I, Hilary E Wilson-Pérez, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Neuroscience/Medical Science Scholars Interdisciplinary.

It is entitled: Effects and Mechanisms of Bariatric Surgery: Altered Food Choice and the Role of Glucagon-Like Peptide-1

Student’s name: Hilary E Wilson-Pérez

This work and its defense approved by:

Committee chair: Randy Seeley, PhD
Committee member: Stephen Benoit, PhD
Committee member: James Herman, PhD
Committee member: Randall Sakai, PhD
Committee member: Yvonne Ulrich-Lai, PhD
Committee member: Stephen Woods, PhD
Effects and Mechanisms of Bariatric Surgery:
Altered Food Choice and the Role of Glucagon-Like Peptide-1

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By

Hilary Wilson-Pérez

B.S. Indiana University, Bloomington, IN, 2004

Dissertation Committee:

Stephen C. Benoit, Ph.D., chair
Randy J. Seeley, Ph.D., advisor
James P. Herman, Ph.D.
Randall R. Sakai, Ph.D.
Yvonne M. Ulrich-Lai, Ph.D.
Stephen C. Woods, Ph.D.
ABSTRACT

With obesity now reaching epidemic proportions, and the associated health risks and economic burden, effective weight loss strategies are critical. Currently, the only highly-effective, long-term treatment for obesity is weight-reduction bariatric surgery. Roux-en-Y Gastric Bypass (RYGB), the most commonly-performed bariatric surgery, decreases stomach capacity and bypasses part of the small intestine, re-routing the flow of nutrients. Vertical Sleeve Gastrectomy (VSG), a newer and lesser-known procedure, involves removal of approximately 80 percent of the stomach, and does not manipulate the intestine. VSG is rapidly gaining in popularity due to its safety and positive outcomes regarding weight loss, glycemic control, and other metabolic endpoints.

Despite the broad success of bariatric surgery, we understand remarkably little about how these procedures actually produce their potent effects. One thing we know is that patients who undergo bariatric surgery frequently change their eating behavior after surgery. In particular, after RYGB, humans and rodents select or prefer foods which are lower in fat content. We investigated whether a bariatric surgical procedure limited to the stomach, VSG, causes a similar reduction of fat intake/preference. We found that VSG-operated rats decreased their intake of fat in several diet choice paradigms, and that this change in food choice is comparable to the changes induced by RYGB.

One candidate to mediate the effects of VSG on food choice and other metabolic outcomes is the peptide hormone Glucagon-Like Peptide-1 (GLP-1). GLP-1 is secreted from the intestine in response to nutrient ingestion, and has potent effects to improve glycemic control and
reduce food intake. Furthermore, GLP-1 secretion is greatly enhanced following VSG and RYGB, and has been widely hypothesized to be at least partially responsible for the metabolic benefits of those surgeries. To test this hypothesis, we performed VSG in mice with genetic deficiency for the GLP-1 receptor. Contrary to our hypothesis, GLP-1 receptor-deficient mice responded normally to VSG in all examined aspects, including weight loss, glycemic control, and altered food choice.

These studies demonstrate for the first time the potent effect of VSG surgery to alter food choice, particularly by decreasing fat intake. These data provide another parallel between the effects of VSG and RYGB, suggesting that these two anatomically-distinct surgeries may share a common mechanism to induce their powerful effects. Regarding the nature of that mechanism, we demonstrated that GLP-1 receptor activity is not necessary for those effects to manifest in VSG-operated mice. Instead, enhanced GLP-1 secretion and changes in food choice may be downstream of more global adaptations of the digestive system in response to the surgical manipulations.
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<td>2-MA</td>
<td>2-Mercaptoacetate</td>
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<td>AGB</td>
<td>Adjustable Gastric Band</td>
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<td>DPP-IV</td>
<td>Dipeptidyl Peptidase IV</td>
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<td>GIP</td>
<td>Glucose-dependent Insulinotropic Peptide</td>
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<td>GLP-1</td>
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<td>Glicentin-related Pancreatic Polypeptide</td>
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<td>HFD</td>
<td>High-fat Diet</td>
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<td>HG</td>
<td>Horizontal Gastroplasty</td>
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<td>intracerebroventricular</td>
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<td>intra-gastric</td>
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<td>ip</td>
<td>intraperitoneal</td>
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<td>LiCl</td>
<td>Lithium Chloride</td>
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<td>NTS</td>
<td>Nucleus of the Solitary Tract</td>
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<td>PYY</td>
<td>Peptide YY</td>
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<td>RYGB</td>
<td>Roux-en-Y Gastric Bypass</td>
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<td>VBG</td>
<td>Vertical Banded Gastroplasty</td>
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CHAPTER 1:

General Introduction
Obesity and Metabolic Syndrome as a Public Health Concern

The worldwide prevalence of obesity has risen rapidly over the past several decades, and is predicted to continue to rise in the coming years (Mokdad et al., 2003; Wild et al., 2004). In the United States, the Center for Disease Control and Prevention reports that in 2008, 33.9% of adults were classified as obese (Freedman, 2011), representing more than a doubling since 1980 (Flegal et al., 1998). The World Health Organization has called obesity the greatest current threat to human health (Kopelman, 2000), now surpassing malnutrition and infectious diseases.

Obesity is strongly associated with the development of many health problems, including type 2 diabetes, cardiovascular disease, and hyperlipidemia, and is associated with increased incidence of some forms of cancer, sleep disorders, and osteoarthritis, among others (Haslam and James, 2005). This translates to increased morbidity and mortality, and an important predictor of decreased longevity (Stein and Colditz, 2004). In addition to the risks to the individual, obesity poses a substantial burden on the health care system (Sturm, 2002). Medical spending attributable to obesity amounts to nearly $150 billion per year, representing almost 10 percent of all medical spending (Finkelstein et al., 2009). Furthermore, indirect costs of obesity, such as decreased productivity, disability, and the value of future income lost by premature death, land the economic burden higher still (Wolf, 2002).

Bariatric Surgery

Most effective obesity treatment

Despite a dire need to reduce excess body weight, therapeutic options are scarce. Conventional treatments are diet and exercise, which can be effective in the short-term, but long-term studies
indicate that only a minority of patients are able to sustain weight loss of greater than 10 percent one year after dieting (Anderson et al., 2001; Kraschnewski et al., 2010). Relatively few studies follow patients greater than five years after a weight loss intervention, but data from the Swedish Obesity Study show that despite early decreases in body weight (up to 1 year), 3-10 year results actually show weight gain compared to before treatment (Figure 1.1) (Sjostrom et al., 2007).

Although popular media touts a multitude of pills and supplements to aid in weight loss, only one pharmaceutical agent is currently approved by the FDA for long-term use for the treatment of obesity. Orlistat works by potently inhibiting pancreatic lipase, thus blocking the uptake and digestion of dietary lipids by the intestine. Two randomized controlled studies demonstrated a maximum body weight loss of approximately 10%, which occurred at 1 year of treatment (Sjostrom et al., 1998; Torgerson et al., 2004). Sibutramine and Rimonabant are two additional drug therapies that cause similar weight loss outcomes (Pi-Sunyer et al., 2006; Wadden et al., 2005), but both have been withdrawn from the market due to safety concerns.

Fortunately, a high-efficacy alternative to lifestyle modifications and drug therapies exists in the treatment of obesity. Bariatric surgery is the general name for several kinds of surgical procedures that modify the digestive system in various ways, usually by restricting the capacity of the stomach and/or by excluding a portion of the small intestine. In contrast to the limited efficacy of lifestyle modifications and drug therapies, bariatric surgery is now widely accepted to be the most effective long-term weight loss treatment currently available (Pories et al., 1995). A large randomized controlled trial (Sjostrom et al., 2007) showed that patients who undergo bariatric surgery achieve an average maximum weight loss of 20-37% (depending on the kind of procedure) one year after surgery, and maintain an average of 15-25 % body weight loss 10 years after surgery (Figure 1.1). Not only do these procedures cause a loss of body
Figure 1.1. Weight Changes in Subjects in the Swedish Obese Subjects Study over a 10-Year Period. All data are for subjects who completed 10 years of the study. The average weight change in the entire group of surgically treated subjects was almost identical to that in the subgroup of subjects who underwent vertical banded gastroplasty. The I bars represent the 95 percent confidence intervals. Reproduced with permission from (Sjostrom et al., 2007).

weight and body fat, but many procedures also dramatically improve other hallmarks of the metabolic syndrome. A 2004 meta-analysis reported that type 2 diabetes was completely resolved in 76.8% of bariatric patients and resolved or improved in 86.0%. Hyperlipidemia, hypertension, and sleep apnea were also resolved or improved in a majority of patients (70%, 78.5%, and 85.7%, respectively) (Buchwald et al., 2004). Although the cost of surgery is considerable to the individual and/or insurance company, multiple studies now report that obesity surgery presents a positive return on investment, with one study indicating that the costs may be recouped in as early as two years (Cremieux et al., 2008; Picot et al., 2009). These and
similar data demonstrate that the efficacy of bariatric surgery far exceeds any other available obesity therapy, and provides clear benefits to both the individual and to society as a whole.

**Historical perspective**

In the 1950s, observations of “short-gut syndrome”, resulting from surgical removal of a large segment of the small intestine for the treatment of cancers or other intestinal diseases, taught physicians that such manipulation of the small intestine causes malabsorption and massive weight loss (Kremen et al., 1954). Similarly, surgeons who performed stomach surgeries such as partial or total gastrectomy for the treatment of ulcers or stomach cancer, also noted substantial weight loss in those patients (Badenoch et al., 1951). These observations paved the way for the first dedicated weight-loss surgery, the jejunoileal bypass (**Figure 1.2A**), which was described in 1953. This procedure excluded a large segment of the distal small intestine, producing malabsorption, weight loss, and a host of detrimental side effects including vitamin deficiencies, protein malnutrition, kidney stones, and even liver failure (Lee and Pryor, 2011). This procedure was modified and refined over the next decades, creating a variety of malabsorptive procedures that excluded different portions and lengths of the small intestine, using various solutions to treat or remove the bypassed segment.

In 1966, building on the findings from these intestinal bypass procedures as well as observed weight loss following gastrectomy surgeries, Mason and Ito performed the first gastric bypass for weight loss, the precursor to the modern Roux-en-Y Gastric Bypass (RYGB, **Figure 1.2B**) (Saber et al., 2008). This was the first procedure that added a stomach manipulation, the creation of a small gastric pouch, to an intestinal bypass component. While the early weight loss procedures were classified as malabsorptive, this new wave of procedures combined
malabsorption with restriction by reducing stomach capacity. The final class of bariatric procedures, which were restrictive only, was the last to be developed.

During the same time period, significant advancements were being made in the surgical treatment of gastric ulcers by longitudinal or segmental gastric resection, although it wasn’t until
1973 that these techniques would be applied as a stand-alone weight loss procedure. Early efforts were plagued by post-operative gastric dilation, resulting in insufficient weight loss, and several strategies were employed to prevent this issue, including clips, rings, and various placements of staple lines. Ultimately, two separate solutions emerged, establishing the modern procedures of Vertical Sleeve Gastrectomy (VSG, sometimes referred to as simply Sleeve Gastrectomy, Figure 1.2C) and Adjustable Gastric Banding (AGB, Figure 1.2D) (Spiegel and Skawran, 2011), procedures which, as described later, have surprisingly different postsurgical outcomes. The various iterations of gastric restriction procedures were used, and continue to be used, either alone or in conjunction with intestinal bypass (malabsorptive) procedures (Figure 1.2E).

**Modern bariatric procedures**

Today, the three most commonly-performed bariatric surgeries worldwide are Roux-en-Y Gastric Bypass, Adjustable Gastric Banding (AGB), and Vertical Sleeve Gastrectomy (VSG). It is estimated that in 2008, RYGB constituted 49% of all bariatric operations, AGB was close behind at 42.3%, and VSG represented 5.3% (Buchwald and Oien, 2009). Compared to 2003, RYGB had decreased somewhat in prevalence, AGB had increased, and VSG represented the newcomer as it had 0% reported worldwide prevalence in 2003. The vast majority of all bariatric procedures are now performed laparoscopically, and safety and complication rates have improved dramatically. These surgeries have a reported mortality rate of 0.3-0.35% in United States Centers of Excellence (Flum et al., 2009; Pories, 2008), similar to the complication rates after gall bladder removal (cholecystectomy), one of the most common outpatient procedures.
RYGB is the most well-studied of all obesity procedures, due to both its current high prevalence among bariatric surgeries, as well as its longer history compared to the relatively modern AGB and VSG procedures. As mentioned previously, RYGB is classified as a combined restrictive and malabsorptive procedure, owing to the reduction in stomach capacity and the bypass of part of the small intestine. In RYGB, a small golf ball-sized pouch is created from the proximal portion of the stomach, and physically separated from the remainder of the stomach by staples or division. The jejunum (middle portion of the small intestine) is then attached to the gastric pouch, creating a passage for the flow of nutrients – “the alimentary limb” – thereby bypassing the remainder of the stomach and the duodenum (first portion of the small intestine). The bypassed duodenum – the “biliopancreatic limb” – transports gastric and biliopancreatic secretions to the point where it is joined to the more distal jejunum, creating a “Y” configuration of the small intestine (Figure 1.2B). Although the procedure is becoming increasingly standardized, variations persist including differences in the size of the gastric pouch, length of the alimentary and biliopancreatic limbs, as well as numerous details of the surgical techniques.

AGB is the second most commonly-performed bariatric surgery, closely following RYGB at 42.3% of all bariatric surgeries performed in 2008. AGB uses a purely restrictive approach by placing a small bracelet-like ring around the upper portion of the stomach, creating a gastric pouch similar in size to a typical RYGB. The band is lined by an inflatable cuff that is attached to a subcutaneous abdominal port that is used for adjusting the inflation of the band, therefore modifying the degree of restriction. The band is initially inserted un-inflated, and after postoperative recovery, is gradually inflated to achieve an appropriate level of restriction, as determined by doctor and patient. This procedure works by limiting the amount of food that can be eaten in an individual meal, causing the patient to feel full more quickly. Therefore, under-
inflation yields too little effect, whereas over-inflation can cause chronic reflux, nausea, and vomiting.

AGB has rapidly gained popularity around the world because of its safety and reversibility, but studies consistently show that RYGB produces greater weight loss than does AGB, approximately 30-37% maximum weight loss with RYGB versus 15-20% with AGB (Angrisani et al., 2007; Colles et al., 2008; Korner et al., 2009; Sjostrom et al., 2007).

Comorbidities, notably type 2 diabetes, are also resolved or improved to a greater degree with RYGB than AGB. Moreover, these differences in metabolic outcomes seem to be independent of the differential degree of weight loss. In AGB patients, diabetes improvement is correlated with weight loss, and develops concurrently with decreasing body weight. In contrast, metabolic improvements in RYGB patients are apparent days or weeks after surgery, before significant weight loss has been achieved, and the amount of weight lost does not correlate with these improvements (Ahn et al., 2010; Korner et al., 2009).

**Vertical Sleeve Gastrectomy – a challenge to the theory of restriction**

VSG was initially introduced as a first-phase staging procedure to promote initial weight loss in patients with extreme obesity, for whom the more-invasive intestinal procedures such as RYGB pose elevated surgical risk. After preliminary weight loss had occurred, and operative risk diminished, a second-phase intestinal procedure would be performed (Cottam et al., 2006; Regan et al., 2003). However, due to the strong positive outcomes observed with this procedure, VSG has now been adopted as a stand-alone weight loss option.
VSG is a relatively simple procedure that involves removing approximately 80 percent of the stomach along the greater curvature, creating a tube-like gastric sleeve connecting the esophagus and the small intestine. Unlike RYGB and AGB, VSG is one of the only bariatric surgeries that involves tissue removal, resulting in the loss of hormone-producing cells from the resected stomach. VSG is frequently categorized with AGB as being a restrictive-only bariatric operation because it does not induce malabsorption.

Weight loss after VSG is generally greater than AGB (Himpens et al., 2006), and may on average be somewhat less than RYGB, although head-to-head comparisons of VSG and RYGB have shown mixed results, with some studies reporting them to be equally efficacious, and others showing superior efficacy of RYGB. Type 2 diabetes and other metabolic co-morbidities resolve more rapidly and to a greater degree than with AGB and, like weight loss, the improvements are similar or somewhat less pronounced than in RYGB (Karamanakos et al., 2008) (Peterli et al., 2009) (Abbatini et al., 2010). Disparate results may be due to variations in surgical technique, including the volume of the stomach remnant, which may affect weight loss and metabolic outcomes, although no consensus exists on this matter (Deitel et al., 2011). In any case, what is clear is that post-operative outcomes of VSG are more similar to those of RYGB than to those of AGB, despite its being more anatomically similar to AGB.

Another challenge to conventional ideas about VSG comes from a rodent study demonstrating that VSG-operated rats are able and willing to over-eat in certain circumstances despite their reduced stomach size (Stefater et al., 2010). This indicates that metabolic consequences at the level of the central nervous system may mediate weight loss rather than gastric restriction alone. These observations have led to challenges to the notion of VSG as a restrictive procedure (Melissas et al., 2007; Sandoval, 2011), and the suggestion that it be added
to the list of so-called “metabolic surgeries”, together with RYGB, based on their global effects on energy balance rather than only weight loss.

**Mechanisms of action, current hypotheses**

The number of publications on bariatric surgery has increased exponentially in recent years (92 publications in 2000 versus 1,484 in 2011 according to a keyword search), indicating a surge of interest in not only the procedures themselves, but a drive to understand the mechanisms that produce such a large effect on body weight and overall energy balance. Despite this swell of interest, and a robust increase in the characterization of surgical outcomes, our understanding of those mechanisms remains limited.

Restriction and malabsorption, while certainly not complete answers the question, still have their place in a discussion of mechanisms of bariatric surgery. Clearly, a dramatic decrease in stomach capacity, such as occurs in VSG, RYGB, and AGB, limits meal size. Even modest quantities of food over a short period of time can cause unpleasant or painful sensations termed food intolerance, which is discussed in more detail on page 31. However, behavioral compensation by eating more frequent meals and/or high-calorie liquids foods such as milkshakes is not only possible, but can in some cases (most often following AGB) be sufficiently robust to abolish or reverse the effects of the surgery. The conundrum is why patients who are able to overeat, despite reduced stomach capacity, choose not to do so in most cases.

There is also no question that certain surgeries, including RYGB, can cause malabsorption of macro- and micronutrients. However, clinically significant calorie malabsorption is not typical in the most common surgical variants of RYGB, and in fact, a long-limb variant has been suggested to enhance malabsorption in cases of extreme obesity (Odstrcil et al., 2010). Clearly,
malabsorption as a mechanism of bariatric surgery pertains only to those procedures that contain an intestinal bypass component, and is not relevant to AGB or VSG, which do not cause malabsorption at all. Furthermore, while restriction and malabsorption may contribute somewhat to weight loss, neither can explain the rapid and weight-independent effects of RYGB and VSG on type 2 diabetes and other metabolic outcomes.

Bariatric procedures that re-route the flow of nutrients by bypassing part of the stomach, specifically RYGB and the now less commonly-practiced biliopancreatic diversion, are well-documented to produce rapid and dramatic improvements in glucose homeostasis. These observations have led to two related hypotheses regarding the mechanisms for such improvements, the so-called hindgut hypothesis and the foregut hypothesis (Rubino and Gagner, 2002). In both procedures, nutrients bypass the proximal segment of the intestine (the “foregut”), and enter directly into a more distal segment of the small intestine (the “hindgut”). The hindgut hypothesis focuses on overstimulation of the distal small intestine due to contact with nutrients that arrive in an under-digested state. The foregut hypothesis focuses on the understimulation of the bypassed proximal small intestine, which is no longer exposed to the flow of nutrients. Both hypotheses suggest that these changes in intestinal stimulation cause alterations in hormonal and/or neuroendocrine output from the gut, which then have downstream effects on glucose homeostasis. The biggest challenge to the foregut/hindgut hypotheses is VSG surgery itself, which does not manipulate the intestine, and therefore should cause no understimulation of the foregut nor overstimulation of the hindgut; yet, post-operative outcomes are remarkably similar to those of RYGB.

Others have foregone the hindgut/foregut framework and have focused more directly on hormonal changes following various bariatric procedures. Some of the most common players
suggested to be involved in metabolic improvements following bariatric surgeries are Glucagon-Like Peptide-1, Peptide YY, and ghrelin.

Glucagon-Like Peptide-1 (GLP-1) and Peptide YY (PYY) are both hormones that are released from enteroendocrine cells in the small intestine in response to nutrient ingestion. Post-prandial secretion of each increases dramatically after RYGB and VSG, but not after AGB (Korner et al., 2006; Peterli et al., 2009), garnering interest as mediators of various effects of bariatric surgery. GLP-1 has been the focus of much research in this context, and is discussed in more detail in the next section. PYY has an inhibitory effect on many digestive functions, including gastric acid secretion, gastric emptying, and intestinal transit time (Ballantyne, 2006), which collectively contribute to a feeling of satiation and may also decrease food intake (Batterham et al., 2003; Boggiano et al., 2005). Only one study has examined a mechanistic role for PYY in surgical outcomes, and found that mice with genetically-induced PYY deficiency lose less weight in response to a modified gastric bypass procedure compared to genetically normal mice (Chandarana et al., 2011). However, this study only examined early post-operative weight loss, and in a non-traditional bypass procedure, such that the role of PYY as a primary driver of post-surgical outcomes in RYGB and VSG has yet to be determined.

Ghrelin is a postulated “hunger hormone” whose exogenous administration augments food intake in humans and animals. Ghrelin is released mainly from the stomach, and to a lesser degree from the duodenum and pancreas. In addition to its effect to increase food intake and adiposity, ghrelin has also been shown to impair glucose tolerance (Karamanakos et al., 2008; van der Lely et al., 2004). Therefore, it would follow that reduction of ghrelin levels – either by removal of ghrelin-producing cells (as in VSG) or by down-regulation of secretion – could decrease hunger and body fat and improve glucose homeostasis. As expected, VSG induces a
clear decrease in ghrelin levels (Karamanakos et al., 2008; Langer et al., 2005; Wang and Liu, 2009); however it is still not known to what degree this change actually drives surgical outcomes. Recent data from our group (Chambers et al, unpublished) has shown that mice with genetically-induced ghrelin deficiency respond normally to VSG, indicating that a drop in ghrelin is not critical for the effects of the surgery. The effect of RYGB on ghrelin is less clear, with studies variously reporting an increase, decrease, or no change in circulating levels (Tymitz et al., 2011). These disparate results may be partially due to methodological challenges for measuring ghrelin, and the regulation between its active and inactive forms, as well as surgical differences between RYGB procedures. In short, the effect of RYGB on ghrelin is controversial, and the role of this hormone to induce post-surgical outcomes untested. Finally, AGB has been shown in several reports to increase circulating ghrelin (Langer et al., 2005; Li et al., 2009; Wang and Liu, 2009), demonstrating that at least in the case of that procedure, ghrelin is not a critical mediator of metabolic improvements.

In addition to restriction, malabsorption, the foregut/hindgut hypothesis, and altered gastrointestinal hormones, a number of other hypotheses have been proposed as mediators of metabolic outcomes of bariatric procedures. These include the role of bile acids, gastric emptying, gut microbiota, intestinal gluconeogenesis, and morphological adaptation of the small intestine. However, these hypotheses remain largely untested for a variety of reasons, and currently represent little more than speculation. The bottom line is that while we have identified an ever-expanding list of physiological changes that occur with various bariatric procedures, we have yet to identify those that are important mediators of weight loss, diabetes resolution, and/or other metabolic improvements.
Glucagon-like Peptide-1

GLP-1 is best-known as a gut-derived incretin hormone, enhancing insulin secretion in response to ingested nutrients (Holst, 2007). However, since its discovery, GLP-1 has been shown to have a multi-faceted role in the regulation of energy balance, with effects on food intake, body weight, gastric emptying, stress responses, visceral illness, and cardiovascular function (Baggio and Drucker, 2007).

**Discovery and physiology**

GLP-1 is a product of the preproglucagon gene and the proglucagon prohormone, which are expressed in the pancreatic alpha cells, the L cells of the distal small intestine, and within a discrete population of neurons in the Nucleus of the Solitary Tract (NTS) in the brainstem. (Han et al., 1986; Jin et al., 1988). Glucagon-Like Peptide-1 was first discovered following identification of endocrine cells in the gastrointestinal mucosa that resembled pancreatic alpha cells (Orci et al., 1968), and which reacted with glucagon antibodies (Unger et al., 1966). Subsequent research led to the identification of several “glucagon-like” molecules produced in these cells, including GLP-1, and the designation of the gastrointestinal endocrine cells as “L-cells” (Holst, 2007). In the pancreas, proglucagon is processed by prohormone convertase 2, resulting in production of glucagon and three additional peptide fragments: glicentin-related pancreatic polypeptide (GRPP), intervening peptide-1, and the major proglucagon fragment. On the other hand, posttranslational proglucagon processing in the gut and brain is mediated by prohormone convertase 1/3, which ultimately yields the peptide products GRPP, oxyntomodulin, GLP-1, intervening peptide-2, and GLP-2.
Like GLP-1 itself, the GLP-1 receptor is expressed both in the periphery and in the central nervous system (CNS), and has widespread expression including the pancreas, intestine, stomach, lungs, and brain (Holst, 2007). The GLP-1 receptor is a G-protein coupled receptor that belongs to the same family as the GIP and glucagon receptors, and is the only known mediator of GLP-1 action. Accordingly, mice with genetic deficiency of the GLP-1 receptor are insensitive to the effects of exogenously-administered GLP-1 (Scrocchi et al., 1996).

**Pancreatic effects of GLP-1**

Plasma glucose levels exhibit a characteristic rise and fall following a meal or oral glucose load. The rise corresponds to glucose transport through the intestine and into systemic circulation, and the subsequent fall represents glucose uptake by the muscles and other peripheral tissues. When glucose is injected intravenously or interperitoneally, thereby bypassing the intestine and entering directly into circulation, the glucose peak is significantly higher, and often maintains the elevated level for a longer period of time (Elrick et al., 1964). Insulin, which stimulates glucose uptake by peripheral tissues, is secreted from the pancreas in response to oral or systemic glucose, and is the primary force which causes the removal of glucose from the blood. As might be expected, then, the insulin peak is earlier and higher in response to oral glucose administration compared to a systemic dose, consequently causing more rapid glucose clearance. This difference between the effects of an oral glucose challenge versus a systemic glucose challenge is called the incretin effect, and is believed to be due entirely to two hormones which are released from the gut, Glucose-dependent Insulinotropic Peptide (GIP, formally called “Gastric Inhibitory Peptide”), and GLP-1, which are known as incretins (Nauck et al., 1993). Both incretins stimulate insulin release from pancreatic β-cells, which then acts on peripheral
tissues to enhance glucose uptake from plasma, thereby enhancing the rate at which glucose is removed from plasma, and dropping those levels more quickly down to baseline. Blocking these hormones by pharmacological or genetic means abrogates the incretin effect (Hansotia and Drucker, 2005; Kolligs et al., 1995), attenuating the insulin response, and causing an oral glucose load to induce a greater plasma glucose excursion, similar to what would normally be caused by systemic administration of the same dose.

Patients with type 2 diabetes exhibit an impaired incretin effect, with a decreased insulin response to oral glucose, and similar glucose excursions after oral and systemic challenges (Nauck et al., 1986). GIP is hypersecreted in type 2 diabetics, however its insulinotropic effect is substantially impaired. Although GIP loses efficacy in these patients, GLP-1 does not, and is equipotent to stimulate insulin secretion along a spectrum of impaired glucose tolerance and type 2 diabetes (Meier et al., 2002). Due to this sustained efficacy and consequent therapeutic potential in the treatment of diabetes, GLP-1 has since been the focus of much research and is the foundation for several new diabetes drugs.

The effect of GLP-1 at the level of the pancreas goes beyond its insulinotropic effect. GLP-1 has also been shown to stimulate beta cell proliferation, inhibit apoptosis, and enhance differentiation of new beta cells from progenitor cells. Type 1 and type 2 diabetes are both associated with increased beta cell apoptosis, and treatment with GLP-1 or GLP-1 analogs has been shown to attenuate or reverse this effect in several rodent models of these diseases (Farilla et al., 2002; Li et al., 2003). GLP-1 also inhibits glucagon secretion. Glucagon is a hormone produced from pancreatic islets that has a principally glucose-elevating effect. Glucagon is an important part of the counter-regulatory response to hypoglycemia, and its secretion is suppressed by meals. Again, diabetes is associated with dysregulation of this hormone, as type 2
diabetes patients exhibit elevated glucagon levels during fasting, and blunted meal-induced glucagon suppression (Muller et al., 1970). Importantly, GLP-1 only inhibits glucagon secretion in normoglycemic and hyperglycemic conditions. If blood glucose falls below the normoglycemic range, GLP-1 treatment does not inhibit glucagon secretion, thus permitting its function to protect from potentially-dangerous hypoglycemia. Therefore, GLP-1 has a physiological role to regulate insulin and glucagon secretion, and the use of GLP-1-related treatments in diabetes counteracts the dysregulation of both of these pancreatic hormones.

**Effects on other tissues**

The effects of GLP-1, physiologically and pharmacologically, are not limited to the pancreas. In the gastrointestinal tract, GLP-1 inhibits gastric acid secretion, delays gastric emptying, and slows intestinal transit time. In muscle, GLP-1 stimulates glucose uptake and metabolism (independently of its effect on insulin), and inhibits glucose production by the liver. Finally, GLP-1 acts in the heart to enhance cardiac output, and has cardioprotective functions, although these effects may be due to the GLP-1 metabolite GLP-1<sub>9-37</sub> (Baggio and Drucker, 2007; Holst, 2007).

A major site of GLP-1 action not yet discussed is the brain. The identification of GLP-1 receptors in the central nervous system prompted researchers to study their function by injecting GLP-1 directly into the brain via the cerebral ventricles (intracerebroventricular, “icv”). Icv-GLP-1 causes a potent reduction in food intake in rodents (Tang-Christensen et al., 1996; Turton et al., 1996), but also induces a feeling of visceral illness as indicated by the acquisition of a conditioned taste aversion, effects that are mediated by distinct populations of GLP-1 receptors (Kinzig et al., 2002). Rodent experiments have also demonstrated a role for GLP-1 in the stress...
response. GLP-1-expressing neurons are responsive to several kinds of stressful stimuli (interoceptive and psychogenic), and central administration of GLP-1 augments secretion of the stress hormone corticosterone and increases anxiety-like behaviors (Kinzig et al., 2003). Other researchers have gone on to show neuroprotective effects of GLP-1, which improves neural function and performance in learning and memory tasks (Holst et al., 2011). In addition, GLP-1 has been shown to decrease levels of Amyloid-beta, a protein linked to the pathogenesis of Alzheimer’s disease, and prevent Amyloid-beta-induced cell death (Bak et al., 2011). Evidence for the physiological relevance of many such findings has been demonstrated using loss-of-function models – pharmacological and genetic – which point to the same conclusions.

Evidence for neuroendocrine action

Understanding how GLP-1 could have physiological actions in the brain – or in other tissues distant from its primary site of secretion in the intestine – has been a challenge in light of its rapid degradation in circulation. Canonical endocrine hormones are secreted from a source organ, causing a build-up of the hormone in the blood, which then carries the hormone to end organs where the effects are manifested. The hormone is metabolized over time, diminishing its circulating concentration as well as its end effects. GLP-1 does not seem to follow these rules. As previously mentioned, GLP-1 is secreted in response to meals; however, this rise is quite minute in comparison with typical hormones including the incretin GIP, and is often undetectable after smaller meals. Furthermore, the vast majority of GLP-1 is metabolized before it even reaches systemic circulation.

After it is secreted from intestinal L-cells, GLP-1 diffuses across the basal lamina, enters the lamina propria, and is taken up by capillaries. These capillaries express the enzyme
Dipeptidyl Peptidase IV (DPP-IV), which cleaves the active form of GLP-1 (GLP-1$_{7-37}$) into its inactive form (GLP-1$_{9-37}$), which has few known effects, except possibly in the case of cardiac function as discussed earlier. GLP-1 continues into the portal vein, where it is then transported to the liver, where it is subject to further degradation by DPP-IV. By the time it leaves the liver, it is estimated that only 5% of GLP-1 remains intact, and it will face further DPP-IV degradation as it travels through systemic circulation toward its target organs.

These observations have led to the hypothesis that GLP-1 acts locally by interacting with GLP-1 receptors on sensory nerve fibers in the intestine and portal vein before such extensive degradation has taken place (D’Alessio, 2011). According to this theory, these sensory fibers then initiate neural reflexes terminating in a number of tissue-specific responses that mediate the blood glucose-lowering effect of GLP-1. This idea has been supported by the identification of sensory nerves that originate in the nodose ganglion with terminals in the portal vein that express the GLP-1 receptor. Infusion of very low concentrations of the GLP-1 receptor agonist Exendin-4 and antagonist Exendin-9 into the portal vein were shown to regulate glucose tolerance. Importantly, the same doses infused into the jugular vein caused no such effects on glucose tolerance, implicating portal GLP-1 receptors – and therefore the neurons that express them – as critical mediators of GLP-1 action (Vahl et al., 2007). More recently, another group demonstrated that local DPP-IV inhibition in the intestine improves glucose tolerance in wild-type but not in GLP-1 receptor-null mice, supporting a role for intestinal GLP-1 receptors to mediate glucose homeostasis (Waget et al., 2011).

However, local action of GLP-1 at the level of the intestine and portal vein is clearly not its sole method of action. GLP-1 receptors on the pancreas respond directly to circulating GLP-1, yet the pancreatic response can also be initiated via the indirect neuroendocrine reflex described
above. These data may indicate that large GLP-1 responses (to large meals or via pharmacology) may be mediated by endocrine and neuroendocrine actions, whereas smaller GLP-1 responses rely exclusively on the neuroendocrine component. Furthermore, none of this clarifies the role of GLP-1 in the brain. As previously mentioned, GLP-1 is synthesized in neurons of the NTS, and brain GLP-1 receptors mediate a variety of physiological effects of the peptide. Indeed, there is strong evidence that both central and peripheral GLP-1 and GLP-1 receptors can each independently regulate feeding and glucose homeostasis (Williams, 2009). Clearly, GLP-1 physiology is a complicated system consisting of secretion and receptors in both the periphery and the brain, and endocrine and neuroendocrine actions.

**GLP-1 therapeutics in the treatment of diabetes and obesity**

The past decade has seen significant interest in GLP-1-based therapeutics for the treatment of diabetes and obesity, which are quickly becoming a standard in diabetes care. GLP-1-based therapies include synthetic GLP-1R agonists as well as DPP-IV inhibitors, all of which have proven effects to improve glycemic control in human patients. Exenatide (synthetic Exendin-4) and Liraglutide, the so-called “incretin mimetics” are potent GLP-1 receptor agonists that are resistant to degradation by DPP-IV. These synthetic agonists have much longer circulating half-lives (60-90 minutes and 10-14 hours, respectively), in comparison to the meager 1-2 minute half-life of native GLP-1. Both drugs have the drawback that they require subcutaneous injection and may cause some gastrointestinal side effects, but convey the benefit of weight loss or prevention of weight gain.

The DPP-IV inhibitors Sitagliptin and Vildagliptin prolong the action of endogenous GLP-1 (Lovshin and Drucker, 2009) by preventing its breakdown, and are preferred by many
patients because they can be taken orally. In contrast to the incretin mimetics, these DPP-IV inhibitors do not promote weight loss, but may prevent weight gain. In addition, DPP-IV inhibitors are associated with few gastrointestinal side effects (Drucker and Nauck, 2006). Novel GLP-1 receptor agonists and DPP-IV inhibitors, including long-acting formulations, continue to be developed, and represent the promise for continuing advancement in GLP-1-based therapeutics.

**GLP-1 and bariatric surgery**

Human (Peterli et al., 2009) and rodent data (Chambers et al., 2011b) indicate that VSG subjects display a dramatic increase in meal-induced GLP-1 levels, and numerous data from the more extensively-studied RYGB show similar results (Beckman et al.). Since the first reports of this elevated GLP-1 response in bariatric surgery, it has been widely hypothesized that it may be a critical mediator of improved glucose tolerance. Data indicating AGB – which is less effective at improving glycemic control and reducing body weight – does not cause a similar GLP-1 increase has lent further credence to this hypothesis.

Furthermore, across the wide range of metabolic effects of RYGB and VSG, many of them are similar to the endogenous or pharmacological effects of GLP-1 action. These include pancreatic effects, such as potentiated insulin response, enhanced meal-induced glucagon suppression, and beta cell hypertrophy, as well as reduced food intake and body weight. However, in contrast to RYGB, GLP-1-based treatments do not commonly result in complete type 2 diabetes remission.

One clinical study examined the role of GLP-1 in RYGB patients by measuring the insulin response to a meal with and without the GLP-1 receptor antagonist Exendin-9. The
authors found that Exendin-9 was more effective to reduce insulin secretion in RYGB patients than in non-operated controls, and they conclude that increased GLP-1-stimulated insulin secretion contributes significantly to hyperinsulinism in RYGB subjects (Salehi et al., 2011). However, beyond this single study, very little has been done to address the mechanistic contribution of GLP-1 to post-bariatric surgery outcomes. In conclusion, despite considerable enthusiasm for the GLP-1 hypothesis, a causative relationship has yet to be established.

The Physiology of Food Choice

Energy can be obtained from food in the form of carbohydrate, protein, or fat. Most food sources contain a mixture of these three macronutrients, and in addition contain a variety of micronutrients in the form of vitamins and minerals. To examine food choice, foods are often broken down into categories (meats, grains, fruits and vegetables, etc.), and/or may be analyzed by their macronutrient content. Classic experiments by Curt Richter and colleagues demonstrated that rats given separate sources of individual macronutrients selected nutritionally adequate diets, and showed no signs of nutritional deficiencies (Richter, 1943; Richter et al., 1938).

The flavor and other orosensory properties of food clearly influence their intake, both in terms of the choice between foods, and also the total amount consumed. Sweet and salty tastes are the most obvious examples, and are intrinsically reinforcing to humans and many other species, even in the absence of prior experience or learned associations with that quality. Fat also has reinforcing properties, although its method of detection (whether by a lingual fat receptor, the orosensory properties of its oily texture, or by learned associations with its post-ingestive consequences) is still an area of investigation (Drewnowski, 1997). But food choices are
conditioned by much more than palatability. Companies that market food products are very aware that properties such as shape, texture, color, and even temperature condition food choices (Asp, 1999). One interesting example is a study that gave people a meal consisting of pasta in only one shape, or of a mix of three different-shaped pastas, and found that people at 14% more when given the choice between the three shapes (Rolls et al., 1982), even though all other properties of the food were held constant. Social cues also have an enormous influence on food intake, conditioning when to eat, how much to eat, and what kinds of foods are preferred (Nestle et al., 1998).

Beyond the properties that are experienced before or during food consumption, the post-ingestive consequences of food also condition our attitudes and behaviors, even if we are not aware of it. Experiments in rodents using an electronic esophagus preparation, most notably by Sclafani and colleagues, have made significant advancements in understanding the post-ingestive detection and discrimination of nutrients. In this preparation, freely feeding rats drink a fluid over the course of the day, and their intake is coupled to the infusion of the same or different fluid via an implanted intragastric catheter. For example, intake of a (non-caloric) cherry-flavored solution could be coupled with an intragastric infusion of sucrose, and on another day, intake of a grape flavor could be coupled with infusion of water or another type of nutrient. These experiments show that rats are capable of detecting calories, and regulate their intake of the solutions in order to obtain appropriate daily intake based on the caloric density of the nutrients infused. Furthermore, they can distinguish between different caloric sources, such as carbohydrate and fat, and alter their preferences based on the kind and density of the nutrient infusions (Sclafani, 2001).
Thus, we know that in addition to cultural and psychological factors that regulate food choices, physiological mechanisms are also in place. However, identifying the molecular basis of those physiological mechanisms has proven challenging. For example, the concept of “one peptide, one macronutrient”, which posits that a given neuropeptide regulates the intake of one macronutrient, has not held up. Nonetheless, research has yielded significant insights into the molecular basis of food choice and macronutrient selection.

**Molecular mediators of food choice**

A variety of endogenous and exogenous factors have been shown to modulate macronutrient intake, particularly those that influence food intake *per se*. Morphine and other opioid agonists have been shown to preferentially increase fat intake over other macronutrients, and neuropeptide Y more potently stimulates carbohydrate intake. However, further studies indicated that the source of the macronutrients as well as the animals’ baseline dietary preference strongly influence those responses (Levine et al., 2003). Ghrelin is another example. One study shows that centrally-administered ghrelin preferentially increases fat intake over carbohydrate intake (Shimbara et al., 2004), and another study demonstrated that ghrelin stimulates the intake of fat-rich palatable foods, whereas genetic or pharmacological inhibition of ghrelin causes a decrease in the intake of palatable foods (Egecioglu et al., 2010).

**A role for GLP-1?**

Very little has been published regarding a potential role for GLP-1 in the regulation of food choice. One 2001 study reported the effects of a GLP-1r agonist and antagonist on rats given the choice between a high-carbohydrate and a high-protein diet (Peters et al., 2001). The authors
reported peripheral administration of the GLP-1r agonist Exendin-4 caused a modest decrease in protein intake, whereas the antagonist Exendin-9 caused a modest decrease in carbohydrate intake. These data indicate that GLP-1 may have a role in macronutrient selection, but fail to address any potential effect on fat intake or preference. Another study examined the effect of nutrient pre-load on food intake inhibition following peripheral injection of Exendin-4. The authors report that Exendin-4 produces greater food intake inhibition following preloads of fat and protein compared to carbohydrate preloads (Aziz and Anderson, 2002). These data are suggestive that GLP-1 action may influence food choice, but fail to elucidate its physiological role.

The role of lipid metabolism

Several lines of evidence link lipid metabolism to food choice. First, inhibition of fatty acid oxidation by 2-mercaptoacetate (2-MA) selectively decreases fat intake relative to carbohydrate and protein (Singer et al., 1998), and this effect is believed to be mediated via vagal afferents (Ritter et al., 2000) originating in the small intestine (Langhans et al., 2011). Secondly, enterostatin, which is co-secreted with pancreatic co-lipase, and whose regulation is thereby coupled to lipid metabolism (Berger et al., 2004), also decreases fat intake relative to other macronutrients (Erlanson-Albertsson et al., 1991; Okada et al., 1991). Finally, genetic ablation of CD36, a fatty acid translocase expressed in both the intestine and lingual papillae, abolishes fat preference in a 2-bottle choice test (Laugerette et al., 2005), although it should be noted that further experiments revealed that this effect is likely mediated via lingual CD36, as a sham-feeding preference test (in which the lipid is infused intra-gastrically) revealed no deficit in preference compared to wild-type controls (Sclafani et al., 2007). Taken together, these studies indicate that lipid processing in the intestine results in signaling events to the CNS that
contribute to food selection. However, the fact that one agent that inhibits fat utilization (2-MA) and two others that are positively associated with fat absorption or breakdown (enterostatin and CD36) all inhibit dietary fat intake creates a challenge for understanding the relationship of lipid metabolism and food choice.

In summary, a variety of neuropeptides, hormones, and receptors have been linked to the regulation of food choice, but none has been demonstrated to entirely mediate the preference for any given macronutrient or food type. The complexity may partly be due to the fact that all three macronutrients – carbohydrate, protein, and fat – can be metabolized to make energy, and animals in the wild may not frequently have the luxury of choosing among multiple acceptable caloric sources. Like many homeostatic systems, the regulation of food choice is clearly a complex process involving interplay between neural, intestinal, and endocrine factors.

**Effect of Bariatric Surgery on Ingestive Behavior**

Numerous reports show that patients who undergo bariatric procedures decrease their food intake and eat smaller meals after surgery (Bobbioni-Harsch et al., 2002; Brolin et al., 1994; Dias et al., 2006; Moize et al., 2003; Naslund et al., 1988; Trostler et al., 1995; Warde-Kamar et al., 2004). This is not surprising considering that RYGB, VSG, and AGB all decrease the size of the stomach, or the portion of the stomach which immediately collects the ingested food. What may be less intuitive is that patients often change their food preferences, selecting different foods after surgery, and reporting loss of interest or aversion to certain kinds of foods.

An important addition to the literature on this topic has been the investigation of food choice in animal models of bariatric surgery, which corroborate the human findings. There are
relatively few reports on this topic to date, but they indicate two important points. First, although the typical methods of measuring food intake in humans (self report and food diary) are prone to considerable error (Klesges et al., 1995; Zhang et al., 2000), the results from human studies are not solely due to bias or reporting errors, as more controlled animal experiments highlight the same trends. Secondly, changes in food choice are due to more than doctor’s orders. Although bariatric patients are given considerable dietary counseling (Mechanick et al., 2009; Parkes, 2006), the replication of altered food choice in animal models indicates a physiological mechanism contributing to dietary changes rather than simply being a result of compliance with postoperative instructions.

**Food choice**

While there is considerable literature describing altered food choice or food preferences following bariatric surgery, the methodology and categorization of foods varies widely from study to study, making it difficult to draw direct comparisons between them. Another caveat to some of the published reports is that while they report changes in intake of certain kinds of foods, they may not report relative intake. For example, a morbidly obese patient may eat 3000 kcal of food per day prior to surgery, and 1500 kcal after (Kenler et al., 1990). While this person may decrease their intake of “sweets”, for example, it may be that the relative intake of sweets (normalized to total caloric intake) is unchanged. Therefore, in the context of decreased caloric intake, increases in intake of a certain kind of food are both absolute and relative, whereas decreases may or may not indicate a true shift in diet choice.

The largest number of published works that examine eating behavior after bariatric surgery have examined RYGB surgery specifically, either quantifying post-operative food
choices, comparing those to pre-surgical food choices or a control group, or comparing RYGB to AGB or other kinds of bariatric surgery. Studies that focus on macronutrient content of food have indicated that RYGB patients decrease their relative intake of fat and correspondingly increase intake of carbohydrate (Brolin et al., 1994; Lindroos et al., 1996; Trostler et al., 1995), whereas others have shown no difference in the percentage of fat intake compared to the pre-operative condition (Bobbioni-Harsch et al., 2002), or the trend did not reach significance (Kenler et al., 1990). Thomas et al (Thomas and Marcus, 2008) reported that RYGB patients select low-fat foods at a higher frequency than high-fat foods, but paradoxically that low-fat foods are more associated with food intolerance. Studies which grouped foods according to other categories have variously reported decreased intake of meat (Halmi et al., 1981), sweets and soda (Ernst et al., 2009; Kenler et al., 1990; Olbers et al., 2006), milk and ice cream (Kenler et al., 1990; Olbers et al., 2006), and increased intake of fruits and vegetables (Olbers et al., 2006; Trostler et al., 1995), milk products (Trostler et al., 1995) poultry, fish, and eggs (Ernst et al., 2009; Trostler et al., 1995). It should be noted that Kenler et. al. and Olbers et al. (Kenler et al., 1990; Olbers et al., 2006) found a decrease in the “milk and ice cream” category, whereas Trostler et al. (Trostler et al., 1995) found an increase in “milk products”, but this difference may be related to both the categorization of foods or that, in the Trostler study, food preferences for RYGB and Vertical Banded Gastroplasty (VBG) were averaged together, although they were reported to be similar between the operations.

Animal studies, which measure food intake in a more controlled setting and without the social changes that accompany large amounts of weight loss, support that RYGB causes a decrease in fat intake, with RYGB rats decreasing their preference for a high-fat diet when given a choice between two or more food sources (Shin et al., 2011a; Zheng et al., 2009). Furthermore,
in a two-bottle choice test, RYGB-operated show a decreased preference for Intralipid®, a fat solution, when compared to sham-operated controls (Le Roux et al., 2011).

Fewer published reports examine food choice following AGB, although reports on (non-adjustable) Gastric Banding (GB), Horizontal Gastroplasty (HG), and Vertical Banded Gastroplasty (VBG), which cause restriction of the stomach similar to AGB, may be useful for supplementing the knowledge base on this procedure. In a large survey study, Ernst et al. (Ernst et al., 2009) found that, compared to obese controls, GB patients ate more poultry and fish; and less pasta, fruit and bread. Compared to RYGB, band patients consumed less fruit, eggs and diet soft drinks; but more chocolate. Two other studies indicated reduced eating of (Hudson et al., 2002) and cravings for sweets (Himpens et al., 2006) after AGB, although none of these studies normalized the reported changes to total caloric intake.

Several studies have compared the food choices of patients which received VBG or HG to RYGB. Regarding relative macronutrient intake, VBG was reported to decrease fat intake (Brolin et al., 1994) whereas HG was not (Kenler et al., 1990). However, when each of these surgeries is compared to RYGB, the reduction in “milk and ice cream” and “sweets and soda” was not as great in VBG/HG as in RYGB (Brolin et al., 1994; Kenler et al., 1990). Accordingly, another study found that VBG patients ate more desserts, cakes and cookies, and candies, but fewer fruits and vegetables than RYGB patients (Olbers et al., 2006). This same study analyzed macronutrient content, and found that VBG patients ate more fat and less carbohydrate than RYGB. Finally, Shai et al (Shai et al., 2002) report that VBG causes decreased intake of carbohydrates and fats (not normalized to total caloric intake), and those patients eat fewer fruits, vegetables, and sweets; and increase their intake of milk, yogurt, cheese, and diet soda.
One limitation of using macronutrient intake to represent food selection patterns, particularly in band patients, is what may be a dissociation between non-sweet carbohydrates such as bread, which are reduced relative to the unoperated condition (Ernst et al., 2009; Olbers et al., 2006; Shai et al., 2002) and relative to RYGB, (Ernst et al., 2009; Olbers et al., 2006), and “sweets”, for which the results are more variable. Therefore, differing effects of gastric banding on sweet versus non-sweet carbohydrates may obscure the relevance of a macronutrient intake analysis.

Taken together, these studies indicate that RYGB is more effective to decrease fat intake than gastric banding or gastroplasty (VBG or HG). Furthermore, RYGB appears to promote the intake of fruits and vegetables, whereas GB/gastroplasty does not. Both types of surgery seem to decrease the intake of sweets and fatty sweets, although it is unclear whether this decrease is simply proportional to the decrease in total caloric intake, or whether it reflects a true shift in dietary preference.

Until now, the effect of VSG on food choice has not been investigated, neither in human patients nor in rodent models, although one report indicated that VSG patients decreased their cravings for sweets (Himpens et al., 2006). Investigation in this area could reveal whether changes in food choice are driven by mechanical restriction of the stomach, or if instead they depend on altered nutrient flow through the intestine.

**Food intolerance**

One explanation for altered food choices following bariatric procedures is the presence of aversive symptoms following the consumption of certain kinds of foods, which then drives
patients to avoid those foods. Collectively, these aversive symptoms are referred to as food intolerance (or poor food tolerance), but may include several different kinds of postprandial distress, including dumping syndrome and vomiting.

Dumping syndrome is a cluster of symptoms that includes gastrointestinal and vasomotor consequences including nausea, abdominal pain, diarrhea, palpitations, and flushing, and which occurs when nutrients reach the small intestine too quickly (Abell and Minocha, 2006; Tack et al., 2009). Dumping syndrome is most commonly associated with RYGB (Mallory et al., 1996; Ward and Prachand, 2009), and does not appear to occur after AGB or gastroplasty procedures (Alamo Alamo et al., 2006; Mallory et al., 1996). VSG has been widely believed not to cause dumping syndrome (Fuks et al., 2009; Iannelli et al., 2008; Snyder-Marlow et al.), although a recent report indicates that when provoked in laboratory conditions, some symptoms may occur in a minority of VSG patients (Tzovaras et al., 2011). However, these findings should be interpreted with caution, as other reports of dumping syndrome do not use this provocation method.

Vomiting is the most common food intolerance complaint after AGB (Broadbent, 1994). AGB patients must eat small meals, and avoid the ingestion of liquids while eating solid food to prevent vomiting. These symptoms cause many patients to shift their caloric intake toward liquid sources (Busetto et al., 1996) (which may be nutritionally maladaptive for weight loss), and in some cases, are sufficiently severe to prompt band removal (Dargent, 2008).

Several studies have compared overall food tolerance between bariatric surgeries. Suter et al (Suter et al., 2007) showed that food tolerance is better in RYGB than AGB in the long-term. Whereas RYGB patients experience the poorest food tolerance in the immediate post-operative
period, and gradually improve over time, AGB patients show the opposite pattern, with gradually deteriorating food tolerance. However, this report assessed only food tolerance as a whole, and did not evaluate reactions to specific foods. Schweiger et al (Schweiger et al.) assessed food tolerance for 8 categories of food in several bariatric procedures, including RYGB, AGB, and VSG. Overall, AGB patients had the poorest food tolerance, the highest frequency of vomiting, and the lowest satisfaction with their eating ability compared to other surgeries. RYGB and VSG were more favorable in each of these measures, and similar to each other. When broken down by food category, AGB had the lowest tolerance in each of the eight food categories compared to other surgeries, with the poorest tolerance for red meat, bread, and pasta. VSG was similar to RYGB, or intermediate between RYGB and AGB, in every category except red meat, for which it had the highest tolerance compared to the other surgeries. This study did not include a control group, and did not assess tolerance for fatty foods. In a comparison of RYGB and VBG, Olbers et al (Olbers et al., 2006) showed that greater than 30% of VBG patients had intolerance for fruits and vegetables, whole meat, and bread, whereas this did not occur in RYGB. Conversely, almost one third of RYGB patients reported intolerance for fat foods, which did not occur in VBG patients.

Animal studies of food intolerance are scarce due to the difficulty of assessing those symptoms in rodents, and the fact that rats cannot vomit. However, one study used a conditioned taste aversion paradigm to examine whether an intra-gastric infusion of a fat stimulus causes aversive consequences in RYGB-operated rats, and showed that, indeed, corn oil caused a modest taste aversion in RYGB-operated rats but not control rats (Le Roux et al., 2011). This study indicates that food intolerance does occur in a rodent model of RYGB, but does not indicate the type of discomfort experienced by the animals. Further experiments will be
necessary to determine the responses to different kinds of food across the various bariatric procedures.

*Taste acuity*

Another factor that may influence patients’ food choices after surgery is their ability to detect taste stimuli. Two studies utilized laboratory taste detection protocols to examine RYGB patients pre- and postoperatively. Both studies found that RYGB patients decreased the detection threshold (increased sensitivity) for certain taste stimuli after surgery. The first reported increased taste acuity for bitter, sour, and a trend for salty stimuli (Scruggs et al., 1994), whereas the second reported increased acuity for sweet but not bitter (Burge et al., 1995). Interestingly, another report which used a survey procedure (i.e. “Have you experienced a decrease in taste for sweet foods?”) found contradictory results. This comparison of RYGB and AGB (which did not include a control group or pre-operative evaluation) indicated that 65% of RYGB patients reported a *decrease* in the taste of sweet foods, whereas 62% of AGB patients reported an *increase* in the same. Responses for detection of other taste stimuli were more mixed. Overall, more RYGB (82%) than AGB patients (46%) reported a change in the taste of food or beverages after surgery (Tichansky et al., 2006). Rat studies of RYGB have also indicated possible changes in taste detection (Hajnal et al., 2010; Le Roux et al., 2011; Shin et al., 2011b; Tichansky et al., 2011), although the procedures used (rapid access lick test and two-bottle choice test) do not distinguish between detection and liking of the stimuli, and are discussed in further detail in the next section. No studies of taste acuity in relation to VSG surgery have been reported.
Food reward

Finally, bariatric patients may decrease intake of certain foods due to decreased food reward; that is, after surgery these patients may like or want those foods less. While this may be a general decrease in food reward related to all caloric sources, it may also vary according to kind of food; and furthermore, these changes may be learned based on experiences with food intolerances or taste acuity.

Using the Power of Food Scale, a questionnaire which measures an individual’s hedonic appetite for highly palatable foods but not the actual consumption of such foods, Shultes et al (Schultes et al., 2010) reported that hedonic hunger, the craving for food in the absence of physiological need, is increased in obese individuals, but reversed by RYGB. Furthermore, this measure was most reduced in RYGB patients who reported frequent episodes of dumping syndrome. Similarly, RYGB patients reported decreased “thinking of food”, as well as several other measures of hunger sensations (Delin et al., 1997). In a comparison of VSG and AGB, more VSG patients indicated a greater loss of hunger and loss of cravings for sweets than AGB patients (Himpens et al., 2006).

Several reports have examined the effects of RYGB on food reward in rat models. One method for examining subjective pleasantness of a taste stimulus is to measure lick rate in a brief access test, with higher lick rates indicating greater liking of that stimulus. However, at low concentrations, lick rates that are similar to the lick rates for water may indicate one of two things: lack of detection, or lack of liking. When rats were examined for their licking response to sucrose, at low concentrations the results are mixed, with one report (Shin et al., 2011b) showing that RYGB rats have an increased lick rate (enhanced detection/liking) compared to sham
controls, while two other reports (Hajnal et al., 2010; Tichansky et al., 2011) find no differences between groups. However, at higher concentrations of sucrose (well above the threshold for detection), lick rate for sweet tastants is uniformly reported to decrease in RYGB-operated rats (Hajnal et al., 2010; Shin et al., 2011b; Tichansky et al., 2011). In the two studies which examined lick rate for a fat stimulus, Shin et al (Shin et al., 2011b) found increased lick rate in RYGB rats compared to sham rats at low concentrations, and decreased lick rate for high concentrations of corn oil. In contrast, le Roux et al (Le Roux et al., 2011) found no differences in lick rate for Intralipid® at any concentration.

Motivation is another important aspect of food reward, in that patients may eat less due to a decreased drive for food, regardless of how much they like the food once they consume it. While the Power of Food Scale (mentioned above) is one method for assessing food-related motivation in humans, in rodents, tests which require an animal to complete a task (pressing a lever or simply moving toward a food source) can be used to examine food-related motivation. Shin et al (Shin et al., 2011b) showed that obese rats had a slower run-way speed toward a food stimulus (less motivation) than lean rats, but that this was reversed by RYGB. These results are surprising because they indicate that RYGB actually increases food-related motivation. Contrary to what might be expected, these studies indicate that RYGB-operated rats show greater or equal motivation than control rats to work for a food rewards, at least in a context in which the total amount of food consumed is limited.

Changes in behavior and perception, such as altered food choice, taste acuity, and food reward, must ultimately stem from changes in the brain. When examined by functional magnetic resonance imaging, RYGB patients exhibited a selective reduction in neuronal responses to high-calorie foods in mesolimbic reward areas (Ochner et al.). Genetically obese rats which received
the same procedure exhibited blunted neuronal responses in the parabrachial nucleus to oral sucrose exposure, indicating altered taste processing with RYGB (Hajnal et al., 2010). Dopamine, a neurotransmitter associated with various kinds of rewarding stimuli including food, has also been reported to change after RYGB. However, two recent studies offer conflicting reports of the direction of change, with one reporting an increased in dopamine type 2 receptor availability (Steele et al.), and the other reporting a decrease (Dunn et al.).

**Objective of this Research**

The purpose of this research is to understand the effects and mechanisms of bariatric surgeries. The reasons for this are manifold. The ultimate goal is to use this knowledge to produce effective therapies that are less invasive for the treatment of obesity and the metabolic syndrome. In addition, by comparing various kinds of bariatric surgeries, we can understand which procedures are most effective for the various endpoints such as weight loss, glycemic improvement, dyslipidemia, and heart disease, and therefore help tailor the selection of procedures for patients based on their unique concerns. Finally, by studying an intervention that has such a broad range of physiological effects, we can begin to tease out which effects are weight-loss dependent, and of those that are not, which physiological systems are altered in order to produce those changes.

VSG is a convenient and useful procedure for examining many of these issues. First of all, it is technically easier and safer than the more often-studied RYGB, and the existing large body of literature on RYGB provides a useful comparison. And most importantly, increasing evidence indicates that VSG is equally or nearly as effective as RYGB in terms of body weight,
glucose homeostasis, and many other surgical outcomes. This similarity of outcomes despite anatomical differences provides an opportunity to compare and contrast, and understand which are the salient features of these procedures that drive the various changes, and which other aspects are less important or even unnecessary.

Food choice is particularly important because of its effect on nutrition, and association with body weight. Elevated fat intake and fat preference is associated with overweight and obesity, but it is not clear whether this is a cause or a consequence of increased body weight. In addition to the actual selection of foods, food reward – the motivation for food or pleasure derived from it – has also been associated with obesity, and may be predictive of weight gain in children. Studying such issues of food choice and food reward in humans entails important cultural and socio-economic caveats, especially in the context of bariatric surgery, in which dramatic weight loss and resulting social influences may condition some choices and attitudes related to food. By investigating food choice in rodent models, we can eliminate the social influences on food-related behaviors, and assess physiological outcomes in a more straightforward manner.

Since the first reports of increased GLP-1 following bariatric surgery (Naslund 1997, 1998), it has been widely hypothesized that GLP-1 is at least partially responsible for the glycemic improvements, and possibly other metabolic endpoints, that result from these procedures. Hundreds of publications now examine this peptide hormone in the context of many bariatric procedures and various indices of glucose homeostasis. However, nearly all of those are correlational, and do not answer whether GLP-1 is truly the cause of such changes, or merely a side effect. Even of those publications using pharmacological blockade of GLP-1 action do not adequately address the long-term consequences caused by such a dramatic change in GLP-1
responsivity seen following RYGB and VSG. Again, animal models offer a convenient solution to this problem, because genetically-modified mice offer the possibility to examine the effects of VSG or other procedures in the absence of GLP-1 signaling.

In summary, this research examines the effects of VSG on food-related behaviors in rodent models, and compares them to RYGB. It also investigates the outcomes of VSG in mice which are unresponsive to GLP-1 in order to elucidate the role of GLP-1 in the metabolic improvements induced by surgery, as well as its role in food choice.
CHAPTER 2:
The Effect of Vertical Sleeve Gastrectomy on Food Choice in Rats
ABSTRACT

Objective: Diets high in fat are implicated in the development and maintenance of obesity, and obese individuals display greater preferences for high-fat foods than do their lean counterparts. Weight-reduction bariatric surgery is associated with changes in food choice. In particular, after Roux-en-Y Gastric Bypass (RYGB), humans and rodents select or prefer foods which are lower in fat content. We asked whether a bariatric surgical procedure limited to the stomach, Vertical Sleeve Gastrectomy (VSG), causes a similar reduction of fat intake/preference.

Research Design and Methods: Rats received VSG or Sham surgery or remained surgically naïve, and were assessed for food preference using three diet-choice paradigms. Using progressive-ratio and conditioned taste aversion paradigms, we further asked whether surgically-induced changes in food choice are secondary to changes in the reward value of food and/or to the formation of a food aversion. Finally, food choice was compared between VSG and RYGB-operated rats.

Results: VSG rats decreased their intake of dietary fat, and shifted their preference toward lower caloric-density foods. This change in food choice was not associated with changes in motivated responding on a progressive-ratio schedule for either a fat or a carbohydrate food reinforcer. When VSG and RYGB were compared directly, both procedures caused comparable changes in food choice. The conditioned taste aversion paradigm revealed that VSG rats form an aversion to an intra-gastric oil administration whereas RYGB rats do not.

Conclusions: VSG and RYGB, two anatomically-distinct bariatric procedures, produce similar changes in food choice.
INTRODUCTION

Diets high in fat are implicated in the development and maintenance of obesity (Hill et al., 2000; Lissner and Heitmann, 1995; Winzell and Ahren, 2004) in humans and animal models, and increasing concentrations of dietary fat cause dose-dependent body-weight gain in some strains of mice (de Wit et al., 2011). Furthermore, obese individuals display greater preferences for high-fat foods than their lean counterparts (Drewnowski et al., 1985; Drewnowski et al., 1992).

Weight-reduction bariatric surgery is the most effective long-term treatment for obesity. Despite its broad success, we understand remarkably little about how these procedures produce their potent effects. What is clear is that patients who undergo certain bariatric procedures, including Roux-en-Y gastric bypass (RYGB), frequently change their eating behavior after surgery. Several studies have demonstrated that humans (Ernst et al., 2009; Lindroos et al., 1996; Thirlby et al., 2006; Thomas and Marcus, 2008) and rodents (Zheng et al., 2009) who receive RYGB select or prefer different foods, particularly foods which are lower in fat. However, the effect of alternative bariatric procedures on food choice is less clear, and in particular, food choice following Vertical Sleeve Gastrectomy (VSG) has not been reported.

Bariatric surgeries have been classified as either restrictive, malabsorptive, or both. RYGB, which includes both gastric and intestinal modifications by shrinking the effective size of the stomach and rerouting the flow of nutrients, is classified as both restrictive and malabsorptive. In contrast, VSG is a comparatively simple procedure in which only the stomach is modified, and is therefore classified as a purely restrictive procedure. VSG involves removing approximately 80% of the stomach along the greater curvature, creating a gastric “sleeve”
connecting the esophagus directly to the pylorus. Like RYGB, this procedure induces loss of weight and fat mass, and improves glucose tolerance in humans and in rodent models (Karamanakos et al., 2008; Peterli et al., 2009; Stefater et al., 2010).

We used a rat model of VSG to investigate whether rats undergoing VSG change their food choice after surgery. We hypothesized that, due to the decreased stomach volume, VSG rats would prefer the most calorically-dense food available in order to maximize caloric intake into their reduced stomach volume and would therefore increase fat intake relative to other macronutrients.

To investigate the mechanism underlying altered food choice, we also asked whether food reward, specifically fat-related food reward, is correspondingly altered. The reward value of food has been reported to increase in obesity (Clark et al., 2010; Saelens and Epstein, 1996; Temple et al., 2008), and is predictive of weight gain in children (Hill et al., 2009). It is therefore important to understand if and how food reward is modulated by bariatric surgery. The flip side may also be true. Many RYGB patients experience “dumping syndrome”, a cluster of aversive symptoms that includes gastrointestinal and vasomotor consequences, and which occurs when nutrients reach the small intestine too quickly (Abell and Minocha, 2006; Tack et al., 2009). We hypothesized that a learned association of fat intake with these aversive consequences drives patients to eat less fat, and that a similar mechanism may operate in VSG as well. Therefore, we investigated whether RYGB and/or VSG would induce a conditioned aversion to food stimuli.

METHODS

Animals. Rats were housed at the Metabolic Diseases Institute of the University of Cincinnati under standard controlled conditions with free access to food and water except where noted. All
procedures for animal use were approved by the University of Cincinnati Institutional Animal Care and Use Committee. These studies used 3 cohorts. Male Long-Evans rats (Harlan Laboratories, Indianapolis, IN; 250-300g) were maintained on a high-fat diet (HFD; Research Diets, New Brunswick, NJ; D03082706; 40% fat; 4.54 kcal/g) for 6-8 weeks prior to receiving VSG, RYGB, or sham surgery, or remaining surgically naïve. Rats were matched for body weight and fat mass before being divided into the appropriate surgical groups. Rats were maintained on HFD before and after surgery except during diet selection testing, and all surgeries were conducted by the same surgeons. Cohort A included sham (n=13), VSG (n=14) and surgically naïve (n=7) rats. Cohort B included sham (n=20) and VSG (n=17) rats. Cohort C included sham (n=17), VSG (n=14) and RYGB (n=9) rats. The sham group was counterbalanced to include rats which received either a sham-VSG or sham-RYGB procedure as described below. No differences in any of the parameters measured were detected between the 2 sham procedures, and their data are therefore presented as a single group.

**Surgical Procedures.** VSG surgery was conducted as previously described (Stefater et al., 2010). Briefly, the lateral 80% of the stomach was excised leaving a tubular gastric remnant in continuity with the esophagus superiorly and the pylorus and duodenum inferiorly. The VSG-sham procedure involved analogous isolation of the stomach followed by manually applying pressure with blunt forceps along a vertical line between the esophageal sphincter and the pylorus. RYGB surgery was conducted as described previously (Chambers et al., 2011a). Briefly, a small pouch was created at the proximal stomach, and physically separated from the remainder of the stomach. The jejunum was then transected, and the open end of the distal jejunum was anastomosed to the new pouch, creating the alimentary limb. The open end of the remaining proximal intestine (biliopancreatic limb) was anastomosed to the jejunum at a point
10-cm distal to the initial transection, creating the classic “Y.” For the RYGB-sham procedure, the jejunum was transected and re-anastomosed. Rats consumed liquid diet (Osmolite OneCal) for the first 3 post-operative days, and were transitioned back to solid diet by Day 5.

**Body Weight, Food Intake, and Body Composition.** Body weight and food intake (Cohort A) were recorded from the day of surgery until food restriction began on Day 27. Magnetic resonance imaging was performed 6-wk after surgery (Cohort A) to determine body composition using a whole-body composition analyzer (EchoMedical Systems, Houston, TX).

**Diet Selection Testing.** Three kinds of diet selection testing were employed. In the first paradigm (Cohorts A and C), three pure macronutrient diets (Harlan Teklad; TD.02521[carbohydrate], TD.02522[fat], and TD02523[protein]) were presented in separate containers simultaneously for 4d. In the second paradigm (Cohort B), rats were given a choice between two novel, nutritionally-complete pelleted diets, a high-fat diet which included sucrose (Research Diets, New Brunswick, NJ, D12331, 58% fat) and a low-fat diet (D12450B, 10% fat). To assess a possible preference for caloric density, rats in Cohort A were offered 2 liquid diets simultaneously: regular Ensure Plus™ (1.41kcal/g; 29% Fat, Abbott Nutrition, OH) and Ensure Plus that had been diluted by 50% with water, and intake of both diets was recorded over 48h.

**Progressive-Ratio Paradigm.** We employed a progressive-ratio lever-pressing paradigm to assess motivated responding for food cues (Cohort A). Prior to surgery, rats were trained to lever press for sucrose (Test Diet, Richmond, IN) and peanut oil (Planters brand/Nabisco, East Hanover, NJ) reinforcers in separate environments distinguished by light color, lever location, and presence or absence of a fan. This paradigm was chosen based on its ability to distinguish the reinforcing values of sucrose and peanut oil (Tracy et al., 2008). Training consisted of
alternating 45min sessions in the sucrose and peanut-oil chambers, beginning with autoshaping and fixed-ratio sessions. Subsequently, rats were tested in a 1h Progressive-Ratio (PR) schedule. The response requirements of the PR schedule increase progressively, minimizing the effects of satiation (Tracy et al., 2008). Beginning 10d post-surgery, rats were retested on the PR or fixed-ratio schedules in an ad libitum or food-restricted condition.

**Conditioned Taste Aversion.** Three months after surgery (Cohort C), food aversion was assessed using a conditioned taste aversion paradigm in which a novel flavor (0.15% sodium saccharin) was paired with an intragastric (ig) infusion of 1 ml peanut oil, 1 ml water, or 1 ml of the malaise-inducing agent, lithium chloride (0.15 M LiCl) as a positive control. Rats were trained for 15 d during which access to water or saccharin was limited to 2 brief exposures per day. The first 3 d consisted of acclimatization, in which rats were given 30-min water access in the morning, followed immediately by ig infusion of 1 ml water, and 45-min water access in the afternoon. On Days 4-15, rats received 0.15% saccharin or water for 30 min in the morning, and water for 45 min in the afternoon. Each rat received 3 “test pairings” (30-min saccharin access followed by ig infusion of the appropriate stimulus), and 3 “control pairings” (30min water access followed by ig water), for a total of 6 pairing sessions alternating with recovery days in which rats drank only water and received no ig infusion. Saccharin intake was recorded for all pairings, and rats which drank less than 2 ml during the 30-min exposure period were orally flushed with 1ml saccharin prior to administering the ig stimulus to ensure adequate flavor-stimulus pairing. On Day 17, following 16-h water deprivation, a saccharin intake test was administered in which rats were given only saccharin to drink for 4 h and their intake recorded.

**Statistics.** Data were analyzed using the appropriate ANOVA or student’s t-test. Where appropriate, Tukey’s post-hoc comparisons were used to determine pair-wise differences
between groups. P<0.05 was considered significant for each of these analyses. All data were
analyzed using GraphPad (Prism, San Diego, CA).

RESULTS

**Body Weight, Body Composition, and Food Intake.** The body weight of Naïve (non-operated)
and Sham rats did not differ significantly at any time-points, although Sham rats exhibited a
trend toward weight loss in the first post-operative week that was subsequently recovered. VSG
rats lost more body weight than both control groups (Naïve vs. VSG, p<0.05 starting at 4d, Sham
vs. VSG p<0.05 starting at 3d) (**Fig 2.1A**). The decreased body weight of VSG rats compared to
controls was sustained until sacrifice at 126 d (Sham vs. VSG, p<0.001, data not depicted). Food
intake was measured starting 4 d post-operatively, when solid food (HFD) was re-introduced.
Rats consumed HFD except during Days 16-20, when they were assessed for macronutrient
selection, as described below. Naïve rats ate more than Shams from Days 4-6 (p<0.05), after
which there were no longer significant differences. VSG induced a transient reduction in food
intake compared to both control groups (Naïve vs. VSG, p<0.05 Days 4-20, Sham vs. VSG
p<0.05 Days 4-12, and Day 20), an effect which was no longer apparent by Day 27 (**Fig 2.1B**).
These results are in agreement with previous reports from our group (Stefater et al., 2010;
Wilson-Perez and Seeley, 2011), and demonstrate a lack of rebound hyperphagia which occurs
following food restriction and discontinuation of many other weight-loss agents (Inoue et al.,
1997; Schreiber et al., 2000; Vickers et al., 2003). Body composition was measured 6 wk after
surgery, and revealed that VSG rats had approximately 50% as much fat mass as controls
(p<0.05 vs. both Naïve and Sham) (**Fig 2.1C**), and also had a modest decrease in lean mass
(p<0.05 vs. Naïve and Sham) (**Fig 2.1D**).
**Food Selection.** Prior to surgery, rats were tested for macronutrient selection, at which time there were no differences among groups (data not shown). Beginning at post-operative Day 16, rats were re-tested over 4d. VSG rats consumed less fat, more carbohydrate, and less protein than compared to Naïve and Sham (p<0.05) (**Fig 2.2A**). Total caloric intake of VSG rats was significantly lower compared to Naïve and Sham (p<0.05) (**Fig 2.2B**). When the intake of the individual macronutrient diets was normalized for total caloric intake, fat intake and carbohydrate intake differed significantly between VSG and control groups (p<0.05 Naïve vs. VSG and Sham vs. VSG), whereas protein intake did not (**Fig 2.2C**). This effect was seen at later time-points relative to surgery (8wk, Cohort B, data not shown) and was also observed in female VSG rats (data not shown).
When rats selected between a novel high-fat diet (HFD) and a low-fat diet (LFD), VSG rats ate less HFD (p<0.05) and more LFD (p<0.05) than Sham rats (Fig 2.2D). When expressed as a preference (kcal of HFD / total kcal), VSG rats had a significant decrease in their preference for HFD (p<0.05) (Fig 2.2F). Total caloric intake of VSG rats was lower than that of Sham rats during the testing period (p<0.05) (Fig 2.2E).

We hypothesized that VSG rats decreased their intake of fat due to the high caloric density of fat relative to the other macronutrients. To test this hypothesis, rats were given a
choice between 2 liquid diets which differed in caloric density but not in relative nutrient composition (diluted[50%] or undiluted[100%] Ensure Plus™). During the first 24 h, rats from all groups consumed the majority of their calories as the undiluted (100% Ensure) diet (Fig 2.3A), and there was no difference in dietary preference among groups (Fig 2.3B). However, during the second 24 h, VSG rats decreased their intake of 100% Ensure (p<0.05 Naïve vs. VSG and Sham vs. VSG), and increased their intake of 50% Ensure (p<0.05 Sham vs. VSG) (Fig 2.3C), resulting in a decreased preference relative to Naïve and Sham rats (p<0.05) (Fig 2.3D).
Total caloric intake was again lower in VSG rats compared to Naïve and Sham (p<0.05) (Fig 2.3E).

Figure 2.4. Rats that received Sham surgery or VSG surgery, or remained surgically naïve, were tested for motivated responding for food cues using a lever-pressing paradigm. No differences were found among groups for motivated responding (progressive-ratio) for sucrose (A) or oil (C) reinforcers under food-restricted (FR) or ad libitum-fed (Ad-lib) conditions. However, on a fixed-ratio schedule, VSG rats earned fewer food reinforcers compared to sham rats when pressing for either sucrose (B) or oil (D).

Food Reward. To test the hypothesis that decreased fat intake was due to decreased reward value of fatty foods, we used the progressive-ratio lever-pressing paradigm which assesses the motivation to work for sucrose or peanut oil food reinforcers. In this context, the continually increasing difficulty to obtain food rewards limits the total number of reinforcers earned, and therefore calories consumed. Rats were trained prior to surgery (Pre-sx) under food-restricted
conditions (FR), at which time there were no differences among groups. Rats were re-tested at 2
and 4 wk post-surgery under *ad libitum*-fed conditions (ad-lib), and again at 5 wk post-surgery
while food-restricted. No differences were found among groups at any of the time-points (**Fig
2.4A, C**). To validate that lever-pressing is a relevant paradigm for assessing food intake
behaviors in VSG-operated rats, we used a fixed-ratio reinforcement schedule as a positive
control. In these conditions, in which rats are easily able to obtain a large number of food
rewards (presumably eating to the point of satiation), VSG rats earned significantly fewer
sucrose and peanut-oil reinforcers than Naïve or Sham rats (P[interation group x time]<0.0001
for sucrose, P=0.0297 for peanut oil) (**Fig 2.4B, D**). These data are in accordance with previous
data that VSG rats eat smaller meals (Stefater et al., 2010).

**Figure 2.5.** Rats that received Sham surgery, VSG surgery, or RYGB surgery were tested for food
choice using a macronutrient-selection paradigm. Intake of each macronutrient (A), total combined
food intake (B), and food intake of each macronutrient normalized to total food intake (C) are
presented. Symbols indicate significant differences when compared to Sham (* p<0.05, ** p<0.01,
and *** p<0.001). **

**Food Selection in VSG and RYGB.** We used the macronutrient selection paradigm to directly
compare food choice of VSG and RYGB-operated rats. We found that VSG and RYGB animals
decreased their fat intake and increased their carbohydrate intake to a comparable degree relative
to sham controls (p<0.05), and were not significantly different from each other (Fig 2.5A). This was the case both when the data are expressed as raw values, as well as when expressed as a percent of total food intake (Fig 2.5C). During the 2-d testing period, total caloric intake was significantly lower for VSG rats (p<0.05), but not for RYGB rats, when compared to Sham controls (Fig 2.5B).

![Figure 2.5](image)

**Figure 2.5.** A comparison of saccharin intake in rats given sham surgery, VSG surgery, or RYGB surgery. Bars indicate mean ± SEM. *p<0.05, **p<0.01 versus Sham controls.

**Conditioned Taste Aversion.** To determine whether the post-ingestive consequences of fat intake may be aversive to rats that have undergone VSG or RYGB, we trained rats to associate a novel flavor, saccharin, with an intra-gastric infusion of water, peanut oil, or the malaise-inducing agent LiCl as a positive control. Sham rats that received LiCl infusions formed a strong conditioned taste aversion, drinking far less saccharin than water-conditioned sham rats on the test day (water vs. LiCl p<0.05). Among rats that were conditioned with oil, Sham and RYGB rats formed no aversion, whereas VSG rats formed a strong aversion (p<0.05, Fig 2.6A-C). To ensure that RYGB surgery does not impair the ability to form associations between flavors and
intra-gastric stimuli, we investigated whether RYGB rats would form a LiCl-induced taste aversion, and found that this was indeed the case (water-conditioned vs. LiCl-conditioned, p<0.01, data not shown).

DISCUSSION

Increasing evidence suggests that RYGB and VSG are both metabolic surgeries, meaning that these surgeries influence metabolic outcomes in ways that are not fully explained by restriction and malabsorption alone. Our data support this hypothesis. If mechanical restriction of meal size was the sole reason animals reduce food intake, we would predict that rats would compensate for this by choosing the most calorically-dense food and thus maximize caloric intake. However, we observed exactly the opposite. In addition to decreasing intake of fat, the most calorically-dense macronutrient, VSG rats shifted their preference toward a less calorically-dense liquid diet, even when the relative proportions of macronutrient content were held constant. Furthermore, despite the anatomical differences between VSG and RYGB, both procedures resulted in the same changes in food selection. This suggests that a common underlying mechanism not involving direct manipulation of the intestine may be shared between VSG and RYGB.

Whether the changes in food choice may be secondary to changes in body weight remains unresolved. Although there are reports in humans that obese individuals have a greater preference for fat than lean individuals (Drewnowski et al., 1985; Drewnowski et al., 1992), it is not clear whether these taste preferences are a cause or consequence of obesity, and whether they may change with body weight gain or loss. In rodents, there are several reports that acute food restriction causes an increase in fat preference (Smith et al., 1997; Thouzeau et al., 1995; Welch
et al., 1994), but they do not address the condition of chronic weight loss. Further studies will be needed to understand whether weight loss itself contributes to the potent effects of VSG and RYGB to alter food preferences.

Food intake is a complex behavior that, while necessary to obtain energy, is subject to numerous non-homeostatic influences, including the rewarding value of the food itself. We hypothesized that decreased fat intake following VSG is due to an impaired motivational drive for fat-related food stimuli. However, in the progressive-ratio lever-pressing paradigm, in which the amount of food acquired is severely limited by the increasing difficulty of the task, we found no deficits in motivational responding. This suggested that perhaps aversive consequences associated with ingestion of larger amounts of fat may instead be the culprit. While our conditioned taste aversion results indicate that this is a possibility following VSG, RYGB rats did not form an aversion to the intra-gastric fat stimulus. These results are in contrast to recent published data indicating that RYGB-operated rats form a taste aversion to an intra-gastric infusion of corn oil (Le Roux et al., 2011). This difference may be due to disparate surgical RYGB procedures, as our procedure includes a much shorter alimentary limb (10 cm versus 50 cm) and a longer common channel (60 cm versus 30 cm), providing more distance during which biliopancreatic secretions mix with chyme, presumably favoring greater fat absorption in our model. Thus, although fat aversion may partially explain the decreased fat intake after VSG, it cannot be the only mechanism, since RYGB rats decrease their fat intake even in the absence of an aversion.

These results are surprising considering clinical reports that RYGB patients, but not VSG patients, experience the aversive symptoms including nausea and abdominal pain known as “dumping syndrome.” One possibility is that the paradigm we employed did not capture the
effects of the classical dumping syndrome, and that the conditioned aversion produced in the VSG rats is indicative of some other kind of gastro-intestinal distress that is not experienced by RYGB rats. Indeed, there is evidence that administering lipids directly into the proximal intestine of unoperated rats can cause an aversion (Ramirez et al., 1997). This would not happen in RYGB rats since the administered oil enters more distally in the intestine than in the VSG procedure, which maintains a normal pyloric-duodenal juncture. Another consideration is that our rat RYGB procedure maintains a proportionally larger gastric pouch (30% of initial volume) than the standard human procedure (10% of initial volume), and we cannot rule out that this difference may explain the lack of the expected fat aversion. Alternatively, separate mechanisms (aversion in VSG vs. an unknown mechanism in RYGB) could operate in the two procedures causing the same food choice changes.

Although altered food choice has been reported following RYGB and some other bariatric procedures (Ernst et al., 2009; Lindroos et al., 1996; Thirlby et al., 2006; Thomas and Marcus, 2008; Zheng et al., 2009), the physiology that underlies this phenomenon has not been identified. However, there are a number of possibilities. The first is altered hormone profiles, particularly those of glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and ghrelin. All three hormones have been reported to modulate intake of certain kinds of foods or the choice between foods, and each has been reported to be altered following VSG and RYGB. Post-prandial GLP-1 and PYY levels are greatly enhanced after both surgeries (Chambers et al., 2011a; Karamanakos et al., 2008; Peterli et al., 2009). One study found that peripheral administration of the GLP-1 receptor agonist Exendin-4 causes a relative increase in carbohydrate intake (Peters et al., 2001), and that it is more effective at reducing food intake following an intra-gastric preload of fat than of carbohydrate (Aziz and Anderson, 2002). Similarly, PYY injected into the paraventricular
nucleus of the hypothalamus was reported to stimulate carbohydrate intake to a greater degree than fat intake (Stanley et al., 1985). Ghrelin is another peptide hormone which several reports have linked to changes in food choice. One study found that centrally-administered ghrelin preferentially increases fat intake over carbohydrate intake (Shimbara et al., 2004), and another study demonstrated that ghrelin stimulates the intake of fat-rich palatable foods, whereas genetic or pharmacological inhibition of ghrelin causes a decrease in the intake of palatable foods (Egecioglu et al., 2010). Regarding bariatric surgery, ghrelin has been reported to decrease following both VSG and RYGB surgery (Li et al., 2009; Peterli et al., 2009), although controversy remains whether these changes are consistently observed in RYGB (Thaler and Cummings, 2009). While the literature on this topic is relatively sparse, each of these hormones has been linked to changes in food choice in normal rodents in a direction that may explain the effects of VSG and RYGB on food choice. However, variation in dose, route of administration (peripheral vs. central), and the specific types of foods used in the food choice studies makes it difficult to predict precisely if and how changes in GLP-1, PYY, and/or ghrelin may account for changes in food choice following bariatric surgery.

Changes in taste acuity and/or neuronal responses to food cues may influence food choice after bariatric surgery. Two studies have found that RYGB patients have enhanced taste acuity, especially for sweet tastants (Burge et al., 1995; Scruggs et al., 1994). Interestingly, when examined by functional magnetic resonance imaging, humans who underwent RYGB displayed reduced activation in mesolimbic reward areas, and this effect was more pronounced in response to high-calorie than for low-calorie foods (Ochner et al., 2011). Animal models will make possible a wider range of the experiments necessary to directly test this hypothesis.
Altered lipid handling by the gastrointestinal tract is another potential explanation for the changes in food choice following VSG and RYGB. Both surgeries decrease plasma triglycerides and increase plasma bile acids (Asztalos et al., 2010; Nguyen et al., 2006; Patti et al., 2009; Stefater et al., 2011; Zlabek et al., 2005). Plasma bile acids are an indicator of intraintestinal bile acid levels, which are involved in the processing of dietary fat. Recent work from our group indicates that in VSG this difference is likely due to decreased secretion of dietary lipid from the intestine. Several lines of evidence link lipid metabolism to food choice. First, inhibition of fatty acid oxidation by 2-mercaptoacetate (2-MA) selectively decreases fat intake relative to carbohydrate and protein (Singer et al., 1998), and this effect is believed to be mediated via vagal afferents (Ritter et al., 2000) originating in the small intestine (Langhans et al., 2011). Second, enterostatin, which is co-secreted with pancreatic co-lipase, and whose regulation is thereby coupled to lipid metabolism (Berger et al., 2004), also decreases fat intake relative to other macronutrients (Erlanson-Albertsson et al., 1991; Okada et al., 1991). Finally, genetic ablation of CD36, a fatty acid translocase expressed in both the intestine and lingual papillae, abolishes fat preference in a 2-bottle choice test (Laugerette et al., 2005).

Taken together, these studies indicate that lipid processing in the intestine results in signaling events to the CNS that contribute to food selection. However, the fact that one agent that inhibits fat utilization (2-MA) and two others that are positively associated with fat absorption or breakdown (enterostatin and CD36) all inhibit dietary fat intake creates a challenge for understanding both the relationship of lipid metabolism and food choice, and also how these effects translate to VSG and RYGB. While there remains considerable uncertainty, altered lipid handling provides a compelling alternative to restriction and malabsorption as a common mediator of a variety of effects caused by VSG and RYGB.
In conclusion, in contrast to our original hypothesis, VSG decreases the preference for high-fat or calorically-dense foods, and neither restriction nor caloric malabsorption can account for these effects. Despite the very different surgical manipulations, VSG and RYGB result in such remarkably similar changes in food choice that it suggests a common underlying mechanism. It is important to note that these changes in food choice are not crucial to the weight-loss effects of these surgical procedures given that weight loss occurs even when rats are maintained with access to only the single high-fat diet that caused their obesity in the first place. Nevertheless, these results demonstrate a compelling effect of VSG to alter ingestive behavior beyond its anorexic effect, and that this altered food choice does not require post-surgical dietary advice and counseling typically provided to human bariatric surgical patients. This highlights the important changes in signaling to the CNS that occur during these bariatric surgical procedures that result in profound changes in behavior. While further experiments will be necessary to delineate the key underlying physiological changes that result in altered signals to the CNS, understanding these mechanisms could lead to development of less invasive targeted therapies to enable more successful strategies to reduce body weight.
CHAPTER 3:

Vertical Sleeve Gastrectomy is Effective in Two Genetic Mouse Models of Glucagon-like Peptide-1 Receptor Deficiency
ABSTRACT

Glucagon-Like Peptide-1 (GLP-1) is a peptide hormone that is released from the gut in response to nutrient ingestion, and has a range of metabolic effects including enhancing insulin secretion and decreasing food intake. Post-prandial GLP-1 secretion is greatly enhanced following some bariatric procedures, including Vertical Sleeve Gastrectomy (VSG), and has been hypothesized to be responsible for reduced intake, weight loss and the improvements in glucose homeostasis. We tested this hypothesis using two separate models of GLP-1 receptor deficiency. We found that VSG-operated GLP-1r deficient mice responded similarly to wild-type controls in terms of body weight and body fat loss, improved glucose tolerance, food intake reduction and altered food selection. We conclude that increased GLP-1 receptor activity is not necessary for the metabolic improvements induced by VSG surgery.

INTRODUCTION

Glucagon-Like Peptide-1 (GLP-1) is a peptide hormone that is released from the L cells of the small intestine in response to nutrient ingestion. GLP-1 is most well known for its effect as an incretin, stimulating insulin secretion from pancreatic β cells, but also has a wide range of other effects, including the reduction of food intake and improvement of glucose homeostasis independent of its ability to augment insulin secretion. Long-acting GLP-1 receptor agonists are used as therapies for type II diabetes, and convey the additional benefit of weight loss in many patients (Drucker and Nauck, 2006).
Weight-reduction bariatric surgery is the most effective weight loss treatment, surpassing drug therapies and lifestyle interventions (Pories, 2008). Vertical Sleeve Gastrectomy (VSG) is a bariatric procedure that involves the removal of approximately 80% of the stomach along the greater curvature, creating a gastric “sleeve” connecting the esophagus and pylorus. Unlike the more commonly-performed Roux-en-Y Gastric Bypass (RYGB), VSG includes no intestinal manipulation, and does not re-route the flow of nutrients through the digestive tract. Both procedures induce loss of body weight and fat mass, and improve glucose tolerance in humans and in rodent models (Abbatini et al.; Karamanakos et al., 2008; Pereferrer et al., 2008; Peterli et al., 2009; Stefater et al.). We have also shown that VSG causes changes in ingestive behavior, including reduction of fat intake and the eating of smaller, more frequent meals. Multiple reports show that weight-reduction bariatric procedures such as VSG and RYGB cause greatly enhanced meal-stimulated GLP-1 secretion (Chambers et al., 2011b; Cummings et al., 2005; Peterli et al., 2009), leading to the hypothesis that GLP-1 action underlies many of the metabolic improvements caused by these surgeries.

GLP-1 has one known receptor (GLP-1r), and mice that lack this receptor (GLP-1r KO) are insensitive to the effects of exogenously-administered GLP-1. These mice have impairments in glucose homeostasis, but are resistant to diet-induced obesity (Ayala et al., 2010; Scrocchi et al., 1996). We tested the hypothesis that GLP-1 action is necessary for the effects of VSG by performing VSG surgery in two separate models of whole body GLP-1r deficiency, and examining their metabolic and behavioral outcomes.

METHODS
All procedures for animal use were approved by the University of Cincinnati Institutional Animal Care and Use Committee. Mice were maintained on a 12h/12h light-dark cycle with light onset at 1am.

**Experiment 1.** A cohort of C57B6 mice, used to determine post-prandial GLP-1 levels following VSG, were bred in-house from breeders purchased from Jackson Labs and maintained on a high-fat butter-based diet (Research Diets, New Brunswick, NJ, D12492; 60% fat; 5.24 kcal/g). Weight-matched mice received either VSG (n=5) or sham (n=7) surgery. At 12 weeks post-operatively, mice were fasted for 4 h in the middle of the light cycle prior to receiving a gavage of 200μl of Ensure Plus liquid diet. 15 min later, mice were placed briefly in a CO2 chamber and then sacrificed by decapitation. Trunk blood was collected in heparinized tubes, and plasma used for measurement of GLP-1 (total, Meso Scale Discovery, Gaithersburg, MD).

**Experiment 2.** GLP-1r wild-type (WT) and knockout (KO) mice (Scrocchi et al., 1996) were generated from homozygous breeding pairs, which were F1 offspring from GLP-1r heterozygote breeders. KO (n=31) and WT (n=24) mice were placed on a high-fat butter-based diet (HFD; Research Diets, New Brunswick, NJ, D03082706; 40% fat; 4.54 kcal/g) for 5 weeks starting at age 4-8 weeks, at which point each was subdivided into 2 body-weight and fat mass-matched groups (VSG and Sham) prior to gastric surgery. Post-operative deaths, mainly within the first week, yielded final group numbers of n=8 WT Sham, n=11 WT VSG, n=12 KO Sham, and n=16 KO VSG. The mice were maintained on high-fat diet after surgery except during the immediate post-operative period, and during diet choice testing, as noted. Body weight and food intake were measured by weighing the mice and their food hoppers daily or weekly. A continuous monitoring system (TSE Systems, Inc, Chesterfield, MO) was used to obtain detailed food intake data from Days 28-31.
Surgery. VSG surgery was performed using isofluorane anesthesia. The lateral 80% of the stomach was excised leaving a tubular gastric remnant in continuity with the esophagus superiorly and the pylorus and duodenum inferiorly. The sham procedure involved analogous isolation of the stomach followed by manually applying pressure with blunt forceps along a vertical line between the esophageal sphincter and the pylorus. Mice consumed liquid diet (Osmolite OneCal) for the first 4 post-operative days, and were re-introduced to solid food (high-fat diet) on Day 4.

Macronutrient Selection. Food choice was assayed from post-operative day 11-17 using a macronutrient selection paradigm, in which 3 pure macronutrient diets (Harlan Teklad; TD.02521 [carbohydrate], TD.02522 [fat], and TD02523 [protein]) were presented simultaneously in separate containers. The first 2 days were acclimation, and data from the final 4 days are presented. Due to diet spillage by some mice, analysis was limited to n=6 WT Sham, n=7 WT VSG, n=9 KO Sham, and n=12 KO VSG mice.

Body Composition. Magnetic resonance imaging was performed on Day 21 to determine body composition using a whole-body composition analyzer (EchoMedical Systems, Houston, TX).

Mixed-meal Tolerance Test. A mixed-meal tolerance test (MMTT) was performed on post-operative Day 18. Mice were fasted for 4 hours beginning 5 hours after the initiation of the light phase of the light-dark cycle. After a baseline blood sample was taken (0-min), 200 μl of Ensure Plus liquid diet (1.41 kcal/g; 29% fat; Abbott Nutrition, Columbus, OH) was delivered by intra-gastric gavage. Blood glucose was measured at 0, 15, 30, 45, 60, and 120 min after glucose administration on duplicate samples using Accu-chek glucometers and test strips (Roche, Indianapolis, IN). All blood samples were obtained from the tip of the tail vein of freely moving
mice. An additional 50 μl of blood was collected in heparinized tubes at time 0-min and 15-min to measure plasma insulin concentration. Blood was cold centrifuged, and plasma was stored at -80°C until insulin was assessed by ELISA (CrystalChem, Inc., Downers Grove, IL).

**Exendin-4 Challenge.** On Day 41, all mice were fasted for 4 h prior to the onset of the dark phase of the light-dark cycle (t=-4 h). Exendin-4 (400 μg/kg, ip) was injected at t=-1 h, and food was returned at t=0 h. Food intake was measured at t=2, 4, and 24 h. On the previous day, a procedurally identical experiment was conducted using an injection of ip saline. Data shown are the difference in food intake per mouse between Exendin-4 treatment and saline treatment.

**Experiment 3.** We generated a separate mouse model of GLP-1 receptor deficiency using a Cre-lox system. Briefly, we generated mice with loxP sites flanking exons 6 and 7 of the GLP-1 receptor gene, and crossed them with mice that express Cre Recombinase driven by the cytomegalovirus minimal (CMV) promoter. In CMV-Cre mice, deletion of loxP-flanked genes occurs in all tissues, including germ cells (Schwenk et al., 1995). Thus, mice that are homozygous for GLP-1r flox and hemizygous for CMV-Cre (GLP-1r flΔCMV) lack the GLP-1 receptor in all tissues. Littermates that were hemizygous for CMV-Cre and wild-type for the GLP-1 receptor (CMV-Cre) were used as controls. Gene expression of GLP-1r exons 6-7 was determined by quantitative PCR, and normalized to expression of L32. Experimental procedures, including surgery, mixed-meal tolerance test, and Exendin-4 challenge were conducted as described for experiment 2. Group sizes were: CMV-Cre Sham n=7 and VSG n=7, and GLP-1r flΔCMV Sham n=11 and VSG n=17. The mixed-meal tolerance test was performed 5 weeks after surgery, and the food intake response to Exendin-4 was conducted 10 weeks later.
Statistics. All data are presented as mean +/- SEM. Data were analyzed using the appropriate t-test, 2-way, or 3-way ANOVA. Where appropriate, Tukey’s post-hoc comparisons were used to determine pair-wise differences between groups. P<0.05 was considered significant for each of these analyses. Data were analyzed using GraphPad (Prism, San Diego, CA) and SigmaStat (Systat Software, Chicago, IL).

RESULTS

Experiment 1: Post-prandial GLP-1 in VSG-operated mice. Fifteen minutes after a mixed-meal gavage, VSG-operated mice exhibited much higher total plasma GLP-1 levels than sham-operated controls (Fig 3.1A, t-test, P = 0.0002), confirming that mice show a similar GLP-1 response to VSG surgery that has been shown in humans and rats.

Experiment 2: VSG surgery in GLP-1r KO mice. KO mice weighed significantly less than WT mice at the time of surgery (2-way ANOVA, main effect of genotype P<0.0001), which occurred following 5 weeks of exposure to high-fat diet. VSG induced weight loss in both WT and KO mice (Fig 3.1B, 3-way ANOVA, main effect of genotype), an effect that was sustained for the duration of the experiment. When expressed as a percent change in body weight (Fig 3.1C), WT Sham and KO Sham groups do not significantly differ, nor do WT VSG and KO VSG. Body composition analysis revealed that VSG caused loss of fat mass in both WT and KO animals, with KO mice having less fat mass than the corresponding WT group (Fig 3.1D, 2-way ANOVA, main effect of genotype p<0.001 and surgery p<0.001). Lean mass was unchanged by
the surgery, but lower in KOs than in WTs (Fig 3.1E, main effect of genotype p < 0.001). Percent change of fat mass was similar between WT and KOs (main effect of surgery p<0.001), and percent change of lean mass was similar among all groups (data not shown).

To verify that the GLP-1r KO mice had functional loss of GLP-1 receptors (in addition to genotyping by PCR), we challenged the mice with an ip injection of the GLP-1r agonist Exendin-4, which potently decreases food intake by acting on the GLP-1 receptor. WT mice had the predicted hypophagic response, decreasing their 24-hr food intake relative to a saline
injection, whereas GLP-1r KO mice were unresponsive to the treatment (Fig 3.1F, t-test P<0.0001)

Blood glucose was measured following oral administration of Ensure Plus liquid diet to assess glucose excursions in response to a mixed meal (Fig 3.2A). KO Sham mice had significantly elevated glucose excursions compared to WT Sham (p<0.001 at 45 min and 60 min), and VSG-operated mice of both genotypes had lower glucose levels than the sham-operated groups, and did not differ from each other (WT Sham vs. WT VSG p<0.01 at 30 min, KO Sham vs. KO VSG p<0.05 at 15-120 min). Fasting glucose (baseline for the mixed meal tolerance test) was significantly lower in VSG-operated mice as compared to sham-operated mice of both genotypes (2-way ANOVA of 0 timepoint, main effect of surgery P<0.0001). Baseline fasting insulin levels revealed an effect of both surgery (P=0.0068) and genotype (P=0.0006), with WT and Sham animals having higher insulin levels than KO and VSG counterparts (Fig 3.2B). At 15 min, WT and KO VSG mice increased insulin levels to a similar extent compared to sham controls (Fig 3.2C, main effect of surgery P=0.0012).

In the first 3 post-operative days, VSG-operated mice exhibited lower food intake than sham-operated mice of both genotypes (Fig 3.2D, 2-way ANOVA, main effect of surgery P<0.0001), and gradually increased their energy intake to control levels by the second post-operative week (data not shown). Three-day food intake was measured from Day 28-31 (Fig 3.2D), at which time KO mice ate less than WT mice (2-way ANOVA, main effect of genotype P=0.008), likely due to the decreased body mass of the KO animals. A macronutrient selection paradigm, in which mice were allowed to self-select their diet from pure macronutrient sources, was used to assess food choice during the second post-operative week (Fig 3.2E). VSG altered food choice in WT mice by decreasing fat intake (p<0.01), increasing carbohydrate intake
(p<0.01), and increasing protein intake (p<0.01), an effect that we have recently demonstrated in rats (Wilson-Perez et al., 2012). VSG had a similar effect in KO mice (fat P<0.001, carbohydrate p<0.01), but did not alter protein intake (3-way ANOVA surgery x diet P<0.001, genotype x diet P=0.208).

![Graphs showing metabolic effects of VSG in GLP-1r KO mice.](image)

**Figure 3.2. Metabolic effects of VSG in GLP-1r KO mice.** GLP-1r WT and KO mice that received VSG or Sham surgery underwent a mixed-meal tolerance test on post-operative day 18. Blood glucose (A) was measured following gavage of a standard liquid diet, and plasma insulin was determined at baseline (B) and 15 min (C). Food intake (D) was significantly lower in VSG-operated mice of both genotypes from day 0-3, but by day 28-31, there was no effect of surgery, and KO mice consumed less than WT controls. Food choice was assessed by macronutrient selection, showing that VSG mice of both genotypes decrease fat intake and increase carbohydrate intake (E). Data are represented as mean +/- SEM. *p<0.05, **p<0.01, ***p<0.001.

**Experiment 3: VSG surgery in a new model of global GLP-1r deficiency.** GLP-1r flΔCMV mice and CMV-Cre littermate controls were investigated for expression of the loxP-floxed segment of the GLP-1r gene (exons 6 and 7), and normalized to expression of L32. Compared to
CMV-Cre controls, GLP-1r flΔCMV mice had negligible GLP-1r expression in pancreas, lung, and hypothalamus (Fig 3.3A) (t-test, P<0.001 in all cases). A separate cohort of GLP-1r flΔCMV mice and CMV-Cre littermate controls were challenged with an ip injection of Exendin-4 to determine whether this model of global GLP-1r deficiency was truly insensitive to the hypophagic effect of a potent GLP-1r antagonist. Similar to the results obtained in Experiment 2, the control mice significantly decreased their food intake compared to a saline injection, whereas the GLP-1r flΔCMV mice showed no hypophagic response (Fig 3.3B, t-test P<0.0001).
VSG induced weight loss compared to sham-operated mice in both CMV-Cre and GLP-1r fl^{ACMV} mice (Fig 3.3C, 3-way ANOVA, main effect of genotype P<0.001 and surgery P<0.001), but there was no statistical interaction of genotype and surgery (P=0.326), indicating that VSG is equally effective in both genotypes.

The results of the mixed meal tolerance test (Fig 3.3D) are similar to the results obtained using the established GLP-1r KO mice (Fig 3.2A). VSG-operated mice of both genotypes exhibited a significant improvement in blood glucose excursions, and sham GLP-1r fl^{ACMV} mice tended to be more glucose intolerant than the sham controls (3-way ANOVA, main effect of surgery P<0.001 and genotype P=0.015, and surgery x genotype interaction P=0.679).

DISCUSSION

Here we show that mice lacking GLP-1r respond normally to VSG surgery in terms of body weight and body fat loss, improvements in glucose homeostasis, and altered food choice. Furthermore, VSG overcomes the pre-surgical glucose intolerance of the GLP-1r deficient mice, decreasing the glucose excursions to WT VSG levels. Given that GLP-1 has been widely hypothesized to be responsible for VSG-induced improvements in glucose tolerance, it is especially surprising that mice that lack GLP-1 action show a paradoxically greater magnitude of improvement than wild-type animals. Our data demonstrate that large magnitude improvements in glucose tolerance occur even in the absence of GLP-1r signaling, and therefore that GLP-1 action is not necessary to manifest the effects of VSG surgery.

These data are in contrast to our original hypothesis, and do not directly support the conclusions of several other studies that implicate GLP-1 in the outcomes of RYGB surgery. For
example, one study showed that meal-induced GLP-1 (and PYY) excursions are higher in RYGB patients with superior weight loss compared to those that did not achieve sufficient weight loss (le Roux et al., 2007). Moreover, the same study showed that somatostatin, which inhibits the release of those hormones from the intestine, increased food intake in RYGB but not in AGB patients (AGB patients did not have elevated GLP-1 or PYY responses). The implication is that more robust GLP-1 and PYY excursions directly reduce food intake and lead to body weight loss.

Three additional reports, including one from our own group, have used the GLP-1r antagonist Exendin-9 to demonstrate that surgery-induced improvements in glucose homeostasis can be attenuated when GLP-1 action is blocked. Kindel et al. demonstrated that Goto-Kakizaki rats that undergo Duodenal-Jejunal Exclusion exhibit improved glucose tolerance, but that the effect is abolished following a subcutaneous injection of Exendin-9 (Kindel et al., 2009). Similarly, we showed that Exendin-9 undermined the improvements in glucose tolerance of RYGB and VSG-operated diet-induced obese rats (Chambers et al., 2011b). Finally, a human study of weight-stable RYGB patients demonstrated that meal-stimulated insulin secretion, which is higher in RYGB compared to control subjects, was suppressed to a greater degree by Exendin-9 in those patients (Salehi et al., 2011).

The use of genetic knock-out models to study the role of GLP-1 in bariatric surgery offers clear advantages over a pharmacological approach. Exogenous administration of the GLP-1r antagonist Exendin-9 always carries several uncertainties, including the degree of GLP-1r blockade, potential non-specific effects of the antagonist, partial agonist activity, and the temporal period for which the receptor blockade is achieved. The genetic approaches used here provide unequivocal, long-term gene disruption in all tissues. A caveat of using whole-body
genetic knock-out models of any kind is the potential for developmental compensation. At least two cases of compensation in GLP-1r KO mice have been reported, indicating enhanced action of GLP-2 (Lovshin et al., 2001) and Glucose-dependent Insulinotropic Peptide (Pederson et al., 1998) in these mice compared to wild-type controls. We believe these examples underscore the body’s metabolic flexibility to appropriately regulate energy balance even in the absence of GLP-1 signaling, an effect which is maintained following VSG surgery. Thus, despite the potential caveat of developmental compensation, the use of targeted genetic loss-of-function models is a powerful approach to dissect the molecular pathways involved in the effects of VSG and other bariatric surgeries. Using two independent models of genetic disruption of the GLP-1 receptor, our data clearly demonstrate that GLP-1 receptor activity is not necessary for VSG exert its potent effects on a range of metabolic and behavioral endpoints. Hence, the primacy of GLP-1 as a key mediator of the pleiotropic metabolic benefits of bariatric surgery requires reconsideration.

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CHAPTER 4:

General Discussion
Advances in our understanding of bariatric surgery have clearly demonstrated that the traditional ideas of restriction and malabsorption are not adequate to classify or describe modern bariatric procedures. VSG, which is both less restrictive and less malabsorptive than RYGB, is among the most effective bariatric procedures, causing potent weight reduction and metabolic improvements in ways that are not explained by the restriction/malabsorption framework. Replacing this antiquated terminology is the new concept of “metabolic surgery” that highlights more global metabolic changes including improvements in glucose homeostasis, cardiovascular function, circulating lipids and cholesterol, and many others.

Not to be overlooked are changes in eating behavior. The studies described in Chapter 2 demonstrate for the first time the potent effect of VSG surgery to alter food choice, particularly by decreasing fat intake. These data provide another parallel between the effects of VSG and RYGB, suggesting that these two anatomically-distinct surgeries may share a common mechanism to induce their powerful effects. Furthermore, this effect on food choice (as well as many other effects of VSG and RYGB) is not shared with AGB, indicating that AGB is fundamentally dissimilar from the other two, and does not fall into the category of metabolic surgery.

When it comes to understanding the mechanisms by which bariatric surgery exerts its powerful effects, the data in Chapter 3 suggest that we know even less than we thought. Although widely hypothesized that GLP-1 is an important component of VSG and RYGB-induced metabolic improvements, we demonstrated that GLP-1 action is not necessary for those effects to manifest in VSG-operated mice. Despite what seemed like convincing evidence – GLP-1 secretion increases dramatically after VSG and RYGB, and the effects of GLP-1 parallel many of the effects of those surgeries – it appears that GLP-1 is not the answer to the
mechanistic question. The notion that any single hormone or other signaling molecule could be responsible for such a dramatic change in energy balance may have been short-sighted. An alternative hypothesis (as discussed on Page 86) is that changes in any of those individual components may be downstream of a more global adaptation of the digestive system in response to the surgical manipulations.

Altered food choice with bariatric surgery: What does it mean?

Many questions remain about the nature and the significance of altered food choice in bariatric surgery. One may ask, does altered eating behavior drive weight loss in these surgeries? Curiously, data from our rodent experiments indicate that the answer is definitely not. Even when rodents are maintained on a high-fat diet after surgery (incidentally, the same surgery that induced their obesity in the first place), and therefore not allowed to choose lower-fat foods, weight loss ensues. Another possibility is that, if food preferences don’t drive weight loss, then perhaps they are a consequence of it. In other words, leanness itself may cause a decrease in fat preference compared to the status of being overweight or obese. The validity of this remains unclear. It is true that obese individuals have a greater preference for high-fat foods than do lean individuals, but whether this is a cause or consequence of their obesity has not been determined. Furthermore, the possibility that these preferences may be modulated by weight loss or weight gain is also unknown.

A third possibility is that changes in food choice are a weight-independent effect of VSG and RYGB – a metabolic “side effect” that is unrelated to body weight loss. In support of this, fat preference in VSG-operated rats does not correlate with body weight loss. Among the range of
body weight and food choice responses in those rats (chapter 2’s “cohort A”), those two variables are not statistically related (Figure 4.1).

![Figure 4.1](image)

*Figure 4.1. VSG-operated rats show no correlation between body weight loss and fat preference. Fat preference is calculated as the percentage of total calories ingested as fat during a 4-day macronutrient selection test.*

However, the best evidence that body weight loss is not necessary for altered food choice comes from an experiment that actually failed its originally-intended purpose. Among the dozens of cohorts of sham and VSG-operated rats now produced by our lab’s surgical team, one experiment yielded aberrant weight loss data. Although weight loss occurred initially in the VSG rats as expected, after many weeks of behavioral testing, the body weight difference between groups had disappeared. The effect appears to be in the sham-operated animals, which did not gain weight as expected and, at 11 weeks after surgery, achieved an average body weight and fat mass which was not different than that of the VSG animals. The reason for this is unknown – whether it was a differential response to the behavioral testing or some other factor, but in the end it yielded an ideal opportunity to test whether altered food choice is body weight-dependent. The result was clear: VSG rats decreased fat intake compared to controls in a manner identical to the changes seen in other cohorts, and this difference occurred despite the fact that the experimental groups had the same body weight and fat content (Figure 4.2).
Figure 4.2. VSG rats display reduced fat intake in a macronutrient selection paradigm despite similar body weight and fat mass compared to sham-operated controls.

Finally, and perhaps most troubling, is the possibility that altered food choices may even be maladaptive to weight loss for bariatric patients. Data from a recent study from our group (Chambers et al, unpublished) indicate that when sham and VSG-operated rats were maintained on a high-fat liquid diet (Ensure), VSG rats consistently ate approximately 10% less than the sham controls. However, when they were switched to a low-fat chow diet, the difference in caloric intake between groups completely disappeared. At the same time, sham-operated rats lost body weight whereas VSG animals remained relatively weight stable. The rats were subsequently switched back onto the high-fat Ensure, and the trends reverted to their original patterns, with sham rats eating more and gaining more body weight. Thus, in this study, the maximal effect of VSG is apparent when those animals are consuming high-fat diet.

This is consistent with the idea that bariatric patients avoid eating fat because it causes gastrointestinal discomfort. Aversive symptoms may include nausea, vomiting, and the cluster of symptoms termed dumping syndrome. The aversive consequences of high-fat foods may drive patients to avoid them when other options are available, selecting instead lower-fat alternatives. However, when no alternatives are available, such as in the study just described, VSG rodents compensate by eating less of the high-fat diet, or rather failing to overeat in the manner of the
Figure 4.3. The effect of diet switch on food intake (A) and body weight (B) in sham and VSG-operated rats starting 100 days after surgery. Maximal differences between groups occur when maintained on high-fat Ensure diet compared to low-fat chow diet, and VSG rats are more resistant to diet-induced changes in feeding and body weight.

control animals. Thus, altered food choices may actually allow bariatric subjects to eat more than they would if they were constrained to only aversive high-fat foods.

What do these findings imply for the nutritional counseling of post-bariatric patients? First, they demonstrate that changes in food choice are not a result of compliance with the surgeon’s orders. Dietary counseling may help prepare patients for new eating habits, but our data suggest that their new altered physiology would likely lead them to the same endpoint regardless. As for what kind of diet is conducive to the greatest weight loss, our data unfortunately do not provide a sufficient answer to that question. If anything, data from the Chambers study described above (figure 4.3) suggest that VSG renders individuals more resistant to diet-induced changes in body weight, and therefore that the kind of diet may be of minimal importance. While the control animals adapted their food intake and body weight to the changing diets, the patterns of VSG rats remained relatively stable across the testing period. Thus, although the difference between VSG and control may be the greatest in the context of a high-fat diet, that difference is due to an effect of diet on the control animals rather than the
VSGs, which in free-living humans would never be constrained to the same diet choices. Furthermore, willfully-imposed constrained eating habits are extremely difficult to maintain in the long-term, and it is unlikely that a doctor’s advice to eat foods which are counter to the patients’ own preferences would be effective in the long term. Therefore, I would speculate that nutritional counseling for the purpose of maximizing weight loss in bariatric patients is both unnecessary and misguided.

Nutritional advice for maintaining general health is another matter. RYGB, and to a lesser extent VSG, are both associated with nutrient deficiencies, and supplements and/or diet counseling can help avoid those complications (Snyder-Marlow et al., 2010; Toh et al., 2009). Furthermore, excess fat intake is associated with cardiovascular disease (Shay et al., 2011), and should be avoided regardless of surgical status. Thus, advice to consume nutrient-rich foods and to avoid high-fat foods may be as valuable for bariatric patients as it is for the general population.

Limitations of the current literature on food choice

While food choice research is growing, particularly in the context of bariatric surgery, variations in methodology make comparisons between studies challenging. In human research, food choice is usually assessed by having patients maintain a food diary for a period of time, then analyzing their diet by categorizing foods in a number of ways. While total calorie intake and macronutrient intake can be calculated in a straightforward manner, there is no consensus for grouping foods into other categories. For example, ice cream is differentially classified in four
different studies as “dessert”, “sweets”, “milk and ice cream”, and “energy dense” (Ernst et al., 2009; Kenler et al., 1990; Ochner et al., 2011; Olbers et al., 2006).

Among the rodent literature, food choices are limited to a narrower range of options (often only two or three), and therefore categorization of foods is unnecessary. However, the content of those choices also varies widely from study to study. One of the most common paradigms used for assessing food choice is macronutrient selection (the choice between pure sources of carbohydrate, fat, and protein), which is why it was chosen for the studies performed in Chapter 2. However, this paradigm is also not standardized among research groups, and different or mixed sources of the macronutrients are reported. Furthermore, this paradigm has its drawbacks. First of all, the textural properties of the diets are different from each other (and unlike standard chow or other common foods), with the carbohydrate and protein diets presented as powders, and the fat diet in the form of Crisco-like “dough”. Secondly, some reports indicate anecdotally that rats do not readily accept casein (the most commonly-used protein source in these experiments) as a food source, and suggest methods for adapting the animals to the protocol (White et al., 1988). Finally, using only one source of each macronutrient type does not represent the free-living situation of either rodents or humans, and lacks the resolution to assess the effects of various sources of the individual macronutrients. In short, the ability to generalize the results from the macronutrient selection paradigm is suspect.

One study (Welch et al., 1994) addressed several experimental issues facing food choice research in rodents by assessing the food choice outcomes of several different stimuli (NPY, Norepinephrine, morphine, 24- or 48-hour food deprivation, and caloric restriction) using two different food choice paradigms: the three-choice macronutrient selection, and the two-choice high-fat diet versus high-carbohydrate diet. The authors also addressed another issue
confounding much of the food choice literature, which is the role of individual variation in baseline food preference, by thoroughly acclimating and assessing food choice in both paradigms before the testing period, then including those results as a covariate in their analysis. The results are enlightening in several aspects. First, baseline preferences significantly influenced outcome of all stimuli in the three-choice paradigm, and of four out of six stimuli in the two-choice paradigm. Secondly, the results of each stimulus across the two paradigms conflicted in three out of six conditions. That is, each of three stimuli increased fat intake in one paradigm, but decreased it in the other.

One approach to understanding the regulation of macronutrient selection is the so-called geometric framework proposed by Stephen Simpson (Simpson and Raubenheimer, 1999), which considers the regulation of each macronutrient in an integrated manner along with changes in body weight and body composition. In animals, this is usually tested by providing a series of two-choice tests with isocaloric diets varying in the relative ratio of macronutrients. While studied most extensively in insects, this methodology has also been applied to rodents (Sorensen et al., 2008) and more recently, humans (Simpson et al., 2003). Although this approach provides a more integrated understanding of macronutrient selection, it fails to consider a number of other aspects of food choice, including the source of macronutrients, palatability, dietary history, and sensory properties of the food, among others.

In summary, there are a multitude of factors that influence food choices that go far beyond nutrition, and nearly every investigator has taken a different approach to isolate or analyze a particular aspect of those choices. Although no one testing scheme can answer every question relating to food choice regulation, standardization is necessary. In human research, there is a major need for the adoption of a standardized classification of foods. This would not
prevent alternative categorization/analysis according to the investigator’s interests, and would facilitate comparisons between studies. In rodents, the adoption of one or two standardized procedures using diets of defined compositions would also represent a significant advancement for the same reasons. Similarly, adoption of a standardized paradigm does not preclude use of other hypothesis-specific testing procedures, and would bring the field to a common framework for investigating the biology of food choice.

The Role of Varied Surgical Technique in Bariatric Surgery Outcomes

Bariatric surgeries have surprisingly little standardization. Because surgeries, unlike drugs, are not regulated by the FDA or similar governing agency, surgeons have the liberty to perform each surgery as they see fit. Many investigative reports of bariatric surgery, for example those comparing the weight loss outcomes of RYGB and VSG, show conflicting results, with some reporting greater weight loss and metabolic improvements with RYGB, and others indicating no difference between the procedures or even a superiority of VSG. An open question is the degree to which surgical variations in one of both of those surgeries may influence outcomes.

RYGB, being a more complex procedure than VSG or AGB, has many sources of technical variation, which were recently reported in a survey of bariatric surgeons (Madan et al., 2008). As already mentioned, the length of the alimentary and biliopancreatic (intestinal) limbs is one source of variation. Approximately 10% of RYGB surgeries performed are classified as “long-limb” or “very long limb” (Buchwald and Oien, 2009), which refers to the comparatively

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longer bypassed biliopancreatic limb; however, even among “normal” RYGBs, there is no
consensus for standard limb length. Some surgeons even individualize the procedure for each
patient, using a formula to calculate limb length based on the patient’s BMI, although such
practice has not been validated.

Regarding the gastric pouch, it is in some cases completely transected and physically
separated from the remaining stomach, whereas in other cases it is merely divided by a staple
line. The division – transected or otherwise – may be accomplished by stapling (using a circular
or linear stapler), or hand-sewn using sutures. The pouch size varies, with an average of 25 cubic
cm, and a range of 1-250. The stoma size, which refers to the width of the junction of the gastric
pouch and new alimentary limb, also varies. Some surgeons carefully calibrate these dimensions
(limb length, pouch, and stoma size) using one of several techniques, whereas others merely
estimate. Finally, the manner in which the intestinal limbs are navigated around the large
intestine (antecolic versus retrocolic) and attached to the stomach (antegastric versus
retrogastric) is another source of variation, although most surgeries use the antecolic and
antegastric configurations.

Technical variations also exist in VSG surgery (Deitel et al., 2011), most notably in the
size of the newly-created gastric sleeve. Most VSG surgeons use a rubber tube called a bougie to
size the stomach, and use bougies that range in size from 28-60 French (unit of width). The
position of gastric resection relative to the pylorus also varies somewhat, as do methods for
ensuring the integrity of the staple line.

The effect of these various surgical techniques has been largely ignored. Many assume
that smaller stomach capacity leads to greater weight loss; however, the data do not bear that out,
with at least two studies reporting no correlation between weight loss and bougie size in VSG patients (Frezza et al., 2008; Parikh et al., 2008). Furthermore AGB, which yields the most restricted stomach capacity, induces the least amount of average weight loss compared to other surgeries. In RYGB, intestinal limb length has been reported to influence weight loss outcomes (with long alimentary limbs reported to cause greater weight loss) (Orci et al., 2011), although not all reports support this notion (Feng et al., 2003), and the length of the biliopancreatic limb and common channel are less clear (Savassi-Rocha et al., 2008).

While not detailed in most surgical methods, another source of variation that is relevant to all bariatric surgeries is the degree of enteric nerve damage. The stomach and intestines are densely innervated with both sensory and motor fibres, pertaining mainly to the vagus nerve. Vagal and spinal afferent nerve fibres are important for several aspects of gut-brain communication, including the response to gut hormones (such as GLP-1) (Abbott et al., 2005; Smith et al., 1985) and gastric distention, and contribute to the regulation of meal size and gastrointestinal transit time (Kissileff et al., 2003). Also, visceral afferents display morphological remodeling in response to physical insults (Phillips and Powley, 2005; Powley et al., 2005), suggesting that bariatric surgeries are likely to produce a reorganization of visceral networks as a result of both deafferentation and regeneration processes. RYGB almost certainly causes denervation of the bypassed stomach remnant, as the major vagal and sympathetic innervations are supplied through the gastroesophageal junction and left gastric artery (Berthoud and Neuhuber, 2000). VSG also induces some vagal denervation, at a minimum from the excised gastric fundus, but the extent and anatomical specificity differ from RYGB.

This issue was addressed recently by a group that examined the role of sensory innervation of the hepatic portal vein in the outcomes of a modified RYGB procedure in mice.
The authors used local treatment of capsaicin, which damages sensory nerves, and found that capsaicin treatment strongly attenuated the weight-loss and food intake effects of the surgery (Troy et al., 2008). These data suggest that preservation of those nerves is critical for the effects of at least some bariatric procedures. However, another group found that RYGB is equally effective in rats with and without partial vagotomy (transaction of the common hepatic brach) (Shin et al., 2012).

In summary, disruption and regeneration of enteric nerve fibers may play a role in the outcomes of bariatric procedures, but not all surgeries or surgeons cause the same degree of disruption, and these variations are among many other surgical variations that may contribute to disparate post-surgical outcomes in individual patients and across trials.

**Beyond GLP-1: new hypotheses for the mechanisms of VSG and RYGB**

In an effort to understand the role of gut hormones in bariatric surgery outcomes, these procedures have now been performed in several kids of genetic knock-out mice, including ghrelin, PYY, and now the GLP-1 receptor. As shown in Chapter 3, mice lacking the GLP-1 receptor respond normally to VSG surgery, as do mice lacking the orexigenic hormone ghrelin (Chambers et al, unpublished). PYY knock-out mice have also been studied in this capacity, using an adapted gastric bypass surgery the authors term “entero-gastro anastomosis”. These mice were only studied up to 10 days after surgery, at which time the PYY knock-out mice lost less weight compared to controls. Due to the short timeline and use of a different weight loss procedure, the role of PYY in bariatric surgical outcomes is still uncertain. What is clear is that VSG and RYGB induce a coordinated response in a variety of neuroendocrine factors. We
believe that the next advances in bariatric surgery research (and in energy balance research in general) will be understanding how the gastrointestinal (GI) system orchestrates this coordinated response, instead of looking at any one factor individually.

The gastrointestinal system has the complex task of delivering nutrients to the body while at the same time preventing the potential dangers of nutrient overload (Woods, 1991). On a chronic basis, elevated glucose, cholesterol, and other kinds of fats in the blood are risk factors for metabolic disorders including type 2 diabetes and cardiovascular disease, as well as stroke and some cancers (Danaei et al., 2006). Chronic hyperglycemia is also associated with increased stress and anxiety, impaired performance on neurological tests, and even impaired sexual function. On a more acute basis, elevated blood glucose is associated with an increase in reactive oxygen species, endoplasmic reticulum stress, DNA damage (Yang et al., 2005), and dysfunction of neurotransmitter metabolism, likely contributing to many of the long-term impairments already mentioned (Rowland and Bellush, 1989).

In the context of normal GI physiology, the body’s first defense against these dangers is by temporarily sequestering ingested nutrients in the stomach, then emptying them slowly into the intestine where they are gradually introduced into circulation. After this controlled release, the next task is to get those nutrients out of systemic circulation and safely stored in the tissues. This is accomplished in large part by insulin, as well as other GI hormones, that stimulate nutrient uptake by those tissues.

However, following VSG and RYGB, gastric storage capacity is greatly reduced and gastric emptying occurs almost immediately. A recent study by Sandoval et al (unpublished) compared gastric emptying in rats that received RYGB, VSG, or Sham surgery, and found that 5
minutes after a bolus of nutrients, nearly 100% had been emptied in the RYGB and VSG animals, whereas only 5% had emptied in the sham-operated controls. Hence, these procedures bypass the body’s first defense against nutrient overload, which is controlled gastric emptying. This renders the GI system less able to tolerate nutrient influx, resulting in a compensatory response (a coordinated change in neuroendocrine signals) aimed at decreasing food intake and preventing nutrient overload. Thus, the GI system becomes hypersensitive to nutrient influx.

Data from meal-feeding studies indicate that those compensatory responses can also be anticipatory, occurring before food ingestion. Rats whose intake is limited to one predictable four-hour period each day quickly learn to increase their intake during this period in order to maintain sufficient caloric intake. Like VSG/RYGB-operated rats, meal-fed animals have accelerated gastric emptying and improved glucose tolerance. Interestingly, plasma insulin and GLP-1 increase just before the scheduled meal time, an effect which theoretically should help clear the rapidly-delivered nutrients from circulation (Drazen et al., 2006; Vahl et al., 2010). Overall, there are a number of similarities between the effects of meal feeding and VSG/RYGB. In both cases, rats initially lose weight, then achieve and maintain a normal weight trajectory (which is significantly below that of non-operated or non-meal fed controls). Gastric emptying is profoundly accelerated in both cases, and glucose tolerance is improved. Finally, GI hormone responses are enhanced, although in meal-fed rats this has been studied mainly before meals, whereas in surgical animals, the data refer to post-meal responses.
Taken together, these data constitute strong evidence that accelerated gastric emptying is a primary event that induces many downstream effects of VSG and RYGB, including decreased food intake and body weight, and improved glycemic control. However, this interpretation has its challenges, specifically that a seemingly-maladaptive phenomenon, accelerated gastric emptying, could trigger so many beneficial responses that then outweigh the initial drawbacks. The answer may be that this whole series of events has a net benefit only in the context of nutrient excess, both internally and externally. Weight loss and improved glucose tolerance are only desirable when the starting point is excess weight and poor glycemic control, factors which each confer
detrimental consequences of their own. In a lean, healthy individual, weight loss and/or more rapid glucose clearance would have little significance; therefore accelerated gastric emptying would have only drawbacks. Furthermore, the adaptive response of eating smaller, more frequent meals is only possible when those meals are continuously available, something to which our physiology is not well-adapted, evolutionarily speaking. In summary, this author’s belief is that VSG and RYGB are beneficial in obese, glucose-intolerant individuals because of a favorable cost:benefit ratio of accelerated gastric emptying and its consequences in these individuals.

Figure 4.5 Theoretical model for the mechanisms of VSG and RYGB.
One question that remains unanswered is why accelerated gastric emptying, if it is indeed the primary effecter of the other consequences of VSG and RYGB, would cause a shift in food choice away from fat intake. One possibility is that fat itself is not being avoided, but rather the ingestion of calorically-dense foods. By shifting food intake toward less calorie-dense sources (carbohydrate and protein), a given volume of food would present a smaller nutrient influx, and therefore a smaller homeostatic challenge. Indeed, data from chapter 2 showing that VSG rats shift their preference toward a diluted liquid diet supports this hypothesis.

A major implication of this model is that recreating accelerated gastric emptying should recapitulate all the effects of VSG and RYGB. Surgical or pharmacological interventions that target pyloric function may be one way to test this hypothesis. Another possibility is to infuse nutrients directly into the duodenum, therefore bypassing the pylorus and simulating rapid gastric emptying, and quantify the effects on weight change and glucose tolerance. We would predict that chronic use of this technique would cause outcomes similar to VSG and RYGB.

**Conclusions from this research**

Overall, the data presented here highlight two important points. First, altered food choice is a true physiological response to VSG and RYGB surgeries. Not only do human patients change their food preferences – which could be related to a myriad of social, cultural, and lifestyle factors, but rats that undergo the same surgeries show a comparable effect. Moreover, the fact that food choice can be modulated so profoundly by any manipulation, surgical or otherwise, indicates that food choice is a regulated process. Thus, the body can sense and
differentiate food sources, alter its preference, and induce a behavioral response to manifest a change in ingested nutrients.

The second important point is that GLP-1 is not necessary for the effects of VSG, and more broadly, that it is unlikely that any single neural or endocrine factor underlies the broad range of effects induced by both VSG and RYGB. Instead, future research will determine the role of more global adaptations of the GI system, in particular the responses to accelerated gastric emptying, in the dramatic weight loss and health benefits conferred to bariatric patients.
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