Relationships between leptin levels, body composition, and glucometabolic parameters as a function of sex and bariatric surgery procedure type

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A Master's Thesis

Presented to the Faculty of Graduate Programs in Human Nutrition and Oregon Health & Science University School of Medicine

In partial fulfillment of the requirements for the degree of

Master of Science in Human Nutrition

December 2019

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Specific Aims and Hypotheses

With obesity rates steadily increasing over time, a thorough understanding of the causes and factors that perpetuate this condition is essential to finding effective treatment options. The difficulties people experience with inducing and maintaining weight loss belie the complex nature of the homeostatic mechanisms governing body weight and the need for treatment modalities that extend beyond lifestyle modification, pharmacologic treatment, and bariatric surgery.

Leptin is an adipocyte-derived hormone that circulates in the blood at levels proportional to the amount of adipose tissue in the body. As such, leptin is thought to play a key role in weight regulation by providing a feedback signal to the hypothalamus reflecting fat mass. As a feedback hormone, increased leptin concentrations would normally result in central nervous system inhibition of food intake, thereby limiting weight gain and restoring weight back to its set point. On the other hand, lower leptin levels would trigger central signals that lead to greater food intake so as to limit weight loss and restore baseline body weight. In patients with obesity, it is therefore believed that they experience leptin resistance, similar to insulin resistance, wherein increased leptin does not decrease food intake and, instead, contributes to further weight gain and maintenance of the obese state.

Sexual dimorphism in leptin concentrations in which women have higher leptin levels than men at the same BMI and total fat mass has been previously reported. One interpretation of this observation would be that women have greater central leptin resistance than men, necessitating a higher leptin level to maintain similar weights. Alternatively, leptin levels may play a more complex role in weight homeostasis than simply an “adiposity factor” by reflecting body composition (e.g. percent body fat) rather than absolute fat mass. Evidence regarding the relationship between percent body fat and circulating leptin levels in men and women is conflicting. Some studies indicate that even after accounting for greater percent body fat,

women's leptin levels continue to remain higher than men's, while others suggest that the difference between sexes disappears. This is an important research question to address because if leptin is truly acting as a signal of body composition (fat in proportion to lean mass) and not simply as a marker of total fat mass, then other, as of yet undiscovered, hormonal signals reflecting lean mass must also exist for the brain to properly integrate leptin levels during weight regulation. On the other hand, if women maintain greater leptin levels to account for differences in central leptin signaling compared to men, then are the actions of leptin in peripheral tissues also more leptin "resistant" in women than men? This has very important implications for normal liver, muscle, pancreatic, and immune function.

Bariatric surgery is a very effective treatment for severe obesity and its complications, including type 2 diabetes mellitus. Several preliminary research studies have shown that leptin levels post-surgery are lower than predicted for the amount of weight lost, especially after Roux-en-Y gastric bypass (RYGB) compared to the laparoscopic gastric banding (LAGB). If true, this observation would have important implications for differences in both central and peripheral leptin signaling following these two mechanistically very different surgical procedures.

To address these evidence gaps, we plan to use a large, prospective cohort of well-characterized men and women before and after bariatric surgery from the Longitudinal Assessment of Bariatric Surgery (LABS) study to address the following aims:

Specific Aim 1: To examine serum leptin concentration differences between men and women as a function of BMI, total fat mass, and percent body fat.

Hypothesis: Serum leptin concentrations will be higher in women than men matched for BMI and total fat mass but will not be statistically different between sexes after adjusting for percent body fat.

Specific Aim 2: To determine the change in serum leptin concentrations adjusted for fat mass and percent body fat for up to 3 years after Roux-en-Y gastric bypass (RYGB) versus laparoscopic gastric banding (LAGB).

Hypothesis: Adjusted serum leptin will decrease more in patients who received a RYGB than in those who received LAGB (in both men and women).

Specific Aim 3: To determine the relationship between adjusted leptin levels and glucometabolic parameters, namely HOMA-IR, HOMA-%S, and HOMA-%B, up to 3 years after RYGB and LAGB.

Hypothesis: Lower adjusted leptin levels following bariatric surgery will be associated with improved glucometabolic parameters, especially insulin secretion.

Completion of these aims will help to address key knowledge deficiencies regarding leptin's relationship to body composition in its function as an integrative signal to the hypothalamic centers that regulate body weight and how bariatric surgery alters these relationships, possibly to explain greater glucometabolic improvements after RYGB than LAGB. By gaining more insight into the pathophysiology of obesity, its interplay with leptin, and how leptin impacts weight outcomes post-bariatric surgery, clinicians can better understand, manage, and treat obesity effectively.

Background

Introduction to Homeostatic Weight Regulation

More than one-third of American adults have obesity, with this statistic steadily increasing since 1999.[1,2] Comorbidities of obesity include hypertension, type 2 diabetes mellitus (T2DM), dyslipidemia, coronary heart disease, sleep apnea, stroke, and others, which increase disease burden and have economic, medical, and psychological implications.[1,3] A number of factors influence a person's weight. Family, adoption, and twin studies have demonstrated the heritability of obesity: identical twins raised in the same or different environments tend to have similar body mass indices (BMIs), and children who are adopted have weights more similar to their biological parents than their adoptive parents.[4,5] It is estimated that the heritability of BMI ranges between 40-70% with 10-20% of genetic variants being associated with obesity, further indicating a strong genetic component to attained adult weight.[5,6] Increased availability and intake of calorie-dense, nutrient-poor foods in concert with decreased physical activity also increase a person's risk of developing obesity.[2] The interplay of genetics, environment, and lifestyle can increase a person's susceptibility to becoming obese.[2,4]

Treatment modalities for obesity include lifestyle interventions (i.e. diet and physical activity), pharmacologic treatment, and bariatric surgery.[3,7] Lifestyle interventions can attenuate obesity-related comorbidities even with modest weight reductions of 5-10%.[7,8] However, these have not been shown to be effective for long-term weight maintenance, with two-thirds of lost weight being regained after one year and the remaining weight being regained 5 years later.[8] Additional weight loss has been shown to further improve comorbidities.[7] Pharmacotherapy is recommended as an adjunct therapy to lifestyle interventions and promotes additional weight loss when compared to lifestyle changes alone.[8] Finally, bariatric surgery is an option for individuals with a BMI over 40 kg/m^2 (Obesity Class II) or with a BMI over 35 kg/m^2 (Obesity Class I) with comorbidities. These procedures alter the nutrient path flow through the

gastrointestinal (GI) tract leading to reduced food intake for long-term weight maintenance that has been demonstrated to be more effective than either lifestyle or pharmacological intervention.[9,10] Additional details on bariatric surgery are described in a later section.

The need for interventions that allow people to lose weight and keep it off beyond simple caloric restriction ("eat less") and increased activity ("exercise more") belie the complex systems involved in weight regulation. In fact, governance of body weight is now known to be homeostatically controlled by physiological mechanisms that maintain body weight within a particular set point (or range) by influencing appetite, food intake, and energy expenditure.[11,12] These mechanisms tend to be most robustly activated in response to starvation, reduced food intake, and weight loss, as opposed to counter-regulating against unwanted weight gain, suggesting a physiological tendency toward weight gain as proposed by Schwartz et al.[13] For patients who become overweight or obese, this system becomes further dysregulated, causing unwanted weight gain and increased risk of comorbidities. Insight into the mechanisms and hormones involved in weight homeostasis is essential for better understanding how and why people gain weight.

Leptin is one such hormone that is synthesized by adipocytes and circulates in concentrations proportional to adipose tissue.[6,13,14] Leptin is able to cross the blood brain barrier to act as a feedback hormone to the hypothalamus reflecting energy stores, thus leptin is referred to as an adiposity signal.[6,15] In the absence of impaired leptin signaling, increases in leptin levels trigger efferent signals that reduce appetite and food intake and increase energy expenditure, while decreases in leptin trigger signals that increase appetite and food intake and decrease energy expenditure, with each response intent on restoring weight to the body's set point.[4,6]

The genes coding for leptin and its receptor in humans, LEP and LEPR, respectively, have been studied at length in the *ob* genes of mice and rat models, which are similar to the LEP genes of humans. Inactivating mutations of either of these genes results in hyperphagia,

hypometabolism, and obesity.[4,14] Obesity caused by a mutation of the LEP gene can be reversed via exogenous leptin administration, but leptin replacement is ineffective in rodents with leptin receptor gene (*db* and *fa*) mutations.[16] It is important to note that obesity caused by mutations of the LEP or LEPR genes are very rare in humans and are not the cause of the increasing rates of obesity observed over the past thirty years. Instead, leptin resistance has been proposed as the primary mechanism leading to increasing obesity rates. Worrisomely, it has been suggested that as leptin levels continue to increase with increasing adipose tissue deposition, leptin transport across the blood brain barrier may be diminished, further reducing central signaling with the hypothalamus, creating a feed-forward mechanism that worsens leptin resistance and further contributes to unwanted weight gain.[17,18]

Exogenous leptin administration has been studied as a potential weight loss therapy in humans, but for reasons beyond the scope of this review, results have shown little success.[15] However, there is evidence that leptin administration may be useful in individuals with no or decreased leptin production and in individuals who have previously lost weight as a method for maintaining weight loss.[15,16] Having a more thorough understanding of leptin's action is critical to better understanding its essential role in weight homeostasis, the development of obesity, and the treatment thereof. Regulation of its secretion, how its blood levels are influenced by body composition, its central and peripheral effects, and how it influences bariatric surgery outcomes are covered in this review to illustrate what is known and what requires additional research.

Regulation of Leptin Secretion

Leptin secretion is mediated by a number of factors, such as weight (adiposity) gain or loss, food availability, the action of glucose and insulin, hormones, and macronutrient composition of the diet. At its most fundamental level, leptin levels track closely with the amount of fat mass (fat cell size, number of adipocytes). For example, with weight gain, there is

increased lipid deposition in adipocytes and, in some cases, a proliferation of adipocytes.[4] As a result, leptin gene expression and leptin secretion increases in proportion to total adipose, thus leading to consistently higher leptin levels in individuals with obesity than those who are lean.[4,6,14]

In addition to changes in fat mass, leptin levels are influenced by meal timing and acute changes in food availability, even before weight change occurs. Decreased food availability in the form of energy restriction and fasting lowers leptin. Decreasing caloric intake to 630 kcal/day in women and 840 kcal/day in men for the duration of one week resulted in significant leptin decreases out of proportion to decreases in body weight, percent body fat, and total body fat.[19] Another study demonstrated a 53% decrease in serum leptin following 10% weight loss.[14] In a complete fasting state with no caloric intake over 52 hours, leptin was shown to decrease by 72% in subjects with obesity and 64% of those who are normal-weight without significant weight loss.[20] The lack of weight loss accompanying the decreases in leptin indicates that changes in fat mass alone are not entirely responsible for leptin secretion. In the same study by Boden et al, a second study group was also put on a multi-day fast, but their blood glucose was held constant using an infusion of 5% glucose. Serum leptin and insulin remained stable and did not change with glucose infusion despite an absence of food intake during the study period.[20] This study confirmed the key role that insulin and glucose play in mediating the disproportionate drop in leptin levels during prolonged fasting.

Conversely, and as might be predicted, increases in leptin levels of approximately 40% have also been observed within 12 hours of overfeeding using a 120 kilocalorie/kilogram (kcal/kg) body weight diet, prior to any weight gain.[21] Overfeeding over longer periods of time, such as 5-7 weeks, with accompanying weight gain have also resulted in 3-fold increases in serum leptin concentrations, which exceeded what would be expected with increases in adipose tissue stores.[21]

Additional research highlights the importance of glucose and hormones in leptin secretion. Increases in LEP mRNA are closely tied to the rise in glucose levels, and decreases in leptin levels as a result of fasting are better correlated to glucose than insulin levels.[22] In an animal model, Mueller et al showed that when insulin was added to rat adipocytes, the uptake and metabolism of glucose produced a proportional release of leptin. The addition of the glucose transport blockers 2-DG, phloretin, and cytochalasin B all produced dose-dependent decreases in leptin secretion. Similarly, the addition of glycolysis inhibitors iodoacetate and NaFl also resulted in decreases in leptin secretion. These glucose transport and metabolism inhibitors also reduced *ob* gene expression within the adipocytes. These findings suggest that the metabolism of glucose in rat adipocytes is an important regulator of leptin gene expression and secretion. Hormones also influence leptin levels. Glucocorticoids, such as cortisol, and pro-inflammatory cytokines increase leptin concentrations while catecholamines decrease leptin levels.[17] Sex hormones, such as estrogen and testosterone, also influence leptin's sensitivity in the brain and its synthesis in peripheral adipocytes, which will be described in the next section.

In addition, macronutrient composition of the diet also has an impact on leptin responses. More specifically, altering the proportion of protein and fat in the diet appears to favorably influence leptin signaling to allow for successful weight loss without compensatory increases in appetite typically observed in weight loss accomplished with hypocaloric diets. One study examined the impact of a high-protein diet, wherein protein comprised 30% of total calories, carbohydrates comprised 50% of total calories (unchanged from a standard diet), and fat intake was reduced to 20% of calories. [23] The study design involved participants consuming a baseline "typical American" diet containing 15% calories as protein for 2 weeks, followed by an isocaloric (wherein they consumed their estimated daily caloric needs to maintain weight stability) high-protein diet for 2 weeks, which was then followed by *ad libitum* consumption of the high-protein diet for 12 weeks. Participants reported increased satiety during the isocaloric, high protein stage of the study, while their 24 hour leptin area-under-the-curve (AUC) did not change

significantly from baseline.[23] Once they transitioned to the *ad libitum* phase of the study, participants experienced a significant and consistent decrease in spontaneous caloric intake of approximately 441 ± 63 kcal/day.[23] This decrease in caloric intake resulted in significant weight loss over the 12 weeks.[23] Leptin AUC decreased significantly from the isocaloric stage to the *ad libitum* stage without any indication of increased appetite from participants, which would normally be expected.[23] In the absence of this reaction, the authors suggest that the increased protein in the study diet have may enhanced leptin sensitivity in the brain.[23] They also suggest that substituting protein for fat may be conducive to greater weight loss than by replacing carbohydrates with fat.[23]

A similar study examined the effect of a low-fat, high-carbohydrate diet, which was comprised of 15% of calories from fat, 65% of calories from carbohydrates, and 20% of calories from protein. The isocaloric stage of this study also resulted in markedly increased satiety reported by participants accompanied by no significant changes in leptin measurements.[24] The *ad libitum* phase of the study also produced decreases in spontaneous caloric intake of 16% within 24 hours of initiation as well as consistent weight loss throughout this phase.[24] Leptin AUC decreased significantly from baseline after the 12-week *ad libitum* phase, again without participants indicating any increases in appetite or caloric intake.[24] Ghrelin AUC was another measure in this study, and after the *ad libitum* phase, no increase was observed.[24] Normally, increased ghrelin works synergistically with decreased leptin to stimulate appetite and food intake for weight regain. The authors suggest that these findings indicate that a low-fat, high-carbohydrate diet may also contribute to increased central leptin sensitivity, which is supported in part by past research indicating the development of leptin resistance in proportion to dietary fat intake.[24] Taken together, these studies suggest that dietary fat restriction with replacement either by protein or carbohydrate calories can enhance weight loss effectiveness through enhanced central leptin signaling. Another feeding study by Havel et al demonstrated significantly higher 24-hour leptin secretion in response to a low-fat, high-carbohydrate,

isocaloric diet, which contrasts from the results in both of the previous studies where the isocaloric periods did not change leptin secretion.[25] These data suggest that the length of feeding study (2 weeks vs. 24 hours) may have an impact on the observed changes in leptin secretion.

While a diet low in fat appears to improve leptin sensitivity and perhaps leptin secretion in the short-term, the type of fat may also be important. Evidence suggests that unsaturated, omega-3 fatty acids specifically may enhance central leptin sensitivity. In a 4-week intervention by Payahoo et al of daily polyunsaturated omega-3 supplementation in people with obesity, participants experienced increased feelings of satiety with concurrent decreased caloric intake.[26] Additionally, the intervention group experienced a significant decrease in BMI and non-significant increase in leptin levels.[26] Another study by Kratz et al examined the effects of olive, rapeseed, and sunflower oils in participants over a 4-week period. Women consuming rapeseed oil, which is high in linolenic acid (another omega-3 fatty acid), experienced significant decreases in leptin levels while the men in the group experienced a slight increase in leptin.[27] Both of these findings were observed in the absence of change to participants' body weight, body fat, or BMI. Interestingly, the authors observed decreased energy intake in the women of this group despite their decreased leptin levels.[27] Although these studies by Payahoo et al and Kratz et al used different sources of dietary fat, they both suggest some benefits related to the unsaturated nature of these omega-3 fatty acids. Kratz et al suggest that the beneficial effects they observed could be explained by omega-3's impact on glucose tolerance and insulin sensitivity, by their ability to increase leptin gene expression in both rat and human models, and by potentially affecting the blood-brain barrier, which could positively impact leptin transport and signaling.[27] However, some of the conflicting results from these studies, such as varying effects of omega-3 supplementation on leptin levels, indicate a need for further research with study designs that are more easily comparable.

Results from studies looking into optimal macronutrient composition are reflected in the dietary recommendations for people who have undergone bariatric surgery: adequate carbohydrates providing approximately 50% of calories, slightly increased protein at 1.2 g/kg of body weight during active weight loss, and the remaining dietary needs being met mainly by unsaturated fats and not exceeding 35% of calories.[9]

Leptin secretion is influenced by a number of dietary factors, including food availability and macronutrient composition. The mechanisms underlying these factors are not fully understood, but glucose and insulin appear to play important roles. The data also indicate that restricting dietary fat and replacing calories with protein or carbohydrates improves central leptin signaling. While the factors discussed in this section affect leptin secretion, circulating concentrations of leptin may be further influenced by body composition, body fat distribution, and sex hormones.

Determinants of Blood Leptin Levels: Body Weight and Parameters of Composition

Some studies have reported higher leptin levels in women than men, even when adjusting for body mass index (BMI) and differences in fat mass.[14,28-30] Factors that may be influencing this discrepancy in leptin levels include the ratio of subcutaneous fat to visceral fat and sex hormones, which vary between men and women. However, this sexual dimorphism is not well understood, and there is evidence that the difference in leptin levels between men and women disappears when adjusting for other body composition measures, namely percent body fat.

Women typically have more fat mass than men as well as more subcutaneous fat than visceral or omental fat.[30-32] Montague et al found that human subcutaneous adipocytes had a significantly greater amount of leptin mRNA than omental adipocytes.[32] Leptin mRNA correlates well with circulating leptin levels, suggesting that subcutaneous adipocytes produce more leptin than omental adipocytes.[32] Similarly, leptin concentrations were significantly correlated with

subcutaneous fat thickness in women in another study by Minocci et al.[30] If women consistently have greater amounts of subcutaneous than omental adipose tissue as compared to men, fat distribution could potentially be part of the mechanism behind women's elevated leptin levels.

Another explanation may be in the different levels of estrogen and testosterone in men and women, their impact on adipocytes' ability to synthesize leptin, and their effects on the brain's sensitivity to leptin. Vettor et al suggest that free testosterone has an inhibitory effect on leptin production in adipocytes. In their study, free testosterone levels were significantly inversely correlated with plasma leptin levels in men.[33] Free testosterone has been shown to increase the density of β-adrenergic receptors on adipocytes, thereby increasing their sensitivity to catecholamines. In rats, catecholamine signaling increases cAMP and decreases *ob* gene expression and leptin release.[33] The authors thus suggest that the inverse association between free testosterone and blood leptin levels may be explained by enhanced catecholamine signaling.[33] These findings may also help explain why men have lower levels of leptin compared to women: their relatively higher testosterone levels may be inhibiting their maximal leptin production.

This peripheral action of testosterone on leptin levels is contrasted with the findings of Clegg et al, which found that estradiol promotes greater hypothalamic sensitivity to leptin. In this study, rats with higher levels of estrogen (either intact females or females post-ovariectomy and intact males administered estradiol) were more sensitive to leptin's anorexigenic effects than to the effects of insulin, meaning they demonstrated significant decreases in food intake and body weight.[31] Additionally, female rats post-ovariectomy and intact males given exogenous estradiol developed body fat distribution patterns similar to those found in the intact females.[31]

An important test of this hypothesis in humans is the effect of menopause and hormone replacement therapy (HRT) on leptin levels in women. If estrogen increases central sensitivity to leptin, this sensitivity may be lost with menopause onset. Indeed, Kristensen et al found that post-menopausal women not treated with HRT experience 2.4-fold higher accumulation of body

fat when compared to women treated with HRT for 5 years, indicating decreased sensitivity to leptin and perhaps indicating a state of leptin resistance in the context of decreased estrogen. This is supported by the finding that the control group also experienced a significant increase in leptin levels over the course of the study.[34] However, leptin levels also increased significantly in the HRT group, though they were significantly lower than levels in the group not treated with HRT. Additionally, weight gain was not completely avoided in the HRT group: those women still experienced a significant increase in body fat, albeit still significantly less than the control group.[34] These findings suggest that, in contrast to rodent models, leptin sensitivity in the brain does not rely on estrogen alone in humans.

Due to increased amounts of subcutaneous fat and its increased capacity to produce and secrete leptin, it is logical to conclude that women have increased circulating leptin levels when compared to men. It would also be logical to assume that women are more sensitive to leptin due to their greater relative amount of estrogen. However, we do not see leptin's effect of decreasing women's fat mass to amounts similar in men: women continue to have, on average, greater amounts of fat mass. These discrepancies raise important questions as to whether women are naturally more leptin resistant than men and whether leptin levels are always greater in women when looking at various measures of body composition.

A number of body composition measures have been used to compare leptin levels between men and women. In a study by Saad et al, women had leptin levels approximately 3 times higher than men without any adjustments. This trend continued after adjusting for weight, percent body fat, BMI, and fat mass.[28] Other research supports the difference in leptin levels in women and men in some but not all of these anthropometric measures. Nicklas et al found this trend to be true only when examining fat mass: at any given fat mass, women had approximately 3 times more leptin.[29] Considine et al also found this trend when matching women and men by BMI.[14] However, these last two papers in addition to another by Maffei et al report that after adjusting for percent body fat, the difference in leptin concentrations between

men and women disappeared.[14,29,35] Whether leptin is a marker of total body composition or whether it truly differs between women and men remains unclear. Thus, one aim of this study is to explore the notion that leptin does not simply reflect adipose stores, but that it signals a more nuanced picture of body composition (fat amount in relation to body size or non-fat mass) to the hypothalamus. When adjusting for this more nuanced picture of total body composition, that is percent body fat, our hypothesis is that leptin levels will no longer be different between men and women.

Leptin Signaling

Central Leptin Signaling

Leptin can bind to five different leptin receptors (LepR), which are found on a number of tissues both centrally and peripherally, including the hypothalamus, pituitary gland, lymphoid tissue, pancreas, ovaries, and skeletal muscle.[17] In the brain, the only LepR isoform expressed is the long form (LepRb), which is also the only isoform that interacts with STAT3, a signal transducer and transcription activator that will be discussed shortly.[6,12]

Once leptin binds the LepRb, 5 possible signaling pathways may be stimulated: 1) JAK2/STAT3, 2) JAK2/STAT5, 3) IRS/PI3K, 4) SHP2/MAPK, and 5) AMPK/ACC, with Janus kinase 2 (JAK2) being the initial trigger for the first four. In the first and second pathways, when leptin binds to its long-form receptor, LepRb, JAK2 is activated and phosphorylates 3 tyrosine residues ($Tyr^{985, 1077, 1138}$) in the cytoplasmic region of LepRb. Phospho-Tyr^{1077} and phospho-Tyr^{1138} phosphorylate STAT3 and STAT5, which translocate into the nucleus and bind to the POMC and AgRP promoter regions. This binding stimulates POMC expression and inhibits AgRP expression, which stimulate appetite and suppress energy expenditure. One pathway that inhibits leptin signaling is with suppressor of cytokine signaling 3 (SOCS3), which is the target gene of STAT3. SOCS3 acts as a negative feedback to inhibit the JAK2/STAT3 pathway by

interacting specifically with phospho-Tyr^{985} of JAK2. Tyrosine phosphatase 1B (PTP1B) also inhibits leptin signaling by dephosphorylating JAK2.[17]

JAK2 activates insulin receptor substrate (IRS), which, in turn, activates phosphatidylinositol 3 kinase (PI3K) in the IRS/PI3K pathway. PI3K activates phosphodiesterase 3B (PDE3B) and protein kinase B (Akt), decreasing intracellular cyclic adenosine monophosphate (cAMP) and activating mammalian target of rapamycin (mTOR), respectively. Akt also stimulates POMC neurons via ATP-sensitive potassium channels and voltage-gated calcium channels. Finally, Akt inhibits Forkhead box O1 (FoxO1), which normally stimulates AgRP and inhibits POMC expression. All of these effects decrease appetite and increase energy expenditure in response to leptin.

The SHP2/MAPK pathway begins with JAK2 activating Tyr^{985}, which phosphorylates SH2-containing protein tyrosine phosphatase 2 (SHP2). SHP2 recruits growth factor receptor-bound protein 2 (Grb-2) before finally activating mitogen-activated protein kinase (MAPK). MAPK can also be activated directly by JAK2 to decrease food intake and increase energy expenditure.

The binding of leptin inhibits adenosine monophosphate-activated protein kinase (AMPK), which activates acetyl-CoA carboxylase, leading to decreased food intake. When AMPK is active (i.e. when leptin is not bound to its receptor), in the context of weight loss or fasting, for example, it increases food intake and weight. This particular pathway occurs both centrally in the brain and in peripheral tissues but in opposing directions.[17] Knowing the pathways impacted by leptin provides details into its effects on weight homeostasis and overall health.

Leptin and CNS Regulation of Body Weight

Leptin has a number of central functions in humans including weight homeostasis through regulation of feeding and energy expenditure, modulation of several neuroendocrine

axes, and memory and learning.[17] The arcuate nucleus (ARC) in the hypothalamus is the main integration center for the afferent leptin signals, which directly target two neuron populations found there: POMC/CART (pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript) and AgRP/NPY (Agouti-related peptide/neuropeptide Y) neurons.[6,12,17] Leptin activates POMC/CART-containing neurons via the action of STAT3 and STAT5 to increase expression of alpha-melanocyte-stimulating hormone (α-MSH), which activates downstream melanocortin pathways and decreases appetite and increases energy expenditure.[6,12,17,18] This anorexigenic pathway involves α-MSH binding to melanocortin 4 receptor (MC4R), a specific G-protein coupled melanocortin receptor found in the paraventricular nucleus (PVN) of the hypothalamus.[6,12] Additional inhibition of feeding has been demonstrated by leptin's stimulation of neurotrophic factor and steroidogenic factor-1 (SF-1).[17] In the orexigenic pathway of the ARC, AgRP/NPY neurons secrete the hormones AgRP and NPY, which work together to increase food intake and decrease energy expenditure in response to decreased leptin levels.[6,18] AgRP accomplishes this by acting as an antagonist of MC4R, thus blocking α-MSH's action in the PVN.[12] NPY binds a number of receptors that stimulate appetite. Y1R and Y5R receptors in the lateral hypothalamic area (LHA) are stimulated by NPY and contain orexins and melanin-concentrating hormone that increase appetite and encourage weight gain.[12,17] The Y2 receptor increases appetite upon binding of NPY, but inhibits feeding upon stimulation by peptide-YY (PYY).[36] GABA is also an appetite stimulant, and AgRP/NPY neurons express GABA synthesizing enzymes and transporters, likely serving as an additional influence on appetite.[37]

The reduction in fat mass that accompanies weight loss decreases serum leptin levels, activating the orexigenic AgRP/NPY neuronal response. Thus, the CNS compensates for the lost body mass, conserving fat mass through reductions in energy expenditure and enhanced food-seeking behaviors, such as delayed satiation, decreased food restraint, and decreased perception of the quantity of food eaten, so as to facilitate return to the previous body weight, referred to as the body's set point.[15]

When losing weight, healthy subjects tend to lose 15-30% fat free mass (FFM) and 70-85% fat mass (FM), although there can be a great deal of variation between individuals.[11] Loss of FFM, which is more metabolically active than FM, leads to a decrease in total energy expenditure (TEE).[18] This decrease in TEE is primarily accomplished by increases in skeletal muscle efficiency, which have been shown to increase by 20% with a 10% weight loss and is responsible for 75% of the drop in non-resting energy expenditure.[15] Thus, when comparing one individual who has lost weight via diet and exercise to a person of the same weight who has not undergone any weight loss, the former will have a lower TEE than the person who is weight stable.[18] It has been hypothesized that decreased levels of thyroid hormones as well as decreased sympathetic tone may play into this effect as well.[15] These adaptations to weight loss prevent the maintenance of a lower body weight through lifestyle alone in most people.

Some studies have investigated feeding behaviors observed in individuals who are attempting to maintain their weight loss. Findings include persistent feelings of hunger, delayed and decreased satiation with meals, and altered perception of the quantity of food consumed.[15] Some studies evaluating brain activity of individuals who have lost weight have demonstrated increased emotional and cognitive responses in the orbitofrontal cortex to seeing food with decreased activity observed in the hypothalamus, where leptin's effect is largely mediated.[15] However, leptin receptor expression is found not just in the hypothalamus, but also in regions of the pre-frontal cortex, hippocampus, and amygdala.[15] Receptors at these locations may help understand how regulation of eating goes beyond basic, physiological responses, and how it can be influenced by learned behaviors, memory, and reward systems in the brain.[15] Indeed, modern eating behaviors are thought to be influenced by social factors, environment, cognition, and opportunity.[11] These "hedonic" influences are not likely the root cause of the obesity epidemic, but they most certainly contribute to unwanted weight gain and the dysregulation of homeostatic mechanisms. All these factors, physiological, social, and environmental, contribute to the regain of weight lost and demonstrate the multi-faceted difficulty people encounter when

trying to maintain their weight loss.[12,13,15]

Leptin and Fertility

Leptin appears to have another central role in regulating fertility. Leptin is expressed in a number of tissues associated with fertility, including the placenta and ovaries in women.[38] At either end of the weight spectrum, both in those who are overweight and obese as well as those who are underweight, women may experience infertility due to lack of leptin response, as seen in leptin resistance or in leptin deficiency.[39] Congenital leptin deficiency is also associated with inadequate gonadotropic-releasing hormone (GnRH) secretion, which can result in hypogonadotropic hypogonadism as well as a failure to reach puberty.[38] In normal subjects, leptin and insulin promote the secretion of gonadotropin-releasing hormone (GnRH), a key mediator of reproductive function.[39] It follows, then, that ovulatory function improves in women with leptin deficiency and hypothalamic amenorrhea who have been given exogenous leptin.[39] Additionally, fertility in leptin-resistant obese mice is restored after exogenous leptin administration, indicating a potential fertility treatment for women with obesity.[39]

While leptin stimulates the release of GnRH, GnRH neurons do not express LepRb, so leptin's effect must be indirect.[38] One possible explanation for leptin's indirect effect on GnRH is that neurons in the ARC that control appetite and energy expenditure (i.e. POMC and AgRP/NPY) are anatomically associated with GnRH neurons, thereby creating a connection between appetite, energy expenditure, and fertility.[38] A second possibility involves the interplay of leptin and kisspeptins. Kisspeptins have been shown to stimulate the release of GnRH, luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone in a number of animal models.[38] In humans, mutations causing kisspeptin receptor dysfunction result in hypogonadotropic hypogonadism.[38] The interplay between leptin and kisspeptins has been shown in 40% of cells in the ARC expressing *Kiss1* (the gene encoding for kisspeptins) mRNA

that also express LepRb mRNA.[38] In mouse studies, *ob/ob* mice treated with leptin responded with increased levels of *Kiss1* mRNA, which helps elucidate the connection between leptin and reproductive function.[38] Finally, a third explanation for leptin's indirect effect on GnRH lies in a subpopulation of neurons in the ARC that express hormones, including kisspeptins, that influence the feedback mechanisms of GnRH and that also have LepRb, which demonstrates the interplay of neurohormonal regulation of fertility as well as appetite and energy expenditure.

It has been suggested that fertility is one of the main reasons why leptin levels in women are higher than in men, and that higher leptin levels may allow for preservation of reproductive function in the context of caloric deficit.[28] Chan et al found that lean women preserved their leptin levels more effectively than men during a fast by decreasing overall leptin production while maintaining normal rates of leptin clearance, whereas lean men's leptin levels decreased and leptin clearance rates increased.[40] The authors suggest this could be indicative of women's state of pseudo leptin resistance when compared to men as well as a strategy to preserve fertility in times of decreased food availability.[40] However, it is important to question the extent to which it is evolutionarily advantageous to preserve reproductive capacity in the context of decreased food availability and access.

Peripheral Leptin Signaling

Beyond central functions on weight and appetite regulation, leptin also impacts peripheral organs, including skeletal muscles, liver, pancreas, the immune system, and nutrient absorption. In skeletal muscle, leptin activates AMPK (as opposed to inhibiting it in the hypothalamus), which phosphorylates and inhibits acetyl-CoA carboxylase (ACC), causing a decrease in malonyl-CoA and stimulating the carnitine shuttle system, resulting in increased fatty acid oxidation.[17,41] The activation of AMPK also stimulates glucose uptake by skeletal muscles.[17,41] The subsequent utilization of glucose and fatty acids for energy production as

opposed to converting and storing them as triglycerides may help prevent the build-up of intracellular fat and may thus prevent metabolic and functional impairments of muscles.[38,41,42] By preventing an excess of fat accumulation in the muscle, this pathway has been proposed as a mechanism by which intramuscular triglyceride stores are maintained within a set range to perform normal signaling and structural functions.[42] However, decreased leptin signaling or impairment thereof would disrupt this homeostatic mechanism, which could cause abnormal fat accumulation in skeletal muscle. Additionally, the leptin inhibits hepatic gluconeogenesis as well as insulin production and secretion in the pancreas, which positively impacts blood glucose control.[17]

Similar to skeletal muscle, the liver also experiences increases in fatty acid oxidation when stimulated by leptin, which reduces intrahepatic lipid concentrations, fasting blood glucose, and decreases the risk of T2DM.[43] On the other hand, as described above, leptin has been shown to suppress insulin production in the pancreas via the JAK2/STAT/SOCS3 pathway.[17] A resulting reduction in insulin levels might actually worsen glucose control if leptin levels increase to a degree where glycemia cannot be adequately controlled.

Leptin also impacts the innate and adaptive immune systems. In the innate immune system, STAT3 activation by leptin signaling promotes natural killer cells, while leptin directly stimulates neutrophil chemotaxis and macrophage phagocytosis. Pro-inflammatory cytokines such interleukin-6 and -12 are released in response to leptin and also prevents apoptosis in neutrophils and regulates maturation of dendritic cells.[17] In the adaptive immune system, leptin increases the amount the native T cells as well as facilitating the transition to using helper T cells in an immune response. It also helps activate B cells, which secrete cytokines. All of the immune-related leptin effect mentioned above suggest an important role for the hormone in mediating inflammation and immunity.[17]

Leptin has been shown to have impacts on nutrient transport in the GI tract. Leptin receptors have been found on both apical and basolateral membranes of enterocytes, with

gastric leptin binding to apical receptors and circulating leptin binding to basolateral receptors.[44] Luminal leptin (secreted as gastric leptin) has been shown to inhibit and reduce the number of SGLT-1, which transport galactose and glucose, while increasing the activity of GLUT2 and GLUT5, which transports glucose and fructose, respectively.[44] Luminal leptin may also impact amino acid and peptide absorption via PEPT1, though there are confounding results as to whether leptin positively or negatively influences their transport.[44] Leptin from the general circulation stimulates leptin receptors on the basolateral membrane of enterocytes to decrease triglyceride export as chylomicrons, apolipoproteins B-100 and B-48, and low-density lipoproteins (LDL).[44]

Novel physiological actions of leptin have been emerging, with one example being aldosterone secretion and its impact on blood pressure regulation. Aldosterone has been shown to increase parallel with adiposity, but its normal regulatory activators (plasma potassium, angiotensin II, and adrenocorticotropin) do not, suggesting that another factor is involved.[45] Immunostaining of human adrenal cortex cross-sections revealed leptin receptors on zona glomerulosa cells, which produce aldosterone.[46] This finding has been used to propose that leptin has a direct stimulatory effect on aldosterone, but the exact mechanism of how this occurs is unclear.[45] Blood pressure has important implications in cardiovascular health, and Faulkner et al suggest that increases in adiposity, leptin, and thus aldosterone may help explain hypertension in individuals with obesity, though they also add that the mechanisms and the effects of this pathway likely differ between sexes and is more pronounced in women.[45]

Central leptin signaling and the implications of leptin resistance have been discussed at length in the literature. Peripheral leptin resistance and its mechanisms have not been addressed as thoroughly. With what we know about the development of obesity, increasingly elevated leptin levels appear to desensitize some peripheral organ systems while potentially stimulating others. For example, it is known that the development of obesity includes the accumulation of fat not only in adipose tissue, but also in muscle and the liver. Leptin normally

increases fatty acid oxidation in both of these organs, so perhaps leptin resistance is taking place in people with obesity, desensitizing these organs and allowing for accumulation of fat outside of normal depots. If this desensitization also occurs in the pancreas, leptin would no longer inhibit insulin production and secretion, potentially exhausting β-cells in the long-term. Additionally, leptin normally increases glucose uptake by skeletal muscle indirectly through the stimulation of AMPK and subsequent inhibition of ACC, so in the context of leptin resistance, blood glucose may not be as effectively controlled, leading to further insulin release, β-cell exhaustion, and the development of insulin-dependent T2DM overtime. In the case of leptin increasing aldosterone secretion, this may be an example of increasingly higher leptin acting in a positive feedback loop with aldosterone secretion, thereby promoting the development of hypertension. Finally, the effects of high leptin levels are not as clear-cut in the GI tract, though there is evidence of maladaptive changes to nutrient absorption. For example, a 4- week hypercaloric diet decreased PEPT1 transporters by 46% and decreased the total number of leptin receptors in the GI tracts of mice, which the authors suggest may be a sign of leptin desensitization.[44] Taken together, when altered, these proposed roles of peripheral action could significantly impact the severity of obesity expression and the development of obesity-related complications.

Leptin Levels after Bariatric Surgery

Several bariatric surgical procedures result in meaningful and sustained weight loss. Two examples of these are the Roux-en-Y gastric bypass (RYGB) and the laparoscopic gastric banding (LAGB). RYGB involves reducing the size of the stomach to hold approximately 30 milliliters and then attaching the jejunum, which is disconnected from the duodenum, to the newly formed gastric pouch, which forms the Roux, or alimentary limb.[47-50] As a result, the distal stomach, duodenum, and proximal jejunum are bypassed, and together they create the “Y” in Roux-en-Y.[50] LAGB is a restrictive procedure and involves a band being placed around the

opening to the stomach that can be tightened or loosened by injecting or removing saline, respectively.[18]

Weight loss following these procedures has been demonstrated to be both significant and maintained long-term. After 7 years, Courcoulas et al found that 75% of RYGB recipients maintained at least 20% of their total weight loss, and 50% of LAGB recipients maintained at least 16% of their weight loss in the same time period.[51] In an analysis of the Swedish Obese Subjects Study (SOS) evaluating weight loss and other health outcomes up to 10 years post-bariatric surgery, Sjortrom et al found that RYGB recipients experienced a 38±7% weight loss at 6 months post-operatively and maintained a 25±11% weight loss at 10 years.[52] LAGB experienced a 21±10% weight loss at 6 months and maintained 13.2±13% weight loss 10 years after surgery.[52]

A number of GI hormones change after RYGB that are thought to uniquely influence weight loss success via appetite and energy expenditure compared to the purely restrictive procedure LAGB. For example, satiety signals such as glucagon-like-peptide 1 (GLP-1) and peptide-YY (PYY), which are secreted in response to a meal to delay gastric emptying and increase feelings of satiety, increase after RYGB.[50] GLP-1 is also increased post-RYGB but not in patients with LAGB.[50] Ghrelin, which increases hunger and GI motility prior to meals, decreases shortly post-RYGB with this effect persisting for greater than 1 year post-operatively.[12,50] On the other hand, ghrelin levels increase with weight loss after LAGB.[50] It is these changes in GI hormones and others, as opposed to the physical changes made to the size of the gastric pouch, that are believed to drive the weight loss post-operatively, especially in the case of RYGB since the changes in these hormones is more pronounced than with LAGB.[12,50,53]

Bariatric surgery also impacts circulating leptin levels. Decreases in adipocyte area and increases in leptin receptor gene expression have been observed with weight loss following Roux-en-Y gastric bypass (RYGB), which could explain the decrease in leptin levels accompanying weight loss and potentially an increase in leptin sensitivity as well.[54] A number of

studies and meta-analyses suggest that leptin concentrations decrease significantly by 6 and 12 months post-RYGB when compared to pre-RYGB levels.[50,55] Many studies have also found that decreases in leptin post-RYGB correlate positively with change in weight, fat mass, and BMI.[50] This persists across varying levels of glucose tolerance from normal to T2DM, indicating that glucose tolerance does not impact leptin reduction post-operatively.[56] There have also been reports of leptin decreasing rapidly after RYGB before any meaningful weight loss has occurred, leading some to hypothesize that this may be due to significantly decreased oral intake.[19,20,55] Chief cells and endocrine cells of the gastric mucosa secrete leptin into the gastric lumen and into circulation, respectively, so without food intake stimulating gastric leptin in RYGB, this may further contribute to the decrease in serum leptin concentrations post-operatively.[44,55,57]

Subjects undergoing LAGB also experience a significant decrease in leptin post-operatively. In a study by Ram et al, subjects' plasma leptin concentrations decreased significantly by 54% from baseline to 6 months post-operatively (119.3 ± 53.1 ng/mL to 67.3 ± 40.5 ng/mL).[58] From 6 to 14 months, leptin decreased by another 15% to 57.4 ± 34.5 ng/mL.[58] Similar to the correlations in the RYGB studies, the authors also found a strong correlation between plasma leptin concentration and BMI at baseline and 6 and 14 months post-operatively.[58] Another study by Urbanavicius et al compared leptin 1 year post-LAGB in diabetic and non-diabetic subjects and found similar results in both groups: leptin in the diabetic subjects decreased significantly from 34.91 to 25.17 ng/mL, and leptin decreased significantly from 38.74 to 29.07 ng/mL in the non-diabetic subjects.[59] While the decreases in leptin within each group were statistically significant, the difference between groups was not.[59]

Although both RYGB and LAGB result in lowered leptin concentrations, RYGB appears to decrease levels to a greater degree than LAGB when comparing people with similar anthropometric measurements. In a study by Korner et al, lean women, overweight women, women with LAGB, and women with RYGB were compared at weight stability. Despite being matched for BMI, age, and post-operative period, women with LAGB had significantly higher

leptin levels than women with RYGB, who had leptin levels similar to lean women.[53] The authors suggested that this finding in women with RYGB may indicate increased leptin sensitivity, because the decrease in leptin levels was greater than what would be expected with the amount of weight loss that occurred in the RYGB group.[53] Other research have examined the role of changing adipocytes following weight loss on leptin levels. In a study by Löfgren et al, women who lost weight via vertical gastric banding, another purely restrictive weight-loss procedure, or conventional lifestyle intervention were compared to weight-matched controls once they reached weight stability. In women who lost enough weight to no longer be considered obese, leptin levels and adipocyte leptin production were 54% and 68% lower than controls, respectively, despite being matched for percent body fat, body fat distribution, and BMI.[60] These leptin measures in the treatment group displayed a linear relationship with fat cell volume as well, meaning that women who were previously obese had many more subcutaneous fat cells that were smaller compared to controls.[60] The authors suggest that this adipose hyperplasia is likely the cause of the significantly lower leptin levels in women post-weight loss.[60]

This trend of larger reductions in leptin than predicted in response to weight loss has also been demonstrated in people with T2DM. In a study by Purnell et al, leptin levels were much lower than expected based on subjects' fat mass after RYGB but not LAGB surgery, and a baseline lower leptin-to-fat mass ratio was predictive of T2DM remission.[61] Our research will use the same original dataset, but expanded to include participants both with and without T2DM to examine the change in leptin post-RYGB versus post-LAGB, with and without adjustment for changes in body composition, and the relationships between leptin adjusted for fat mass and the improvement in various glucometabolic parameters, such as HOMA-IR, HOMA-%S, and HOMA-%B.

Health Improvements Post-Bariatric Surgery

Accompanying this weight loss following bariatric surgery, comorbidities such as T2DM or glucose intolerance, dyslipidemia, hypertension, and sleep apnea, also show improvement post-operatively. In a study comparing RYGB to sleeve gastrectomy (SG), pooled results from both groups indicate drastic improvement in incidence of T2DM from 40% of subjects to 3.3% post-operatively.[55] Additionally, insulin, fasting glucose, insulin resistance index, and HbA1c were also significantly reduced post-operatively and decreased in parallel with weight.[55]

Specifically with RYGB, glycemia appears to improve markedly post-operatively before significant weight loss occurs.[61] In the Longitudinal Assessment of Bariatric Surgery (LABS), 71% of subjects who underwent RYGB experienced remission of diabetes at one year post-operatively and 68.7% still experienced remission after 3 years.[61] On the other hand, LAGB recipients experienced diabetes remission rates of 29.9% at one year post-op and 30.2% after 3 years.[61] Subjects who were more likely to achieve diabetes remission included those who were younger, used fewer non-insulin diabetes medications, had a shorter duration of diabetes diagnosis, had a higher percentage of body fat, and had greater reductions in body weight, waist circumference, and neck circumference.[61] Both RYGB and LAGB increased insulin sensitivity significantly but only subjects undergoing RYGB demonstrated a decrease in insulin secretion post-operatively.[61]

In another analysis of the LABS dataset, Courcoulas et al assessed health and weight outcomes 7 years post-operatively in subjects who underwent either RYGB or LAGB. Overall diabetes prevalence in the RYGB group decreased from 28.3% to 11.6% at 7 years while no significant differences were observed in the LAGB group.[51] From years 3 to 7, diabetes prevalence increased slightly in this group, though not due to increased numbers of new cases of diabetes but instead due to decreased rates of remission of diabetes over that time period.[51]

The Swedish Obese Subjects Study (SOS) was a prospective trial that followed individuals with obesity up to 10 years who either underwent bariatric surgery or who were treated conventionally with lifestyle and behavior modification and served as the control group. In this

study, recipients of both RYGB and LAGB has significantly lower incidence of diabetes and higher rates of recovery from diabetes compared to the control group.[52]

Dyslipidemia is also greatly impacted by bariatric surgery. In the LABS dataset analysis by Courcoulas et al, RYGB significantly decreased the prevalence of high LDL (33.3 to 14.3%), low HDL (34.9 to 5.8%), and high triglycerides (23.7 to 4.9%) at 7 years post-operatively, whereas subjects undergoing LAGB experienced significant decreases in the prevalence of low HDL (33 to 16.3%) and high triglycerides (21.3 to 9.7%) only.[51] The rates of prevalence, incidence, and remission did not change significantly in these markers over years 3 to 7 in either surgical group except for triglycerides in the LAGB group, which had increasing remission rates from 66.2 to 80.8% over this period.[51]

In the SOS study, the incidence of subjects with high triglycerides were significantly lower in subjects who had undergone bariatric surgery as opposed to the controls.[52] Similarly, subjects receiving either RYGB or LAGB experienced higher rates in recovery from low HDL and high cholesterol than the control subjects.[52]

While bariatric surgery has significant effects on diabetes and dyslipidemia, it has demonstrated small impacts on hypertension. Seven years after RYGB in the LABS analysis, the prevalence of hypertension decreased from 67.6 to 51.6%.[51] When examining the period between 3 and 7 years post-operatively, hypertension prevalence increased slightly due to both increased incidence as well as decreased remission rates. While there were no significant changes in prevalence of hypertension in the LAGB group from baseline to 7 years, the period between 3 and 7 years showed an increase in prevalence of approximately 56.4%.[51] Interestingly, the authors note that incident hypertension is not uncommon following either RYGB or LAGB.[51] In the SOS study, recovery from hypertension was more frequent in the surgical group as compared to the controls, but the incidence of hypertension did not differ significantly across both surgical and control groups over the course of the study.[52]

Finally, evidence suggests that bariatric surgery improves or resolves sleep apnea. In a meta-analysis by Buchwald et al, recipients of both gastric bypass and gastric banding demonstrated significant improvements in sleep apnea, with 94.6% of banding patients and 86.6% of bypass patients experiencing resolution of their symptoms.[62]

Summary

Obesity and unwanted gain are growing concerns due to their impacts on chronic disease and quality of life. Lifestyle, pharmacologic, and surgical interventions are all used to reduce excess fat mass and improve comorbidities. With weight loss being exceptionally difficult to maintain, research is being done to better understand the components of weight homeostasis. Leptin is a hormone secreted by adipocytes that is implicated in body weight homeostasis that has both central and peripheral effects to regulate appetite, food intake, energy expenditure, muscular and hepatic fatty acid oxidation, pancreatic function, immune function, nutrient absorption, and more. Some studies indicate that women have higher leptin levels compared to men, but when adjusting for different anthropometric measures, this difference may disappear. In obesity, leptin levels are high. Normally, the central nervous system reacts to elevated leptin to decrease appetite and food intake to reduce body weight. However, leptin resistance is a phenomenon wherein the hypothalamus loses sensitivity to the action of leptin, thereby contributing to unwanted weight gain. Bariatric surgery is one treatment modality of obesity that has been shown to induce significant, lasting weight loss while also reducing leptin levels toward normal without the unwanted weight regain. It also positively impacts obesity-related comorbidities such as type 2 diabetes. In the present proposal, we will use a database of subjects to examine relationships between leptin and anthropometric measures in men and women and to examine the leptin response after undergoing bariatric surgery. We aim to address the discrepancy in leptin levels in men and women by examining

leptin as a function of percent body fat. We will also compare the degree of leptin decrease after Roux-en-Y gastric bypass and laparoscopic gastric banding and the association of this decrease in leptin to improved glucometabolic parameters post-operatively. With this research, we hope to elucidate leptin's relationship to body composition and how bariatric surgery changes these relationships to gain more insight into the pathophysiology of obesity and the treatment thereof.

Methods

General Design

The Longitudinal Assessment of Bariatric Surgery (LABS) consortium is a large, multicenter, prospective database used to plan, develop, and conduct coordinated clinical, epidemiological, and behavioral research around bariatric surgery, specifically the Roux-en-Y gastric bypass (RYGB) and laparoscopic adjustable gastric banding (LAGB) procedures. The LABS-2 dataset is an observational cohort of approximately 2400 participants collected between 2006 and 2009. For the purposes of this thesis, demographic, anthropometric, clinical, behavioral, surgical, and postoperative data collected before and 1, 2, and 3 years after bariatric surgery were analyzed. Blood specimens were collected at baseline and postoperatively at 12 months and annually thereafter.

Participants' biochemical and anthropometric data from this dataset were studied to determine the relationship between serum leptin and body composition and how leptin changes post-RYGB and post-LAGB. The institutional review board at each study center approved the protocol and consent forms, and all participants provided informed consent before enrollment.

Biochemical Analyses

Serum leptin, glucose, and insulin were used in this analysis. Serum leptin levels were determined by radioimmunoassay kit (EMD Millipore, Inc. St. Charles, MO). Glucose was measured by Roche autoanalyzer (Roche Diagnostics Inc., Indianapolis, IN). Insulin was measured by a two-site immunoenzymometeric assay using Tosoh 2000 autoanalyzer (Tosoh Bioscience, South San Francisco, CA). Additionally, HOMA-%B, HOMA-%S, and HOMA-IR were used in the analysis. HOMA-IR, a measure of insulin resistance, is calculated by multiplying fasting glucose by fasting insulin and dividing by 405. HOMA-%S, a measure of insulin sensitivity, is calculated by multiplying fasting insulin by fasting glucose and dividing the

result by 22.5. HOMA-%B, a measure of insulin secretion, is calculated by multiplying 20 by fasting insulin, dividing by fasting glucose, and then subtracting 3.5.

Anthropometric Variables

Body weights were measured on a study-purchased standard scale (Tanita® Body Composition Analyzer, model TBF-310). Participants' body mass indices (BMIs) were calculated by dividing participants' weight in kilograms by their height in meters squared. Percent body fat (%BF) was measured by bioelectrical impedance analysis (BIA) using a Tanita scale model TBF-310 (Tanita Corporation, Arlington Heights, IL). Fat mass (FM) was calculated by multiplying percent body fat by the participants' body weight in kilograms.

Statistical Analyses

We used descriptive statistics to characterize baseline demographic, anthropomorphic and laboratory measures, and diabetes status for our study population overall, by sex (men and women) and by surgery type (RYGB and LAGB). Lab results reported well below the normal range (e.g. c-peptide of <0.05 or glucose of <2) were left out of the analysis. Two waist circumference measurements were taken at each study visit, and a third was taken if the first two measurements differed by more than 2 cm. For our analyses, all waist circumference measures were averaged for each study visit. The race and ethnicity variables were combined to create a smaller total number of race/ethnicity variables: Non-Hispanic White, Non-Hispanic Black, Hispanic, and Other, which includes Non-Hispanic Asian, Non-Hispanic American Indian or Alaska Native, Non-Hispanic Native Hawaiian or Pacific Islander, and Non-Hispanic Other. For the baseline descriptive statistics, diabetes status had three levels: no diabetes, pre-diabetes, or diabetes. For subsequent analyses, subjects with no diabetes diagnosis and those with a pre-diabetes diagnosis were combined.

Normality of the continuous variables was assessed using the Shapiro-Wilk test, which showed our variables to be non-normally distributed. To normalize leptin, HOMA-IR, HOMA-%S, and HOMA-%B, the natural log was taken, and confidence intervals in subsequent statistical analyses were bootstrapped. When variables are log-transformed, the resulting differences are multiplicative as opposed to additive, so the results were back-transformed for clearer interpretation. Medians and interquartile ranges (IQR) were computed for the continuous variables; frequencies and percentages were computed for the categorical variables. Differences in subject characteristics between sex and surgery types were tested using Mann-Whitney Rank Sum tests for continuous variables and chi-square analyses for the categorical variables.

Specific Aim 1, Hypothesis 1: To test the hypothesis that leptin levels would be higher in women than in men at baseline while controlling for body weight (kg), BMI, fat mass (FM), but would not be different between sexes after adjusting for percent body fat (%BF), we used multiple linear regression to assess the association between sex and leptin levels at baseline, controlling separately for body weight, BMI, FM, and %BF. A series of nested models were built to examine these relationships. The first was an unadjusted model with only sex as the independent variable. Next, four minimally adjusted models were created by separately adding in body weight, BMI, FM, and %BF to the sex-only model. Last, age, waist circumference, and diabetes status were added to create four fully adjusted models. Model fit diagnostics, including AIC/BIC, the normality of the residuals, r-squared, and homoscedasticity (the variability of the variables within the model), were also assessed for each model.

Specific Aim 2, Hypothesis 1: We tested the hypothesis that leptin levels divided by fat mass would be lower after RYGB compared to after LAGB at each post-surgery time point (1, 2, and 3 years post-surgery). We used mixed effects regression to examine the effect of surgery and time on post-surgery leptin levels, controlling for baseline diabetes diagnosis. Our variables of interest were surgery type, visit (discrete), and an interaction of surgery type by visit. This

interaction term allowed us to test if leptin levels were different over time according to surgery type. To examine the relationship between leptin levels and surgery type further, we assessed how sex affected this relationship. We incorporated a three-way interaction into our mixed effects regression (visit x surgery type x sex), which allowed us to test if sex modified any differences previously seen in leptin levels over time according to surgery type.

Specific Aim 3, Hypothesis 1: We tested the hypothesis that leptin levels divided by fat mass following bariatric surgery would be associated with improved glucometabolic parameters, especially insulin secretion (HOMA-%B). Glucometabolic parameters examined were HOMA-IR, HOMA-%S, and HOMA-%B. The natural log of each of these measures was used in the analysis due to their non-normal distribution. We tested these associations using mixed effects regression with the three HOMA measurements as the outcomes. Leptin levels divided by fat mass, time, and an interaction between leptin levels and time were the primary predictors. We created our regression models and then stratified by surgery type (RYGB and LAGB) in order to assess the relationship between glucometabolic parameters, leptin levels, and time according to surgery type.

Results

Baseline Characteristics

At baseline, 2264 subjects were included in the analysis, with the majority of subjects being female (79.02%) (Tables 1 and 2). By design, participants were predominately in the severely obese or greater category with a median (IQR) BMI of 45.62 (41.62-50.99) kg/m^2. In general, men and women had significantly different baseline measurements for all variables except for HOMA-%B (Table 3). Prior to surgery, men had larger BMIs (BMI 46.48 kg/m^2 (42.36-53.37) versus 45.35 kg/m^2 (41.5-50.51) in women, $p < 0.001$) and had more fat mass (FM) than women (65.42 kg (53.32-83.33) versus 63.70 kg (55.78-73.52), $p = 0.023$), but had lower percent body fat (%BF) (45% (38.4-50.2) versus 51.7% (49.5-53.9), $p < 0.001$) (Table 3). The majority of subjects (74.12%, n = 1678) underwent Roux-en-Y gastric bypass (RYGB) while 25.88% (n = 586) received laparoscopic adjustable gastric banding (LAGB).

The surgery groups also differed in several baseline variables (Table 4). Subjects who went on to get RYGB had higher baseline BMI (46.36 kg/m^2 (42.28-51.69) versus 43.76 kg/m^2 (40.28-47.97)), FM (65.06 kg (56.61-76.42) versus 60.84 kg (52.91-70.64)), and %BF (51.3% (48.3-53.9) versus 50.5% (47.1-53.1)) compared to subjects who would go on to receive LAGB.

Subjects identifying as non-Hispanic White comprised the majority of the study population at 82.15% (Table 2). At baseline, nearly half (49.53%) of the subjects did not have a type 2 diabetes mellitus (T2DM) diagnosis whereas 16.67% had a diagnosis of pre-diabetes, and 33.79% were diagnosed with T2DM (Table 2). There was a significant association between sex and diabetes diagnosis (χ^2 (2) = 44.003, $p < 0.001$), where women made up the majority of subjects with T2DM at baseline (71.24% women vs. 28.76% men) (Table 5). There was an additional significant association between sex and racial/ethnic group identification (χ^2 (3) = 15.933, $p = 0.001$), where women made up the largest percentage of minority groups (Table 2). There was also a significant association between procedure type and diabetes status (χ^2 (2) = 8.998, $p = 0.011$), where recipients of RYGB had slightly higher rates of T2DM at baseline

compared to those who would receive LAGB (35.5% vs. 28.92%), and between procedure type and racial/ethnic group identification (χ^2 (3) = 8.749, p = 0.033), where non-Hispanic Whites were more likely to choose LAGB and all other racial/ethnic groups were more likely to choose RYGB.

Table 1 displays the baseline measures of continuous variables' medians and interquartile ranges (IQR).

Table 1 – Baseline Characteristics (continuous)

	Total (n)	Median	IQR
Age (years)	2264	46	37-54
Weight (kg)	2264	127.89	114.74-145.58
Height (m)	2264	1.68	1.63-1.73
BMI	2264	45.62	41.62-50.99
WC (cm)	2205	130.75	121-142.25
% BF	1944	51.1	48.05-53.7
FM (kg)	1944	64.06	55.4-75.38
Leptin (ng/mL)	2215	56.8	42.2-73.2
Leptin/FM	1905	0.87	0.67-1.10
Leptin/%BF	1905	1.13	0.86-1.41
Insulin (uU/mL)	2254	20	13.2-31.1
C-peptide (ng/mL)	2232	3.81	2.87-5.02
Glucose (mg/dL)	2241	97	89-114
HbA1c (%)	2237	5.6	5.2-6.3
HOMA-IR	1775	2.44	1.65-3.56
HOMA-%S	1775	41	28.1-60.7
HOMA-%B	1775	140	100.8-185.6

BMI = body mass index, measured in kg/m^2
WC = waist circumference, measured in cm
% BF = percent body fat
FM = fat mass, calculated by multiplying body fat percentage by weight in kg
HOMA-IR = homeostatic model assessment of insulin resistance; measure of insulin resistance
HOMA-%S = homeostatic model assessment of insulin sensitivity; measure of insulin sensitivity
HOMA-%B = homeostatic model assessment of insulin secretion; measure of insulin secretion

Table 2 displays the frequencies and percentages of baseline categorical variables as the percentage of the entire study population and then broken down by men and women.

Table 2 – Baseline Characteristics (categorical)				
	Total (n)	Percent	Men (%)	Women (%)
Sex	2264			
Men	475	20.98%	-	-
Women	1789	79.02%	-	-
Procedure	2264			
Roux-en-Y	1678	74.12%	20.14%	79.86%
Laparoscopic gastric banding	586	25.88%	23.38%	76.62%
Diabetes Status	2243			
No diabetes	1111	49.53%	16.02%	83.98%
Pre-diabetes	374	16.67%	20.59%	79.41%
Diabetes	758	33.79%	28.76%	71.24%
Ethnicity	2262			
Non-Hispanic	2155	95.27%	21.21%	78.79%
Hispanic	107	4.73%	15.89%	84.11%
Race	2264			
White/Caucasian	1988	87.42%	22.06%	79.02%
Black/African American	252	11.08%	12.85%	87.15%
American Indian/Alaska Native	48	2.11%	8.33%	91.67%
Asian	10	0.44%	30%	70%
Native Hawaiian/Pacific Islander	9	0.39%	11.11%	88.89%
Race/Ethnicity	2259			
Non-Hispanic White	1864	82.15%	22.51%	77.49%
Non-Hispanic Black	235	10.36%	13.36%	86.64%
Hispanic	107	4.72%	15.89%	84.11%
Other	63	2.78%	11.11%	88.89%
Fasting for blood draw visit 1	2264			
Yes	1924	84.61%	21.32%	78.68%
No	332	14.59%	18.98%	81.02%
Unknown/NA	18	0.80%	22.22%	77.78%

Table 3 displays the results of analyzing the continuous variables at baseline using Mann-Whitney Rank Sum test and comparing by sex.

Table 3 – Comparison of Baseline Variables by Sex

	Men (at baseline)			Women (at baseline)			
	Total (n)	Median	IQR	Total (n)	Median	IQR	p-value
Age (years)	475	48	39-57	1789	45	36-53	< 0.001*
Weight (kg)	475	151.02	133.79-170.07	1789	123.81	111.56-137.87	< 0.001*
Height (m)	475	1.78	1.75-1.85	1789	1.65	1.6-1.7	< 0.001*
BMI	475	46.48	42.36-52.37	1789	45.35	41.5-50.51	< 0.001*
WC (cm)	462	145.38	136-155.25	1743	127.45	118.6-137	< 0.001*
%BF	405	45	38.4-50.2	1539	51.7	49.5-53.9	< 0.001*
FM (kg)	405	65.42	53.32-83.33	1539	63.70	55.78-73.52	0.023*
Leptin (ng/mL)	471	38.8	27.2-52.4	1744	61.4	48-77	< 0.001*
Leptin/FM	401	0.55	0.39-0.75	1504	0.94	0.76-1.15	< 0.001*
Leptin/%BF	401	0.85	0.62-1.16	1504	1.18	0.95-1.47	< 0.001*
Insulin (uU/mL)	472	25.1	16.3-39.35	1782	18.7	12.6-29.3	< 0.001*
C-peptide (ng/mL)	471	4.2	3.29-5.53	1761	3.72	2.81-4.88	< 0.001*
Glucose (mg/dL)	472	101	90.5-125	1769	97	88-111	< 0.001*
HbA1c (%)	469	5.8	5.3-6.9	1768	5.5	5.2-6.1	< 0.001*
HOMA-IR	362	2.98	2.04-4.15	1413	2.34	1.59-3.39	< 0.001*
HOMA-%S	362	33.45	24.1-48.9	1413	42.8	29.5-63	< 0.001*
HOMA-%B	362	146.15	102-191.9	1413	138.7	100.8-184.6	0.059

IQR = interquartile ranges
BMI = body mass index, measured in kg/m^2
WC = waist circumference, measured in cm
%BF = percent body fat
FM = fat mass, calculated by multiplying body fat percentage by weight in kg
HOMA-IR = measure of insulin resistance

HOMA-%S = measure of insulin sensitivity
HOMA-%B = measure of insulin secretion
* denotes significance of $p < 0.001$

Table 4 displays the results of analyzing the continuous variables at baseline using Mann-Whitney Rank Sum tests and comparing by surgical procedure.

Table 4 – Comparison of Baseline Variables by Surgery type

	RYGB (at baseline)			LAGB (at baseline)			
	Total (n)	Median	IQR	Total (n)	Median	IQR	p-value
Age (years)	1678	45	37-54	586	47	37-56	< 0.001*
Weight (kg)	1678	130.16	116.1-149.21	586	122.45	111.11-138.78	< 0.001*
Height (m)	1678	1.68	1.63-1.73	586	1.68	1.63-1.73	0.989
BMI	1678	46.36	42.28-51.69	586	43.76	40.28-47.97	< 0.001*
WC (cm)	1637	132.15	122.5-143.65	568	126.5	117.45-136.83	< 0.001*
%BF	1428	51.3	48.3-53.9	516	50.5	47.1-53.1	< 0.001*
FM (kg)	1428	65.06	56.61-76.42	516	60.84	52.91-70.64	< 0.001*
Leptin (ng/mL)	1639	57.8	43.2-73.6	576	53.1	39.7-71.3	< 0.001*
Leptin/FM	1398	0.86	0.67-1.09	507	0.87	0.64-1.13	< 0.001*
Leptin/%BF	1398	1.14	0.89-1.42	507	1.09	0.8-1.4	< 0.001*
Insulin (uU/mL)	1672	20.35	13.5-31.1	582	18.95	12.8-30.3	< 0.001*
C-peptide (ng/mL)	1651	3.85	2.91-5.04	581	3.75	2.77-4.98	< 0.001*
Glucose (mg/dL)	1660	98	89-114	581	97	89-113	< 0.001*
HbA1c (%)	1656	5.6	5.2-6.35	581	5.5	5.2-6.2	< 0.001*
HOMA-IR	1323	2.49	1.68-3.6	452	2.3	1.58-3.48	< 0.001*
HOMA-%S	1323	40.1	27.8-59.6	452	43.5	28.75-63.5	< 0.001*
HOMA-%B	1323	141.9	100.8-187.1	452	135.9	100.75-180.55	< 0.001*

IQR = interquartile ranges

BMI = body mass index, measured in kg/m^2
WC = waist circumference, measured in cm
%BF = percent body fat
FM = fat mass, calculated by multiplying body fat percentage by weight in kg
HOMA-IR = measure of insulin resistance
HOMA-%S = measure of insulin sensitivity
HOMA-%B = measure of insulin secretion
* denotes significance of $p < 0.001$

Table 5 shows the results of the chi-square analyses comparing the categorical variables between sexes at baseline. In these analyses, the combined race/ethnicity variable was used.

Table 5 – Comparison of categorical variables by sex at baseline

	df	chi2	p-value
Procedure	1	3.163	0.075
Diabetes status	2	44.003	<0.001*
Race/Ethnicity	3	15.933	0.001*

CI = confidence interval
* denotes significance of $p < 0.05$

Comparison of Leptin Levels between Men and Women

Women had 1.63 times higher leptin levels than men at baseline (61.4 ng/dL versus 38.8 ng/dL) ($p < 0.05$) (Tables 3 and 6). After adjusting for weight in kilograms, BMI, total FM, or BF%, women still had higher leptin than men. Regardless of body composition adjustment, the differences in leptin levels between men and women remained significant. The addition of age, waist circumference, and diabetes status to the statistical models resulted in small but significant increases in these differences (weight: 2.06; BMI: 1.72; FM: 1.76; and %BF: 1.65) (Table 6).

Table 6 displays the coefficients and confidence intervals for the unadjusted, minimally adjusted, and fully adjusted models comparing the natural log of leptin between men and women as a function of various measures of body composition at baseline.

Table 6 - Difference in the natural log of leptin between men and women at baseline

	Unadjusted[1]	Minimally-adjusted[2]	Fully-adjusted[3]
	0.492 (0.442, 0.542)	-	-
Body Weight (kg)	-	0.697 (0.649, 0.745)	0.724 (0.673, 0.774)
BMI	-	0.522 (0.480, 0.563)	0.545 (0.494, 0.597)
Fat Mass	-	0.524 (0.475, 0.573)	0.568 (0.514, 0.622)
Percent Body Fat	-	0.320 (0.259, 0.381)	0.501 (0.433, 0.569)

[1] Sex is the only independent variable in this model.
[2] Model contains both sex and the variable listed in the left-most column (measures of body composition) as independent variables.
[3] Model contains the variables in the minimally adjusted model as well as age, waist circumference, and diabetes status.
Interpretation of these coefficients to compare leptin between men and women can be determined by exponentiating the β-coefficient. For example, when controlling for body weight in kilograms, women have 2.01 times (or exp(0.697)) greater leptin levels than men at baseline.

Comparison of Leptin/FM between Procedures and Sex Post-operatively

After surgery, those who underwent RYGB lost more weight at every time point than those who underwent LAGB (Figure 1). Likewise, both FM (Figure 2) and absolute leptin (Figure 3) levels were also significantly lower at every post-operative visit, particularly following RYGB.

Figure 1 shows mean weight (kg) overtime post-operatively by surgery type.

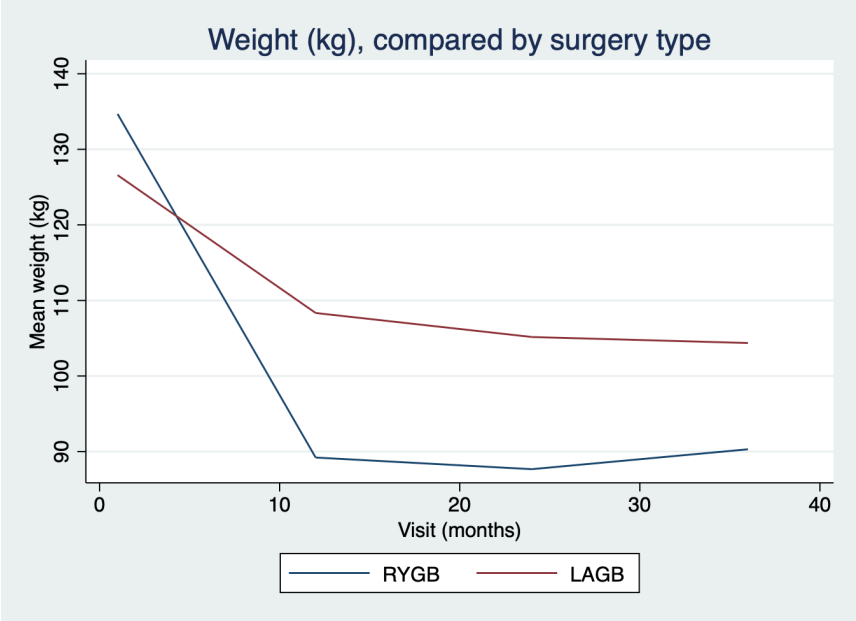


Figure 2 shows mean fat mass (kg) overtime post-operatively by surgery type.

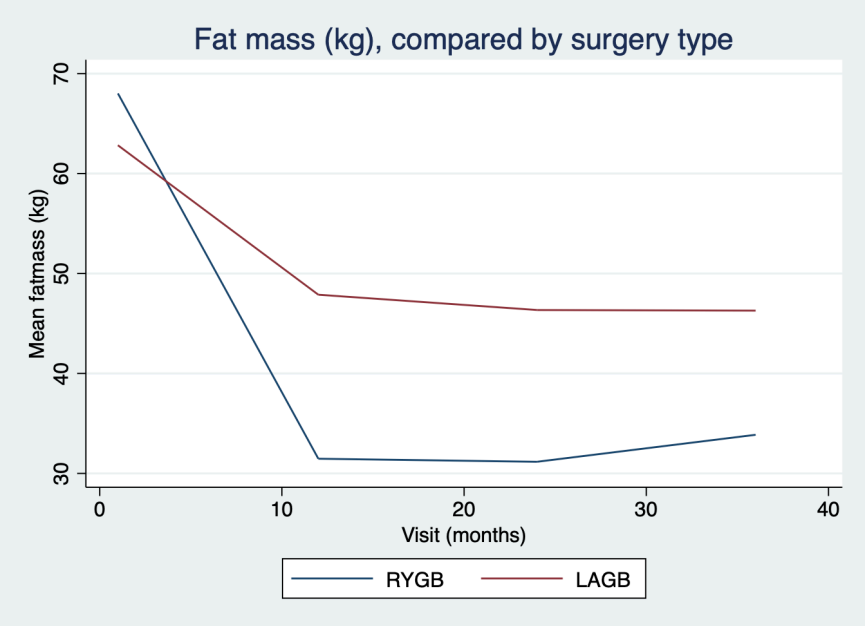
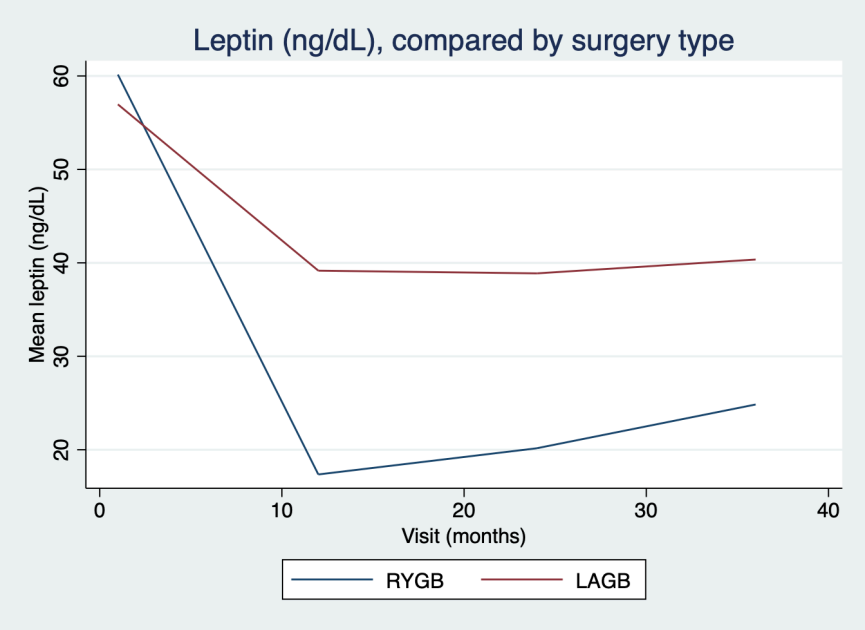


Figure 3 shows mean leptin (ng/dL) levels overtime post-operatively by surgery type.



To test if the lower leptin levels after RYGB compared to LAGB were a function of the greater loss of fat mass or differences in body composition, absolute leptin levels were adjusted for both FM and then %BF. To examine the relationship between leptin/FM and procedure type overtime, a mixed effects regression model including procedure type, visit, and an interaction between procedure and visit was used. We also controlled for baseline diabetes status in all of our models. In this model, baseline leptin/FM was not significantly different between surgical groups (0.007 unit difference, CI [-0.035, 0.049], $p = 0.776$). The ratio of leptin/FM decreased more in subjects after RYGB compared to subjects after LABG (surgery type main effect $p < 0.05$) at all post-operative time points (time $p < 0.05$). The rate of change over time was different by surgery type (interaction $p < 0.05$) with a more pronounced decrease in subjects post-RYGB, suggesting that even though the subjects with RYGB lost more total fat mass post-operatively, the remaining adipose tissue secreted less leptin per kilogram of fat mass than adipose tissue of subjects post-LAGB (Table 7 and Figure 4).

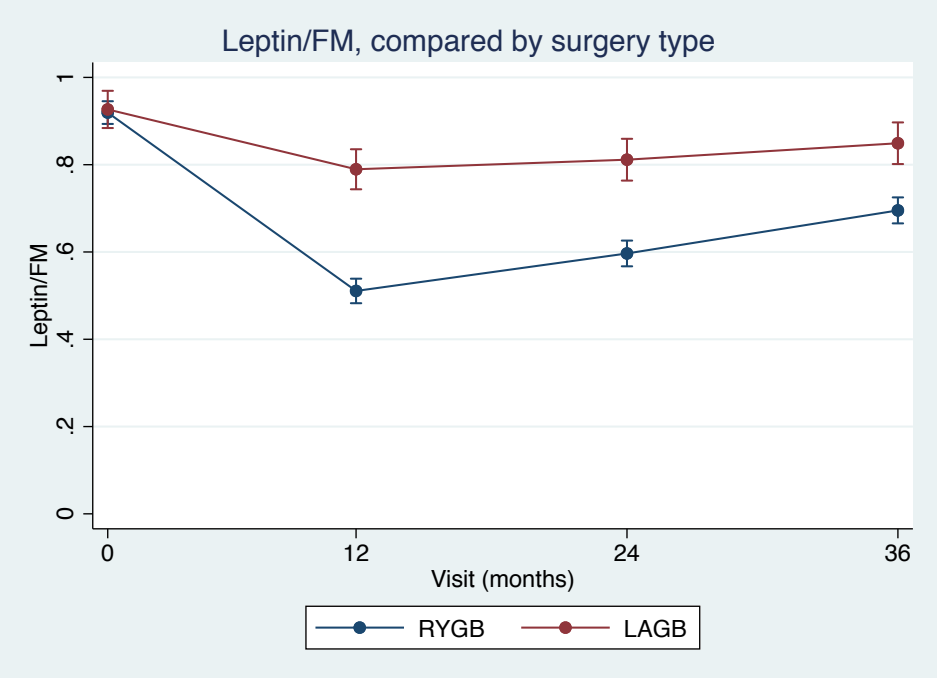
In both surgical groups, there were slight increases in leptin/FM from 12 to 24 months and from 24 to 36 months (Table 7 and Figure 4). These increases were not significant at either time point in the LAGB group, but they were significant in the RYGB group ($p < 0.05$).

Table 7 displays the estimated means of leptin/fat mass and 95% confidence intervals for both surgery types at each post-operative time point, controlling for baseline diabetes status.

Table 7 - Leptin/FM at each time point post-operatively, by surgery type

Time	LAGB	RYGB
Baseline	0.927 (0.888, 0.965)	0.919 (0.901, 0.938)
12 months	0.790 (0.756, 0.823)	0.512 (0.488, 0.534)
24 months	0.811 (0.775, 0.848)	0.597 (0.569, 0.624)
36 months	0.849 (0.813, 0.886)	0.695 (0.640, 0.751)

Figure 4 shows the estimated means and 95% confidence intervals of the leptin/fat mass over time post-RYGB and post-LAGB.



We then examined whether these changes in leptin/FM differed post-operatively in men and women by adding sex to the model. The overall effect of surgery, visit, and sex was significant ($p < 0.05$), meaning that surgery, time, and sex all influence leptin/FM.

The leptin/FM ratio in men was not significantly different between surgery groups at baseline ($p = 0.743$). After surgery, leptin/FM in men with RYGB was significantly lower at all post-surgical time points compared to men with LAGB ($p < 0.05$) (Table 8 and Figure 5). Women had a similar pattern as men: the surgery groups at baseline did not differ significantly in leptin/FM ($p = 0.225$). Women receiving RYGB had significantly lower leptin/FM ratios than those receiving LAGB at each time point post-operatively ($p < 0.05$). In both men and women, leptin/FM ratios appear to rebound slightly from 12 to 36 months (Figure 5): men who underwent RYGB experienced a significant increase from 24 to 36 months ($p < 0.05$), while women in both surgery groups experienced significant increases from 12 to 24 months and from 24 to 36 months ($p < 0.05$).

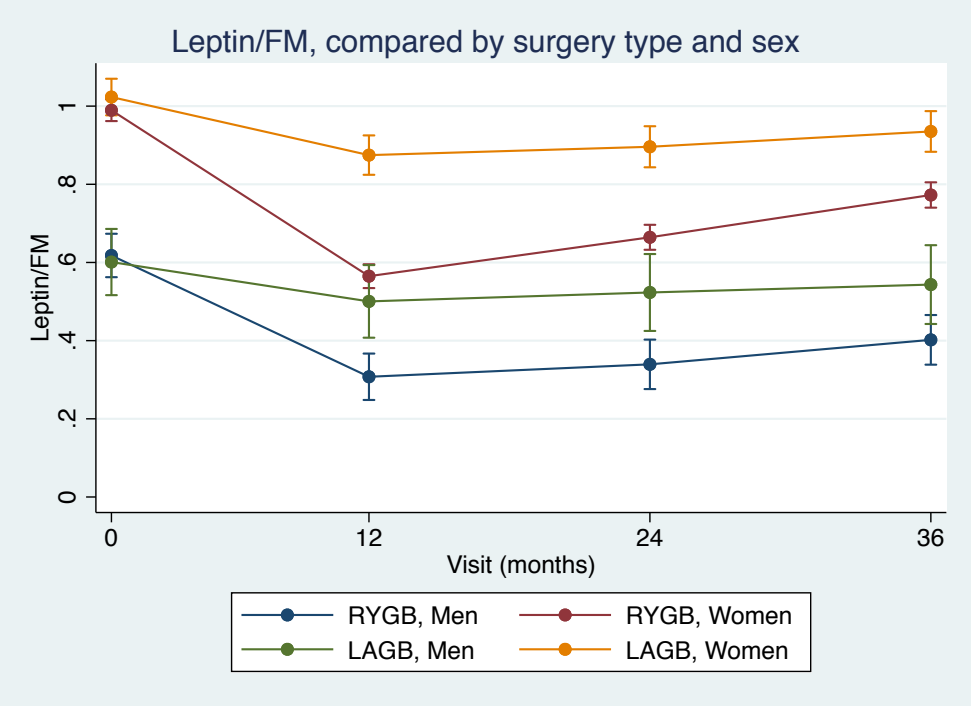
Table 8 displays the estimated means of leptin/fat mass and 95% confidence intervals for both surgery types at each post-operative time point according to sex, controlling for baseline diabetes status.

Table 8 – Leptin/FM at each time point post-op, by surgery type and sex

Time	Surgery Type	Sex	Mean Leptin/FM (95% CI)
Baseline	LAGB	Male	0.601 (0.549, 0.653)
		Female	1.023 (0.979, 1.067)
	RYGB	Male	0.618 (0.586, 0.649)
		Female	0.990 (0.970, 1.009)
12 months	LAGB	Male	0.500 (0.455, 0.546)
		Female	0.875 (0.837, 0.912)
	RYGB	Male	0.308 (0.275, 0.340)
		Female	0.565 (0.538, 0.592)
24 months	LAGB	Male	0.523 (0.466, 0.581)
		Female	0.896 (0.856, 0.936)
	RYGB	Male	0.339 (0.313, 0.366)

		Female	0.664 (0.632, 0.697)
36 months	LAGB	Male	0.543 (0.481, 0.606)
		Female	0.935 (0.895, 0.975)
	RYGB	Male	0.402 (0.371, 0.433)
		Female	0.773 (0.705, 0.841)

Figure 5 shows the means and 95% confidence intervals of leptin/fat mass by sex and surgery type from baseline to 36 months post-operatively.



Similar results were found when using leptin/%BF. At baseline in our model, leptin/%BF was not significantly different between surgery groups ($p = 0.085$) (Table 9 and Figure 6). At every time point post-operatively, subjects who received RYGB had significantly lower leptin/%BF than those who received LAGB ($p < 0.05$). Additionally, increases in leptin/%BF at 24 and 36 months were only significant in subjects who had received RYGB ($p < 0.05$). In the model that added sex as a covariate, leptin/%BF at baseline in men was not statistically different between surgery types ($p = 0.0547$) (Table 10 and Figure 7). At each time point post-

operatively, men who had received RYGB had significantly lower leptin/%BF than those who had received LAGB ($p < 0.05$). This same pattern was shown in women: there was no difference at baseline between the surgery groups ($p = 0.552$), but post-operatively, women who had received RYGB had lower leptin/%BF than those who received LAGB ($p < 0.05$). The increases observed in leptin/%BF at 24 and 36 months in both surgery groups and in men and women were only significant in women who had received RYGB ($p < 0.05$).

Table 9 displays the estimated means of leptin/percent body fat and 95% confidence intervals for both surgery types at each post-operative time point, controlling for baseline diabetes status.

Table 9 - Leptin/%BF at each time point post-operatively, by surgery type

Visit	LAGB	RYGB
Baseline	1.153 (1.102, 1.204)	1.201 (1.179, 1.224)
12 months	0.854 (0.813, 0.896)	0.463 (0.442, 0.484)
24 months	0.866 (0.823, 0.909)	0.531 (0.507, 0.554)
36 months	0.898 (0.854, 0.942)	0.629 (0.593, 0.666)

Figure 6 shows the means and 95% confidence intervals of leptin/percent body fat over time post-RYGB and post-LAGB.

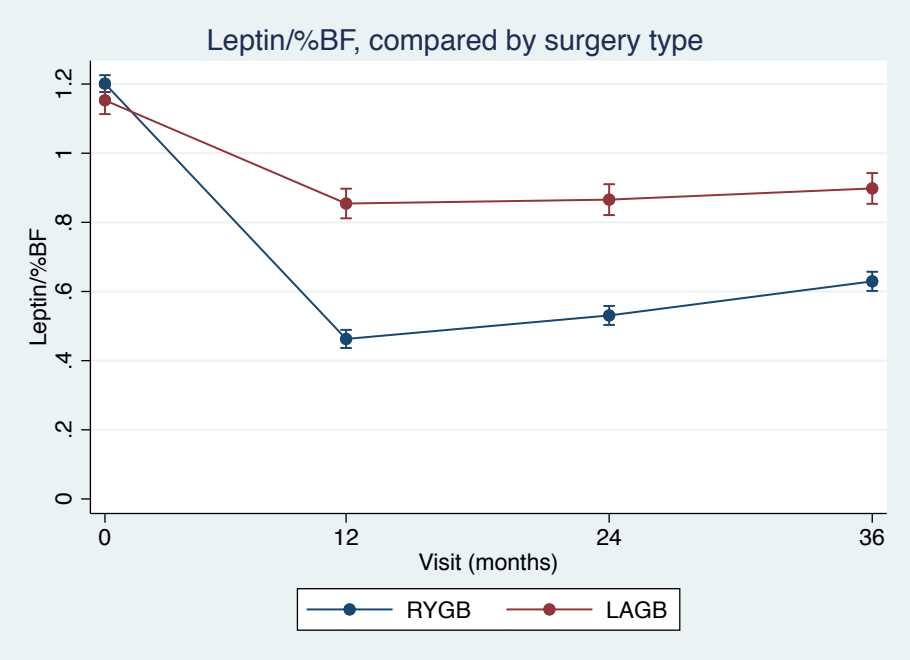
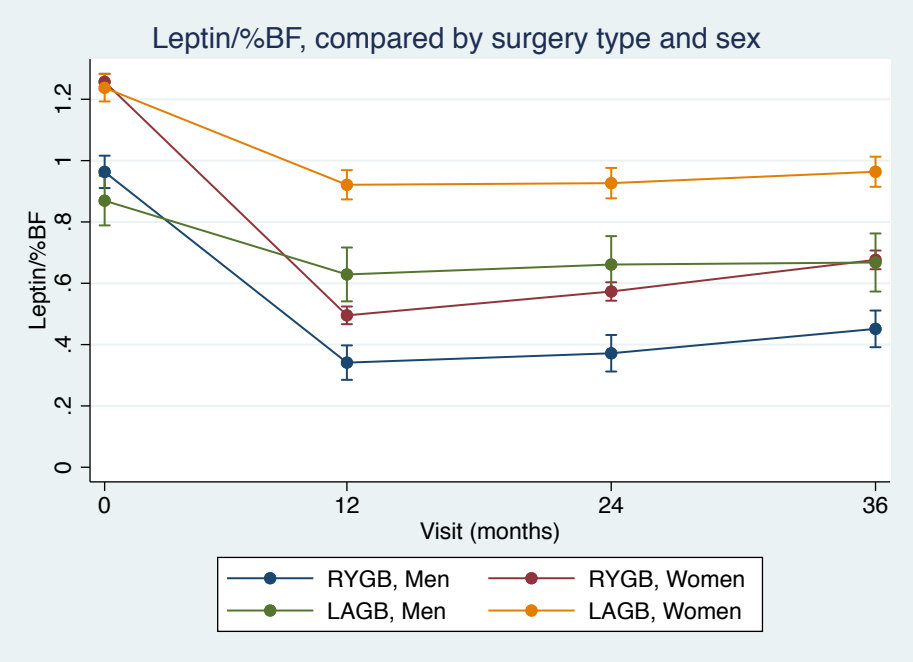


Table 10 displays the estimated means of leptin/percent body fat and 95% confidence intervals for surgery type at each post-surgery time point according to sex, controlling for baseline diabetes status.

Table 10 – Leptin/%BF at each time point post-op, by surgery type and sex

Time	Surgery Type	Sex	Mean Leptin/%BF (95% CI)
Baseline	LAGB	Male	0.869 (0.790, 0.948)
		Female	1.238 (1.178, 1.297)
	RYGB	Male	0.963 (0.912, 1.015)
		Female	1.257 (1.233, 1.281)
12 months	LAGB	Male	0.629 (0.565, 0.692)
		Female	0.921 (0.872, 0.970)
	RYGB	Male	0.341 (0.301, 0.382)
		Female	0.495 (0.472, 0.519)
24 months	LAGB	Male	0.661 (0.581, 0.742)
		Female	0.927 (0.878, 0.975)
	RYGB	Male	0.372 (0.338, 0.406)
		Female	0.573 (0.546, 0.601)
36 months	LAGB	Male	0.668 (0.591, 0.744)
		Female	0.964 (0.914, 1.013)
	RYGB	Male	0.451 (0.412, 0.491)
		Female	0.676 (0.632, 0.720)

Figure 7 displays the means and 95% confidence intervals of the leptin/percent body fat by sex and surgery type from baseline to 36 months post-operatively.



Regardless of whether leptin was indexed to FM or %BF, the ratios at every post-operative time point were lower after RYGB compared to LAGB. The same results were found when comparing men and women by surgery type: RYGB consistently resulted in lower ratios of leptin to FM and to %BF.

Relationships between Leptin and Glucometabolic Measures Post-operatively

HOMA-IR

Insulin resistance, as measured by HOMA-IR, decreased in the entire study group post-operatively, with the majority of this improvement taking place within the first 12 months after surgery in parallel with the decreases in FM and leptin levels (Figures 2 and 3), and continued to decrease at each time point (Figure 8a and Table 11). The leptin/FM ratio was related to the calculated HOMA-IR value at all time points. At baseline, for every unit of leptin/FM decrease

within the study population, HOMA-IR changed by a factor of 0.879 ($p = 0.001$). At each time point post-operatively, similar relationships between leptin/FM and HOMA-IR were observed but they became stronger. As leptin/FM decreased, HOMA-IR decreased by a factor of 0.662, 0.731, and 0.691, respectively (Table 12). While all of these associations were significant, the difference in association from baseline only reached significance at 36 months ($p < 0.001$), suggesting that surgery may have changed the relationship between leptin/FM and HOMA-IR by this time point.

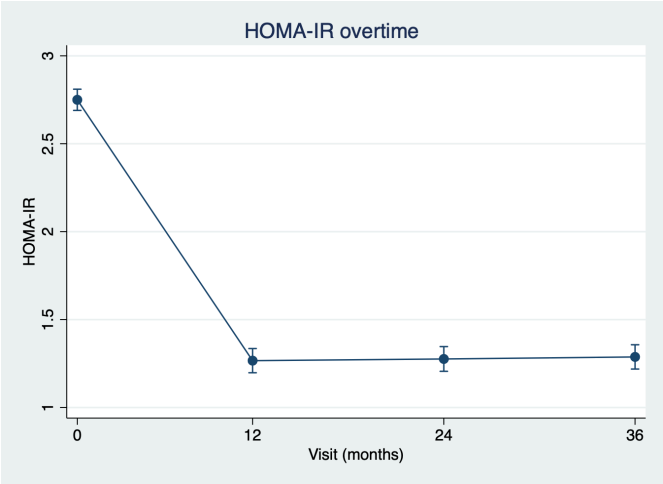
We then looked at the effect of surgery type on the relationship between leptin/FM and HOMA-IR. Changes in HOMA-IR post-RYGB showed a similar pattern as that observed in the entire study group's: the greatest decrease in insulin resistance occurred 12 months after surgery, but slight increases in mean ln(HOMA-IR) were noted at 24 and 36 months post-op (Figure 8b and Table 11). At baseline before undergoing RYGB, leptin/FM was related to HOMA-IR such that for every unit change of leptin/FM within the population translated to HOMA-IR changing by a factor of 0.917 ($p = 0.049$). After RYGB, there was still a relationship between leptin/FM and HOMA-IR such that as leptin/FM decreased, HOMA-IR decreased by a factor of 0.799, 0.837, and 0.815 at 12, 24, and 36 months, respectively, though the association between leptin/FM and HOMA-IR was only significant at 36 months ($p < 0.001$) (Table 12). Additionally, while associations between leptin/FM and HOMA-IR after RYGB were stronger compared to baseline, the difference in association from baseline did not reach significance at any time point, making it unclear whether RYGB changed the relationship between the two variables (Table 12).

The change in HOMA-IR after LABG was less than after RYGB, but the pattern of change was similar (Figure 8c and Table 11). At baseline before undergoing LAGB, there was a relationship between leptin/FM and HOMA-IR such that as leptin/FM changed within the population, there was a change in HOMA-IR by a factor of 0.850 ($p = 0.018$). After LAGB, the relationship between the factors remained and was stronger than at baseline such that for every

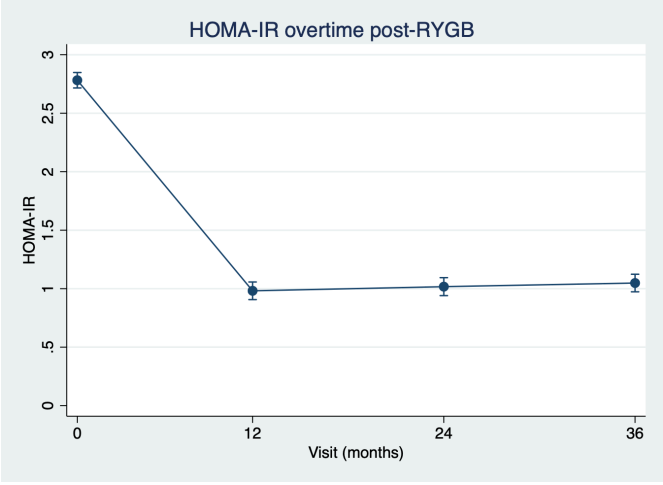
unit decrease in leptin/FM, HOMA-IR decreased by a factor of 0.618, 0.671, and 0.614 at each post-op time point, respectively ($p < 0.001$) (Table 12). The differences in associations from baseline were also significant at each time point, suggesting that LAGB changed the relationship between leptin/FM and HOMA-IR.

Figures 8a, b, and c display the means of HOMA-IR (insulin resistance) and 95% confidence intervals overtime post-operatively in the entire study group (a), in subjects who received RYGB (b), and in subjects who received LAGB (c).

a.



b.



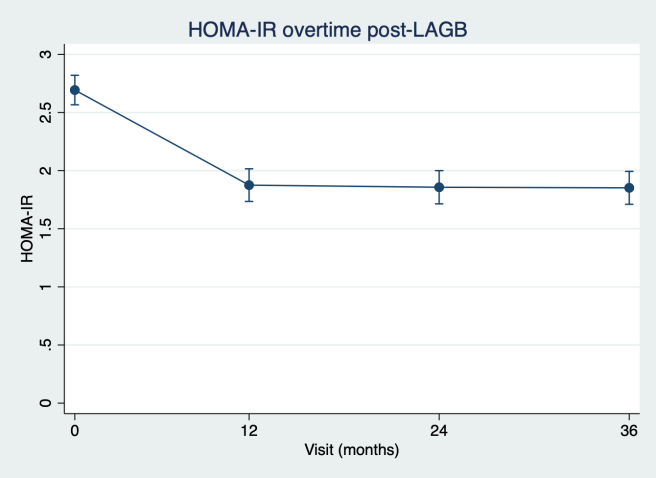
c. 

Table 11 displays the mean ln(HOMA-IR) values at baseline and 12, 24, and 36 months post-operatively in the entire cohort and in the RYGB and LAGB groups separately.

Table 11 – Mean ln(HOMA-IR) (95% CI)

	Visit (months)	Mean ln(HOMA-IR)
Entire cohort	Baseline	0.857 (0.828, 0.887)
	12	0.052 (-0.012, 0.115)
	24	0.039 (-0.003, 0.080)
	36	0.037 (0.002, 0.072)
RYGB	Baseline	0.872 (0.836, 0.908)
	12	-0.143 (-0.209, -0.077)
	24	-0.122 (-0.168, -0.075)
	36	-0.107 (-0.143, -0.072)
LAGB	Baseline	0.839 (0.788, 0.889)
	12	0.470 (0.417, 0.524)
	24	0.406 (0.342, 0.470)
	36	0.387 (0.318, 0.456)

Table 12 displays the association between leptin/FM and the natural log of HOMA-IR at baseline and at each time point post-operatively and the difference in these associations from baseline, each with 95% confidence intervals.

Table 12 – Association between ln(HOMA-IR) and leptin/FM at baseline (pre-op) and overtime (post-op).

	Visit (months)	Association between ln(HOMA-IR) and leptin/FM[1]	p-value[2]	Difference from baseline[3]	p-value[4]
Entire cohort	Baseline	-0.128 (-0.202, -0.052)	0.001	-	ref
	12	-0.412 (-0.732, -0.091)	0.012	-0.284 (-0.599, 0.030)	0.072
	24	-0.313 (-0.574, -0.053)	0.021	-0.185 (-0.443, 0.070)	0.158
	36	-0.369 (-0.483, -0.254)	< 0.001	-0.241 (-0.367, -0.116)	< 0.001
RYGB	Baseline	-0.087 (-0.169, -0.003)	0.049	-	ref
	12	-0.224 (-0.498, 0.050)	0.107	-0.137 (-0.423, 0.147)	0.342
	24	-0.178 (-0.431, 0.075)	0.172	-0.091 (-0.358, 0.174)	0.505
	36	-0.205 (-0.308, -0.101)	< 0.001	-0.118 (-0.248, 0.010)	0.082
LAGB	Baseline	-0.163 (-0.299, -0.028)	0.018	-	ref
	12	-0.482 (-0.632, -0.332)	< 0.001	-0.319 (-0.473, -0.165)	< 0.001
	24	-0.399 (-0.544, -0.255)	< 0.001	-0.236 (-0.382, -0.091)	0.001
	36	-0.487 (-0.625, -0.349)	< 0.001	-0.324 (-0.482, -0.165)	< 0.001

All β-coefficients were estimated using a mixed effects linear regression

[1] Estimated association between ln(HOMA-IR) and leptin/FM. Interpretation of this coefficient on the original HOMA-IR scale can be determined by exponentiating the β-coefficient. For example, at baseline in the entire cohort, for every 1 unit of leptin/FM decrease, HOMA-IR increases by a factor of 0.879 or exp(-0.128).

[2] p-value for the association between ln(HOMA-IR) and leptin/FM.

[3] Estimated difference in association between ln(HOMA-IR) and leptin/FM at each follow-up visit and baseline (reference). Interpretation of this coefficient on the original HOMA-IR scale: The magnitude of the association between HOMA-IR and leptin/FM in the entire cohort changed by a factor of 0.753 (exp(-0.284)) from baseline to 12 months post-op, resulting in an association that was stronger than at pre-op.

[4] p-value for the difference in association between ln(HOMA-IR) and leptin/FM at each follow-up visit and baseline (reference).

Overall, HOMA-IR decreased after bariatric surgery, suggesting insulin resistance decreased post-operatively. The leptin/FM ratio was related to HOMA-IR, and this relationship became stronger over time after both surgeries. The difference in the association between leptin/FM and HOMA-IR was significant at 36 months in the entire cohort and at every post-op time point in the LAGB group but not in the RYGB group. Our data suggest that surgery

changed the relationship between leptin/FM and HOMA-IR in the entire cohort and in the LAGB group. These observations do not prove a causal relationship between leptin/FM and HOMA-IR but do suggest that changes in leptin sensitivity and/or leptin secretion may influence whole body insulin resistance.

HOMA-%S

Insulin sensitivity, or HOMA-%S, behaved reciprocally to HOMA-IR and increased sharply in the entire study group between baseline and 12 months post-operatively and continued to increase at each time point (Figure 9a and Table 13). Leptin/FM was related to HOMA-%S at every time point. At baseline, for every unit decrease in leptin/FM, HOMA-%S changed by a factor of 1.136 ($p = 0.001$). At each post-op time point, for every unit decrease in leptin/FM, HOMA-%S increased by a factor of 1.509, 1.367, and 1.446, respectively (Table 14). The relationship between the variables became stronger than at baseline for all follow-up time points while the difference in the relationship from baseline reached significance at 36 months.

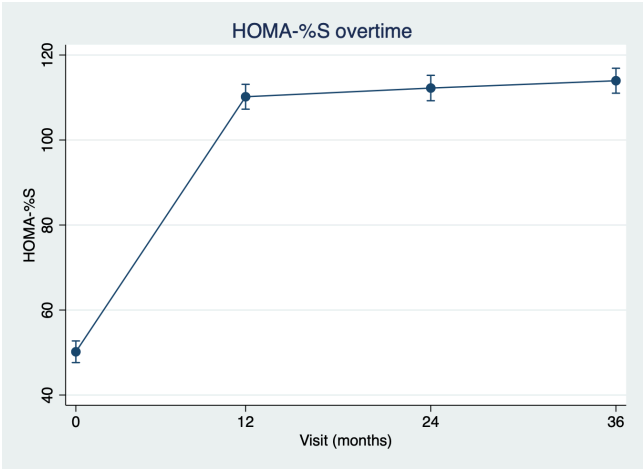
In the RYGB group, HOMA-%S also improved most during the first 12 months, after which little additional improvement can be observed (Figure 9b and Table 13). At baseline, every unit decrease in leptin/FM translated to a change in HOMA-%S by a factor of 1.091 ($p = 0.059$). At each time point post-op, for every unit decrease in adjusted leptin levels, HOMA-%S increased by a factor of 1.251, 1.195, and 1.228, respectively (Table 14). The associations between leptin/FM and HOMA-%S after RYGB were stronger than at baseline, but the difference in association from baseline did not reach significance at any post-op time point. Due to this result, it is unclear that RYGB changed the relationship between these variables.

HOMA-%S after LAGB did not increase as much as in the RYGB group by 12 months post-op, though it continued to increase by smaller margins at the 24 and 36 months (Figure 9c and Table 13). At baseline, every unit decrease in leptin/FM translated to a change in HOMA-

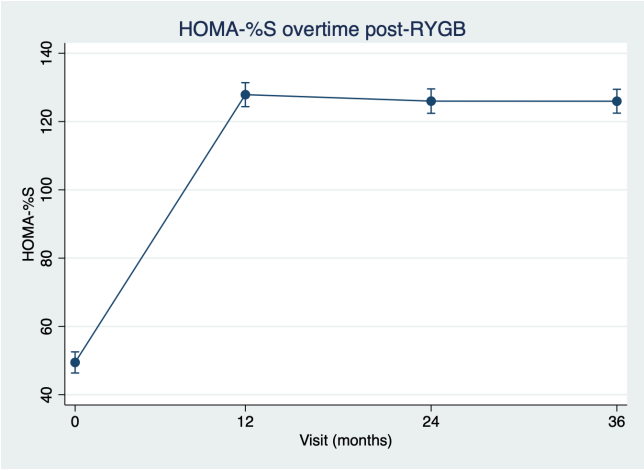
%S by a factor of 1.177 (p = 0.018). After LAGB, for every unit decrease in leptin/FM, HOMA-%S increased by a factor of 1.619, 1.490, and 1.627 at each post-operative time point, respectively (p < 0.001) (Table 14). The associations between leptin/FM and HOMA-%S after LAGB were stronger than at baseline, and the difference in associations from baseline were all statistically significant, suggesting that LAGB changed the relationship between these variables.

Figures 9a, b, and c display the means of HOMA-%S and 95% confidence intervals overtime post-operatively in the entire study group (a), in subjects who received RYGB (b), and in subjects who received LAGB.

a.



b.



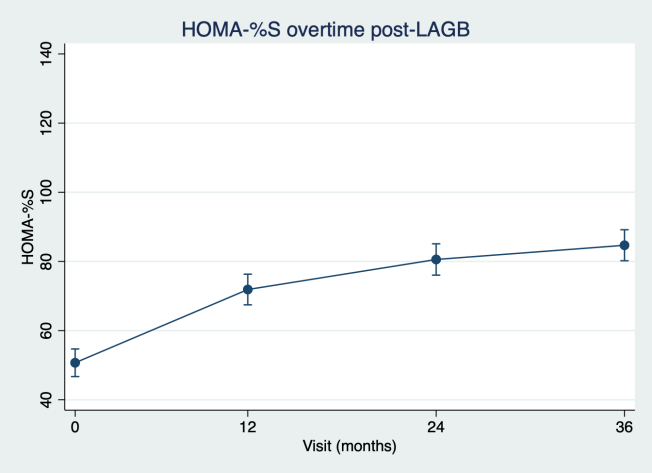
c. 

Table 13 displays the mean ln(HOMA-%S) values at baseline and 12, 24, and 36 months post-operatively in the entire cohort and in the RYGB and LAGB groups separately.

Table 13 – Mean ln(HOMA-%S) (95% CI)

	Visit (months)	Mean ln(HOMA-%S)
Entire cohort	Baseline	3.748 (3.718, 3.778)
	12	4.554 (4.490, 4.617)
	24	4.566 (4.525, 4.608)
	36	4.568 (4.533, 4.603)
RYGB	Baseline	3.733 (3.697, 3.769)
	12	4.748 (4.682, 4.814)
	24	4.727 (4.681, 4.773)
	36	4.713 (4.677, 4.748)
LAGB	Baseline	3.766 (3.716, 3.817)
	12	4.135 (4.081, 4.189)
	24	4.199 (4.135, 4.263)
	36	4.218 (4.149, 4.287)

Table 14 displays the association between leptin/FM and the natural log of HOMA-%S at baseline and at each time point post-operatively with 95% and the difference in these associations from baseline, each with 95% confidence intervals.

Table 14 – Association between ln(HOMA-%S) and leptin/FM at baseline (pre-op) and overtime (post-op).

	Visit (months)	Association between ln(HOMA-%S) and leptin/FM[1]	p-value[2]	Difference from baseline[3]	p-value[4]
Entire cohort	Baseline	0.128 (0.052, 0.202)	0.001	-	ref
	12	0.412 (0.091, 0.732)	0.012	0.284 (-0.030, 0.599)	0.072
	24	0.313 (0.053, 0.574)	0.021	0.185 (-0.070, 0.443)	0.158
	36	0.369 (0.254, 0.483)	< 0.001	0.241 (0.116, 0.367)	< 0.001
RYGB	Baseline	0.087 (0.003, 0.169)	0.049	-	ref
	12	0.224 (0.050, 0.498)	0.107	0.137 (-0.147, 0.423)	0.342
	24	0.178 (0.075, 0.431)	0.172	0.091 (-0.174, 0.358)	0.505
	36	0.205 (0.101, 0.308)	< 0.001	0.118 (-0.010, 0.248)	0.082
LAGB	Baseline	0.163 (0.028, 0.299)	0.018	-	ref
	12	0.482 (0.332, 0.632)	< 0.001	0.319 (0.165, 0.473)	< 0.001
	24	0.399 (0.255, 0.544)	< 0.001	0.236 (0.091, 0.382)	0.001
	36	0.487 (0.349, 0.625)	< 0.001	0.324 (0.165, 0.482)	< 0.001

All β-coefficients were estimated using a mixed effects linear regression

[1] Estimated association between ln(HOMA-%S) and leptin/FM. Interpretation of this coefficient on the original HOMA-%S scale can be determined by exponentiating the β-coefficient. For example, at baseline in the entire cohort, for every 1 unit of leptin/FM decrease, HOMA-%S increases by a factor of 1.136 or exp(0.128).

[2] p-value for the association between ln(HOMA-%S) and leptin/FM.

[3] Estimated difference in association between ln(HOMA-%S) and leptin/FM at each follow-up visit and baseline (reference). Interpretation of this coefficient on the original HOMA-%S scale: The magnitude of the association between HOMA-%S and leptin/FM in the entire cohort changed by a factor of 1.328 (exp(0.284)) from baseline to 12 months post-op, resulting in an association that was stronger than at pre-op.

[4] p-value for the difference in association between ln(HOMA-%S) and leptin/FM at each follow-up visit and baseline (reference).

Overall, HOMA-%S increased after bariatric surgery, suggesting insulin sensitivity improved post-operatively. (This measure is the opposite of the HOMA-IR measure, and thus it seems logical the relationships would mirror the changes in HOMA-IR). The leptin/FM ratio was related to HOMA-%S, and this relationship became stronger over time after surgery. The difference in the association between these two variables was significant at 36 months in the

entire cohort and at every post-op time point in the LAGB group but not in the RYGB group. Once again, our data suggest that surgery changed the relationship between leptin/FM and HOMA-IR in the entire cohort and in the LAGB group. These observations do not prove a causal relationship between leptin/FM and HOMA-%S but do suggest that changes in leptin sensitivity and/or leptin secretion may improve whole body insulin sensitivity.

HOMA-%B

Insulin secretion in fat fasting state, as measured by HOMA-%B, decreased sharply between baseline and 12 months post-operatively when examining the entire study group (Figure 10a and Table 15). At baseline, every unit decrease of leptin/FM in the entire population translated to a change in HOMA-%B by a factor of 0.809 ($p < 0.001$). At each time point post-operatively, for every unit decrease in leptin/FM, HOMA-%B decreased by a factor of 0.801, 0.837, and 0.781, respectively. The strength in the associations between leptin/FM and HOMA-%B varied from baseline to 36 months post-op, and the difference in the associations from baseline did not reach significance at any time point (Table 16). Due to this variation, it is unclear if surgery changed the relationship between HOMA-%B and leptin/FM.

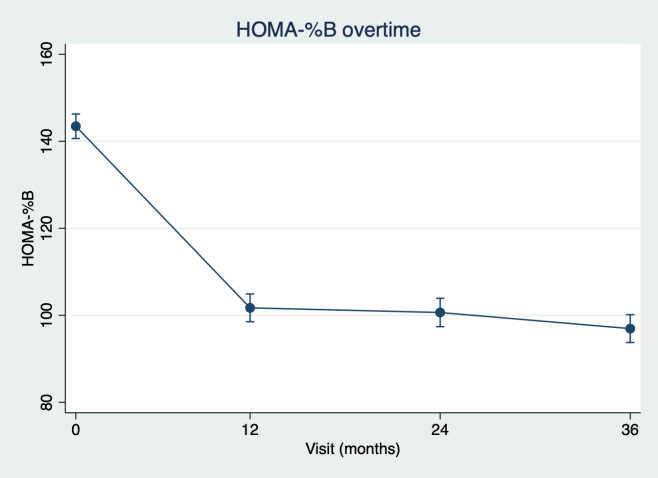
Similar to the entire study group, the RYGB group had a sharp decrease in HOMA-%B by 12 months post-op, and HOMA-%B decreased steadily at each time point (Figure 10b and Table 15). At baseline, every unit decrease in leptin/FM in the RYGB group translated to a change in HOMA-%B by a factor of 0.804 ($p < 0.001$). For every unit decrease of leptin/FM at each of the post-op time points, HOMA-%B decreased by a factor of 0.859, 0.884, and 0.874, respectively (Table 16). The associations between leptin/FM and HOMA-%B post-RYGB became weaker compared to baseline, and, similar to the entire cohort, the difference in associations from baseline did not reach significance.

HOMA-%B did not decrease as much among subjects post-LAGB at any time point when compared to the RYGB group (Figure 10c and Table 15). At baseline, each unit decrease

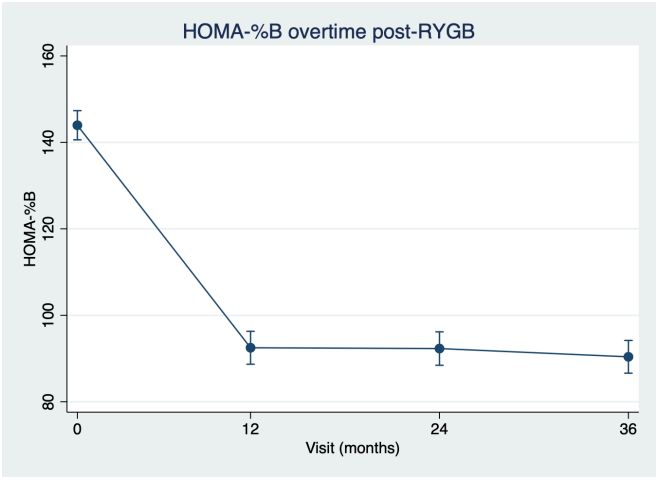
in leptin/FM in the LAGB group translated to a change in HOMA-%B by a factor of 0.842 (p = 0.038). At each post-op time point, HOMA-%B decreased by a factor of 0.803, 0.837, and 0.681, respectively, for every unit decrease in leptin/FM (p < 0.001) (Table 16). Similar to the entire study group, the strength of the associations between leptin/FM and ln(HOMA-%B) post-LAGB varied. Due to this variation, it is unclear if LAGB changed the relationship between leptin/FM and HOMA-%B.

Figures 10a, b, and c display the means of HOMA-%B and 95% confidence intervals overtime post-operatively in the entire study group (a), in subjects who received RYGB (b), and in subjects who received LAGB.

a.



b.



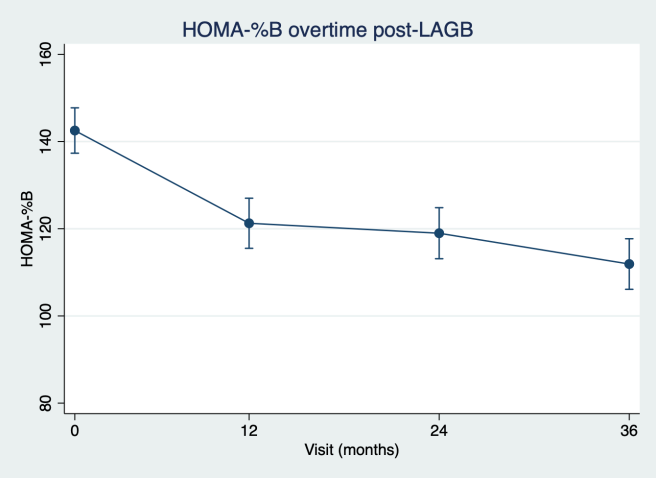
c. 

Table 15 displays the mean ln(HOMA-%B) values at baseline and 12, 24, and 36 months post-operatively in the entire cohort and in the RYGB and LAGB groups separately.

Table 15 – Mean ln(HOMA-%B) (95% CI)

	Visit (months)	Mean ln(HOMA-%B)
Entire cohort	Baseline	4.831 (4.799, 4.863)
	12	4.536 (4.498, 4.574)
	24	4.513 (4.486, 4.541)
	36	4.470 (4.444, 4.497)
RYGB	Baseline	4.828 (4.790, 4.867)
	12	4.456 (4.407, 4.501)
	24	4.439 (4.405, 4.745)
	36	4.413 (4.385, 4.441)
LAGB	Baseline	4.838 (4.789, 4.889)
	12	4.709 (4.663, 4.755)
	24	4.679 (4.634, 4.725)
	36	4.607 (4.557, 4.658)

Table 16 displays the association between leptin/FM and the natural log of HOMA-%B at baseline and at each time point post-operatively and the difference in these associations from baseline, each with 95% confidence intervals.

Table 16 – Association between ln(HOMA-%B) and leptin/FM at baseline (pre-op) and overtime (post-op).

	Visit (months)	Association between ln(HOMA-%B) and leptin/FM[1]	p-value[2]	Difference from baseline[3]	p-value[4]
Entire cohort	Baseline	-0.211 (0.124, 0.295)	< 0.001	-	ref
	12	-0.221 (0.047, 0.394)	0.014	-0.010 (-0.190, 0.167)	0.907
	24	-0.177 (0.024, 0.330)	0.026	0.033 (-0.131, 0.195)	0.679
	36	-0.246 (0.166, 0.325)	< 0.001	-0.035 (-0.140, 0.068)	0.496
RYGB	Baseline	-0.218 (-0.302, -0.132)	< 0.001	-	ref
	12	-0.151 (-0.341, 0.039)	0.118	0.067 (-0.128, 0.260)	0.503
	24	-0.123 (-0.298, 0.053)	0.170	0.096 (-0.087, 0.276)	0.309
	36	-0.135 (-0.221, -0.049)	0.002	0.083 (-0.028, 0.191)	0.151
LAGB	Baseline	-0.172 (-0.335, -0.010)	0.038	-	ref
	12	-0.219 (-0.341, -0.098)	< 0.001	-0.047 (-0.219, 0.125)	0.591
	24	-0.178 (-0.281, -0.080)	0.001	-0.006 (-0.177, 0.160)	0.946
	36	-0.384 (-0.507, -0.260)	< 0.001	-0.212 (-0.381, -0.041)	0.015

All β-coefficients were estimated using a mixed effects linear regression

[1] Estimated association between ln(HOMA-%B) and leptin/FM. Interpretation of this coefficient on the original HOMA-%B scale can be determined by exponentiating the β-coefficient. For example, at baseline in the entire cohort, for every 1 unit of leptin/FM decrease, HOMA-%B decreases by a factor of 0.809 or exp(-0.211).

[2] p-value for the association between ln(HOMA-%B) and leptin/FM.

[3] Estimated difference in association between ln(HOMA-%B) and leptin/FM at each follow-up visit and baseline (reference). Interpretation of this coefficient on the original HOMA-%B scale: The magnitude of the association between HOMA-%B and leptin/FM in the entire cohort changed by a factor of 0.990 (exp(-0.010)) from baseline to 12 months post-op, resulting in an association that was stronger than at pre-op.

[4] p-value for the difference in association between ln(HOMA-%B) and leptin/FM at each follow-up visit and baseline (reference).

Unlike the relationships between HOMA-IR and HOMA-%S, the relationship between leptin/FM and HOMA-%B is variable and inconsistent. Taken together, this may suggest that the effects of changes in leptin sensitivity as measured by changes leptin/FM after bariatric surgery have a greater influence on whole body insulin sensitivity than on insulin secretion from the pancreas.

Discussion

The overall goal of this proposal was to examine relationships between leptin levels and body composition in men and women, before and after bariatric surgery. We specifically wanted to determine whether, in this population primarily consisting of patients with severe obesity, differences in leptin levels between men and women could be explained by differences in body composition, namely by percent body fat. We also wanted to compare how leptin levels change after RYGB and LAGB. Finally, we wanted to determine the relationship between leptin and glucometabolic parameters, specifically HOMA-IR, HOMA-%S, and HOMA-%B, after RYGB and after LAGB.

To accomplish these goals, we used data from Longitudinal Assessment of Bariatric Surgery (LABS) consortium, which is a database that includes pre- and post-operative data on subjects undergoing bariatric surgery. More specifically, we used LABS-2 dataset, which has data on approximately 2400 participants, the majority of whom underwent either RYGB or LAGB. Outcomes included measurements of body composition, leptin levels, and fasting parameters of glucose metabolism up to 3 years after their bariatric surgical procedures.

Leptin is a hormone secreted by adipocytes that circulates in proportion to adipose tissue.[6,13,14] It circulates in the blood and acts as an adiposity feedback signal to the hypothalamus, which then sends afferent signals controlling appetite and energy expenditure.[6,15] Though leptin circulates in proportion to adipose tissue, there appears to be a non-linear relationship between the two, and women have been found to have higher leptin levels than men,[14,28-30] a difference that persists even after controlling for fat mass.[28] It is possible that leptin may reflect total body composition in the form of percent body fat, which also tends to be higher in women than men.[30,32] This was found to be the case in a recent study that included participants from a spectrum of body weights from normal to obese.[63] In that study, following adjustments for percent body fat and other covariates, differences in leptin levels between women and men were no longer significant.

Therefore, we hypothesized that controlling for percent body fat would similarly eliminate the differences in leptin levels between men and women, as was demonstrated in past research.[35,63] At baseline (prior to surgery) and without any adjustments for body composition, we found that women had significantly higher leptin levels than men. Controlling for body composition measurements did not attenuate this difference, including both fat mass and percent body fat. Our finding is at odds with the previously cited study.[63] However, their analyses were heavily controlled for factors such as age, ethnicity, smoking status, education level, and C-reactive protein.[63] It is possible that such extensive adjustments overfit the models to remove a difference in leptin levels between sexes that truly exists. In contrast, instead of reducing the difference between men and women, the adjustments we made (age, waist circumference, and diabetes status) in our models increased the difference in leptin between sexes. If, in fact, the difference in leptin levels between sexes can be attributed to differences in body composition or diabetes status, we would expect this difference to be at least partially attenuated, but this was not the case. Our results are consistent with the conclusion that there is a difference in circulating leptin concentrations between men and women independent of body composition in agreement with findings from other studies.[28-30]

The physiological reasons behind the difference in leptin levels in men and women remain unclear, though a number of hypotheses exist. Women tend to have different fat distribution patterns and more subcutaneous fat than men, which has been shown to produce more leptin compared to omental fat.[32] However, we used waist circumference as a surrogate for increased visceral fat accumulation and found that adjusting for this variable did not impact our findings. Additionally, free testosterone has been shown to impair leptin production, which could explain how men might have lower leptin levels.[33] Estrogen has also been found to enhance central leptin sensitivity, but the absence of change in body composition in response to higher leptin levels to induce body compositions similar to men's suggests that women may be naturally

more leptin resistant.[31] However, the mechanism for this is not currently known and warrants additional research.

Leptin levels decrease after bariatric surgery, with greater decreases that accompany the greater overall weight loss after RYGB than after LAGB.[50,53,55] Some authors suggest that this greater decrease in leptin levels after RYGB points to a regain in leptin sensitivity with the subsequent need for lower leptin levels, which is critical for long-term maintained weight loss.[53,61] Indeed, in a previous study using only subjects with T2DM from the LABS 2 dataset, leptin levels adjusted for fat mass (leptin/FM) were found to be lower in subjects who received RYGB compared to LAGB.[61]

We wanted to expand this data to include the entire LABS 2 cohort, not just those with T2DM, and hypothesized that, similar to the previous findings in the smaller cohort, leptin/FM levels after RYGB would be lower than after LAGB at all post-operative time points. We found that all subjects, regardless of procedure type, experienced decreases in leptin/FM after surgery. This is consistent with research examining changes in leptin following both weight loss via diet and exercise interventions as well as bariatric surgery.[14,50,53-55,58-61] Subjects who received RYGB had significantly lower leptin/FM at each time point post-operatively, which is consistent with past research.[53] Women who underwent RYGB also experienced larger decreases in leptin/FM than men, particularly in the first 12 months post-operatively. This has also been demonstrated in other non-surgical weight loss trials.[29]

These proportional decreases in leptin (as a function of fat mass) are compatible with a regain in leptin sensitivity, as previously suggested.[20,61] Increased leptin sensitivity may thus contribute to sustained weight loss after bariatric surgery through a number of leptin-mediated systems, including improved appetite control. There is evidence that changes to gut hormones after RYGB contributes to these beneficial changes and work alongside improved leptin sensitivity. For example, postprandial GLP-1 and PYY levels have been shown to increase dramatically after RYGB, leading to early satiety.[50] Furthermore, another study showed

significantly increased satiety in subjects post-RYGB compared to subjects post-LAGB.[53] This recovery of central leptin sensitivity may help avoid compensatory mechanisms related to decreased leptin levels that would otherwise prevent sustained weight loss and lead to weight regain.

In the research done by Purnell et al mentioned earlier, they also found that baseline leptin/FM was predictive of T2DM remission following RYGB.[61] Indeed, other studies confirm that bariatric surgery is an effective way to improve glycemia, and most subjects after RYGB go into remission from T2DM.[51,52,55] Therefore, for our third aim we hypothesized that decreased leptin/FM would be associated with improved markers of glucose control, namely HOMA-IR, HOMA-%S, and HOMA-%B. We also examined the association between leptin/FM and each HOMA measurement in each surgery group independently. Concurrent with decreases in leptin and leptin/FM levels, improvements in the means of all three of these parameters at each time point were observed after both procedures, mainly within the first 12 months post-operatively, which has been demonstrated in previous research.[55,58,59] Subjects who received RYGB experienced greater improvements in all of these measures in comparison to subjects who received LAGB in addition to experiencing greater weight loss, though we did not compare the surgery types directly in our analyses.

In our analyses of HOMA-IR and HOMA-%S in the entire cohort, we found that post-surgical decreases in leptin/FM translated to greater decreases in insulin resistance as well as increases in insulin sensitivity compared to before surgery. The absolute change in HOMA-IR and HOMA-%S was greater after RYGB than after LAGB. However, the change HOMA-IR and HOMA-%S was more closely related to the change in leptin/FM after LAGB than after RYGB. Improved insulin sensitivity after RYGB may be related to factors other than leptin/FM. Additional research with this data is warranted to see what factors may be influencing these results, such as group differences based on surgical choice and/or procedure-independent mechanisms in the RYGB vs. LAGB group, especially since it was the larger of the surgery

groups. Our findings for the relationship between leptin/FM and HOMA-%B were not clear. While HOMA-%B decreased in all groups, the association between leptin/FM and HOMA-%B varied from baseline to 36 months post-op.

The variation in the strength of the associations suggests that leptin/FM may not be as tightly associated with insulin secretion as it was with insulin resistance and insulin sensitivity. It is also not clear as to how our current findings fit in with findings of previous studies that baseline leptin/FM was predictive of T2DM remission post-RYGB and that RYGB induces greater rates of T2DM remission compared to LAGB.[51,61] We did not address this question directly, but if diabetes remission rates simply reflected improvements in HOMA IR / %S / %B, then given the greater magnitude of changes in leptin levels and T2DM remission rates after RYGB, we expected relationships between these HOMA variables and leptin/FM to be stronger after RYGB than LAGB. However, if anything, these relationships were stronger over time after LAGB. Possibilities that explain this include potential actions of weight-independent mechanisms influencing glucose metabolism that occur after RYGP but not LAGB (e.g., increases in bile acids, increases in GLP-1, changes in microbiome) and paradoxical responses in HOMA-%B that reflect recovery of islet cell-function even while demand on secretion is dropping with weight loss and improved insulin sensitivity.[50] This was shown in the subset of present study population with T2DM by Purnell et al who found that while HOMA-%S increased significantly after both procedures, HOMA-%B changed significantly only after RYGB.[61] This would mean that the results in the LAGB group were driven largely by weight loss, which resulted in stronger relationships in these variables compared to the RYGB group in which weight-independent changes that affect glucose metabolism are occurring.

Our results may suggest some regain in peripheral leptin sensitivity is occurring after both procedures. In normal conditions, leptin inhibits insulin production and secretion in the pancreas.[17] In our subjects, we observed both leptin and HOMA-%B (insulin secretion) decrease significantly post-operatively in conjunction with improved insulin sensitivity (HOMA-

%S). The absence of an increase in HOMA-%B that would normally be triggered by decreased levels of leptin suggests a shift toward both normal glucose control as well as leptin sensitivity at the level of the pancreas.

Limitations

Analysis of this data includes several limitations. The first limitation includes the possibility that non-steady state conditions for body weight (including fat mass and percent body fat) and leptin were occurring in patients at baseline. Prior to bariatric surgery, candidates are often required to lose weight, though there is likely some variability in achieved weight loss. Our baseline measurements were taken within 1 month of surgery, so it is likely that at least some of our subjects were in a low-calorie catabolic state, which would affect these baseline anthropometric and laboratory values. Additionally, using BIA with individuals with BMI's greater than 35 kg/m^2 (such as those in our cohort) may result in inaccurate measurements due to increases in total body water, which can affect fat free mass hydration.[64] One study found that compared to DEXA, BIA (measured with a Tanita scale) yielded higher fat mass and percent body fat and lower fat free mass results in subjects with BMI's greater than 35 kg/m^2.[64] In this BMI category, differences in weight of up to 10 kg were observed.[64] The manual for the model of Tanita scale used in this study indicates that based on weight alone, our subjects would be able to use the scale.[65] However, the manual indicates that the scale was designed for "standard and athletic individuals," which suggests that our subject population may not meet the criteria to use this Tanita scale with the greatest accuracy.[65] Furthermore, due to the size of the scales in relation to our subjects, pieces of foam were placed between subjects' thighs to avoid shortening the BIA current. All these factors could contribute to inaccurate measurements of body composition.

This could also explain the large spread variation of baseline leptin values (from <0.5 to greater than 500 ng/mL) that we found. Other studies have also noted the wide range of leptin

values that are present in populations of greater weights. For example, in a study by Maffei et al, there was as much as a 12-fold difference between subjects with a BMI greater than 40.[35] With so much variation in leptin levels, it was difficult to ascertain whether an observation was an outlier and whether or not to keep it in the analysis. In the end, we decided to keep all subjects in the analyses rather than risk bias resulting from inappropriate exclusions.

Another limitation of this study was that while we found improvements in HOMA-IR, HOMA-%S, and HOMA-%B post-operatively, we did not statistically compare the results between the two surgery groups. We also did not relate the improvements in HOMA measures to diabetes remission, which we expounded on in the previous section.

Another limitation is the lack of randomization of the patients to their surgical procedures. Subjects were able to choose which procedure they would receive based on what they wanted in consultation with the surgeon, and is reflected in the differences in baseline characteristics between the two cohorts as RYGB was preferentially recommended to patients with greater BMI's, more severe complications, and greater numbers of comorbidities. A final limitation to our study relates to the fact that our study population almost entirely consisted of patients with severe obesity and findings are not generalizable or directly comparable to studies of populations that included participants who were normal weight or had lower BMI's.

Conclusion

In conclusion, we found that in patients almost exclusively within the category of severe obesity, leptin levels remained significantly higher in women than in men even after adjusting for body composition difference including percent body fat. This finding differs from a recent report consisting of mostly normal and overweight individuals and suggests that the difference in leptin between men and women may be exaggerated at extremes in body weight and composition. We also found that leptin/FM as well as leptin/%BF were lower in patients who underwent both

RYGB and LAGB, but more so after the former. This differs from our hypothesis that leptin levels after LAGB would solely be reflective of the loss in fat mass and suggests that both weight loss and RYGB-specific mechanisms are affecting leptin levels. Finally, we found that with decreases in leptin, measures of insulin resistance and sensitivity improved significantly particularly after LAGB, while the relationship between leptin and insulin secretion was less clear. Our results also suggest that these procedures produce improvements in both central and peripheral leptin sensitivity. Future research to more clearly elucidate the physiologic reasons behind the difference in leptin levels in men and women is needed. Thorough knowledge of leptin and how it impacts other physiologic processes is critical to understanding the development and pathophysiology of obesity as well as finding targeted and effective long-term treatments for this condition.

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