. Physiologic changes occurring within the intestine after VSG include enhanced postprandial GLP-1 release (Adam Chambers, unpublished data). Based on these data and on the report that GLP-1 action may attenuate intestinal triglyceride production\textsuperscript{235}, we hypothesized that enhanced GLP-1 secretion might mediate lower postprandial lipid excursions after VSG surgery. Our results did not support this hypothesis, however (Figure 5). One potential limitation to this study might be an inappropriate dose of the GLP-1 receptor antagonist, EX-9. This dose (50 $\mu$g/kg, i.p.) has been shown to abrogate VSG-induced improvements to glucose tolerance (Adam Chambers, personal communication), but it is unclear whether this dose of EX-9 is large enough to completely normalize GLP-1 action across groups. In other words, the possibility of residual enhancement to GLP-1 action in VSG animals after EX-9 administration may explain why EX-9 did not worsen postprandial lipid tolerance in VSG-operated animals. We also did not observe further impairments to plasma lipid excursions in SHAM and PF animals that were pretreated with EX-9, indicating that the dose used was not sufficient to block all endogenous GLP-1 action. Another limitation to this study was that we did not measure plasma GLP-1 levels after the lipid gavage. Although the gavaged lipid dose used for this study (average: 0.29 g fat, or 2.8 kcal) is in a range which has been reported to
stimulate GLP-1 secretion\textsuperscript{236}, we have not verified whether this dose elicits differential GLP-1 secretion in VSG, PF, and SHAM animals. An equivalent dose of fat in the form of olive oil does not enhance preproglucagon (PPG) expression in VSG animals in any of the four quartiles of the intestine (Supplemental Figure 8), but we cannot eliminate the possibility that olive oil and intralipid, which differ substantially in lipid composition, have differential effects on intestinal GLP-1 production. It is clear that additional study of GLP-1 biology after VSG surgery is important and that many questions remain unanswered. However, despite the limitations of our study, we argue that enhanced GLP-1 action alone is unlikely to explain the profound changes to lipid biology observed after VSG.

Consistent with comparable rates of triglyceride appearance in the blood in fasted rats from each group following i.p. P-407, we report no changes in the expression of either the LPL activator, ApoC2, or the LPL inhibitor, ApoC3, in liver (Figure 6) or intestine (figure 8). Based on the rate of plasma triglyceride appearance following P-407 in the fasted state (Figure 4), we assert that hepatic VLDL secretion is unaffected by VSG. This statement is supported by a lack of ApoB regulation at the transcriptional level in VSG animals.

Despite evidence indicating no change to hepatic VLDL production and secretion following VSG, we did detect several alterations to hepatic physiology after VSG. First, we report increased ApoB48R expression in VSG versus PF animals, which may indicate weight-independent upregulation of hepatic chylomicron uptake in the fed state. Second, although we did not observe any changes in circulating lipoprotein particle size after VSG, we did observe a potential decrease in the number of HDL particles in the plasma (Figure 3). Due to the use of pooled plasma samples for this experiment, we cannot determine whether this reduction is statistically significant. However, recent evidence linking HDL cholesterol to ghrelin, a
gastrically-produced hormone\textsuperscript{77, 78} which is reduced in the circulation after VSG\textsuperscript{84-88}, raises the possibility that the reduced ghrelin levels after VSG might provide a mechanism for reduced circulating HDL levels.

Our data show, for the first time, that VSG enhances plasma bile acid levels (Figure 6). This enhancement appears to be weight-dependent. We do not define a mechanism for weight-related elevation in plasma bile acids, but we do report reduced expression of hepatic CYP7A1, the rate-limiting step in hepatic bile acid synthesis, in VSG animals (Figure 6). Plasma bile acid levels are influenced by a number of variables, including hepatic bile acid synthesis and ileal bile acid reabsorption efficiency. Elevated plasma bile acids in VSG vs. CHOW or PF animals might arise due to distinct mechanisms, such as enhanced ileal reabsorption secondary to accelerated delivery of bile acids to ileal transporters, but these data do not provide sufficient evidence to either support or refute this hypothesis. However, these data uncover a new area for future investigation of a link between weight loss and bile acids.

Collectively, these data provide exciting insight into an important secondary benefit to one model of bariatric surgery. Atherosclerosis, a common obesity-associated comorbidity, can be caused by high postprandial lipid levels\textsuperscript{251}. In fact, ApoB48 has been identified in atherosclerotic plaques\textsuperscript{252}, highlighting the role that intestinal chylomicron secretion may play in the genesis of cardiovascular disease. Significantly, the improvements to lipid homeostasis observed in VSG-operated animals are most dramatic postprandially. Understanding mechanisms by which VSG may elicit this improvement may thereby lead to the development of novel therapies for atherosclerotic cardiovascular disease.
Figure Legends

**Figure 1: VSG reduces plasma lipids in a weight-independent manner.** A: Plasma triglycerides were reduced in VSG and CHOW animals as compared with SHAM (P<0.01 vs. VSG, P<0.001 vs. CHOW) and PF (P<0.01 vs. VSG, P<0.001 vs. CHOW) rats. B: Plasma cholesterol was reduced by VSG as compared with SHAM (P<0.001) and PF (P<0.05). C: Phospholipids were reduced in plasma from VSG and CHOW animals as compared with either SHAM or PF rats (P<0.001 for all comparisons). D: Plasma NEFA were reduced in CHOW animals as compared to PF (P<0.05) but were not reduced significantly by VSG.

**Figure 2: Reductions in plasma lipids are most dramatic during short periods of fasting.** Triglycerides and cholesterol were measured in plasma sampled at 0, 4, 8, and 24 hours of fasting. A: In unfasted blood, triglycerides were reduced by VSG as compared with SHAM (P<0.001) and PF (P<0.01). Triglycerides were also reduced in VSG animals after 4 and 8 hours of fasting (P<0.001 vs. SHAM at each time point), but this reduction was not weight-independent (P>0.05 vs. PF). B: Area under the curve for plasma triglycerides across 24 hours of fasting was reduced for VSG animals as compared with either SHAM (P<0.001) or PF (P<0.05). C: Plasma cholesterol did not differ significantly between groups across the 24-hour fast (P=0.5953). D: Area under the curve for plasma cholesterol across the 24-hour study was unchanged by surgery or weight loss (P=0.8806).

**Figure 3: VSG does not alter lipoprotein composition of plasma.** A: VSG (P<0.001), CHOW (P<0.01), and PF (P<0.05) animals weighed less than SHAM animals on the day of blood
collection. B: Cholesterol in fractionated plasma from VSG rats did not reveal a shift in the lipoprotein composition of the plasma. However, cholesterol content was lower in the VLDL peak for PF, CHOW, and VSG animals than for SHAM animals. VSG animals also have reduced cholesterol content in the HDL peak. C: Plasma cholesterol is reduced in VSG animals as compared with CHOW animals (P<0.05). D: Triglycerides are reduced in plasma from VSG animals as compared with SHAM (P<0.05).

**Figure 4: VSG impairs intestinal triglyceride secretion without affecting hepatic triglyceride secretion.** A: Under fasted conditions, plasma triglycerides were increased along a similar trajectory in all animals after a 1 g/kg i.p. dose of P-407 (treatment X time, interaction P=0.6684; effect of treatment, P=0.7272; effect of time, P<0.0001). B: VSG did not affect the rate of appearance of triglycerides in the blood across the 24-hour experiment (P=0.6053). C: After i.p. P=407, a 0.5 g/kg lipid gavage resulted in an attenuated appearance of triglycerides in the plasma of VSG animals as compared with PF (P<0.001 at 4- and 6-hour timepoints) and SHAM (P<0.01 at 4-hour timepoint and P<0.001 at 6-hour timepoint). Interaction of treatment and time, P<0.0001. D: The rate of appearance of triglycerides in the plasma during the 6-hour experiment was reduced by VSG as compared with SHAM (P<0.01) and PF (P<0.001) animals. E: Plasma ApoB48 (P=0.1193), ApoB100 (P=0.8792), and total ApoB (P=0.6486) content were unchanged at the 6-hour time point.

**Figure 5: GLP-1 does not mediate impaired chylomicron synthesis in VSG rats after a lipid meal.** A, B, and C: EX-9 does not alter plasma triglyceride excursion or clearance in VSG rats
after a gavage of 0.5 g/kg lipid. D: Area under the curve for plasma triglycerides across the 6-hour experiment in all groups was unaffected by EX-9.

**Figure 6: Hepatic lipid content is unaffected by VSG.** A: Bile acid content was increased in plasma from lean animals (vs. SHAM: P<0.05 for VSG, P<0.05 for PF, and P<0.001 for CHOW; ANOVA: P=0.0006). B: Plasma bilirubin levels did not differ among groups (P=0.0776). C: No significant differences in the expression of hepatic CYP7A1 (P=0.1687), CYP27A1 (P=0.4755) or SCARB1 (P=0.2892) were detected between groups. Although an ANOVA comparing HMGCR expression between groups did not detect an overall effect (P=0.0528), Tukey’s post-hoc analysis revealed a significant difference (P<0.05) between VSG and PF animals. D: Hepatic triglyceride content was unaffected by VSG (P=0.3902). E: Cholesterol content was unchanged in livers from VSG animals (P=0.3920). F: No differences in the ratio of hepatic cholesterol ester to total cholesterol content were detected (P=0.7771). G: Wet liver weight (expressed as a ratio to body weight) did not differ among groups (0.2831). H: Expression of genes known to affect hepatic lipid uptake. Only ApoB48R was affected by VSG (ANOVA, P=0.0104; PF vs. VSG, P<0.01). (CD36, P=0.0975; FATP4, P=0.1329; L-FABP, P=0.1598; ACAT2, P=0.0981). I: Expression of hepatic DGAT1, MGAT2, and MTP were unaffected by treatment (DGAT1, P=0.0656; MGAT2, P=0.0637; MTP, P=0.4932). J: Expression of ApoA2 was unaffected by treatment (P=0.7921), but ApoB expression was reduced in PF animals as compared with VSG (ANOVA, P=0.0181; PF vs. SHAM, P<0.05). K: No changes in the expression of hepatic ApoC2 (P=0.4287) and ApoC3 (P=0.0801) were detected.
Figure 7: VSG does not affect the size of intestinal lipid storage pools.  A: $^{13}$C enrichment in M1 intestinal samples after $^{13}$C-Triolein gavage did not differ between groups ($P=0.1852$).  B: Weight of neither M1 ($P=0.5527$), M2 ($P=0.7453$), M3 ($P=0.9788$), nor M4 ($P=0.8089$) was affected by VSG surgery or by weight loss.  C: Triglyceride content did not differ among groups for any quartile of the small intestine (M1, $P=0.6202$; M2, $P=0.9445$; M3, $P=0.2394$; M4, $P=0.4164$).  D: Differences in cholesterol content were not detected in any gut region (M1, $P=0.5310$; M2, $P=0.8361$; M3, $P=0.3177$; M4, $P=0.7376$).  E: Oil Red O staining did not reveal any qualitative differences between groups.  No obvious differences in the morphology of intestinal villi were observed.

Figure 8. Expression of genes affecting intestinal lipid metabolism are unaffected by VSG.  A: No changes were detected in the expression of genes related to intestinal lipid uptake in M1 (CD36, $P=0.3469$; FATP4, $P=0.6533$; L-FABP, $P=0.3477$; I-FABP, $P=0.7320$; ACAT2, $P=0.9084$) and M2 (CD36, $P=0.2392$; FATP4, $P=0.8235$; L-FABP, $P=0.4131$; I-FABP, $P=0.3653$; ACAT2, $P=0.3200$).  B: DGAT1 (M1, $P=0.4614$; M2, $P=0.6997$), MGAT2 (M1, $P=0.2234$; M2, $P=0.6455$), and MTP (M1, $P=0.2356$; M2, $P=0.9531$) expression were unaffected by surgery.  C: ApoAIV (M1, $P=0.6469$; M2, $P=0.7584$) and ApoB (M1, $P=0.6143$; M2, $P=0.5927$) expression was unaltered by treatment.  D: VSG was not associated with changes to the transcription of either ApoC2 (M1, $P=0.4229$; M2, $P=0.9388$) or ApoC3 (M1, $P=0.3952$; M2, $P=0.6156$).  E: ObRb, MC4R, AgRP, NPY, and L32 mRNA but not POMC mRNA were detected in intestinal samples from SHAM animals.  All six transcripts were detected in hypothalamic samples from SHAM animals.  F: No significant changes to the expression of AgRP ($P=0.9478$) and ObRb ($P=0.6748$) in M2 were detected after VSG.
Supplemental Table 1: Overview of experimental groups. Experimental groups used for each of the reported experiments.

Supplemental Table 2: Taqman gene expression assays.

Supplemental Table 3: Primer sequences.

Supplemental Figure 1: Body weight and composition parameters for animals described in Figure 1. Blood was sampled on post-operative day 50. A: At this time, VSG animals weighed less than SHAM (P<0.05) animals. CHOW rats were lighter than SHAM (P<0.001), VSG (P<0.05), and PF (P<0.01) rats on the day of study. B: Fat mass was reduced in VSG (P<0.001) and PF (P<0.05) animals as compared with SHAM. CHOW animals had reduced fat mass as compared with SHAM (P<0.001), VSG (P<0.05), and PF (P<0.001) animals. C: Lean mass was unaffected by surgery, as compared with SHAM and PF groups. CHOW animals had reduced lean mass as compared with SHAM (P<0.05) and VSG (P<0.05) animals.

Supplemental Figure 2: Body weight, food intake, and body composition following VSG. A: VSG elicits body weight loss which is comparable to that which is produced by pair-feeding (PF). Body weight change for VSG-operated animals was significantly different from SHAM by postsurgical day 12 (P<0.001), an effect which persisted for the duration of the study. Body weight change for PF animals was significantly different from SHAM by day 12 (P<0.01) and for all days thereafter except day 76. Interaction of time and treatment, P<0.0001. B:
Presurgical body weight did not differ between groups. At the time of sacrifice, VSG (P<0.01) and PF (P<0.05) animals had lost weight relative to SHAM controls. Interaction of treatment and time, P<0.0001. C: Prior to surgery, treatment groups were matched for fat mass. At the termination of the study, VSG (P<0.001) and PF (P<0.01) animals had significantly reduced fat mass as compared with SHAM controls. Interaction of treatment and time, P=0.001. D: Lean mass did not differ among groups either postsurgically or at the end of the study (interaction of treatment and time, P=0.1406). E: VSG produced transient postsurgical food intake reduction, but daily food intake thereafter was equivalent to SHAM.

Supplemental Figure 3: 1 g/kg lipid gavage induces fecal fat loss in VSG animals. A: A similar rate of appearance of triglycerides in the plasma is observed during the first two hours following a gavage containing 1 g/kg lipid. Plasma triglycerides reach an early, reduced peak in VSG animals, and plasma triglycerides 3 (P<0.001) and 6 (P<0.05) hours after the gavage are lower for VSG than SHAM rats. B: Enhanced clearance in VSG animals is reflected by reduced area under the curve for plasma triglycerides during the 6-hour experiment (P<0.05 for SHAM vs. VSG). C: Fecal lipid loss in VSG animals is nearly 3-fold higher than in SHAM animals (P<0.05; 92.1313% absorption for SHAM vs. 77.8161% absorption for VSG). Absorption was also lower in VSG animals as compared with PF animals (87.7338% absorption), although not significantly.

Supplemental Figure 4: Oral fat tolerance test for 0.5 g/kg olive oil gavage. A: Plasma triglycerides over 6 hours following an intragastric gavage containing 0.5 g/kg olive oil. Interaction of surgical/weight loss treatment and time, P=0.0385. Effect of surgery/weight loss,
P=0.1711. B: Area under the curve shown in (B). Reduced AUC for VSG animals does not reach statistical significance via Tukey’s post-hoc analysis following one-way ANOVA (ANOVA: P=0.1950), but a t-test comparing AUC for VSG and SHAM animals reveals a significant difference (P=0.0170).

Supplemental Figure 5: Triglyceride content in fasted intestinal samples. In 24-h fasted samples from proximal M1 and distal M4, triglyceride content did not differ between groups (M1, P=0.3218; M4, P=0.5794).

Supplemental Figure 6: Plasma lipid levels following $^{13}$C-Triolein-enriched olive oil gavage. A: No significant differences in plasma triglycerides were detected between groups at the time of sacrifice (P=0.5953). B: Plasma cholesterol did not differ between groups (P=0.8806).

Supplemental Figure 7: M4 DGAT1 expression. Postprandial DGAT1 expression in M4 (terminal ileum) was unaffected by VSG (P=0.9399).

Supplemental Figure 8: Intestinal PPG expression. A: No differences were detected in the expression of PPG in any of 4 postprandially-sampled regions of the intestine (M1, P=0.5117; M2, P=0.0454; M3, P=0.1222; M4, P=0.9999). B: Expression of PYY in M4 was unchanged by VSG (P=0.5802). C: CCK expression in M1 was not different between groups (P=0.3025).
Figures

Figure 1. VSG reduces plasma lipids in a weight-independent manner.
Figure 2. Reductions in plasma lipids are most dramatic during short periods of fasting.
Figure 3. VSG does not alter lipoprotein composition of plasma.
Figure 4. VSG impairs intestinal triglyceride secretion without affecting hepatic triglyceride secretion.
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<th>Cohort</th>
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<td>• Fasting plasma timecourse triglyceride and cholesterol (Figure 2) • Hepatic and intestinal triglyceride secretion (Figure 4) • ApoB western blot (N=6/group; Figure 4E) • Effect of EX-9 on oral lipid tolerance (N=5-7 for VEH or EX-9 groups for each treatment group; Figure 5) • Wet liver weight (Figure 6) • Postprandial liver triglyceride, cholesterol, and cholesterol ester content (Figure 6) • Liver gene expression studies (Figure 6) • Stable isotope study (Figure 7) • Wet intestinal weight (Figure 7) • Postprandial intestinal triglyceride and cholesterol content (Figure 7) • Postprandial intestinal Oil Red O staining (Figure 7) • Intestinal gene expression studies (Figure 8 and Supplemental Figures 7 and 8) • Body weight, food intake, and body composition (Supplemental Figure 2) • Plasma triglyceride and cholesterol content (Supplemental Figure 5) • Oral fat tolerance test and dietary lipid absorption study (Supplemental Figure 3)</td>
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Supplemental Table 1. Overview of experimental groups.
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Supplemental Table 2. Taqman gene expression assays.
### Supplemental Table 3. Primer sequences.

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Supplemental Figure 7. M4 DGAT1 expression.

Supplemental Figure 8. Intestinal PPG, PYY, and CCK expression.
CHAPTER 4

Discussion and Future Directions
The data presented in the preceding chapters support a nonrestrictive mechanism for body weight reduction and metabolic improvement following VSG. The surgery also improves postprandial lipid levels by a weight-independent mechanism at the level of the intestine. In the past, VSG was thought to act via a restrictive mechanism to reduce body weight by attenuating food intake. These data, however, demonstrate that this is not the case. Instead, VSG leads to the defense of a lower body weight by “resetting” the individual’s homeostatic machinery. This is supported by the fact that VSG animals appear to have the ability, but not the drive, to overeat in response to weight loss. The hyperphagic ability of VSG-operated rats is uncovered only after additional body weight loss induced by long-term caloric restriction. Strikingly, these animals overeat only to regain postsurgical body weight and do not continue to overeat to attain presurgical body weight levels. Chapter 3 provides further support for homeostatic rewiring after VSG, demonstrating that the surgery improves postprandial plasma lipid levels in a much more dramatic way than weight loss due to caloric restriction. Our data provide only a small piece of the mechanistic explanation for the changes observed after VSG, but we propose that common mechanisms may underlie changes to body weight and lipid homeostasis. Below, we discuss several key questions which arise from the data presented in this thesis.

*What role might the CNS play to mediate the effects of VSG?*

Leptin resistance is a feature of obesity which can make weight loss by dieting very difficult, and so we initially hypothesized that the magnitude and durability of weight loss after VSG might be associated with improved hypothalamic leptin sensitivity. Enhanced sensitivity to lower leptin levels, secreted by smaller fat stores after surgery, might eliminate the hyperphagia which usually occurs following dieting in obese, leptin-resistant individuals. This is not the case,
however. Although VSG was associated with an improved anorectic response to exogenous leptin, as compared with obese, sham-operated controls, this augmentation appeared to be due to weight loss alone; the behavioral response to leptin was comparable in VSG and pair-fed groups (Chapter 2, Figure 7). A strong hyperphagic drive distinguishes pair-fed from VSG animals, and so it is unlikely that leptin sensitivity is playing a role to suppress this drive in VSG animals. Because leptin acts via CNS melanocortin circuitry to reduce food intake and because leptin sensitivity relies on the function of this system, we also investigated whether tonic melanocortin activation was altered in our animals. Few changes to the expression of AgRP, POMC, and NPY were detected in mediobasal hypothalamic lysates from VSG-operated animals.

The data presented in Chapter 2 argue that the hypothalamic melanocortin axis is unlikely to underlie changes to energy balance after surgery. Changes to the function of this axis are also unlikely to mediate VSG-induced improvements to lipid homeostasis. Recently, several reports have demonstrated a role for elements of the CNS melanocortin system to control peripheral lipid metabolism\endnote{253-255}. Central NPY increases hepatic VLDL secretion\endnote{255} and activation of central MC4 receptors enhances hepatic HDL cholesterol uptake\endnote{254}. Because the CNS melanocortin axis does not appear to respond differentially to fasting in VSG animals as compared with PF or obese (SHAM) controls, we argue that the system is also unlikely to mediate changes to lipid homeostasis. Additionally, while the possibility for a role of the CNS to control intestinal lipid secretion has not been eliminated, existing reports describe a role for the CNS to control hepatic lipid secretion, a variable which does not appear to be altered following VSG (Chapter 3, Figure 4).

The observation that VSG-induced homeostatic changes are independent of the CNS melanocortin axis has a number of implications. An important consequence is that the action of
any satiety factor which may suppress hyperphagia after VSG probably acts in extrahypothalamic brain regions. These areas include the brainstem, known to mediate anorectic responses to GLP-1 and leptin among other hormones, and brain reward centers.

Among factors which may elicit satiety via extrahypothalamic mechanisms are lipids. Intraintestinal lipids induce satiety by activating brainstem nuclei including the locus ceruleus complex (LCC), NTS, area postrema (AP), and PVN. Both the satiety and neuronal responses to luminal lipids are attenuated by long-term HFD consumption. Because our data indicate that VSG might reduce hyperphagic drive through extrahypothalamic brain regions, an attractive hypothesis is that VSG might accentuate meal-induced satiety via more robust neuronal activation in the brainstem. Enhanced vagal sensitivity is one possible mechanism by which this might occur. However, because the response does not depend on the caloric content of the food, it is also possible that increased meal frequency leads to more robust satiety in VSG-operated rats via recurrent activation of this pathway in the absence of changes to vagal sensitivity. One way by which dietary nutrients may elicit satiety is through intestinal production of oleoylethanolamide (OEA), produced in the duodenum and jejunum in response to feeding. The production of OEA in the small intestine is a specific response to lipid and is not observed following a protein- or carbohydrate-only meal. OEA reduces food intake in a PPARα- and vagus-dependent manner. In addition to eliciting hypophagia, PPARα agonism by OEA also promotes the expression of genes important for fat catabolism, such as FATP and FAT/CD36. A link between VSG and intestinal OEA has not been investigated, but more consistent mucosal OEA levels entrained by frequent meal ingestion might contribute to enhanced satiety after VSG.
Alternatively, accentuation of lipid-induced satiety in VSG animals, if relevant, might arise from hormonal changes originating in the intestine. Neuronal activation in response to luminal lipids may depend at least in part on vagal activation by CCK which is released in response to lipid-rich foods\textsuperscript{107, 108, 256}. The pattern of brainstem c-fos immunoreactivity in response to intraduodenal lipid infusion mimics the pattern of activation observed after peripherally-administered CCK and is diminished either by perivagal capsaicin or by peripheral injection of CCK-A or CCK-B receptor antagonists\textsuperscript{256}. Future studies should explore not only postprandial plasma CCK levels after VSG, but neuronal and behavioral sensitivity to CCK.

**What other hormones may mediate the observed effects of VSG?**

The data presented in this dissertation argue that leptin is unlikely to mediate the effects of VSG. However, it is increasingly apparent that VSG alters intestinal biology, thereby raising the possibility that the action of intestinally derived hormones might be altered to underlie these effects. One attractive candidate is CCK, particularly because this hormone reduces meal size\textsuperscript{110}. We did not find any changes to the expression of CCK in the duodenum after VSG (Chapter 3, Supplemental Figure 8), arguing against but not eliminating the possibility of increased CCK secretion. Increased CCK action might arise as a result of either increased secretion or increased sensitivity of vagally mediated pathways for satiety.

Interestingly, CCK’s satiating effects are potentiated by subthreshold doses of leptin\textsuperscript{115-117, 269}. Although leptin levels after VSG are low and are perhaps disproportionately low (Chapter 2, Supplemental Figure 3) due to the removal of leptin-producing cells in the gastric fundus\textsuperscript{184} and although hypothalamic leptin sensitivity is unchanged by VSG (Chapter 2, Figure 7), intestinal and/or brainstem sensitivity to leptin might be altered in order to enhance CCK
action. We failed to detect any changes in jejunal AgRP and ObRb expression (Chapter 3, Figure 8), but it is unknown whether these messages are important for the interaction of CCK and leptin at the level of the vagus nerve. Future studies should explore behavioral and neuronal responses to leptin and/or CCK after VSG, in the presence and absence of vagal integrity.

Ghrelin-producing mucosa is removed during VSG, and so a tempting hypothesis is that reduced ghrelin levels underlie the dramatic weight loss observed after the surgery. Our data, however, argue against a role for ghrelin as a sole mediator of this weight loss. Ghrelin-null animals display increased energy expenditure\textsuperscript{270}, an effect not observed after VSG (Chapter 2, Figure 2).

Recently, RYGB has been shown to enhance postprandial GLP-1 release\textsuperscript{94, 95, 138}, an effect which is possibly linked to the rapid effects of the surgery on glucose homeostasis. Because amelioration of diabetes after VSG is on a similar timecourse and of comparable magnitude, GLP-1 has been hypothesized to play an important role to elicit metabolic improvement after VSG. This may include sustained weight loss as well as improved glucose tolerance. Emerging evidence also describes a dynamic interplay between intestinal lipid absorption and GLP-1 release. Ablation of intestinal enteroendocrine cells impairs lipid absorption\textsuperscript{271} and the accumulation of products of triglyceride synthesis, such as diacylglycerol, may augment GLP-1 secretion. The data presented in this thesis, however, do not address the effects of VSG on GLP-1 secretion but do argue against a role for GLP-1 to mediate improvement to postprandial lipid homeostasis (Chapter 3, Figure 5). Our data also seem to argue against a role for increased PPG expression to mediate a putative increase in postprandial GLP-1 release in VSG animals. We failed to detect any differences in PPG expression in samples from any of 4 subsections of intestine. This finding highlights a key difference between
VSG and IT, which is associated with enhanced ileal PPG expression. The interpretation of our data is limited because we did not measure plasma GLP-1 in these animals, but GLP-1 secretion might be altered postprandially despite unchanged PPG expression. The same logic may apply to GLP-2, also a product of the PPG gene, but this anorectic hormone would be unlikely to mediate improvements to plasma lipid levels because it increases intestinal lipid absorption and chylomicron production.

We did not address the possibility that GLP-1 sensitivity at the level of the pancreas and/or CNS may be altered in response to VSG, perhaps as a consequence of vagal remodeling following surgery. This is an important area for future research. Because GLP-1 secretion is potently augmented following RYGB, understanding GLP-1 biology following VSG will elucidate whether common effects of the two surgeries are rooted in common mechanisms.

GLP-1 acts in the pancreas to stimulate insulin secretion. Insulin, in addition to its actions to control glucose homeostasis, acts in the CNS to induce satiety and in the intestine to influence lipid absorption. Hyperinsulinemia is associated in humans and in animals with increased circulating levels of intestinally-derived triglyceride-rich particles. Insulin resistance has been demonstrated at the level of the enterocyte, suggesting a direct effect of insulin to regulate intestinal lipid production. It is unlikely, however, that improved insulin sensitivity is a sole mediator of the effects observed after VSG. Unpublished data from our laboratory (Adam Chambers, unpublished) suggests that VSG and pair-feeding elicit comparable improvements to insulin sensitivity. Because VSG-related improvements to lipid homeostasis appear not to be as dramatic in PF animals, we argue that these changes are unlikely to be secondary to changes in insulin sensitivity.
**Does VSG enhance intestinal lipid utilization?**

Although VSG does not alter energy expenditure, we did detect differences in respiratory quotient (RQ) relative to SHAM and PF controls (Chapter 2). Although overall RQ after VSG is intermediate relative to SHAM and PF, the effects of VSG are most apparent when light phase and dark phase RQ values are considered independently. During both phases, the RQ for PF animals is much lower than for SHAM animals, reflecting a preference in lean animals for fat oxidation. It should be noted that these measurements were taken when daily intake was isocaloric for animals from all three groups. While RQ is equivalent during the light phase for VSG-operated and PF animals, RQ is elevated in VSG-operated animals during the dark phase. Because rats including VSG-operated rats consume the majority of their daily caloric load during the dark cycle, it is likely that differences in RQ values between PF and VSG animals are due to differences in meal patterns. PF and VSG animals were comparable in both body weight and fat mass but daily caloric intake was consumed on a very different schedule for rats in these two groups. Whereas VSG-operated rats eat many, small meals, PF animals were fed one time per day, usually during the light phase. PF animals usually consumed the fed amount very quickly, within minutes. Thus, while VSG animals are “grazers,” PF animals are “gorgers.” It is likely that this pattern of grazing alters the RQ such that carbohydrate oxidation is favored over the oxidation of lipids.

Caloric restriction reduces RQ$^{384}$. While PF animals consume calories equivalent to ad libitum-fed, SHAM animals, PF animals are calorically restricted due to a strong hyperphagic drive to regain lost body weight. VSG-operated animals, on the other hand, do not have this drive. Ad libitum consumption remains isocaloric to, and does not exceed, intake of SHAM
animals. Increased dark phase RQ (relative to PF) indicates that VSG animals do not exhibit the preferential oxidation of fats normally observed in calorically restricted animals. Is it possible that enhanced RQ might also attenuate the drive to overeat after weight loss due to VSG? Feeding may be acutely elicited under either lipoprivic or glucoprivic conditions, but it is unclear how chronically-altered fatty acid oxidation may affect ingestive behavior in VSG rats. To address this question, future studies should begin by focusing on the sensitivity of VSG-operated rats to lipoprovic and glucoprivic signals such as mercaptoacetate and 2-deoxy-D-glucose (2-DG), respectively.

Fatty acid oxidation in enterocytes has been speculated to control food intake. Might enhanced intestinal fatty acid oxidation explain improvements to lipid homeostasis and reduced hyperphagia after VSG? We find that this possibility is unlikely for a number of reasons. First, we do not observe any changes to intestinal lipid storage pool size after VSG (Chapter 3, Figure 8). Second, increased dark-phase RQ in VSG-operated rats relative to PF rats indicates reduced whole-body fatty acid oxidation. Third, a similar plasma triglyceride response to i.p. P-407 in fasted PF, SHAM, and VSG animals leads to the conclusion that VSG must not alter lipase activity.

**What role, if any, may bile acids play to alter energy homeostasis after VSG?**

Chapter 3 demonstrates, for the first time, that VSG increases plasma bile acid levels. This is a weight-dependent phenomenon, perhaps reflecting the ability for weight loss to reverse HFD-induced changes to bile acid synthesis or reabsorption. These data highlight recent reports that bile acids may modulate whole-body energy metabolism. Bile acids have been shown to combat obesity by reversing insulin resistance and by increasing energy expenditure. The
latter is mediated at least in part by TGR5 agonism, which increases the expression of intracellular type 2 thyroid hormone deiodinase^{289}. These data suggest that some of the metabolic improvements associated with weight loss might be due to enhanced bile acid signaling. Although an independent role for bile acids to control food intake has not yet been explored, increased plasma bile acid levels might enhance satiety by enhancing CNS insulin sensitivity. Plasma bile acids also improve lipid homeostasis by reducing hepatic triglyceride synthesis via an FXR-dependent pathway^{291, 292} and enhance GLP-1 secretion via TGR5 agonism^{293}.

Plasma bile acids in our study were elevated in a weight-dependent manner (i.e. increased in CHOW, PF, and VSG groups as compared with SHAM), but this measurement reflected total plasma bile acid pools under fasting conditions and did not consider composition of the pool or responsiveness of the pool to a nutrient load. While changes to total plasma bile acid levels cannot explain the weight-independent effects of VSG on defended body weight and lipid homeostasis, altered composition of the circulating bile acid pool or enhanced production of bile acids after a meal might suggest bile acid-mediated mechanisms for these effects. Differential bile acid pool composition may be relevant since TGR5 has differing affinity for various bile acid species^{238}. For example, enhanced TGR5 agonism after VSG could enhance plasma GLP-1 levels, as GLP-1 secretion is linked to the activation of TGR5 by bile acids^{294}. Additionally, because feeding enhances bile acid production^{295}, VSG-operated animals may have very different patterns of bile acid synthesis and action. Although it is possible that weight- and/or VSG-related changes to lipid metabolism might be mediated by altered plasma bile acid levels, the relationship between VSG and bile acids remains undefined.
Because bile acid-mediated TGR5 agonism in the intestine has been linked to GLP-1 secretion, enhanced plasma bile acid levels also raise the possibility that enhanced GLP-1 release following VSG may be related to larger circulating bile acid pools. Although total plasma bile acid levels were comparable among VSG, PF, and CHOW groups in our study (Chapter 3, Figure 6), it is unknown whether the composition of this pool is affected by VSG. Because TGR5 altered composition of circulating bile acid pools could cause weight-independent changes to GLP-1 secretion.

*Does the altered meal pattern of VSG-operated rats determine the metabolic benefit of the surgery?*

As mentioned above, a key finding from these studies was that VSG elicits the consumption of smaller, more frequent meals than those which are consumed by control rats (Chapter 2, Figure 5). These data raise several questions. First, can altered meal patterns explain the lack of hyperphagia observed after VSG? Smaller, more frequent meals are often considered to produce superior weight loss and metabolic benefit to humans than traditional eating schedules built around only a few, larger meals. In one study, humans who replaced breakfast with five smaller, hourly meals consumed fewer calories at lunch when permitted to eat *ad libitum* than those who consumed the single, large meal296. A mechanism for this phenomenon is unknown. One hypothesis is that satiety factors from the previous meal may persist during a second meal, reducing its size. However, it has been reported that premeal interval does not correlate with the size of a meal297. Alternatively, satiety after a meal might delay the onset of the next meal. If a meal elicits a certain satiety response regardless of the ingested calorie content, then after VSG animals might experience enhanced satiety due to more frequent meals.
A second consequence of the pattern of meals associated with VSG might be altered intestinal lipid handling. Specifically, we propose in Chapter 3 that smaller meals might limit postprandial intestinal lipid secretion. Postprandial intestinal lipid extrusion is associated with the size of a meal, perhaps providing a mechanism for reduced plasma lipid levels in free-fed VSG animals. This phenomenon does not account for reduced intestinal triglyceride production following an isocaloric intragastric gavage (Chapter 3, Figure 4), but we propose that small meal size after VSG might limit the secreted lipid pool at the time of the gavage. This is a hypothesis that should be tested using yoked-feeding cages to “entrain” the meal patterns of an obese, sham-operated group to those of a VSG-operated group. If meal patterns are responsible for weight maintenance and metabolic improvement after VSG, then the yoked SHAM animal should exhibit these effects.

Lastly, if meal frequency rather than size is the critical determinant of nutrient-induced enteroendocrine hormone release, then meal patterns might enhance circulating levels of GLP-1, CCK, and other intestinally derived peptides. To support this hypothesis, we did not observe any changes to these hormones at the transcriptional level (Chapter 3, Figure 8). Together with a failure to detect changes in the expression of several candidate genes controlling hepatic and intestinal lipid metabolism, these data argue strongly that the effects of VSG on postprandial hormone and lipid release are due not to transcriptional reprogramming but, rather, to more transient mechanisms effected by changes to patterns of intestinal nutrient delivery.

Conclusions: current understanding and future directions

In conclusion, we present a model whereby smaller, more frequent meals after VSG are the primary mechanism by which the surgery elicits its effects. This meal pattern is enforced by
the restrictive aspect of the surgery. Although animals learn quickly to increase daily caloric intake, presumably by increasing meal frequency, they do not overeat to regain body weight. Absence of hyperphagia is due to reduced drive to eat, probably accomplished via a combination of enhanced CNS sensitivity to luminal lipids and increased enteroendocrine hormone release. According to this hypothesis, small meal size also limits intestinal triglyceride secretion, thereby improving postprandial lipid levels. Although this theory is not directly tested by the work presented in this dissertation, experiments should be designed to explore this idea. Use of a yoked-feeding apparatus to entrain the meal pattern of an obese rat to that of the VSG-operated rat would be the ideal way to test whether the VSG-induced meal pattern can independently induce the improvements described here.

Another critical aspect of the surgery that must be further explored is the role that each candidate hormone (described above and in Chapter 1) might play to mediate the effects of the surgery. As rodent models for VSG are developed, including use of the surgery in murine knockout and/or transgenic models, a more complete understanding of the mechanisms behind VSG-induced changes will hopefully emerge.

In summary, this dissertation provides evidence that VSG elicits, via a non-restrictive mechanism, sustained weight loss without compensatory hyperphagia. Through an undefined mechanism in the intestine, VSG also improves lipid homeostasis by reducing postprandial lipid levels. These data highlight VSG as a potential treatment not only for obesity but also for obesity-related comorbidities such as hyperlipidemia and atherosclerosis.
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