University of Alberta

The Impact of Sleeve Gastrectomy as compared to Adjustable Gastric Band on Active and Total Ghrelin and other GI Hormones and their influence on Satiety and Hunger

by

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Abstract

Laparoscopic sleeve gastrectomy (LSG) is a relatively new bariatric procedure that has been proposed as a potential definitive treatment for morbid obesity. The mechanism of action of LSG remains unclear, but may include increased restriction to meal portion size, alterations in gastric emptying or increased satiety related to alterations in serum ghrelin (endogenous orexigenic hormone) or other active endogenous satiety hormones. Initial reports have suggested that LSG produces dramatic reductions in hunger and augmented satiety. However, the data on the effect of this procedure on satiety hormones and their influence on satiety and hunger are still limited. This project is a cross-sectional study of 30 matched obese patients following LSG (10), Laparoscopic Adjustable Gastric Banding (LAGB) and obese controls. Satiety hormones and satiety scores were analyzed after a standardized test meal. The result of this work showed that both active and total ghrelin were lower following LSG as compared to LAGB. Also, both PYY and GLP-1 were higher in the LSG cohort. These changes were associated with an improved satiety scores suggesting that LSG is a metabolic procedure that affects multiple gut hormones in a favorable way.

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List of Abbreviations

AGB	Adjustable gastric banding
AGRP	Agouti-related peptide
ARC	Arcuate nucleus
AUC	Area under the curve
BPD	Biliopancreatic diversion
BMI	Body Mass Index
BPDDS	Biliopancreatic diversion with duodenal switch
CCK	Cholecystokinin
CNS	Central nervous system
CV	Coefficient of variation
DPP	Dipeptidyl-peptidase
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EWL	Excess weight loss
GI	Gastro-Intestine
GHRH	Growth hormone releasing hormone
GHS-R	Growth hormone secretagogue receptor
GLP	Glucagon-like peptide
GOAT	Ghrelin-O-acyltransferase
HOMA	Homeostatic model assessment
LAGB	Laparoscopic adjustable gastric banding
LRYGB	Laparoscopic Roux-en-Y gastric bypass
LSG	Laparoscopic sleeve gastrectomy

NIH	National Institute of Health
NPY	Neuropeptide Y
PYY	Peptide YY
RIA	Radioimmune assay
RYGB	Roux-en-Y gastric bypass
SD	Standard deviation
SEM	Standard error of means
SG	Sleeve gastrectomy
STM	Satanderdized test meal
T2DM	Type 2 diabetes mellitus
VAS	Visual analogue scale

Chapter 1: Introduction

1.1. Introduction to Obesity

1.1.1. Definition, Epidemiology and the Economic Burden

Obesity is defined as an abnormal or excessive fat (adipose tissue) accumulation that presents a risk to health. Many sophisticated laboratory procedures have been applied to the direct measurement of body fat content in humans and experimental animals. These include compartmental analysis by measurement of the in vivo dilution of isotopes such as tritiated or deuterated water (1) and determination of cytoplasmic mass from the number of naturally occurring isotopes such as potassium-40 (2). Unfortunately, these methods are based on assumptions and equations that are not valid across the spectrum of body weight. The newer techniques that are now used such as dual x-ray absorptiometry (DEXA) and computed tomography (CT) or magnetic resonance imaging (MRI) scanning offer additional accuracy (3). Still, the most widely used estimator of obesity is simply the body weight. This method is a valid way of measurement of body "fatness". However, estimates are made more accurate by a consideration of height.

A simple mathematical manipulation of ordinary height and weight data is now generally used. The body mass index (BMI), (weight in kilograms)/(height in meters)^2 tends to be constant for individuals of a given degree of leanness or fatness within wide ranges of heights. Thus, a change in the weight/height^2 ratio is useful as a measure of fatness, independent of height. The World Health Organization (WHO) has classified BMI into categories that are meant to represent distinct levels of health risk (Table 1-1). A BMI of 18.5 to 24.9 is considered normal, 25 or more is considered overweighed, 30 or more obesity and 35 or more is considered severe or morbid obesity (8).

Classification	BMI (kg/m2)	Risk of comorbidities
Underweight	< 18.5	Low
Normal range	18.5 to 24.9	Average
Overweight	≥ 25	
Pre-obese	*25.0 to 29.9*	*Increased*
Obese class 1	*30.0 to 34.9*	*Moderate*
Obese class 2	*35.0 to 39.9*	*Severe*
Obese class 3	*40 or more*	*Very severe*

Table 1-1 WHO classification of obesity and risk for comorbidities. Adapted from Philip T. James, The Worldwide Obesity Epidemic. OBESITY RESEARCH Vol. 9 Suppl. 4 November 2001

Overall body fat distribution in obese persons is another factor of concern. Central distribution of adipose tissue (abdominal distribution) in either sex, characterized by an increase in the ratio of the waist to hip (W/H) circumference, or in the abdominal sagittal diameter, has been associated with an increased risk of medical complications (4).

Based on the World Health Organization classification of obesity (5), it has been shown that individuals in each obesity class are at increased risk of obesity-related illness as compared to those with a normal BMI (18.5-24.9) (6). Morbid obesity (class II or III), also referred to as "clinically severe obesity" or "extreme obesity," is estimated to afflict 20% of the obese population, or over 8 million in the US and 1.6 million in Canada. The relative rise of morbid obesity over an already exponential increase in obesity over the past 25 years can be characterized as an epidemic within an epidemic. Morbid obesity is the precursor of many diseases that affect essentially every organ system (table 1-2)

Cardiovascular

- Hypertension
- Atherosclerotic heart
- Peripheral vascular disease
- Myocardial infarction
- Cerebral vascular accidents
- Peripheral venous insufficiency
- Thrombophlebitis
- Pulmonary embolism

Respiratory

- Asthma
- Obstructive sleep apnea
- Obesity-hypoventilation syndrome

Metabolic

- Type 2 diabetes
- Impaired glucose tolerance
- Hyperlipidemia

Musculoskeletal

- Back strain
- Disc disease
- Weight-bearing osteoarthritis (hips, knees, ankles and feet)

Gastrointestinal

- Cholelithiasis
- Gastroesophageal reflux disease
- Nonalcoholic fatty liver disease
- Hepatic cirrhosis
- Hepatic carcinoma
- Colorectal carcinoma

Urologic (stress incontinence)

Urologic

- Stress incontinence

Endocrine and reproductive

- Polycystic ovary syndrome
- Increased risk of pregnancy and fetal abnormalities
- Male hypogonadism
- Cancer of the endometrium, breast, ovary and prostate

Dermatologic

- Intertriginous dermatitis

Neurologic

- Pseudotumor cerebri
- Carpal tunnel syndrome

Psychologic

- Depression,
- Eating disorders
- Body image disturbance

Table 1-2 The impact of obesity on different body systems

In the United States, It has been estimated that obesity is responsible for nearly 300,000 deaths each year (7). As body weight increases, there is a curvilinear increase in mortality mostly attributable to increased deaths from heart disease, diabetes, hypertension, and cancer.

The percentage of Canadians who are overweight or obese has risen dramatically in recent years, mirroring a worldwide phenomenon (8). The 2004 Canadian Community Health Survey found that 23% of Canadians aged 18 or older (an estimated 5.5 million adults) are obese. In Alberta, 25% of the population are obese and 3% are morbidly obese (9).

There have been multiple studies looking at the cost of obesity in North America (10). In the United States, health care cost directly attributable to obesity amount to approximately $68 billion per year, and an additional $30 billion per year is spent on weight reduction programs and special foods (11). In Canada, the 2004 Canadian Community Health Survey (CCHS), estimated the total cost of obesity (direct and indirect cost) at $4.3 billion annually (9). In Alberta, The Cost of Obesity in Alberta Report, estimated total costs (direct and indirect) attributable to overweight and obesity (classes 1, 2, and 3), during the year 2005 totaled $1.27 billion ($2005) (12).

1.1.2. Obesity, Genetics, and Pathophysiology

The etiology of obesity is complex. Many studies have evaluated the contribution of genetics and environment in the development of obesity. Genes are currently thought to explain 25%–40% of the difference in BMI. Environmental factors are responsible for the other 60% to 75% (13). In a given population, certain individuals appear to be born with a genetic predisposition to obesity. However, not every genetically predisposed person develops obesity, with the genotype expressed only under certain adverse environmental conditions, such as high-fat diets and sedentary lifestyles (14).

The development of obesity occurs when the caloric intake is disproportionate to the energy expended. Body weight is regulated within a narrow, individualized range by a process known as “energy homeostasis”. This process precisely matches overall energy intake and expenditure over long periods (15). This is done through multiple pathways which incorporate mechanical, neural, and humoral signals that arise from the gut and are relayed to the brain primarily via the hypothalamus and brainstem.

Peripheral signals involved in regulation of body weight and ingestive behavior are often categorized as long-acting adiposity signals and short-acting gastrointestinal (GI) factors. Long-acting signals characteristically reflect the levels of energy stores and regulate body weight and the amount of energy stored as fat over the long term. Short acting gastrointestinal signals are characterized by gut hormones and mechanical factors, such as gastric distension, which characteristically relay a sense of "fullness" resulting in postprandial satiation and meal termination (16)

The adiposity "negative feedback model" of energy homeostasis is founded on the premise that circulating signals inform the brain of changes in body fat mass and that in response to this input, the brain mounts adaptive adjustments of energy balance to stabilize fat stores (17). These negative-feedback signals should fulfill established criteria for a hormone contributing to long term body-weight regulation. These criteria include the following: circulating levels are found in proportion to body fat content, its ability to cross the blood brain barrier, ability to interact with receptors in well-characterized hypothalamic and brainstem centers of body weight control, and exerts long-acting catabolic effects by decreasing food intake and increasing energy expenditure (18). The blockade of the central neuronal actions of these signals will increase food intake and body weight. Although many nutrients (free fatty acids and glucose) and hormones (glucocorticoids) fulfill some of these criteria, only leptin and insulin satisfy all of them (15).

Leptin is a peptide hormone mainly produced by adipose tissue. It is a product of the *ob* gene, which was first identified and cloned in a severely hyperphagic and obese strain of mutant mouse. Reduction in leptin levels as a result of weight loss is associated with increased hunger in humans (19). Leptin has pleiotropic effects on food intake, hypothalamic neuroendocrine regulation, reproductive function, immune function and energy expenditure. Leptin circulates in

proportion to body fat content, crosses the blood brain barrier, interacts with receptors in well-characterized hypothalamic and brainstem centers of body weight control, and exerts long-acting catabolic effects by decreasing food intake and increasing energy expenditure (20). Insulin is also an afferent signal that circulates in proportion to adipose tissue mass and exerts many central actions similar to those of leptin. Insulin act as anabolic hormone in the periphery while it functions as a catabolic hormone in the central nervous system (CNS) (21). To regulate overall food intake, long-acting adiposity signals ultimately affect the number and size of individual meals. They, therefore, influence decisions regarding when to start and stop eating.

1.2. Fundamentals of Appetite Regulation

1.2.1. Hunger, Satiety and Satiation

Hunger is a sensation experienced when one feels the physiological need to eat food (also known as the homeostatic hunger). The sensation of hunger and the decision to start a meal involve complex and poorly understood interactions between genetic, social, learned, environmental, circadian, and humoral signals (22, 23). While multiple endogenous peptides have been recognized with the ability to stimulate feeding, a unifying, physiological explanation for the experience of hunger and the decision to commence eating is still lacking. However, the current thinking is that most meals are initiated at times that are convenient or habitual and thus are based on social or learned factors as opposed to adjustments of energy within the body. Because of this, the regulatory control over food intake is manifest over how much food is consumed once a meal begins rather than on when the meal will be initiated (24), and this is generally associated with the phenomenon of satiety.

The control of energy intake is vital to energy balance, and satiation and satiety are part of a complex system of appetite control, which regulates how much we consume. “Satiation” refers to processes that promote meal termination, thereby limiting meal size (25). "Satiety" refers to the feeling of fullness that persists after eating, potentially suppressing further energy intake until hunger returns. Satiation and satiety result from a coordinated series of neural and humoral signals that originate from the gut in response to the mechanical and chemical properties of ingested food.

Satiation and satiety is a multi-staged process. Initially, sensory and cognitive factors including expectations about what is to be consumed, the taste, texture and smell of the food or drink, play important roles (26). Numerous studies have shown that the food consumed during a meal is positively related to the sensory pleasantness of the food (27). Apart from that, humans eat more when meals include a variety of foods than when meals contain a single food (28). This phenomenon is caused by sensory-specific satiety, which was defined by Rolls et al (29, 30) as a greater decrease in the pleasantness of an eaten food than in the pleasantness of an uneaten food. Sensory-specific satiety can be conceived as an important driver for meal termination and the variety in food choices that humans make from meal to meal and from day to day (28, 31).

Ingested food induces satiation by two primary effects on the GI tract — gastric distention and release of peptides from enteroendocrine cells. GI signals that influence the brain to stop an ongoing meal are collectively called satiety signals. Satiation signals arise from multiple sites, including the stomach, proximal small intestine, distal small intestine, colon, and pancreas. Although most GI-generated signals (Table 1-3) that influence meal size cause less food to be eaten, consistent with the term *satiety signals*, an exception has recently been discovered. Ghrelin, a peptide hormone mostly made in the stomach, will be discussed later.

Peptide	Cell Type	Effect on food intake
CCK	Proximal intestinal I cells	↓
Amylin	Pancreatic b cells	↓
GLP-1	Distal-intestinal L cells	↓
PYY (3-36)	Distal-intestinal L cells	↓
APO-AIV	Villus epithelia	↓
PP	Pancreatic F cells	↓
Enterostatin	Exocrine pancreas	↓
Oxyntomodulin	Distal-intestinal L cells	↓
Ghrelin	X/A-like cells	↑

Table 1-3 Gastrointestinal Hormones That Affect Satiety. Adapted from Strader AD, Woods SC. Gastrointestinal hormones and food intake. Gastroenterology 2005; 128: 175-91.

1.2.2. Gastric satiation signals

When food or drink reaches the stomach, nerves communicate an increase in gastric volume to the brain (32). The stomach is densely innervated by sensory vagal and splanchnic nerves. It appears that gastric distension promotes satiation, independently of nutrient content (33). The stomach wall is provided with discrete neural sensors of tension, stretch, and volume. Output from these mechanoreceptors is relayed to the brain by vagal and spinal sensory nerves

using a complex array of neurotransmitters and neuromodulators, including glutamate, acetylcholine, nitric oxide, calcitonin- gene-related peptide, and substance P (32).

Experiments from animal models demonstrate that animals overeat with voluminous meals if food is drained from their stomach (through cannula) as they eat (sham feeding) (34). Also studies using animal models involving cuffs that can reversibly close the pylorus and prevent passage of food downstream, demonstrate that major gastric distention alone is sufficient to terminate ingestion. However, the amount of food required for this termination of ingestion exceeds that eaten in a typical meal (32). This can be explained as normal postprandial gastric distention does contribute to satiation when acting in concert with pregastric and postgastric stimuli. Oral and gastric stimuli happen concurrently during eating, and up to 40% of a meal empties into the intestine before meal termination (35). Therefore, pregastric, gastric, and intestinal satiation signals commence almost simultaneously, and they function simultaneously, augmenting each other's satiating effects.

Although the stomach can sense nutrients (for example, to regulate gastrin release), this does not seem to contribute to satiation. This conclusion came from animal studies where researchers filled a cuffed stomach with loads of isotonic saline (2.5–10 ml) and they found volume-related reductions of intake equivalent to comparable loads of liquid diet. In addition, they found that varying nutrient concentration, osmotic concentration or pH, also had no effect on the reduction of intake, independent of the volume (36, 37).

1.2.3. Intestinal satiation signals

Intestinal satiation is nutritive, and there is a limited role for intestinal distension in regulating satiety (36). Intestinal nutrient infusions reduce food intake in many species, including humans. Many intestinal satiation signals inhibit gastric emptying, and this probably helps limit ingestion by enhancing gastric mechanoreceptor stimulation. Mediators of intestinal satiation include many gut peptides that are secreted from enteroendocrine cells in response to ingested food. These mediators diffuse through interstitial fluids to activate nearby nerve fibers and/or enter the bloodstream to function as hormones. In conjunction with gastric distention, satiation peptides produce the perception of GI fullness, promoting meal termination.

Cholecystokinin (CCK)

The gut hormone cholecystokinin (CCK) appears to be involved in satiation. CCK is mainly synthesised by the endocrine L cells in the duodenum and jejunum (38). It is rapidly released into the circulation in response to the presence of nutrients in the gut. Specifically, it is stimulated by fat and protein in the chyme (39). CCK's effects on satiety appear to be mediated via receptors in the vagus nerve and are blocked when this nerve is removed in rats (40). CCK elicits multiple effects on the GI system, including the regulation of gut motility, contraction of the gallbladder, pancreatic enzyme secretion, gastric emptying, and gastric acid secretion.

Glucagon-Like Peptide-1 (GLP-1)

Glucagon-like peptide-1 (GLP-1) is derived from posttranslational modification of the larger precursor molecule proglucagon. Proglucagon is synthesized within the enteroendocrine L cells in the intestines, primarily the ileum and colon. GLP-1 is secreted as GLP-1 (7-37) and

GLP-1 (7-36) However, within a very short time (around 2 minutes), most plasma GLP-1 is degraded by the enzyme dipeptidyl-peptidase IV (DPPIV), yielding the inactive analogs GLP-1 (9-36) and GLP-1 (9-37) (41). Ingested nutrients, especially fats and carbohydrates, stimulate GLP-1 secretion by indirect, duodenally activated neurohumoral mechanisms, as well as by direct contact within the distal intestine. The GLP-1 receptor (GLP-1R) is found in the periphery (gut and endocrine pancreas) and is widespread throughout the central nervous system.

To understand how GLP-1 works, we have to recognize the "ileal brake" phenomenon whereby ingested food activates distal-intestinal signals that inhibit proximal GI motility and gastric emptying (42). It is mediated by neural mechanisms and this is probably also the mechanism by which GLP-1 exerts its effect on appetite (43). In addition to engaging the ileal brake, GLP-1 also induces anorexia. The anorectic actions of GLP-1 are probably mediated through both peripheral and central mechanisms, and a population of neurons that synthesize GLP-1 is located in the brainstem and projects to hypothalamic and brainstem areas important in the control of energy homeostasis. GLP-1 is also one of the incretin hormones that acts on pancreatic β-cells to accentuate growth of these cells and glucose-dependent insulin release. Also, GLP-1 acts on pancreatic α cells to inhibit glucagon secretion (44).

Oxyntomodulin

Like GLP-1, oxyntomodulin is a proglucagon-derived peptide secreted from distal-intestinal L cells in proportion to ingested calories. It reduces food intake and although a specific receptor for oxyntomodulin has yet to be identified, it has been suggested that oxyntomodulin exerts its anorectic effects through the GLP-1R. This is supported by the finding that

oxyntomodulin does not alter feeding in GLP-1R-deficient mice, and the GLP-1R antagonist exendin 9–39 blocks oxyntomodulin-induced anorexia (45, 46).

Peptide tyrosine-tyrosine (PYY)

Peptide tyrosine-tyrosine (PYY) is a member of the pancreatic polypeptide (PP) family that also includes PP and neuropeptide-Y (NPY). PYY is produced mainly by distal-intestinal L cells, most of which also secrete GLP-1. PYY is secreted as PYY (1-36) and is degraded to PYY (3-36) by Dipeptidyl Peptidase (DPP)-IV. It is secreted postprandially in proportion to caloric load, with a macronutrient potency of lipids being greater than that of carbohydrates, which is greater than that of proteins (47). Members of the PP family mediate their effects via Y-receptors. Five Y-receptors (Y1, Y2, Y4, Y5 and Y6) mediate the effects of PYY, NPY and PP. These receptors differ in distribution and function and are classified according to their affinity for PYY, NPY and PP (48). Whereas, PYY1–36 binds to all known Y-receptor subtypes, PYY3–36 shows high affinity for the Y2-receptor subtype and some affinity for the Y1-receptor and Y5-receptor subtypes (49). As with GLP-1, postprandial secretion is biphasic, initially stimulated by atropine-sensitive neural projections from the foregut, followed by direct nutrient stimulation in the hindgut (50). Studies in human and animal models on PYY have shown paradoxically both anorexic and increasing food intake effects. To explain this, a model was formulated, based on Y receptor subtype selectivity and accessibility. PYY1–36 activates all Y receptors, and it acts as an orexigenic hormone from its interactions with Y1R and Y5R, which are expressed in the hypothalamic paraventricular nucleus. This was proved by attenuation of the feeding effects of central PYY in both Y1R-deficient and Y5R-deficient mice (51).

PYY3–36 selectively activates Y2R and Y5R and circulating PYY3–36, was hypothesized to gain access selectively to Y2R in the hypothalamic arcuate nucleus. Y2R act as a presynaptic autoinhibitory receptor on orexigenic neurons. Therefore, the model proposes that circulating PYY3–36 reduces food intake by inhibiting the effect of orexigenic neurons through Y2R, and consistent with this model, the feeding effects of PYY3–36 are abolished by pharmacologic or genetic blockade of Y2R (52).

Despite these findings supporting a hypothalamic mechanism of action of peripherally administered PYY3–36, Y2R is also expressed by vagal-afferent terminals, and some investigators hypothesize vagal mediation. Supporting this assertion, anorectic effects and arcuate neuronal activation elicited by peripheral PYY3–36 were eliminated by either subdiaphragmatic vagotomy or transection of hindbrain-hypothalamic pathways (53).

1.2.4. Pancreatic Satiation Peptides

Pancreatic Polypeptide (PP) and Amylin

PP is produced in specialized islet cells under vagal control, and its secretion is stimulated postprandially in proportion to caloric load (54). Acting primarily on peripheral and central Y4R and Y5R, it influences biliary and exocrine pancreatic function, gastric acid secretion, and GI motility. The role of PP in energy homeostasis is controversial, in part because peripheral administration decreases feeding, whereas central administration increases it. Circulating PP decreasing food intake through Y4R while, central PP increasing it through Y5R deeper in the brain.

Amylin, a peptide co-secreted with insulin postprandially by pancreatic β-cells, inhibits gastric emptying, gastric acid, and glucagon secretion. It can also decrease meal size and food intake after peripheral or central administration (55).

1.2.5. Integration of Satiety Signals with Adiposity Signals

For the satiety control system to be maximally efficient, the information from satiety signals must be integrated with signals related to numerous other factors including learning, the social situation, stress, and signals indicating total body fat. An example of this integration, the inhibitory signals related to body fat and meal ingestion can easily be overridden by environmental events. This pattern can be seen when satiety signals might indicate that no more food should be eaten during an ongoing meal, however, the sight, smell and perceived palatability of an offered dessert can stimulate further intake. Similarly, although an individual is severely underweight and plenty of food is available, the influence of stressors can prevent significant ingestion.

Another important integration occurs when the adiposity signals (insulin and leptin) act by changing the sensitivity to satiety signals. This can be seen when administration of insulin or leptin enhance central sensitivity to input from short-acting peripheral satiation signals, such as CCK (15). When an individual gains excess weight, more insulin and leptin are secreted and consequently stimulate the brain, rendering CCK more efficacious at reducing meal size (56). Another example is the effect of adiposity signals on GLP-1. Leptin and insulin receptors are expressed on L cells (which is resposible for the secretion of GLP-1), and activation of these receptors augments GLP-1 secretion. On the other hand, and similarly to what occurs in the hypothalamus, L cells display diet-induced leptin and insulin resistance, with diminished GLP-1

release. These findings suggest that long- and short-acting anorectic signals cooperate at the level of gut-peptide secretion (57).

Similar interactions occur at the level of vagal sensitivity to gut peptides. The leptin receptor is co-expressed with the CCK receptor at the vagal-afferent nerve terminals in the stomach and duodenum. CCK activation of vagal sensory neurons from these regions is enhanced by leptin, and the two peptides function synergistically to increase discharge of vagal afferent fibers (58), just as they potentiate the anorectic actions of each other. Therefore, the enhancement of CCK-induced duodenal vagal-afferent signaling by leptin might reflect a long-acting adiposity hormone (adipocyte leptin) increasing peripheral neural sensitivity to a short-acting GI satiation factor (16).

1.3. Ghrelin: A Distinctive Orexigenic Hormone

1.3.1. Discovery, Structure, Collection and Measurement

Anterior pituitary growth hormone (GH) secretion was initially believed to be stimulated by GH-releasing hormone (GHRH) and inhibited by the hypothalamic hormone, somatostatin. The discovery of ghrelin (from "ghre" in the Proto-Indo-European language meaning "grow," and the suffix "relin" as in "release"), originally reported by Masayasu Kojima and colleagues in 1999, a natural ligand for the growth hormone secretagogue receptor (GHSR), established a novel independent pathway in the regulation of GH release (59). Although ghrelin was initially discovered as an endogenous ligand, subsequent reports showed that it powerfully increases food intake and body weight. This discovery shifted much of the focus of research on this new peptide to its roles in energy homeostasis.

The human ghrelin gene is located on chromosome 3p26-p25, encoding a 117 amino acid peptide termed preproghrelin. Ghrelin is a 28-amino acid peptide cleaved from preproghrelin. The peptide undergoes a post-translational modification in which the enzyme ghrelin O-acyl transferase (60) acylates the serine-3 residue by a medium-chain fatty acid, typically octanoic acid, through an ester bond. This modification is crucial for ghrelin's physiological effects as it is required for the peptide to bind to and activate its classical receptor, the GH secretagogue receptor (GHS-R1a). Consequently, most biological actions of ghrelin, especially those involving endocrine and anabolic effects, require acylated (active) ghrelin. The stomach can only acylate ghrelin with medium-chain, and not with short- or long-chain fatty acids. Because humans do not synthesize medium-chain fatty acids, octanoic acid from dietary sources is probably used for ghrelin acylation (61). Nonacylated ghrelin (also known as des-acyl ghrelin), which is present in human serum in far greater quantities than acylated ghrelin (62), seems to be devoid of any endocrine action. The inactive form does not displace radiolabeled ghrelin from its hypothalamic or pituitary binding sites (63) and has no GH-releasing or other endocrine activities in rat (59, 64). In man also, the administration of des-acyl ghrelin does not induce any change in the hormonal parameters or in glucose levels, indicating that at least in humans nonacylated ghrelin does not possess endocrine activities of human acylated ghrelin (65). Whether the inactive form has physiologic roles is a controversial question. Few papers showed that it does exert some nonendocrine actions including cardiovascular and antiproliferative effects, probably by binding different GHS-R1 subtypes or receptor families (66, 67).

The stomach is the principal site of ghrelin synthesis. The gastric fundus, which is the main source, produces approximately ten times more of the hormone per gram of tissue than does the next richest site, the duodenum. Lesser concentrations are present throughout the small

intestine, generally diminishing with increasing distance from the pylorus (68). The impact of gastrectomy or extensive small-bowel resection on plasma ghrelin levels suggests that at least 3/4 of circulating ghrelin is secreted by the stomach, and roughly the remaining 1/4 arises from the small intestine. Within the stomach, ghrelin is produced by enteroendocrine cells in the oxyntic mucosa that are known as "X/A-like cells". Most of these cells are "closed", meaning that they appose the basolateral membrane adjacent to the vascular supply but do not directly contact the gastric lumen. More distally in the intestine, ghrelin cells become increasingly "open", contacting both the intestinal lumen and blood vessels (69). Minor amounts of ghrelin are produced in diverse additional tissues, often detectable only by polymerase chain reaction (RT-PCR), these sites include the lungs, pancreatic islets, gonads, adrenal cortex, placenta, kidney, brain, and more.

At room temperature, acylated ghrelin is very unstable. Samples must be collected in ethylenediaminetetraacetic acid (EDTA) -aprotinin treated chilled tubes and centrifuged within 30 minutes from blood collection (70). Aprotinin is a protease inhibitor that will prevent the loss of acylation of ghrelin while the plasma is stored. Furthermore, for the preservation of plasma ghrelin it is recommended that one mol/L HCL be added. Unlike acylated ghrelin, des-acyl ghrelin is relatively stable and is not degraded by different storage temperatures. On review of the literature, there are inconsistencies noted in regards to the best method of measuring ghrelin levels. Most studies utilize commercially available radioimmunoassay (RIA) kits. These assays used Iodine 125-labeled ghrelin as a tracer and polyclonal antibodies against either the C-terminal (total ghrelin) or the N-terminal (acyl ghrelin). However, measurement techniques using RIA were found to be inconsistent when compared with measurements using enzyme-linked

immunosorbent assay (ELISA) (71). Additionally, ghrelin concentrations measured by different kits can vary by as much as 10-fold (72).

1.3.2. Roles of Ghrelin In Short-Term Meal Initiation

Most ghrelin is produced by the stomach and duodenum, positioned to detect recently ingested food. Ghrelin's orexigenic actions are extremely rapid and short-lived, as required for a signal influencing individual meal related behavior (73). Studies from animals and humans have shown that exogenous ghrelin injections affects meal patterns by decreasing the latency to feed, leading to one extra bout of eating shortly after ghrelin administration. Ghrelin also stimulates GI motility, gastric acid secretion, and pancreatic exocrine secretion (74, 75), all of which increase in anticipation of meals, preparing the GI tract for effective transport and processing of food.

The hypothesis that endogenous ghrelin is a physiologic meal initiator predicts that circulating levels should rise before, and fall after, every meal, and that peak concentrations should be sufficiently high to stimulate appetite. Ghrelin levels increase before meals to values that have been shown to stimulate appetite and food intake when generated by peripheral ghrelin administration in humans and rodents (74), suggesting that these levels are sufficient to play a physiologic role in normal pre-meal hunger. The level of postprandial ghrelin suppression is proportional to ingested caloric load. However, the recovery of plasma ghrelin levels is not a critical determinant of inter-meal intervals in healthy individuals (76). In healthy subjects, a longer fasting period during the day (i.e. irregular meal pattern typical for several eating disorders) increases ghrelin concentration, but does not affect postprandial ghrelin response to a mixed meal (77). Ghrelin suppression in healthy individuals depends on the macronutrient content of meals (78).

Surprisingly, prandial ghrelin suppression does not require luminal nutrient exposure in the stomach or duodenum, the principal sites of ghrelin production (79). Instead, signals mediating this response originate farther downstream in the intestine and from post-absorptive events. Contributors include changes in plasma insulin, intestinal osmolarity, and enteric neural signaling, whereas gastric distension, the vagus nerve, and GLP-1 are not required (80)

Ghrelin also acts on the CNS. The most clearly documented effect is mediated via central mechanisms located in the arcuate nucleus (ARC) of the hypothalamus. It has been shown that intracerebroventricular injection of ghrelin leads to a significant increase of neuronal activity within ARC as well as in the paraventricular nucleus (PVN), dorsomedial nucleus of the hypothalamus (DMH), in lateral hypothalamic areas (LHA), in the nucleus of the solitary tract (NTS), and in the area postrema (AP) (81). Furthermore, after intravenous ghrelin injection an increase in neuronal activity in the ARC, PVN, as well as in the NTS and AP (82) or activity within ARC, NTS, and AP but not in the PVN and DMH (83) has been reported. Although the complete central mechanism of action remains to be explained, it is well established that the orexigenic effect of ghrelin is mediated via central pathways involving neuropeptide Y (NPY) and agouti-related peptide (AgRP) in the ARC (73, 84, 85). These neuropeptides are implicated in the central control of meal initiation because their expression increases at times of maximal spontaneous feeding in rodents (86) as well as in anticipation of regularly scheduled meals (87). In contrast, expression of other neuropeptides involved in energy balance is relatively constant throughout the day (86). Accordingly, ghrelin does not affect food intake behaviour in NPY-/AgRP-deficient mice (85). These findings and the co-localization of NPY and ghrelin receptor GHS-R1a in neurons of the ARC suggest that NPY- and AgRP-positive neurons are a basic prerequisite for the ghrelin-induced orexigenic effect (88).

1.3.3. Roles of Ghrelin in Long-term Body Weight Regulation

Beyond its proposed role in short-term feeding control, ghrelin functions as a unique, orexigenic counterpart to leptin and insulin in overall energy homeostasis. As mentioned earlier, long term body-weight regulation signals should fulfill certain criteria. One of the main elements of these criteria is that an adiposity hormone should circulate in proportion to body-energy stores and manifest compensatory changes in response to fluctuations in these stores. Ghrelin levels are inversely correlated with adiposity, being low in the obese, higher in lean subjects, and markedly elevated in subjects who are cachectic due to a diverse range of conditions including anorexia nervosa, cancer, and chronic cardiac failure (89, 90). In addition, ghrelin receptor expression in the hypothalamus increases markedly with fasting or chronic food restriction (91) consistent with a feed-forward loop to enhance ghrelin-mediated appetite stimulation during energy deficit. Together, these observations suggest that ghrelin levels respond in a compensatory fashion to bi-directional alterations in body weight; consistent with the hypothesis that ghrelin contributes to the known adaptive metabolic responses to such alterations (92). It's also important to mention that ghrelin levels respond to changes in body weight—not simply to enteric nutrient load. Consistent with this claim, ghrelin levels are low in people receiving only total parenteral nutrition (TPN) compared with those in fasting individuals not on TPN (93). Thus, in these settings of an empty GI tract, ghrelin levels still reflect nutritional status of the body. Although it is not yet clear how ghrelin-producing cells in the gut sense changes in energy stores, evidence suggests that they respond, at least in part, to adiposity-associated fluctuations in levels of insulin (94) but not leptin (95) .

Another important element of an adiposity hormone, is that it should influence neuronal activity in brain centers known to regulate body weight. As previously mentioned, hypothalamic

NPY/AgRP neurons are obvious ghrelin targets. However, ghrelin probably also exerts orexigenic effects via indirect (vagally mediated) and direct signaling in the hindbrain (96), as well as by direct actions in the mesolimbic dopaminergic system (97), which is involved in reward perception. By targeting these neuronal centres, ghrelin undoubtedly satisfies the second criterion for an adiposity signal.

Exogenous administration of an adiposity hormone should alter food intake and/or energy expenditure, and chronic augmentation of its signaling should change body weight. Results from animal studies of peripheral or central ghrelin chronic administration in rodents results in prolonged hyperphagia and weight gain (98), and chronic or repeated infusions increase body weight, this is also observed in humans (84). The weight gain observed is greater than that expected for the degree of hyperphagia, and may reflect several reported actions of ghrelin that could combine to promote weight gain. These include stimulation of adipogenesis, inhibition of apoptosis, transfer from fatty acid oxidation to glycolysis for energy expenditure, and inhibition of the sympathetic nervous system activity.

1.4. Bariatric surgery

1.4.1. Background, Types and Indication

Despite recent scientific advances, no currently recommended dietary program or medication results in long-term weight loss of more than 10% of body weight for the vast majority of people who attempt these interventions. At present, bariatric surgery is the only treatment associated with documented, substantial, and maintained weight loss as well as the amelioration of obesity related comorbidities (99).

The current selection criteria for bariatric surgery are from the National Institute of Health (NIH) Consensus Development Conference Statement on Gastrointestinal Surgery for Severe Obesity (1991). The most recent publication of it is the 2004 Consensus Conference guidelines (100).

Bariatric surgery is indicated for adult patients with:
1. BMI above 40
2. BMI 35 – 40, with related medical comorbidities
3. BMI 35 – 40, with functional limitations due to body size or joint disease
If, after evaluation by a multidisciplinary team, the patient is judged to:
1. Have a low probability of success with nonoperative weight-loss measures
2. Be well informed about the long- and short-term risks and benefits of surgery
3. Be highly motivated to lose weight through surgery
4. Have an acceptable operative risk
5. Be willing to undergo lifelong medical surveillance

Table 1-4. Patient selection criteria for bariatric surgery. 1991 NIH Consensus Statement. Adapted from: Santry et al. Patient Selection for Bariatric Surgery. In: Buchwald H (ed.). *Surgical Management of Obesity*. Saunders Elsevier: Philadelphia 2007; 93-101 (33)

Although many types of operations for the treatment of clinically severe obesity have been developed over the last four decades, only a few have been considered successful. A successful weight-loss operation has two major goals: significant magnitude and duration of weight loss, and a reasonably low perioperative and long-term complication rate.

Bariatric operations can be classified into restrictive, malabsorptive, and mixed techniques. Two bariatric surgical procedures are performed more frequently, laparoscopic adjustable gastric banding – LAGB (fig-1), and laparoscopic Roux-en-Y gastric bypass – LRYGB (fig-2). The placement of an adjustable gastric band represents a purely restrictive procedure, producing a small gastric pouch and a narrow passage into the remainder of the stomach. Gastric bypass represents a mixed technique that combines the restriction derived from leaving a small stomach pouch near the esophagogastric junction, excluding the greater curvature, together with a metabolic component derived from bypassing most of the stomach and duodenum.

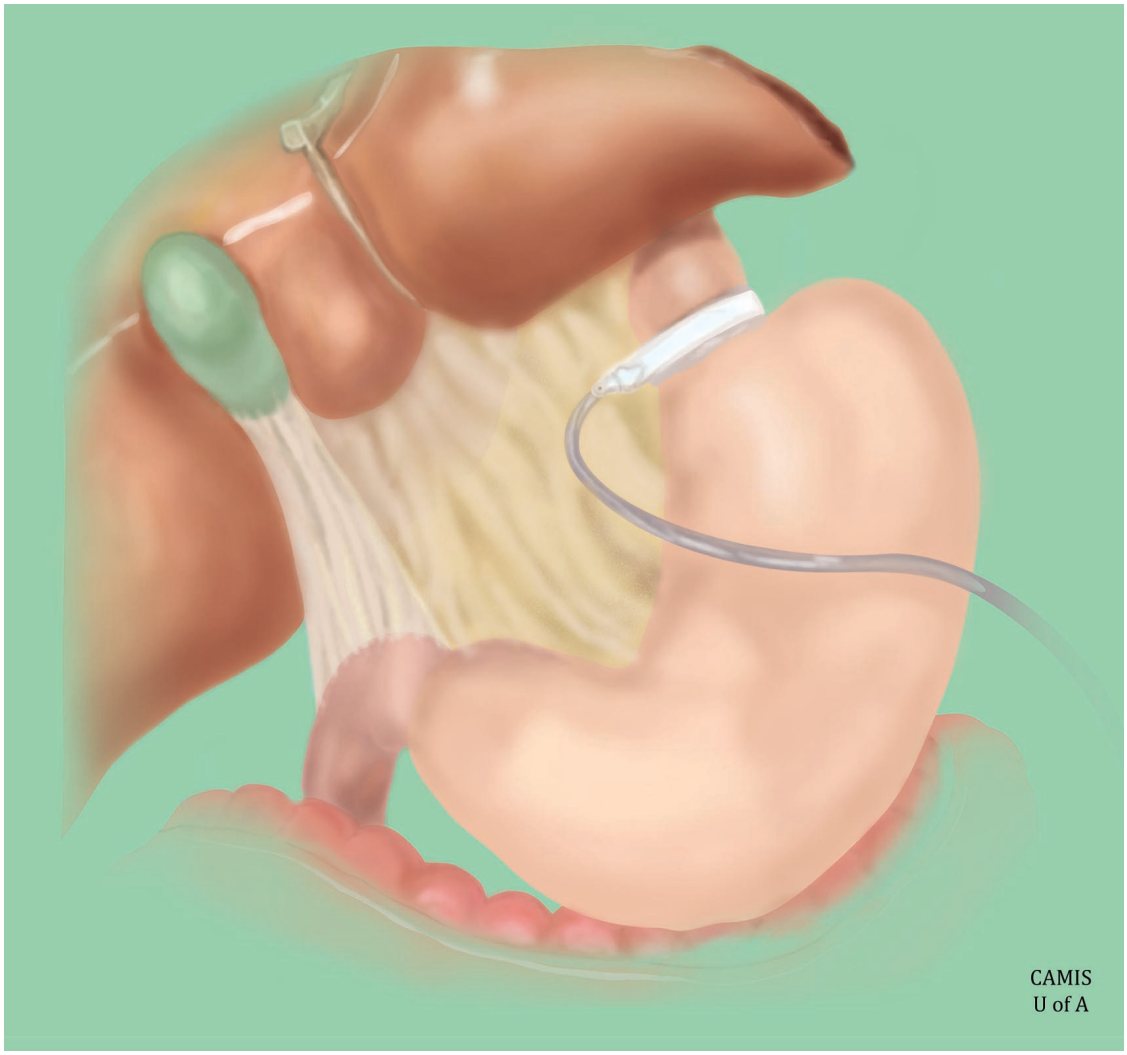

Figure 1-1. Laparoscopic adjustable gastric banding (AGB). Adapted from the collection of images of the Centre for the Advancement of Minimally Invasive Surgery, University of Alberta.

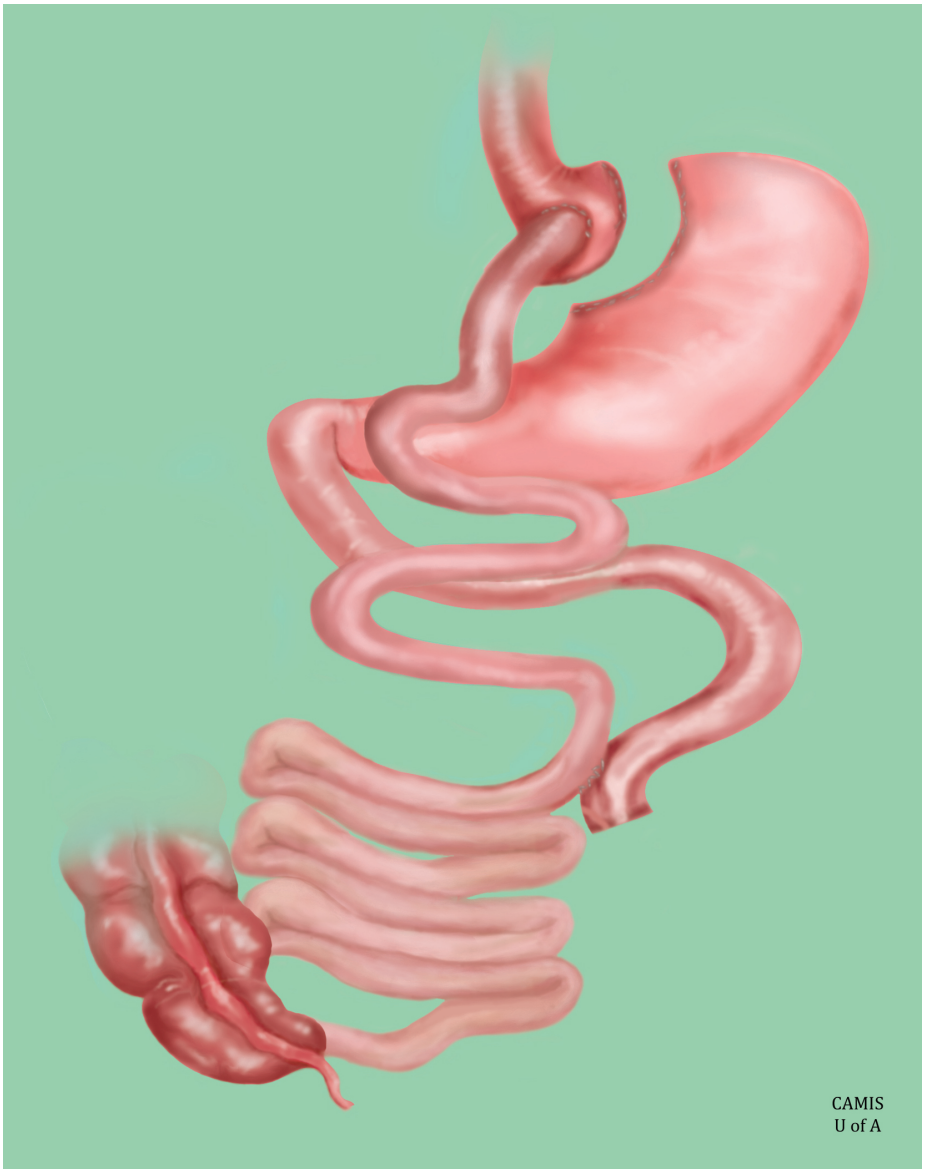

Figure 1-2. Laparoscopic Roux-en-Y gastric bypass (RYGB). Adapted from the collection of images of the Centre for the Advancement of Minimally Invasive Surgery, University of Alberta.

1.4.2. Laparscopic Sleeve Gastrectomy

Laparoscopic sleeve gastrectomy (LSG) has recently been identified as an innovative approach to the surgical management of obesity. It was first described as a modification to the biliopancreatic diversion (BPD) and combined with the restrictive part of duodenal switch (DS) in 1998, and first performed laparoscopically in 1999 (101, 102). It was originally designed to improve the results of biliopancreatic diversion, without distal gastrectomy. In this procedure, the greater curvature of the stomach is resected producing a narrow, tubular stomach (fig.3). Recently SG has been proposed as the first-step in a staged approach to the treatment of the very high BMI patients (>60kg/m^2) or in patients with high operative risk before performing more complicated procedures such as laparoscopic biliopancreatic diversion with duodenal switch (BPD-DS) or LRYGB. The advantages of the LSG are that it confers immediate restriction of caloric intake, does not require placement of a foreign body or require adjustments, and can generally be performed in less time than required for bypass procedures. Early evidence showed that patients experience excellent weight loss after SG alone, and multiple recent reports have documented LSG as single therapy in the treatment of morbid obesity.

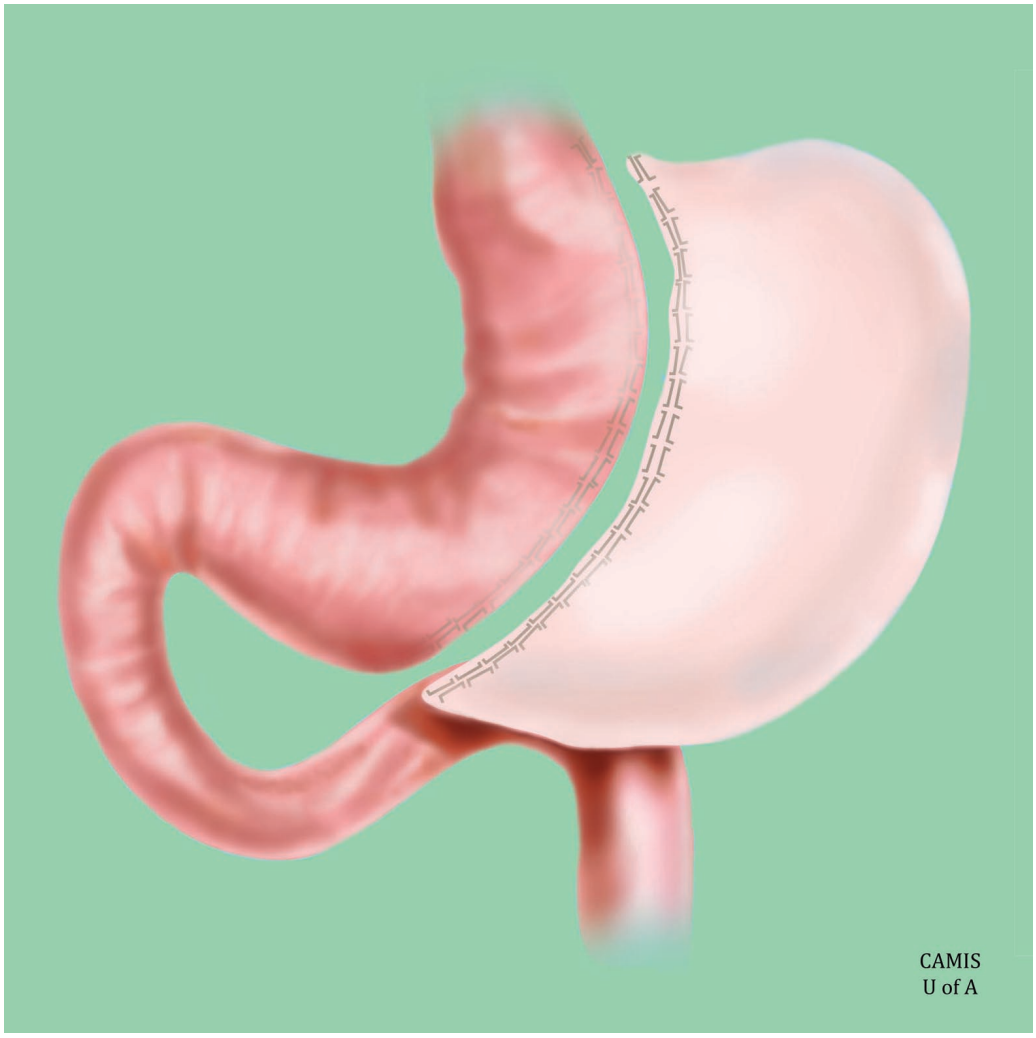

Figure 1-3. Laparoscopic Sleeve Gastrectomy (LSG). Adapted from the collection of images of the Centre for the Advancement of Minimally Invasive Surgery, University of Alberta.

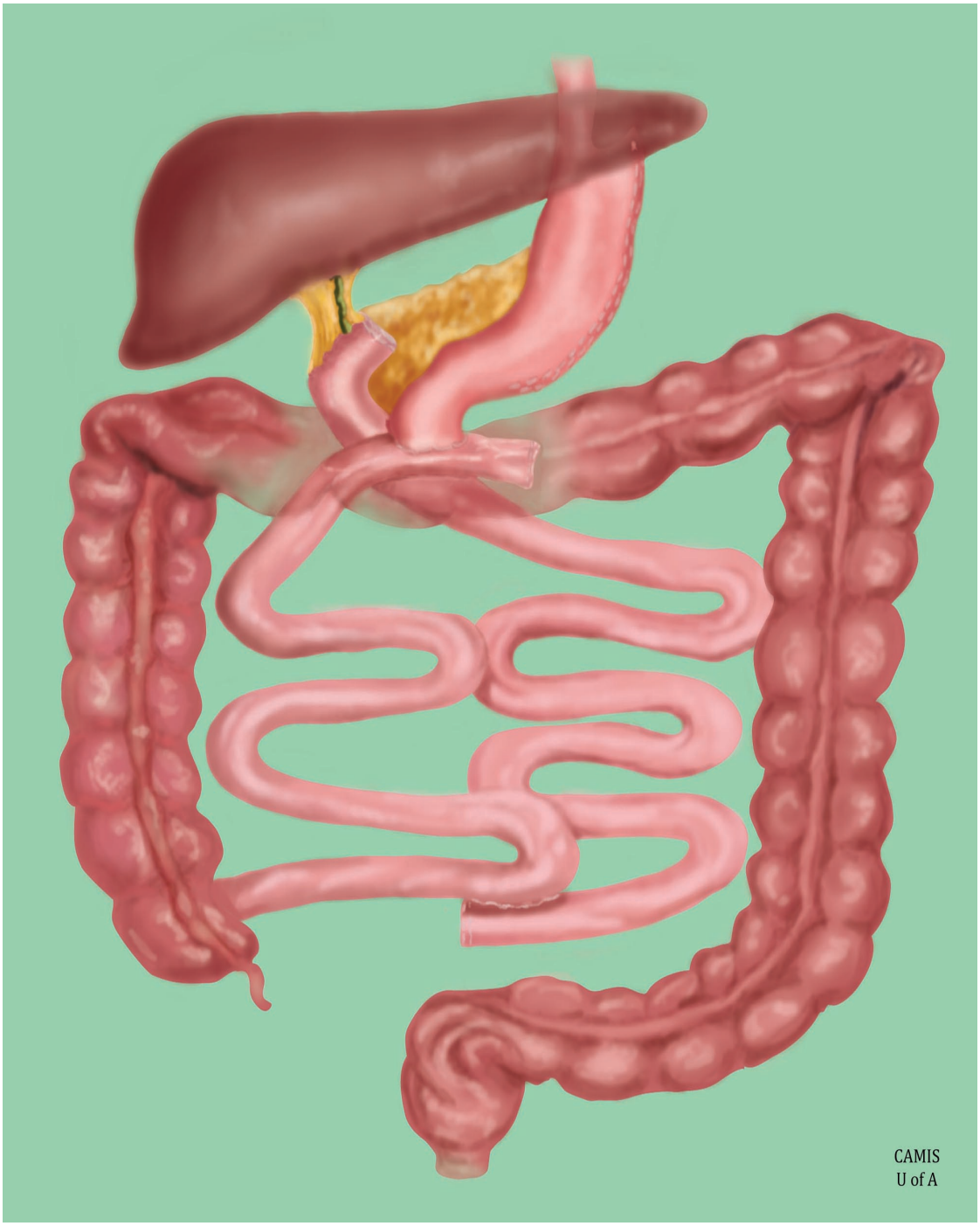

Figure 1-4. Biliopancreatic diversion with duodenal switch (BPDDS). Adapted from the collection of images of the Centre for the Advancement of Minimally Invasive Surgery, University of Alberta.

LSG, however, is a relatively new bariatric procedure with a mechanism of action that remains undefined. Suggested mechanisms include restriction of meal portions, alterations in gastric emptying (103) and increased satiety as a result of changes in serum ghrelin or other active endogenous satiety hormones (104). Short-term data shows that it is effective and safe in producing considerable weight loss (105, 106). However, given the novelty of the approach, there is limited long-term data on the durability of this weight loss.

The mechanisms of restriction involved in weight loss observed after the LSG is based on the dramatic reduction of the capacity of the stomach. The concept of restriction has been widely used in bariatric surgery in vertical banded gastroplasty (VBG) and LAGB. It is generally believed that after restrictive-type surgery, small amount of foods can be consumed and accommodated in the gastric pouch that is constructed in continuation with the esophagus. In LSG, the passage of the food through the created gastric tube into the distal stomach and the gut is mechanically delayed, resulting in energy intake reduction and finally in weight loss (107). Furthermore, the distension of the small gastric tube in the LSG is supposed to account for the feeling of early fullness, enhanced satiety and decreased hunger experienced by a patient after the ingestion of small quantities of food.

There is no consensus regarding the gastric volume that should be created during LSG. In various studies, the gastric tube has been created with different sizes of bougies from 32 Fr to 60 Fr. Some authors suggested using a small calibre bougie to fashion the gastric tube is to increase the restrictive character of the procedure, but it seems to be no short-term weight loss difference in size of the bougie (108). The final volume of the gastric tube has been reported to be as small as 60 mL and as large as 200 mL.

1.4.3. The Effect of LSG on Satiety signals

Ghrelin

A major factor contributing to the long-term success of weight reduction following bariatric procedures may be the profound changes in the eating behaviour postoperatively. As mentioned above, satiety provoked by an adequate meal is the result of a complex array of interacting signals that arise from the brain, GI tract and adipose tissue. Ghrelin seems to play a key role in the complex energy balance loop and it is logical to hypothesize that its changes are involved in the neurophysiologic mechanisms responsible for the changes in appetite observed after bariatric surgery.

Upon reviewing the literature, eight studies reported ghrelin levels before and after LSG. Studies were included involved patients diagnosed with morbid obesity (BMI>40) or BMI between 35 and 40 with severe co-morbidities and underwent SG and the pre and post operative ghrelin levels had been recorded. Studies were excluded if SG was used for treatment of diseases other than morbid obesity, if the SG had been performed as a component of another bariatric procedure and not as a standalone surgical procedure, or if follow-up was not reported. The studies included 3 randomized controlled trials, two prospective and two retrospective case series. Many of these studies measured a single ghrelin level in the morning following an overnight fast, whereas others also assessed the meal-associated suppression of ghrelin. All of the studies measured total (des-acyl) ghrelin, not the the biologically active (acylated) ghrelin.

One of the first studies that looked into ghrelin changes after SG came from Langer et al. from the University of Vienna (104). This prospective study involved a total of 20 patients and compared total plasma ghrelin levels following LSG and LAGB on day one and 1 and 6 months following the operation. Total plasma ghrelin levels were measured by radioimmunoassay using

125-I labeled-ghrelin as a tracer molecule, and a polyclonal antibody against full-length total ghrelin (Peninsula Laboratories, San Carlos CA). Following LSG, patients showed a significant decrease of plasma ghrelin levels at day 1 compared with preoperative levels (35.8 ± 12.3 fmol/ml vs 109.6 ± 32.6 fmol/ml, P=0.005). Plasma ghrelin remained stable at a low level at 1 month (43.7 ± 11.3 fmol/ml) and 6 months (44.8 ± 13.2 fmol/ml) postoperatively.

Of the other studies included, the retrospective Bohdjalian study (109) had the most extensive follow-up of 60 months, with a subgroup of 12 patients having had total plasma ghrelin levels measured both pre-operatively and 5 years postoperatively. In this study, plasma ghrelin was assayed by radioimmunoassay (Peninsula Laboratories, San Carlos, CA, USA) using 125-I labeled ghrelin as the tracer and a rabbit polyclonal antibody against the C-terminal end of human ghrelin. It was noted that the plasma ghrelin levels were reduced from 593 +/- 52 to 219 ± 23 pg/ml ($p<0.05$) 12 months postoperatively, with a slightly, non-significant increase toward the 5-years values of mean 257 ± 23 pg/ml.

Karamanakos et al. (110) did a double blind, prospective, randomized study comparing LRYGB to LSG. Sixteen patients were randomized to each arm. The patients were re-evaluated on the 1st, 3rd, 6th, and 12th postoperative month. Blood samples were collected for analysis of serum total ghrelin in EDTA-containing tubes following an overnight fast and in 6 patients in each group after a standard 420 kcal mixed meal. Serum total ghrelin was then measured in duplicate using a commercial radioimmunoassay (Phoenix Pharmaceuticals), employing iodine 125-labeled bioactive ghrelin tracer and a rabbit polyclonal antibody against the full-length, total human ghrelin. Among the LSG patients in this study, the fasting serum ghrelin level was found to have fallen from 605 ± 185 pg/ml to 399 ± 97 pg/ml ($p<0.001$) over 12 months. An interesting addition to this study was an appetite assessment performed on the patients using visual analogue

scales (VAS) that ranged from 0 to 100 mm. This assessment revealed that the LSG was not only associated with a greater loss of appetite, but also more durable maintenance of this appetite suppression over the entire study period.

Peterli and his colleagues (111) also performed a randomized, prospective, parallel group study. In their study, 13 patients were randomized to LRYGB and 14 to LSG. The patients were evaluated with a liquid 400 kcal test meal after an overnight fast, and blood samples were collected 15 minutes before the meal, then at 0, 15, 30, 45, 60, 120, and 180 minute following the meal. The samples were collected on ice into EDTA tubes. After centrifugation at 4°C, plasma samples were kept frozen at -20°C until analysis. Blood sampling was done pre-operatively, and then 1 week and 3 months after surgery. Samples were assayed for total ghrelin levels using a commercially available kit (Linco Research Inc). Among the patients in the LSG arm of the study, the pre-prandial ghrelin level was found to fall from 1666.5 ± 207.9 pg/ml preoperatively to 894.3 ± 184.8 pg/ml ($p<0.001$) following LSG and while the meal-induced ghrelin release was diminished in both groups of patients, the reduction in ghrelin secretion in the LSG group was more prominent than that in the LRYGB group, both at 1 week and 3 months.

Another prospective study comes from Ramon et al. (112). This randomized parallel group study included 15 patients who were randomized to LRYGB (seven patients) and LSG (eight patients). All patients were evaluated before surgery and at 3 and 12 months postoperatively for serum total ghrelin before and after 10 and 60 min of a standard liquid test meal (Ensure® containing 54% calories as carbohydrates, 16% as protein, and 30%as fat). Blood samples were collected on ice into EDTA tubes. After centrifugation at 4°C, plasma samples were frozen at −20°C until analysis. Total ghrelin was measured with a commercially available

kit (Linco Research Inc, St Charles, MO, USA). In the LSG cohort, the pre-prandial ghrelin fell from 610.15 pg/ml to 342 pg/ml postoperatively.

Basso et al. (113) from University of Rome did a prospective study where they recruited 28 patients and looked into the changes in total ghrelin level before and after 9 days of having SG. Interestingly, ghrelin was measured before and after intravenous glucose tolerance test (IVGTT) and no test meal was used. Plasma ghrelin was measured using a commercially available RIA kit (Phoenix Pharmaceuticals Inc., Phoenix, AZ). The patients were divided into three groups. Group A were patients who had diabetes for less than 10.5 years while group B were those who had diabetes for more than 10.5 years. Group C was non diabetic. The fasting ghrelin was found to be 301.8 ± 63.9, 306.2 ± 58.2 and 215 ± 89.4 pm/ml for the A, B and C group respectively. After SG, the total serum ghrelin level went down to 148.1+/-36.2 pm/ml for group A and B, while in group C, it went down to 92.9 ± 32.2 pm/ml.

The second retrospective series analyzed was a study performed by Wang et al. (114). It involved a review of 10 patients following LSG assessing changes in plasma total ghrelin with surgery. Blood samples were collected into EDTA tubes and centrifuged to get plasma. Overnight fasting plasma ghrelin levels were measured with enzyme immunoassay analysis (Phoenix Pharmaceuticals). The results for these patients after 24 months of follow-up showed that total ghrelin levels were also shown to have fallen from a pre-operative mean of 447.3 ± 71.2 pg/ml to a postoperative level of 319.7 ± 91.9 pg/ml.

The smallest series analyzed was a case series cited in a correspondence by Cohen et al. (115) to the editor of “Obesity Surgery”. The series involved four super-obese female patients whose preoperative and postoperative ghrelin levels were recorded following LSG. The study used a commercial kit (Ghrelin (Total) RIA Kit, Linco Research, Saint Charles, MO, USA), and

the patients were followed for 6 months. The mean preoperative ghrelin levels were 781 ± 96 pg/ml with the postoperative value haven fallen to 589 ± 61 pg/ml.

The method for collecting plasma samples differed among these studies. In their work, Bohdajalian et al. and Peterli et al. used the EDTA/aprotinin tubes, while Ramon et al, Karmanakos et al. and Wang et al. collected ghrelin in EDTA tubes without mentioning aprotinin. If aprotinin was not used, the measured plasma ghrelin will represent the level of the des-acyl ghrelin as the aprotinin is responsible for preventing the loss of acylation of ghrelin while the plasma/serum is stored. This might resulted in lower levels of ghrelin recorded than actually present at the time of sample collection. Each of Langer et al, Basso et al. and Cohen et al. also reported total ghrelin but there was no mention of the type of collecting tube in their publication. Interestingly, none of the papers reviewed here mentioned adding HCL for preservation of the samples. Overall, this heterogeneity makes it particularly challenging to make any solid inferences of the impact of LSG on ghrelin levels.

Another reason for the discrepancies may well be due to the poor sensitivity of the techniques that are widely available to assess ghrelin (71). As mentioned before, ghrelin is found in the circulation in both acylated (biologically active) and des-acyl (inactive) form; however, in all of the above studies, total ghrelin, rather than active ghrelin, was measured. Published data suggest that ghrelin assays using different methodologies do not always agree with one another (116, 117). Many of the published papers in the literature measuring plasma ghrelin levels have used commercial RIAs based on a single recognition site for the primary antibody; however, other assay methods such as ELISAs have also been used (118). Single-site assays can be less specific as a result of interference from peptide fragments or cross-reactivity at a single epitope

(119), whereas sandwich assays require recognition of two epitopes using two different anti-sera, thus eliminating many interferences and lowering nonspecific background (71).

All the studies mentioned earlier measured fasting levels of total ghrelin pre and post operatively. The post operative follow up measurement of ghrelin ranged from 1 month up to five years. Fasting total ghrelin levels did decrease at all time points up to 5 years of follow up. Appetite assessment was reported only by Karamankos et al, however, he compared satiety score for LSG as compared to LRYGB which is a mixed type bariatric procedure. Regarding the test meal, only two studies (111, 112) included this in their assessment and measured ghrelin postprandial at certain time points. The inclusion of a test meal is very important to show the rebound in plasma ghrelin to levels above the preprandial values. Without it, the effect on satiety after bariatric surgery can not be accurately characterized.

Overall, there is a general trend towards decreased ghrelin levels after LSG. However, the discrepancy in the form of ghrelin measured (total versus active), timing of measurement, and the varying study designs precludes a definitive statement on the impact of LSG on biologically active ghrelin levels. To the best of our knowledge, there is no study to date that has measured active ghrelin following LSG and correlated satiety with ghrelin levels post standardized meal.

GLP-1 and PYY

Only three studies have examined the effect of GLP-1 after SG. GLP-1 circulates in many different forms, some of which are biologically active and others are not. Various methods to measure GLP-1 detect different forms of GLP-1, which may cause confusion when comparing results. In his prospective, randomized study, Peterli et al. (111) evaluated changes in the meal-stimulated active GLP-1 (i.e., GLP-1 7-36 amide and GLP-1 7-37) response before and after SG

(1 week and 3 months) and compared them to RYGB group. Following the meal, the SG patients had an augmented GLP-1 response similar to the RYGB patients. Valderas et al. (120) from the University of Chile did a prospective study comparing "total" GLP-1 levels in two groups of non-diabetic obese subjects who underwent SG, and non-diabetic obese subjects who achieved a similar weight loss by medical treatment. Postoperatively, blood samples were drawn before and at 30, 60, 90, 120, and 180 min after the intake of 237 ml of a standard test meal (Ensure Plus®). In the SG group, there were increases in GLP-1 levels at 30 min after the test meal. In the non surgical control group, there were no changes. Finally, the prospective study from Basso et al (113) also reported an increase in the GLP-1 concentrations in the diabetic cohort following SG after IVGTT as well in the non diabetic cohort.

Four studies have evaluated changes in the meal-stimulated PYY response before and after SG. PYY is co-secreted with GLP-1 by L-cells, rises within 15 minutes of initiating a meal and peaks 1–2 hours later, remaining elevated for several hours (121). Further supporting his findings with respect to an increased GLP-1 response to SG, Peterli et al (111) reported increased "active" PYY 1-36 after 1 week and 3 months from surgery in response to test meal. The second study comes from Karamanakos et al (see above) (110). This prospective, randomized study reported the changes in "active" PYY 1-36 before and after SG at 3, 6, 9 and 12 months. The post SG measurements were done in fasting state and two hours following the ingestion of test meal (420 kcal mixed meal) and found significant increases as compared with the fasting levels. Another prospective study from Valderas and colleagues (122) where they compared "total" PYY level in three group of eight patients with similar body mass indexes before and 2 months after weight loss either by surgical treatment (SG group and RYGB group) or medical (obese group who lost weight without surgery). Blood samples were drawn before and at 30, 60, 90,

120, and 180 min after the intake of 237 ml of a standard test meal (Ensure Plus). Following LSG, fasting PYY levels did not change, but there was a significant increase in response to the test meal at 30 and 60 min. The changes in PYY area under the curve (AUC) correlated with increased satiety AUC which was assessed by VAS scores. The last study comes from Basso et al (113) where they demonstrated increased basal and stimulated PYY values after SG after IVGTT as compared to the preoperative values.

Taken together, the changes in GLP-1 and PYY after SG are similar. These finding supports the hypothesis that the enhanced gut hormone response might play a key role in the reduced food intake after SG.

Insulin and Leptin

Serum levels of circulating leptin are proportional to body fat content and BMI, and are reduced by weight loss (123). Leptin influences bodyweight through its effect on energy intake and expenditure. Chronic administration of leptin to rodents causes increased energy expenditure, reduced food intake and loss of bodyweight and fat mass (124). Reduction in leptin levels as a result of weight loss is associated with increased hunger in humans (19). There was only one study that measured leptin before and after SG. Woelnerhanssen and her colleague (125) from Switzerland reported leptin levels were decreased as early as 1 week postoperatively and progressively decreased with weight loss after surgery. The study also reported leptin levels/kg of fat mass ratio which also had significantly decreased at 1 year postoperatively.

Unlike leptin, which does not rise directly in response to food intake, insulin secretion increases rapidly after meals (126) and acts to control blood glucose levels. However, over the longer term, levels of plasma insulin are directly related to changes in adiposity, so that levels increase with obesity. In animal models, experimental administration of insulin results in a

decrease in food intake and loss of bodyweight (127) and inhibition of insulin's actions leads to increased energy intake and weight gain (128).This suggests that insulin contributes to satiety. Several studies (129-132) reported decrease fasting insulin level, reduced homeostasis model assessment-estimated insulin resistance (HOMA-IR) and rapid resolution of diabetes in Type 2 Diabetes Mellitus (T2DM) patients after SG. However, only Peterli and his colleague (111) showed marked increase in postprandial insulin concentrations in response to a test meal at one week and 3 months after LSG. Despite the clear insulin response, subjects did not experience hypoglycemia or symptoms of a dumping syndrome after the test meal.

1.5. Rationale for the Study

LSG is a relatively new bariatric surgical procedure that is now being used as a definitive approach, and in some instances as a preliminary procedure for super-obese, high risk patients prior to a duodenal switch (DS) or LRYGB (133, 134). Although early results on weight loss after LSG have been published, the underlying mechanism of weight loss is still unknown and the long-term efficacy is under investigation. Initially thought to be a purely restrictive operation, some studies reporting changes in gut hormones and the metabolic effects of this procedure have challenged that concept. Ghrelin, an orexigenic hormone produced primarily in the gastric fundus, is significantly decreased after SG and this decrease has been shown to persist 5 years after the procedure. However, most studies have reported only total plasma ghrelin concentrations, thus not distinguishing between the active and inactive forms of ghrelin. The role of total ghrelin is questionable in the physiological and biological activity of this hormone. To our knowledge, there is no study to date that measured total and active ghrelin following LSG. Furthermore, there was only one study that assessed satiety after LSG (110). In this study, satiety

score was measured before and after SG and compared to mixed procedure (RYGB) rather than comparing it to a pure restrictive procedure to assess the effect of restriction on satiety.

Although ghrelin may affect changes in hunger and satiety after SG, it is unlikely that it is the only factor that contributes to the long-term effects of this procedure. It is most likely that other hormones are involved, although these mechanismsremain to be elucidated. The changes in PYY and GLP-1 are likely candidates in the increased long-term satiety observed after SG.

LSG has emerged as a restrictive operation, however, a better understanding of the hormonal modifications raises the promise that it will play a leading role in the future of bariatric surgery especially for those severely obese patients concerned with hunger drive and poor satiation.

1.6. Hypothesis

1. LSG achieves weight loss not only by restricting the amount of food that can be consumed, but also because of reduction in biologically active ghrelin levels due to gastric resection.
2. Decreased active ghrelin is associated with improved satiety scores following LSG.

The aim of this study: To clarify the mechanism of action of weight loss and control of hunger/satiety in LSG as compared to a non resective, purely restrictive procedure LAGB.

1.7. References

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Chapter 2: Materials and Methods

2.1. Study Design and Subjects Selection

This study was approved by the University of Alberta Health Research Ethics Board. It's a cross sectional study comparing patients after LSG and LAGB with preoperative bariatric patients as controls. Participants were recruited from the Weight Wise Clinic at the Royal Alexandra Hospital in Edmonton, Alberta. All surgeries were performed at the Royal Alexandra Hospital by three bariatric surgeons. The surgical patients met the selection criteria for bariatric surgery defined by the NIH Consensus Development Conference Statement on Gastrointestinal Surgery for Severe Obesity, 1991. Three groups of ten consented participants (total thirty) were randomly recruited post-LSG and post-LAGB. All patients were weight stable and at least 12 months following bariatric surgery. Weight stability was defined as the change of no more than 5% of weight in the previous 3 months. The control group included preoperative patients from the wait list for bariatric surgery who were managed without pharmacologic intervention for their obesity. The groups were matched by sex, age (within 5 years), and BMI (within 2 kg/m^2). The inclusion/exclusion criteria as follows:

Inclusion Criteria

1) morbidly obese patients, male and female
2) age 18-60
3) without type 2 DM
4) 12 or more months following LSG or LAGB, or patients waiting for mentioned procedures
5) able to provide written informed consent

Exclusion Criteria

1) chronic liver disease
2) maladaptive eating behavior
3) current pharmacological treatment for obesity
4) for both LSG and LAGB: weight gain after procedure (failure of procedure)
5) for patients following LAGB – ongoing band volume adjustments
6) hypothyroidism
7) undergone or undergoing revision of a previous bariatric procedure
8) any major post-operative gastrointestinal complications, such as an anastamotic leak, outlet obstruction or persistent vomiting,
9) allergy to any component of the study meal
10) renal failure (glomerular filtration rate < 60 ml/min)
11) alcoholism
12) acute illness
13) pregnancy or nursing

2.2. Operative techniques

For LSG, generally, 75%–80% of the greater curvature was excised, leaving a narrow stomach tube. After bougie insertion (size 50–60 French), surgeons commence dissection 6 cm proximal to the pylorus creating a uniform gastric tube (1). The laparoscopic adjustable gastric banding was performed using the standard Swedish adjustable band pars flaccida technique.

2.3. Study Protocol and Blood Sample Collection

Eligible patients were selected and screened for participation from the Weight Wise Program. During their visit to the clinic, eligible patients were asked to participate and informed consent was obtained. Selected participants have been seen by a member of the study team and received all the information about the study, had the opportunity to ask questions and received answers to them to the full his/her satisfaction. After consent, participants had a baseline assessment, consisting of a history (socio-demographic variables, obesity-related comorbid conditions, alcohol/smoking status, medications, and allergies), physical examination (including weight and BMI) and confirmation of eligibility by reviewing inclusion/exclusion criteria. The participants were asked to avoid smoking from the night before and to refrain from strenuous exercise or alcohol in the 24 hour preceding the study and to come fasting to the Clinical Investigation Unit (CIU) at the University of Alberta Hospital, before 8 a.m.

Once the participants arrived at the CIU, an intravenous catheter was inserted to facilitate repeated blood sampling. Immediately before the blood collection, subjects were asked to complete a satiety questionnaire using a validated Visual Analog Scale (VAS) (2) as shown in table 2-1. Subjects were asked to place a vertical mark along a horizontal line 100 mm long for each question asked. The response was converted to mm and analyzed as described below. After

that, the first blood sample was collected (fasting baseline blood) and the time for this samples collection was regarded as time point zero.

On each line below (this varies from 0 to 100), place a vertical mark that corresponds to how you feel about each question asked. For example, if you feel no hunger mark closer to 0; if you are extremely hungry mark closer to 100.

I am hungry	0_____100
I am thirsty	0_____100
I feel nauseous	0_____100
Amount of food I can eat	0_____100
I feel full	0_____100

Table 2-1 The Visual Analog Scale (VAS) used to determine satiety.
Adapted from Nicolas V. Christou: Pre- and Post-prandial Plasma Ghrelin Levels Do Not Correlate with Satiety or Failure to Achieve a Successful Outcome after Roux-en-Y Gastric Bypass. *Obesity Surgery, **15**, 1017-1023.*

Next, subjects were asked to eat the first standardized test meal (STM) which was a fixed energy breakfast(table 2-2). This meal consisted of mixed 450 Kcal (55% carbohydrate, 15-20% protein, 25-30% fat and 2-5 grams of fiber). This meal was chosen as it represents a typical North American style breakfast which was palatable to most tastes and was a suitable meal for the timing of the study. Furthermore, the texture of the content of the test meal was well chosen to be tolerated by post LSG and post LAGB participants.

	KCAL	CHO (g)	Prot (g)	Fat (g)	Fibre (g)
Rice Krispies	105	23.27	1.89	0.19	0.7
1% milk (250ml)	104	12.36	8.34	2.4	0
Blueberry Yogurt	91	13.97	1.33	3.79	1.3
Brown Toast	94	19	3.1	0.95	1.8
Marble Cheese	80	0.02	5	7	0
Total	474	68.62	19.66	14.33	3.8
Percentage		58%	17%	27%	

Table 2-2 Nutrient composition of the standardized test meal (STM)

Following the ingestion of the STM, venous blood samples were collected at 15, 30, 60, 90 and 120 minutes from the test meal ending time. Before each blood collection, subjects were asked to do the written VAS as mentioned above. After the last blood sample collection (i.e. at 120 min), the participants were offered another standardized meal to measure how hungry they were and to correlate the consumption from this meal with the VAS measurement. The second test meal was a pre-packed microwave heated vegetable lasagna (© 2005 Zinetti Food Products Ltd) consisting of 213 Kcal per serving of 277 gram. This meal was weighed before serving and weighed again after serving to calculate the actual amount of food consumed.

The blood samples were analyzed in duplicate for plasma ghrelin (total and active), serum GLP-1(active), PYY 3-36, insulin, glucose and leptin. Leptin was only measured preprandially as it is only useful as a biomarker of satiety in the longer term (3).

For plasma ghrelin, prior to sample collection, 4-[2-aminoethyl benzene] sulfonyl fluoride (AEBSF 200mM: Enzo Life Sciences, Farmingdale, NY); a serine protease inhibitor was added to chilled EDTA lavender Vacutainer tubes. The samples were centrifuged at 1000 rpm at 2-8 °C for 15 minutes. Plasma samples were removed and acidified with 100μL HCl (1N) added to each aliquot. Aliquots were stored frozen at - 80 C till the time of assay (4).

For serum samples, blood was collected into red/gray Vacutainer tubes. Prior to sample collection, aprotinin (40 TIU/ml of blood; Sigma-Aldrich, St. Louis, Missouri) and DPP IV Inhibitor (Millipore, St. Charles, Missouri) were added to each Vacutainer. Samples were allowed to clot on ice for 30 min and each sample was centrifuged at 1,000 rpm for 15 minutes at room temperature, and the serum was removed and stored at -80 C until time of the assay.

Blood samples for glucose measurements were collected into grey top tubes and were taken to the hospital laboratory and analyzed immediately.

2.4 Hormone Assays

2.4.1. Ghrelin

Plasma ghrelin levels were analyzed at the University of Virginia using a very sensitive and specific two site sandwich assay. This assay measures both ghrelin and des-acyl ghrelin with an accuracy greater than can be found with commercial kits (4). The acyl ghrelin assay had a sensitivity of 6.7 pg × ml^{-1} with an intraassay coefficient of variation (CV) of 9.1% at 30 pg × ml^{-1}, 12.6% at 100 pg × ml^{-1}, and 16.8% at 300 pg × ml^{-1}. The interassay CV was 17.8% at 50 pg × ml^{-1}. The desacyl ghrelin assay had a sensitivity of 4.6 pg × ml^{-1} with an intraassay CV of 12.5% at 50 pg × ml^{-1}, 10.7% at 150 pg × ml^{-1}, and 18.0% at 500 pg × ml^{-1}. The interassay CV was 20.8% at 30 pg × ml^{-1}.

2.4.2. GLP-1

GLP-1 was measured with a commercially available ELISA kit (Millipore, St. Charles, Missouri). This kit, as described by Pridal et al. (5) is for nonradioactive quantification of biologically active forms of GLP-1 (i.e. GLP-1 “7-36 amide” and GLP-1 "7-37”) in serum and other biologic media. It is highly specific for the immunologic measurement of active GLP-1 and will not detect other forms of GLP-1 (eg, 1-36 amide, 1-37, 9-36 amide, or 9-37 amide). Briefly, according to the manufacturer's literature, the assay was based on capturing of active GLP-1 from sample by a monoclonal antibody, immobilized in the wells of a microwell plate, that binds specifically to the N-terminal region of active GLP-1 molecule, followed by washing to remove unbound materials and then binding of an anti GLP-1-alkaline phosphatase detection conjugate to the immobilized GLP-1. The quantification of bound detection conjugate is done by adding methyl umbelliferyl phosphate, which in the presence of alkaline phosphatase forms the

fluorescent product umbelliferone. Because the amount of fluorescence generated is directly proportional to the concentration of active GLP-1 in the sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of active GLP-1. When using a 100 μL plasma sample, the lowest level of GLP-1 that could be detected by this assay was 0.25 pmol/L.

2.4.3. PYY3-36

PYY3-36 was measured with a commercially available RIA kit (Millipore, St. Charles, Missouri). This assay utilized 125 I-labeled PYY and a PYY 3-36 antiserum to determine the level of PYY 3-36 in serum by the double antibody/PEG technique. This assay displays 100% cross-reactivity with human PYY3-36, but no cross-reactivity with human pancreatic polypeptide, NPY and unrelated peptides, such as leptin and ghrelin. The lowest level of PYY that could be detected by this assay was 20 pg/mL when using a 100 uL plasma sample.

2.4.4. Leptin

Leptin was measured with a commercially available ELISA kit (Millipore, St. Charles, Missouri). This assay was a direct sandwich ELISA based, sequentially, on first capturing of human leptin by a polyclonal rabbit anti-human leptin antibody immobilized on a 96-well micro-titer plate, and then binding of a biotinylated monoclonal antibody to the captured human leptin, followed by washing away unbound materials and then binding of streptavidin-horseradish peroxidase to the immobilized biotinylated antibodies. This was followed by another wash away to free enzyme conjugates, and then quantification of bound streptavidin-horseradish peroxidase

with the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity was measured spectrophotometrically by the increased absorbency at 450 nm - 590nm after acidification of formed products. The lowest level of human leptin that could be detected by this assay is 0.135ng/mL + 2 SD (25 μL sample size).

2.4.5. Insulin

Insulin levels were determined using a sandwich ELISA with a commercially available human insulin assay kit (ALPCO Diagnostics, Salem, NH). Briefly and according to the manufacture, the capture antibodies against insulin (horseradish peroxidase enzyme-labeled monoclonal antibody) were added to the microplate with the controls and samples. The microplate is then incubated on an orbital microplate shaker at 700-900 rpm. After the first incubation was complete, the wells were washed and blotted dry. TMB substrate was then added and the microplate was incubated a second time on an orbital microplate shaker at 700-900 rpm. Bound to insulin labeled detection antibodies emitted light when a voltage was applied to the electrodes. Emitted light was detected by spectrophotometer at 450 nm with a reference wavelength of 620-650 nm. The intensity of the color generated was directly proportional to the amount of insulin in the sample.

2.5. HOMA/Insulin Resistance

HOMA index of insulin resistance was calculated using the formula described by Matthews and colleagues (6): [insulin (μU/mL) X glucose (mmol/L)] / 22.5. This simple method estimates baseline fasted insulin resistance based on a mathematical model. HOMA index has been validated in multiple studies against physiological methods, such as hyperinsulinemic-

euglycemic clamp (7). HOMA index was found to be reliable in different patient populations including lean and obese, diabetics, both sexes and subjects of different ages (8). A HOMA index above 2.5 indicates insulin resistance (8).

2.6. Statistics

Statistical analysis was carried out using STATA 12 software (StataCorp LP, College Station, TX). Continuous data were presented as means ± standard error of means (SEM) and categorical variables were presented as frequency unless indicated otherwise. Demographic data were summarized with descriptive statistics. Biochemical variables were compared by analyzing the changes of means in different times and area under the curve (AUC). Continuous variable data were tested for normal distribution and statistically significant difference was determined using ANOVA or t-test if the data were normally distributed and Kruskall-Wallis or Mann-Whitney test if the data were not distributed normally. For categorical variables, the data were compared using Fisher's test. Correlations were determined by univariate linear regression (Spearman's rank test). p value <0.05 was considered as a significant difference.

2.7. References

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Chapter 3: Results

3.1. Demographic Characteristics

All of the 30 patients recruited for this project completed the study protocol. No patients were excluded. In the final analysis, group characteristic are presented in table 3-1. All three groups were similar in their weight, BMI, sex and age at the time of the study.

Variable	**LSG** **n = 10**	**LAGB** **n = 10**	**Control** **n = 10**	**p**
Age (yr)	45.3 ± 9.3 (39-60)	44.9 ± 8.9 (36-58)	45.8 ± 9.1 (39-60)	>0.05
BMI current (kg/m^2)	38.7 ± 5.4 (32.7-49.6)	38.4 ± 5 (32.1-47.1)	38.5 ± 4.5 (32-47.6)	>0.05
Weight current (kg)	107.2 ± 17.1 (91-135)	104 ± 13.3 (83.2-133)	106.4 ± 11.2 (88.4-130)	>0.05
Sex (M/F)	0/10	0/10	0/10	>0.05
Waist circumference (cm)	120.8 ± 8	120.4 ± 8.2	121.2 ± 7.6	>0.05

Table 3-1. Patient demographic characteristics, mean ± SD, (range).

3.2. Clinical Results

Weight loss following surgery is shown in table 3-2. Average follow up time for the LSG group was 16.7 months versus 25.2 months for the LAGB group. In the LSG group, the average current weight and BMI was significantly less as compared to the weight and BMI at the time of admission into the obesity program (initial weight) ($p<0.001$). Furthermore, the current BMI was significantly lower than the preoperative BMI for the LSG group ($p<0.001$; fig 3-1). For the LAGB group, current weight and BMI was also significantly lower than the initial weight and BMI. Although the current BMI for the LAGB group was lower than it was preoperativly, this difference did not reach statistical significance ($p=0.34$; fig 3-2).

Weight loss percentage was higher for the LSG group (39 ± 33.54 % of initial weight), whereas the LAGB patients lost 15.8± 13.1 % of initial weight which was significantly less than the LSG group ($p<0.05$). When expressed as percent of excess weight loss (%EWL), the participants lost 39 ± 18.7 % and 20.8 ± 21.4 % in the LSG and LAGB groups, respectively. The %EWL difference between those two groups did not reach statistical difference ($p=0.07$; fig 3-3)

Variable	LSG n = 10	LAGB n = 10	Control n = 10	p value
BMI initial (kg/m^2)	53.4 ± 9.3 (42.5-65.4)	43.6 ± 2.5 (40.8-49.4)	39.5 ± 4 (35-49.2)	<0.0001
Weight initial (kg)	148.5 ± 20 (113-178)	125.5 ± 19.6 (104.5-164)	115.2 ± 19.7 (97-111)	0.0027
BMI Preoperative (kg/m^2)	44.2 ± 6.4 (36-50)	39.6 ±2.7 (35.3-44)	N/A	0.0491
Weight loss (% initial)	39 ± 33.4 (14-44.3)	15.8 ± 13.1 (5-42)	6.5 ± 11.8 (0-36)	0.0162
% Excess Weight Loss	39 ± 18.7 (10-59)	20.8 ± 21.4 (0-48)	11 ± 20.4 (0-63)	0.0154

Table 3-2. Clinical results of treatment, mean ± SD, (range).

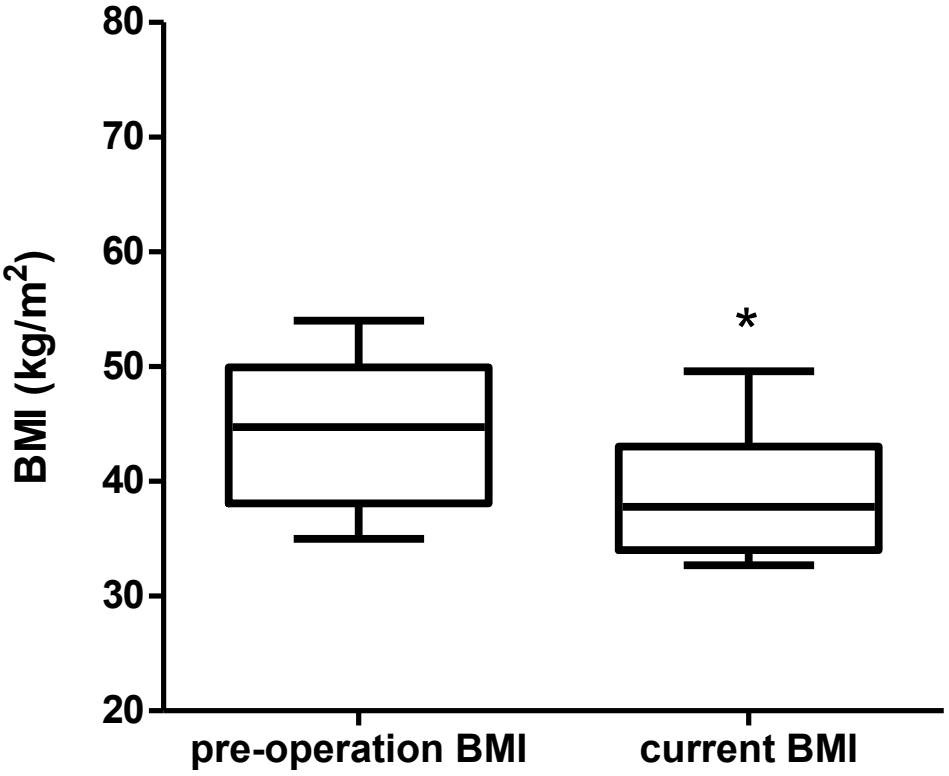

Figure 3-1. Changes in Body Mass Index (BMI) following LSG (* p<0.001)

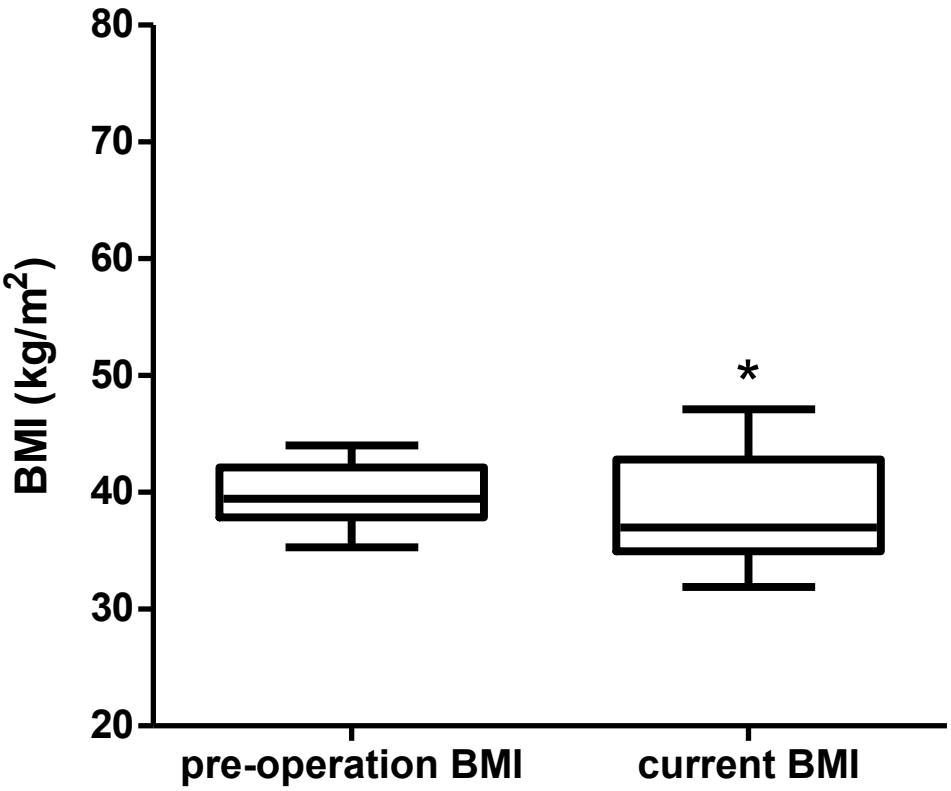

Figure 3-2. Changes in Body Mass Index (BMI) following LAGB (* p=0.34)

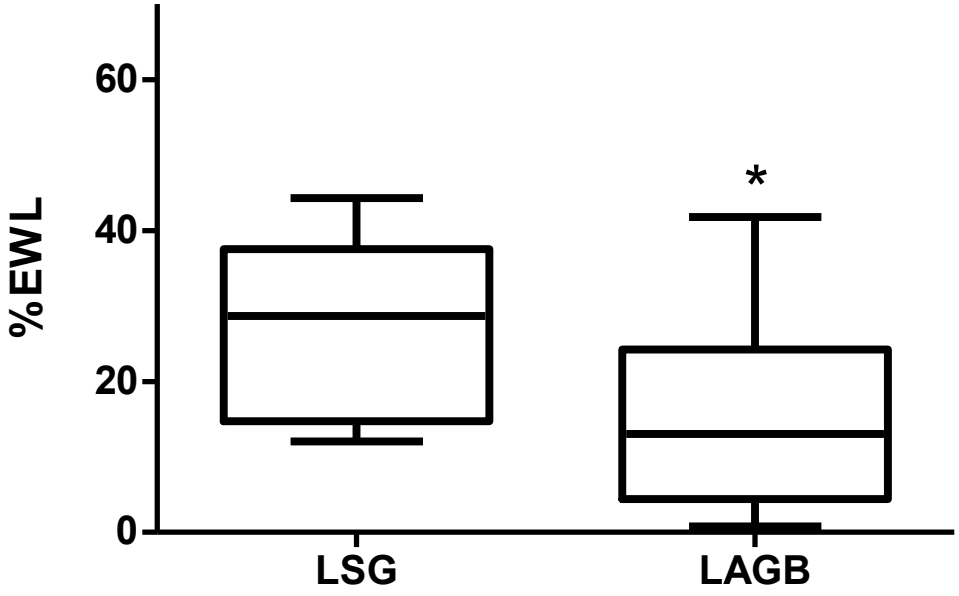

Figure 3-3. Weight loss expressed as excess weight loss percentage (%EWL) following LSG and LAGB (* p=0.07)

3.3. Meal Tolerance

The first test meal was well tolerated by all patients. Mild nausea was reported especially in the medical control group. However, there was no vomiting reported. The second test meal was also well tolerated.

3.4 Satiety Hormones Analysis

3.4.1 Ghrelin

A significant decrease was seen in the fasting plasma acyl ghrelin concentrations in the LSG group compared with the LAGB group and non-operated control group ($p<0.001$). The meal associated changes of acyl ghrelin was also lost after the STM in patients in the LSG group (fig 3-4). A considerable decrease was seen in the plasma acyl ghrelin concentrations at 30 and 60 minutes in the LSG such that the average plasma level was undetectable. For the LAGB and the non operated medical groups, changes in acyl ghrelin were similar. The area under the curve during a 2-hour period (AUC0–120) for of acyl ghrelin (fig 3-5) was 289 ± 303 area units, 2747 ± 1104 area units and 2697 ± 2296 area units in the LSG, LAGB and medical control groups, respectively. The LSG group demonstrated 9.5 fold reduction in AUC 0–120 for acyl ghrelin compared to the LAGB group ($p= 0.0002$). Furthermore, fasting acyl ghrelin concentration was negatively correlated with %EWL for the LSG ($r = -0.50$, $p< 0.05$). There was no correlation between the fasting acyl ghrelin in the LAGB group and the %EWL.

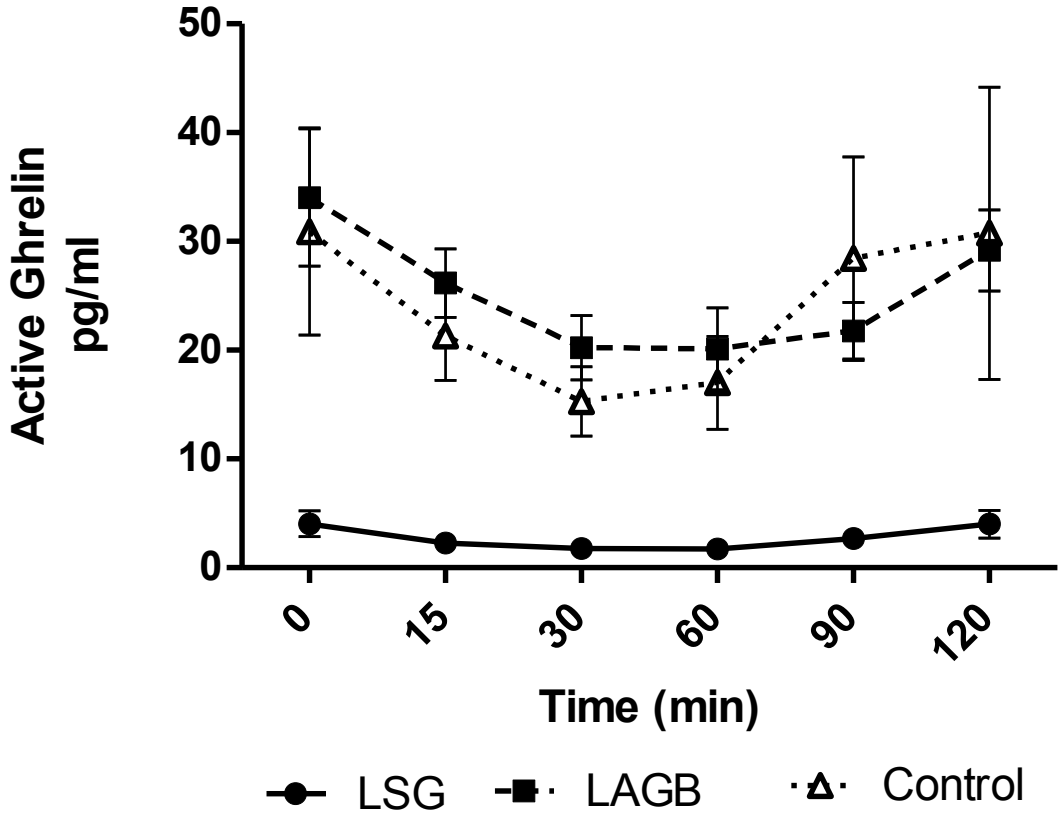

Figure 3-4: Postprandial changes in biologically active (acyl) ghrelin. All data are expressed as mean ± SEM.

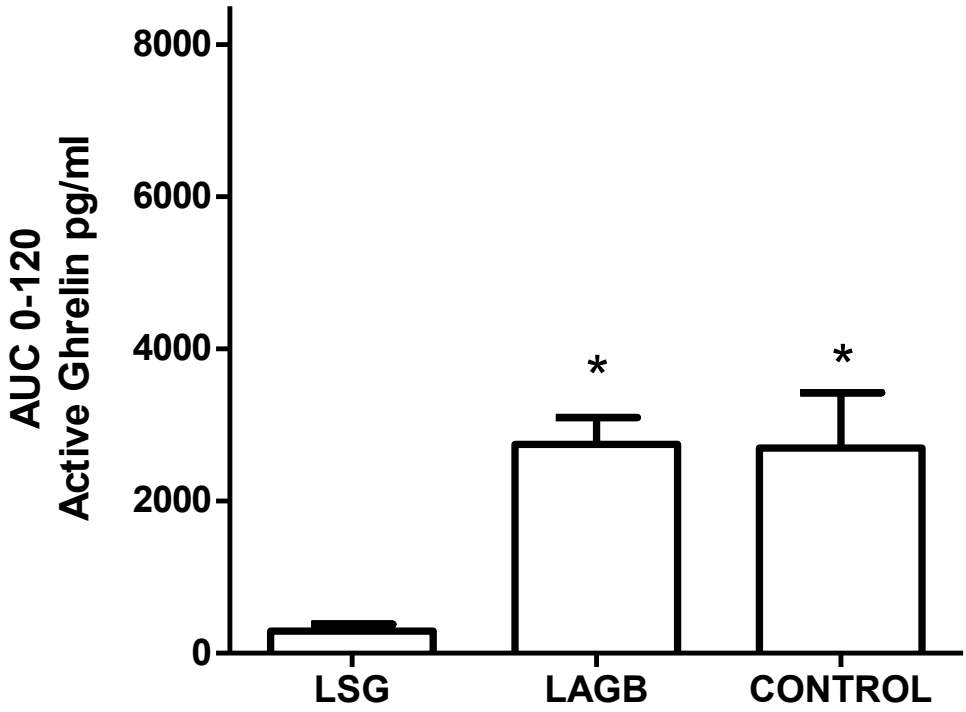

Figure 3-5: Biologically active (acyl) ghrelin, area under the curve (AUC), between groups of treatment, mean ± SEM (* p<0.001)

A similar trend was seen in the des-acyl ghrelin changes (fig 3-6). The AUC 0-120 during a 2-hour period for the des-acyl ghrelin (fig 3-7) was 1540 ± 654 area units and it was significantly smaller (p=0.008) than the LAGB group (4156 ± 1182 area units) and the non operated control group (3907 ± 2017 area units). No correlation was found between the plasma level of fasting des-acyl ghrelin and the EWL% in both surgical groups.

When comparing the two forms of ghrelin (fig 3-8), there was significant difference (p<0.0001) between the AUC 0-120 for Acyl and des acyl ghrelin.

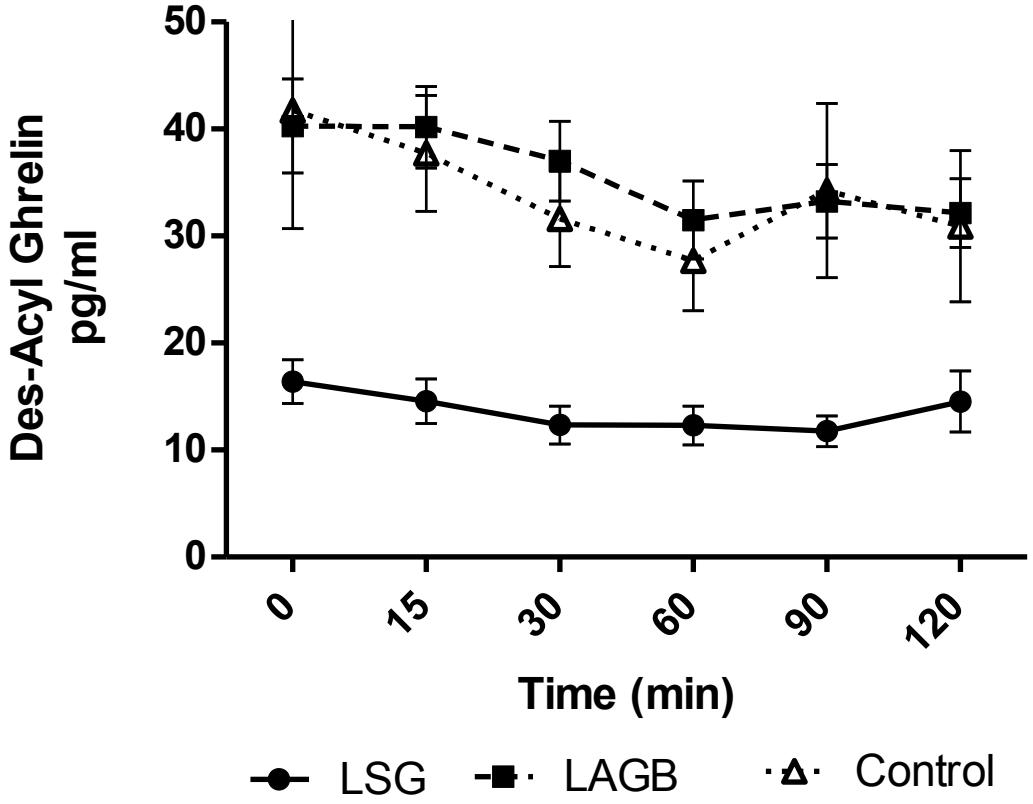

Figure 3-6: Postprandial changes in Des-acyl ghrelin. All data are expressed as mean ± SEM.

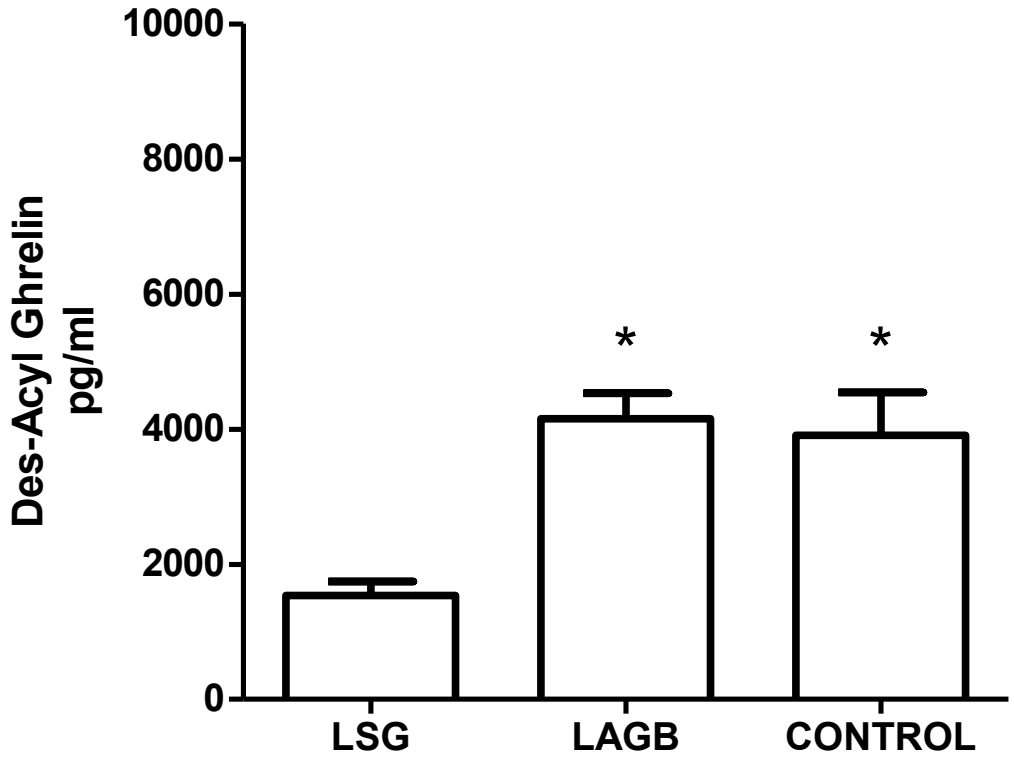

Figure 3-7: Des-acyl Ghrelin, area under the curve (AUC), between groups of treatment, mean ± SEM (* p=0.008)

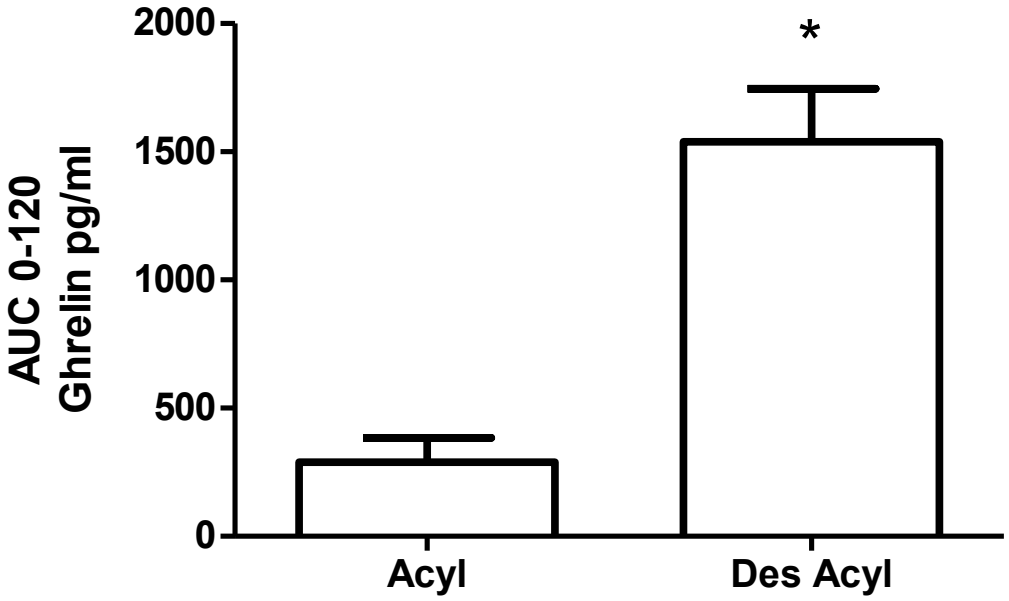

Figure 3-8: Acyl vs Des-acyl ghrelin, area under the curve (AUC), in the LSG group, mean ± SEM (* p<0.001)

3.4.2. GLP-1 and PYY

GLP-1 and PYY exhibited post meal increases. For GLP-1, LSG patients had an increse at 15 and 30 minutes postprandially (fig 3-9). This meal associated increase can also be seen in the PYY response to the test meal in the LSG group (fig 3-10). However, there was a unique pattern of decreasing GLP-1 and PYY levels in the LSG patients at 90 minutes after STM.

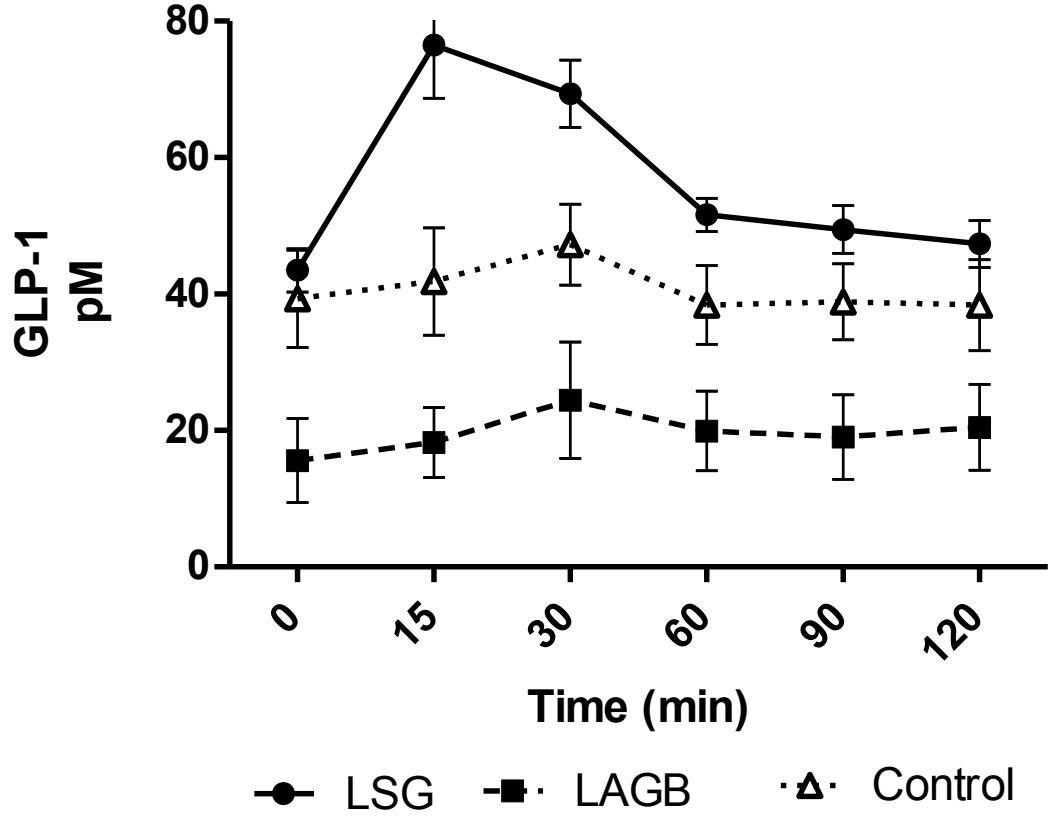

Figure 3-9: Postprandial changes in Active GLP-1. All data are expressed as mean ± SEM.

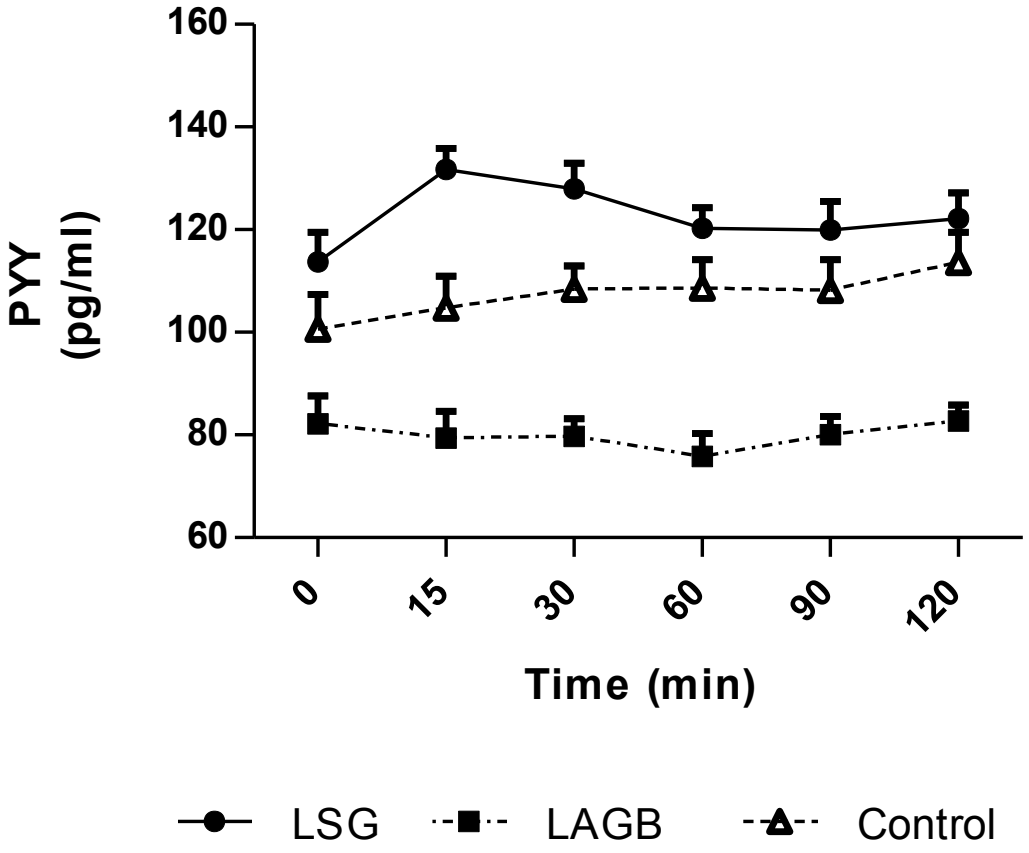

Figure 3-10: Postprandial changes in PYY3-36. All data are expressed as mean ± SEM.

For the LSG group, the AUC0–120 of GLP-1(fig 3-11) in response to the test meal was significantly larger compared with the LAGB and control groups (LSG 6795 ± 1173 area units, LAGB 2424 ± 2310 area units and Control 4890 ± 2286 area units; $p<0.0001$). Likewise, the AUC 0-120 for PYY (fig 3-12) for the LSG patients was significantly larger as compared to LAGB and Control groups (LSG 22184 ± 2739 area units, LAGB 14831 ± 1671 area units and Control group 20199 ± 3027 area units; $p<0.05$).

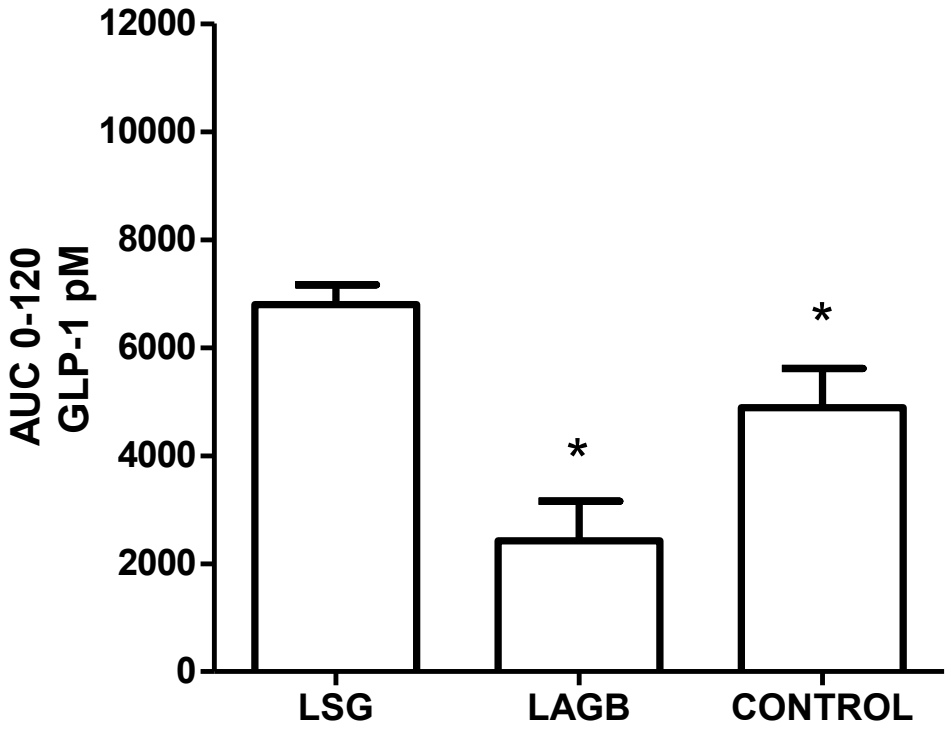

Figure 3-11: Active GLP-1, area under the curve (AUC), between groups of treatment, mean ± SEM (* p<0.0001)

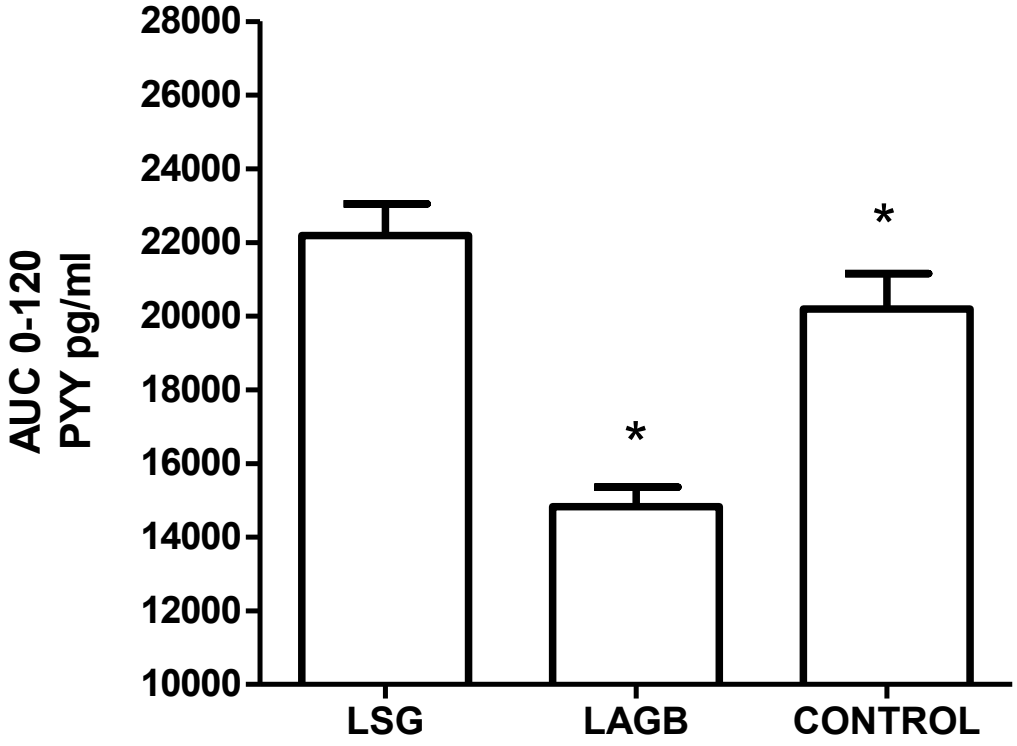

Figure 3-12: PYY 3-36, area under the curve (AUC), between groups of treatment, mean ± SEM (* $p<0.05$)

3.4.3. Leptin, Insulin, Glucose and HOMA Index

No differences ($p>0.05$) were observed between groups in the fasting serum leptin levels (Figure 3-13). The average levels of leptin were 37.5 ± 4.3 ng/ml, 31.5 ± 2.2 ng/ml and 44 ± 6.4 ng/ml for the LSG, LAGB and medical control groups, respectively.

Similarly, fasting insulin concentrations between groups were not significantly different ($p>0.05$). However, there was a significant meal associated increase in the first phase for the LSG group (fig 3-14) and insulin concentration at 15 minute was significantly higher as compared to the LAGB and control patients ($p<0.05$). Overall, there was no significant difference between AUC 0-120 in the three groups ($p>0.05$). The values of insulin AUC 0-120 were 5244 ± 2989 area units, 4829 ± 3127 area units and 2245 ± 888 in the LSG, LAGB and medical control groups, respectively (fig 3-15). Both surgical groups had significantly lower glucose AUC 0-120 (fig 3-16) and HOMA index (respectively $p<0.05$, $p<0.05$) than the medically treated group. However, when comparing the HOMA index between the two surgical groups, the difference was statistically not significant (fig 3-17)

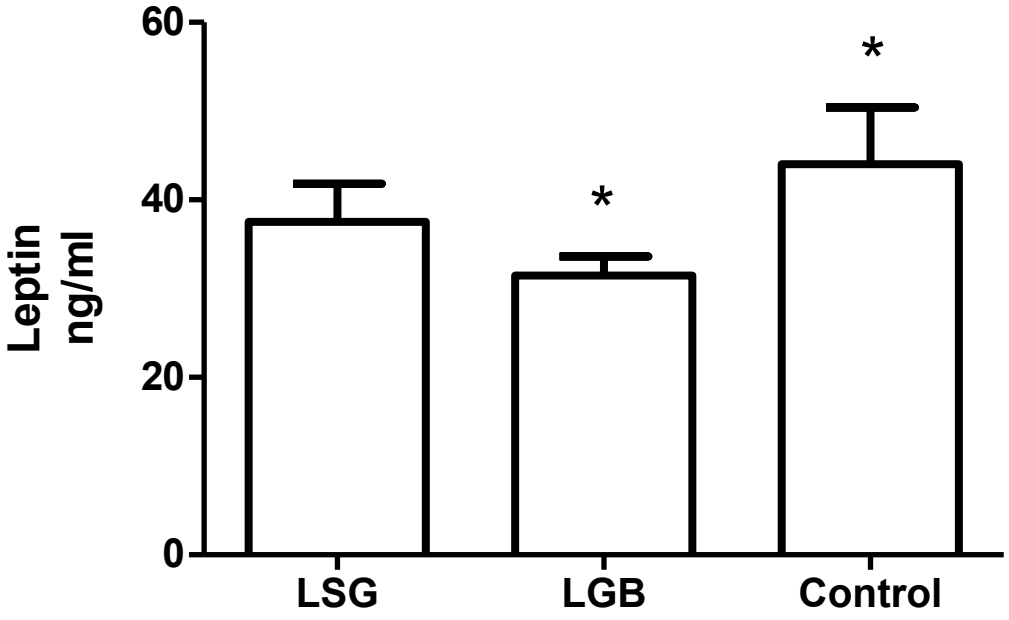

Figure 3-13: Fasting leptin, between groups of treatment, mean ± SEM (* p>0.05)

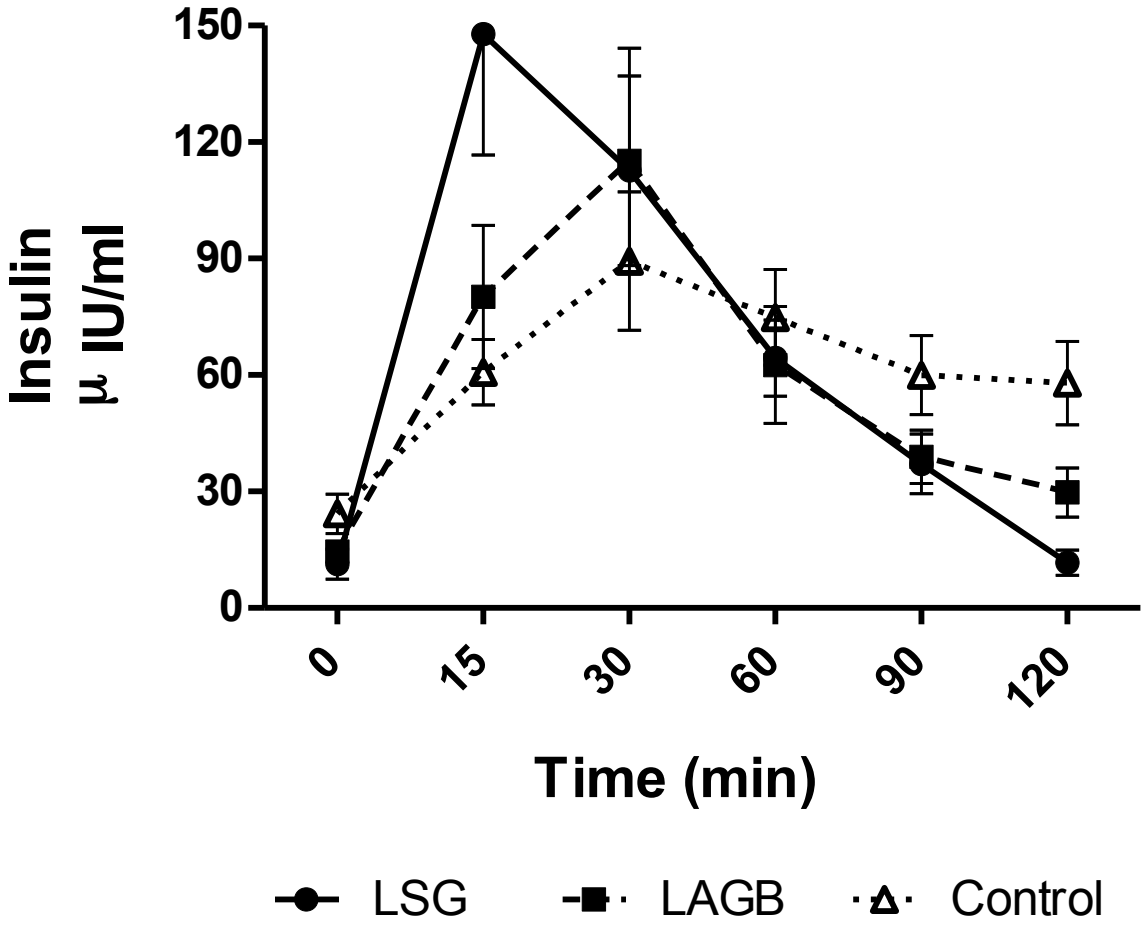

Fig 3-14: Postprandial changes in insulin. All data are expressed as mean ± SEM.

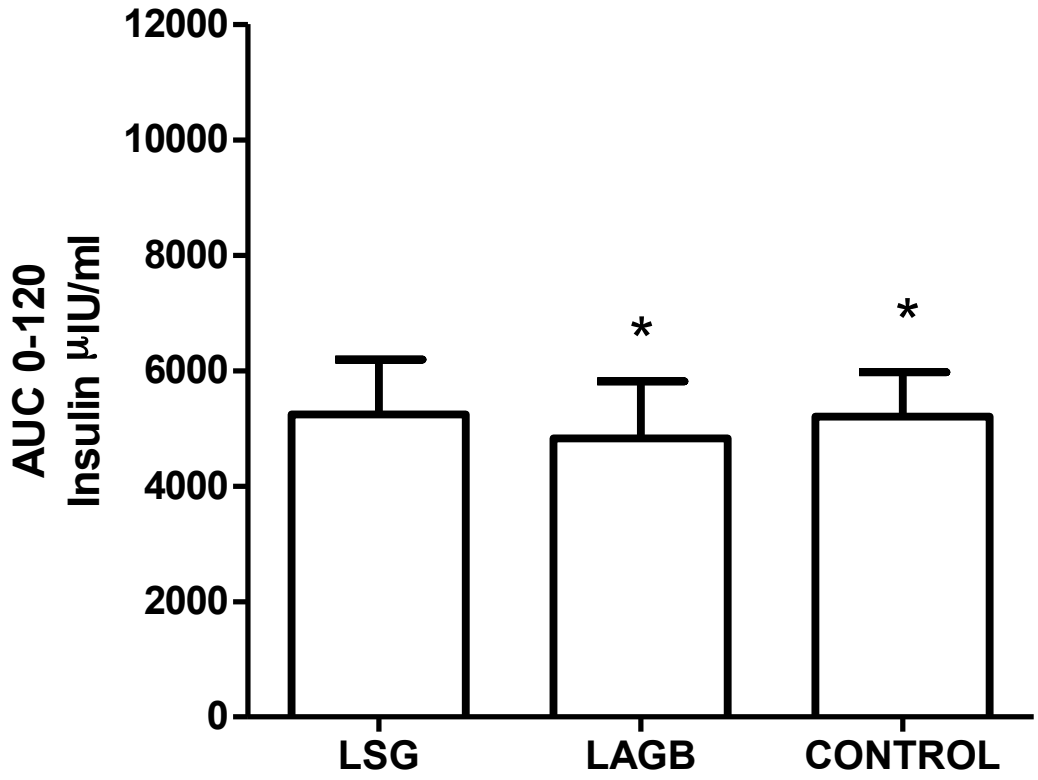

Figure 3-15: Insulin, area under the curve (AUC), between groups of treatment, mean ± SEM (* p>0.05)

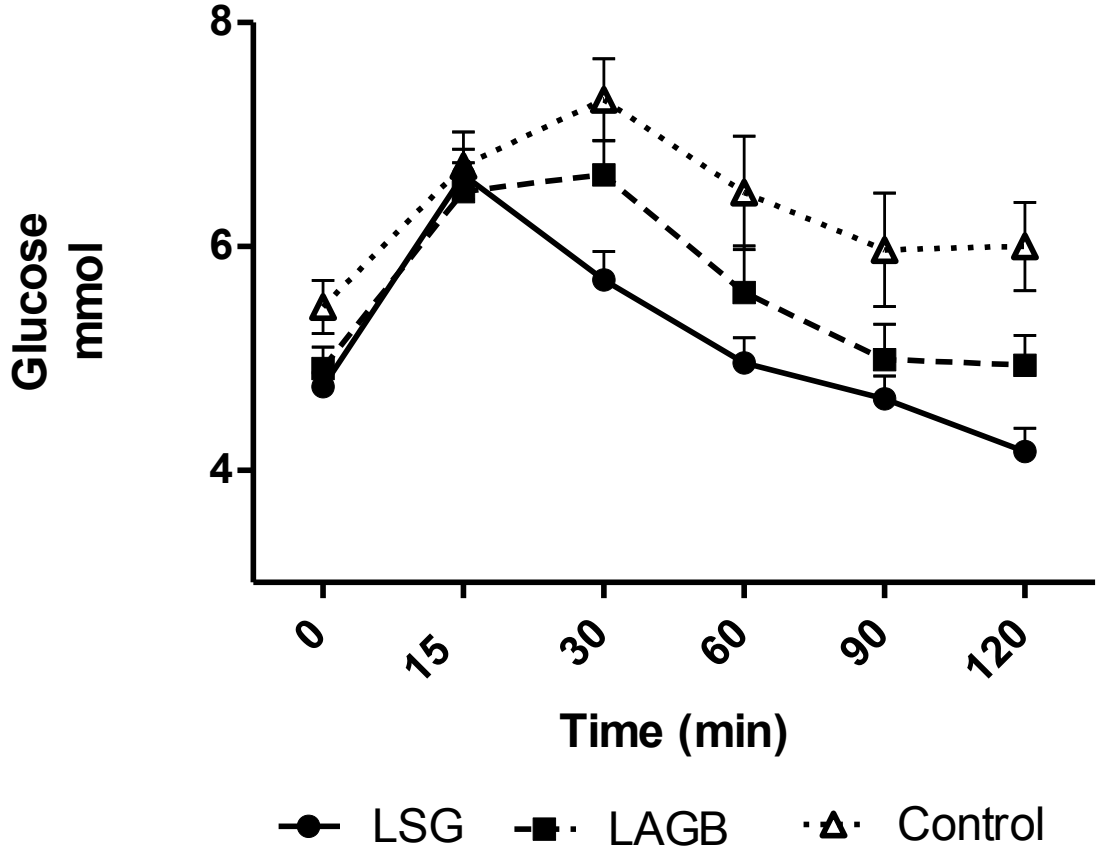

Fig 3-16: Postprandial changes in glucose. All data are expressed as mean ± SEM.

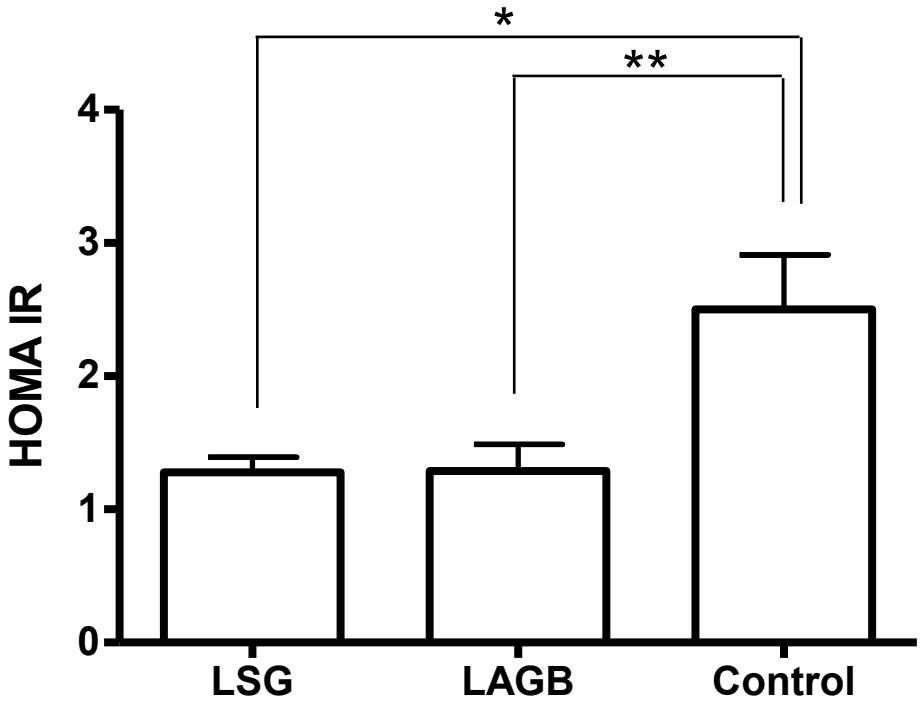

Figure 3-17. Homeostatic model assessment of insulin resistance. All data are expressed as mean ± SEM (* p<0.05 LSG vs Control, ** p<0.05 LAGB vs Control)

3.5 Hunger and satiety VAS scores

Ratings of hunger for the LSG group in the fasting state ($p<0.05$) and after STM ingestion (AUC 0-120, $p<0.05$; Fig. 3-18) were significantly decreased, compared with the LAGB and non operated control groups. Likewise, a greater postprandial satiety (AUC) was reported in the LSG patients after meal intake ($p<0.05$, Fig. 3-19) as compared to the other two groups.

In the LSG group, Acyl ghrelin levels correlated negatively with satiety VAS significantly at time points 15, 30 and 60 minutes postprandially (respectively, $r = 0.75$, 0.75 and

0.64; $p<0.05$). Furthermore, PYY levels correlated positively with satiety VAS significantly at time points 15, 30 and 60 minutes postprandially in the LSG patients (respectively, $r = 0.68$, 0.68 and 0.64; $p<0.05$). In contrast, there was no significant correlation between fasting levels of acyl ghrelin, GLP-1, PYY and the maximal GLP-1 serum concentration in response to meal ingestion and satiety VAS scores.

When comparing the amount of food (meal No. 2) consumed by participants across the 3 groups (fig 3-20), the LSG group consumed the least amount as compared to the other groups ($p=0.006$).

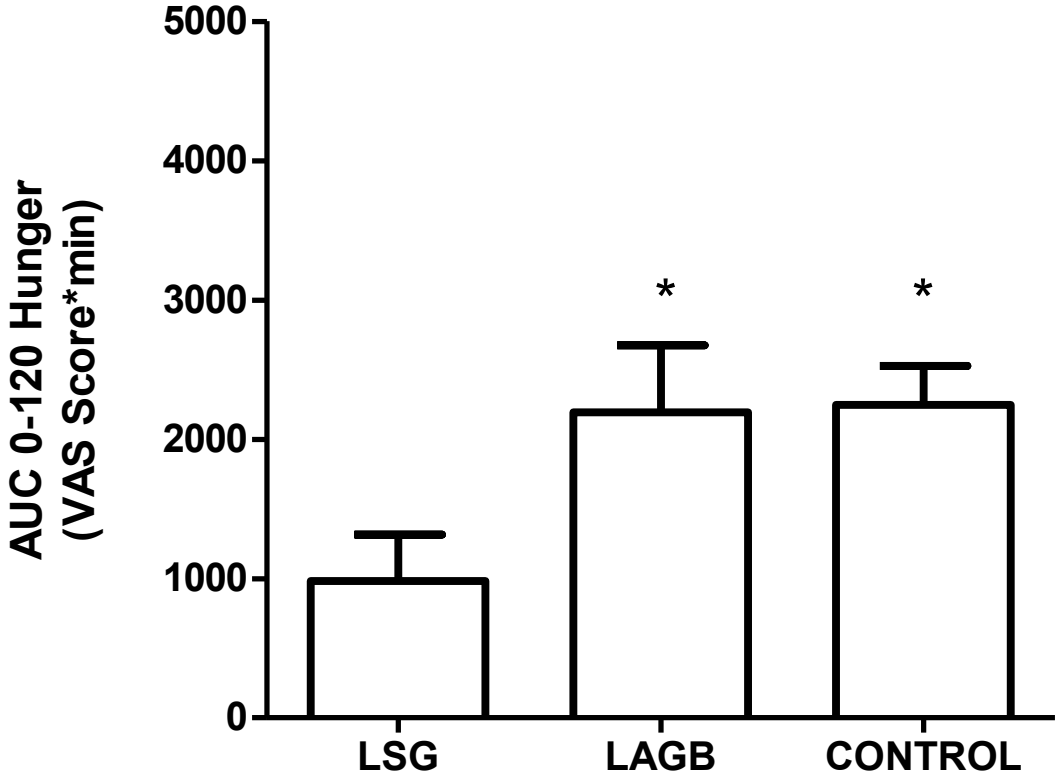

FIG. 3-18: Hunger VAS scores , area under the curve, between groups of treatment, mean ± SEM (* p<0.05)

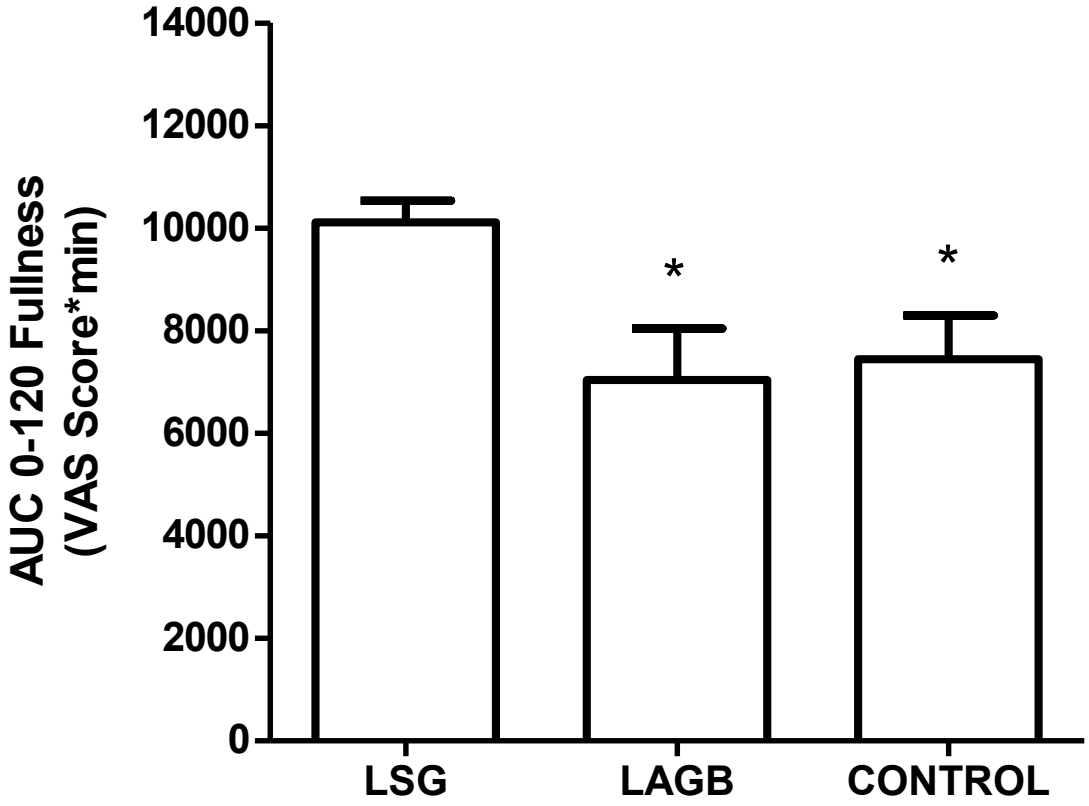

FIG. 3-19: Satiety VAS scores , area under the curve, between groups of treatment, mean ± SEM (* p<0.05)

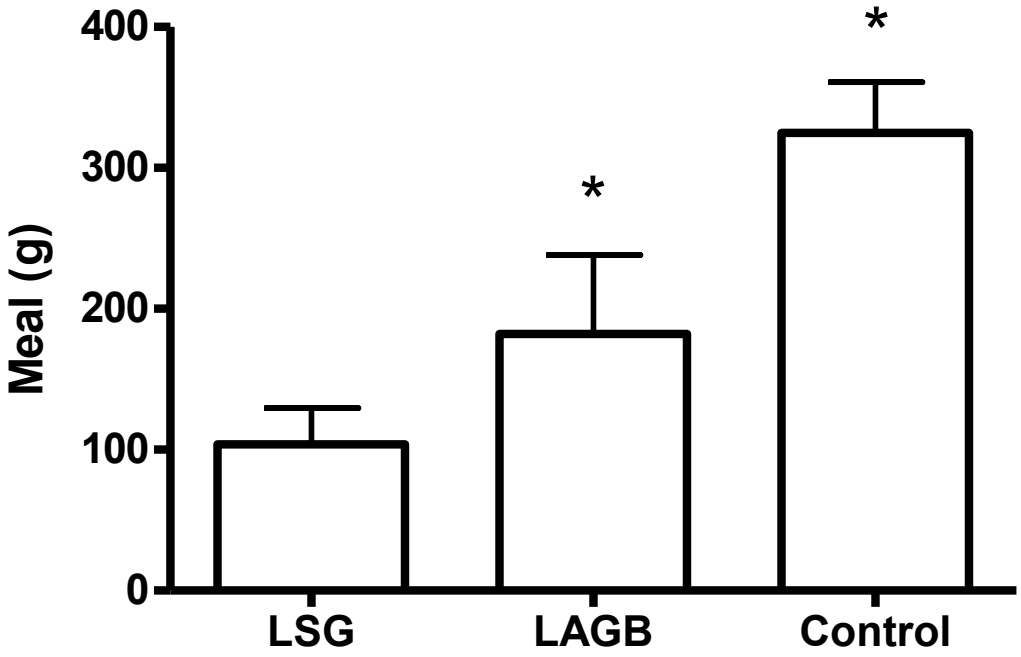

Fig 3-20: Weight of consumed amount from standardized meal No. 2 , between groups of treatment, mean ± SEM (* p=0.006)

Chapter 4: Discussion and Conclusion

The aim of this study was to characterize meal associated changes in satiety hormones and their influence on appetite and hunger in LSG comparing them to another restrictive bariatric procedure, gastric banding, and a non surgical control. The results of our study stronly suggest that LSG cause a reduction in biologically active ghrelin which was associated with significant weight loss and appetite suppression. Our findings of the flat total ghrelin profile was reported in a previous study (1). However, to our knowledge, this is the first study that measured the response of both acyl and total ghrelin levels and correlated their levels with satiety score.

A strength of the present study design is that it compared matched patients treated similarly in all other aspects except the type of procedure they had. Moreover, we completed serial measurements of GI peptides after an overnight fast and after meal stimulation up to 2 hours after the meal. All groups were matched by age, sex and most importantly, BMI. There are multiple confounders in satiety such as age, gender, and behavioural confounders (Habitual diet, alcohol and physical activity). However, the most important confounder is bodyweight (2). Subjects with different weight may respond differently to tests of appetite and energy intake, not least because their energy requirements are different – the energy requirements of obese subjects are generally higher than those of lean subjects. For these reasons, studies must ensure that when measuring satiety, subjects should be matched for weight.

4.1. Active Ghrelin levels after LSG

This study shows, for the first time, a reduction in both fasting and postprandial plasma level of biologically active (acylated) ghrelin after LSG. Ghrelin is a 28-amino acid peptide that undergoes a post-translational modification through acylation of the hydroxyl group of Ser3 by n-octanoic acid (3). To date, the physiology of ghrelin has been defined using the assay for total ghrelin, which mainly reflects levels of des-acyl ghrelin, the major circulating molecule.

However, recent improved methods for measuring acyl ghrelin and des-acyl ghrelin separately have demonstrated that des-acyl ghrelin is regulated differently than acyl ghrelin (4).The acylation is catalyzed by the ghrelin-O-acyltransferase (GOAT) and converts the peptide to the biologically active form (5). Moreover, the fatty acid residue has been found to be essential for the directed transfer via the blood-brain-barrier (6). This suggests that the central oriexigenic effect is a pure function of acylated ghrelin.

In our study, the physiologic fluctuations of acyl ghrelin and des-acyl ghrelin are preserved in LAGB and the control group; however, the normal physiological meal-associated suppression of acyl ghrelin and des-acyl ghrelin is lost after LSG. Furthermore, the AUC during a 2-hour period of plasma acyl ghrelin is significantly reduced in LSG as compared to des-acyl ghrelin.

It is well known that circulating ghrelin levels are negatively associated with BMI; ghrelin secretion is increased in anorexia and cachexia, reduced in obesity, and normalized by recovery of ideal body weight (7). This phenomenon is consistent with a role for ghrelin in the adaptive response to body weight changes, and thus, in long-term energy homeostasis (8). Several studies have found reduced levels of circulating ghrelin in obesity, thus negatively correlating with BMI (9). In our study, we found that postoperative fasting acyl ghrelin levels in LSG were significantly reduced as compared to both comparison groups (LAGB and obese control) and there was a significant negative correlation with %EWL. Additionally, no correlation was found between fasting des acyl ghrelin and %EWL in all groups. Because the restrictive effect on food intake is similar between both LSG and LAGB, the superior effect on weight loss by LSG could be attributed to the sustainably reduced ghrelin levels, preventing an increase in appetite as a compensatory mechanism.

4.2. Increased Hind Gut hormones GLP-1 and PYY

This study shows that LSG is associated with a significant increase in GLP-1 and PYY in response to a test meal. The mechanism behind this elevation is unclear. PYY level is low in the fasting state and is released from L cells located in the distal GI tract in response to food intake, acting to inhibit gastric motility, gastric acid, and insulin secretion . PYY levels are highest 1–2 hours post-ingestion, with this peak level influenced by both the number of calories and the composition of the food consumed (10). Exogenous administration of PYY3-36 induces a dose dependent loss of appetite, both in healthy volunteers and in obese persons, suggesting that the peptide acts as a satiety factor (10, 11)

Several cross-sectional and prospective studies have been performed to evaluate the effects of different surgical procedures on PYY levels during the fasting state and after meal intake. The effects of RYGB, gastric banding, and biliopancreatic diversion on fasting PYY levels that have been observed are diverse. However, PYY response after a meal has been found to increase in all derivative surgeries studied, such as RYGB (12, 13), jejunoileal bypass (14). This increase in postprandial PYY response is not observed in purely restrictive surgeries, such as gastric banding (15, 16) and vertical banded gastroplasty (17). The exception to this rule could be LSG. Both Valderas et al. (18) and Peterli et al. (1) reported similar increase in PYY after LSG with peak level in the immediate postprandial period (30–60 min). Our data are consistent with these studies showing that LSG is associated with a significant increase in PYY that occurs within 15 -30 minutes postprandially. There was no significant increase in PYY response noticed after LAGB.

LSG preserves the integrity of the pylorus and does not include intestinal bypass as part of the technique. Therefore, it would be reasonable not to expect rapid nutrient transit to the distal bowel that can lead to any significant changes in the hind gut hormones. PYY is released from the gut into the circulation in a nutrient-dependent manner. In response to food intake PYY levels increase within 15 min, before nutrients have reached the L-cells (11). Several studies suggested that neural and humoral regulatory systems may also play a role in the regulation of PYY release (19). Intraduodenal infusion of lipids in humans caused a rise in PYY before the nutrients reached the distal GI tract, suggesting the participation of neural or humoral mechanisms in modulation of PYY release (20). Other studies pointed to a vagal role in regulation of PYY release (21, 22). These studies indicate that vasoactive intestinal polypeptide, gastrin, cholecystokinin and the vagus nerve may all modulate meal-stimulated PYY release. To that end, some speculated that a possible mechanism for early PYY surge could be associated with the incomplete digestion in LSG patients due to decreased gastric acid secretion. Delivery to the duodenum of undigested chyme of higher pH could enhance PYY response to the meal (23).

The same gut endocrine cell type that synthesizes PYY also synthesizes a large precursor protein known as preproglucagon. This is processed further to produce a number of biologically active peptides, including GLP-1which is thought to play an important part in the “ileal brake” mechanism (i.e., adjustments of stomach and gut motility after food ingestion) that causes a moderate and stable (digestible) flow of nutrients from the stomach into the small intestines. This is probably also the mechanism by which GLP-1 exerts its effect on appetite (24). In the current study, a clear increase in postprandial GLP-1 levels was observed and the response reached supraphysiological levels in LSG patients compared to both LAGB and obese control subjects.

As with the PYY, the peak response recorded in the early phase after the STM (15-30 min). This finding has been recently described by Peterli et al. (1) and Valderas et al (25) where they reported a similar surge in postprandial GLP-1 levels in the early phase.

GLP-1 is also released rapidly into the circulation after oral nutrient ingestion, and its secretion occurs in a biphasic pattern starting with an early (within 10–15 min) phase that is followed by a longer (30 –60 min) second phase (26, 27). Because the majority of GLP-1 secreting L-cells are located in the distal small intestine, it is unlikely that the early phase of GLP-1 secretion can be mediated by direct nutrient contact with the L-cells. Indeed, several studies have shown that the autonomic nervous system, the neurotransmitters gastrin-releasing peptide (GRP) and acetylcholine, and the glucose-dependent insulinotropic peptide hormone (GIP) all can contribute to the rapid release of GLP-1 after nutrient ingestion. The role of the vagus nerve as an important mediator of nutrient-induced GLP-1 secretion has been established by studies in rats in which it was shown that bilateral subdiaphragmatic vagotomy completely blocks fat-induced GLP-1 secretion, whereas direct electrical stimulation of the celiac branches of the vagus (that innervate the jejunum, ileum, and colon) increases GLP-1 secretion (27). Accordingly, some have speculated that the diminished HCl production induced by the significant reduction of oxyntic cell mass in the LSG patients stimulates the vagally innervated antral mucosa, left intact by SG, to secret GRP and as a consequence, gastrin and GLP-1 (28).

Another possible mechanism of LSG action on hind gut hormones that has been suggested is the possible effect on gastric emptying rate following LSG. To date, few studies have addressed esophago-gastric motility following LSG with conflicting results. Control of gastric emptying is under the influence of duodenal and distal ileum receptors that have a common antro-pyloric target. When the hypertonic meal passes through the distal ileum and the

colon, it stimulates the second phase of L cells secretion (29). However, conflicting reports regarding the gastric emptying after LSG have been published. Braghetto et al. (30) studied 20 obese patients who had LSG (with partially resected antrum) compared to 18 normal controls, and evaluated their gastric emptying to liquid and solids using scintigraphy. They showed a 3 fold acceleration for liquids and twofold for solids (78 to 38 min). This paper confirmed the results of Melissas et al. (31) in which measured gastric emptying to solids before and after LSG (with antrum preservation) with morbid obese patients acting as their own controls was accelerated by two fold (94 to 48 min). However, this is in sharp contrast to the study by Bernstine et al. (32) in which measured gastric emptying in 21 morbid obese patients before and 3 months after LSG with multiple scintigraphic imaging minute intervals. According to this study, LSG performed with antrum preservation has no effect on gastric emptying since both mean time of gastric emptying and retention among the different time intervals failed to show any significant differences (103 and 107 min). A possible reason for these differences may be related to thetechnique used in the LSG. This will produce a pattern of semiobstruction (one or many strictures along the sleeve), which has been observed recently by radiological examinations after sleeve (33). Another reason for changes in gastric emptying between groups of surgeons doing sleeve gastrectomy might be due to the length of antrum left. It has been known that in pylorus-preserving gastrectomy (reasons other than obesity), thegreater amount of antrum left, the faster gastric emptying will occur (34). Therefore, patients who had very long sleeve gastrectomy affecting most of the antrum (i.e. sleeve gastrectomy starting 1 cm from the pylorus) might have slow gastric emptying.

4.3. Improved Insulin Sensitivity after LSG

A number of studies have documented that SG improves T2D (35, 36). SG results in a rapid resolution of diabetes in T2DM patients with decreased fasting insulin levels and a rapid improvement of the first phase insulin response and insulin sensitivity (37). Although our study excluded diabetic patients, the HOMA-Index (indicating insulin resistance) significantly improved for the sleeve group as compared to the obese control. Furthermore, the fasting insulin level after LSG was lower than the LAGB and obese control although it did not reach statistical difference. However, our study reported a significant insulin peak at 15 min postprandially for the LSG cohort that was significant higher the other groups. A similar finding was again reported by Peterli and his colleague (1) at 1 week and 3 months postoperatively. The better glycemic control was explained by decreased caloric intake on the short term and the well-known effect of weight loss to increase insulin sensitivity on the longer term. However, new evidence show that after SG, the improvement of insulin action occurred rapidly and independently of %EWL (38). An explanation for that is probably the identical GLP-1 peak at 15 minutes that we mentioned earlier. GLP-1 is a key incretin hormone with a primary functions of potentiating the glucose-stimulated insulin secretion, enhancement of b-cell growth and survival, and inhibition of glucagon release. Moreover, recent publication proposed that the lack of ghrelin and its insulinostatic activity increase the maximal capacity of glucose-induced insulin release and enable islet to secrete more insulin to meet an increased demand associated with obesity, thereby achieving normoglycemia (39). Interestingly, Broglio and colleagues (40) suggested that ghrelin effect on glucose homeostasis and insulin secretion and sensitivity could depend on its state of acylation. They observed that in healthy humans, the administration of des acyl ghrelin alone did not induce any change in glucose and insulin levels compared to placebo.

4.4. Satiety scores after restrictive surgery

The mechanisms by which LSG is associated with a decrease in meal frequency are multi-factorial. In the present study, we used a validated instrumet to measure hunger and satiety in the fasting state and throughout the standardized test meal. Our data showed that LSG is associated with a decreased feeling of hunger both in the fasting state and after meal intake as well as with an increased satiety after meal ingestion as compared to the matched LAGB and obese control. Furthermore, we reported a correlation between maximal levels of acyl ghrelin and PYY with satiety score. When comparing the amount of food consumed after the last satiety score, the LSG patients consumed the least followed by the LAGB and the obese control. These data matched well with our satiety scores prior to the meal.

Satiety assessment after LSG was reported by Karamanakos et al. (23) who showed a significant attenuation of appetite in LSG as compared to pre operative assessment. Another study from Himpens et al. (41) where they assessed feeling of hunger, sweet eating 1 and 3 years after LSG using questionnaire of 5 grading scale. The author reported a significant loss in hunger and sweet craving. In both of these studies, no correlation with any satiety hormones have been done.

Its well known now that the key to weight loss surgery's success is in providing relief from hunger. This relief from hunger "satiety" and the ability to produce "satiation" feeling is directly related to the amount of weight patients can lose and how successful they are in the long term. In that context, it is reasonable to hypothesize that the success of weight loss and maintenance after LSG could be due to decreased hunger and increased satiety induced not just by volume restriction but also by decreased ghrelin secretion and possible increased hindgut hormons. Consistently, the changes in acyl ghrelin, GLP-1 and PYY concentration after meal

intake are potential explanation for the changes in hunger and satiety seen post-operatively.

4.5. Study Limitations

Our study has several limitations. It is not a prospective randomized trial and patients were not used as their own control. However, we had well-matched control groups in which we were able to find differences in the levels of different satiety hormones and satiety scores. Second, the small sample size may not allow definitive conclusions on the correlation between the changes in satiety signals levels and satiety score. Finally, other satiety signals such as CCK and Oxyntomodulin were not measured which may play important part in the satiety network.

4.6. Concluding Remarks

In summary, we believe that LSG is a metabolic operation that impacts multiple satiety signals. LSG was more effective in appetite suppression and excess weight loss and the effect of LSG on patients' metabolic profile was exerted primarily by caloric restriction and ghrelin reduction after fundus removal. Furthermore, changes in PYY and GLP-1 are also factors in improved satiety. Our result would suggest that for those severely obese patients concerned with hunger drive and poor satiation with meals, LSG is a metabolic procedure that will offer optimal treatment.

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