Changes in Gastrointestinal Hormones, Leptin, and Satiety After Gastric Bypass Surgery

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Lauren Marie Beckman

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ABSTRACT

The Roux-en-Y gastric bypass (RYGB) is a well-accepted tool for the treatment of obesity and compared to conventional weight loss methods (e.g. diet and exercise) and other weight loss surgeries (e.g. gastric banding), it results in considerable weight loss that is maintained long-term. Although successful, the mechanisms for weight loss are not completely understood and it is thought that favorable changes in several gastrointestinal (GI) hormones and satiety play a role. Previous research suggests that the satiety promoting hormones, GLP-1 and PYY increase after RYGB, while the orexigenic GI hormone ghrelin and the adipocytokine leptin decrease. These changes generally occur before substantial weight loss, suggesting that a component of the surgery is responsible. Subjective satiety has also been reported to increase after RYGB, likely because of changes in the GI hormones and due to the reduced stomach capacity after surgery, but it is not clear if this alteration is maintained long-term. In addition, it is currently not well understood what effect different macronutrients have on the GI hormones and subjective satiety in the post-RYGB patient population. From a clinical perspective, there is a need for understanding how various macronutrients affect these parameters, as this is useful information that might allow for improved dietary treatment recommendations after RYGB.

In the first study, changes in the GI hormones and leptin were evaluated after RYGB. This study also assessed if the GI hormones differed after a short-term dose of protein (PRO-BEV) or fat (FAT-BEV). GLP-1, PYY, ghrelin, and leptin were assessed in 16 women before and at 2, 6, 26, and 52 weeks after RYGB. GLP-1 increased at Weeks 6 and 52 in the FAT-BEV group compared to before surgery. PYY remained

elevated at Week 52 in the FAT-BEV group. Ghrelin decreased at Weeks 2, 6, and 52 in the PRO-BEV group compared with Pre-RYGB. Ghrelin was lower in the PRO-BEV group compared with the FAT-BEV group at Week 6. Fasted leptin decreased at all visits in both groups and was lower in the FAT-BEV compared with the PRO-BEV group at Week 52.

In the second study, subjective satiety was evaluated before and after RYGB. This study also assessed if subjective satiety differed after a dose of either protein or fat and investigated if subjective satiety, the GI hormones, and/or weight loss are related. Subjective satiety was not different between treatment groups. Satiety increased at the Week 2 visit compared to before surgery. Satiety scores had generally returned to Pre-RYGB levels after the Week 6 visit. Subjective satiety was not related to any of the GI hormones. Weight loss was unrelated to subjective satiety and the GI hormones.

Results from these studies indicate that favorable changes occur after RYGB for the GI hormones/leptin and subjective satiety; some differences were evident soon after surgery (ghrelin, leptin, subjective satiety) while others were maintained long-term (GLP-1, PYY, ghrelin, leptin). In response to a short-term stimulus, protein suppressed ghrelin and fat stimulated GLP-1 and PYY. Although we did not find a difference in subjective satiety between beverage groups, assessment of the macronutrient effect on satiety is a novel analysis in this patient population, and further work is needed to better define the post-RYGB nutrition recommendations. Continued research in this area that attempts to better understand dietary components that could ultimately lead to successful weight loss/maintenance is needed for proper nutrition care after RYGB.

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LIST OF ABBREVIATIONS

AUC: Area under the curve

Baseline: Study visit about 30-70 days before surgery

BMI: Body mass index

CHO: Carbohydrate

EWL: Excess weight loss

FAT-BEV: Fat-containing beverage

GCRC: General Clinical Research Center

GI: Gastrointestinal

GLP-1: Glucagon-like peptide-1

HbA1c: Glycated hemoglobin

LAGB: Laparoscopic adjustable gastric banding

OGTT: Oral glucose tolerance test

Pre-RYGB: Study visit about 30-70 days before surgery

PRO-BEV: Protein-containing beverage

PYY: Peptide Tyrosine Tyrosine or peptide YY

RYGB: Roux-en-Y gastric bypass

SLIM: Satiety Labeled Intensity Magnitude

Time 0: Fasted blood draw

Week 2: Study visit about 2 weeks after surgery

Week 6: Study visit about 6 weeks after surgery

Week 26: Study visit about 26 weeks after surgery

Week 52: Study visit about 52 weeks after surgery

CHAPTER 1: INTRODUCTION

Overweight and obesity are worldwide epidemics and it is estimated that over one and half billion adults are overweight (Body Mass Index (BMI) $\geq$ 25 kg/m^2) with 600 million classified as obese (BMI $\geq$ 30 kg/m^2).[1] By 2015, the World Health Organization estimates that over 2.3 billion adults will be overweight and 700 million will be obese.[1] Conventional weight loss therapies including low-calorie diets, exercise, behavioral therapy, and pharmacotherapy have been implemented, however these methods have had little long-term success.[2] Alternatively, bariatric surgery is currently the only known method that offers both considerable and long-term weight loss.[2] In fact, bariatric surgery is increasing in prevalence in the United States (US) and worldwide[3] due to: the increasing rate of obesity; lack of effectiveness with conventional therapies; introduction of the laparoscopic method, which has made it less invasive to patients; increased media attention; and greater access to the therapy.[4]

Roux-en-Y gastric bypass (RYGB) is the most commonly performed bariatric surgery in the US and Canada[3] and is considered to be the most effective in terms of long-term weight loss. Compared to conventional weight loss methods (e.g. diet and exercise), which have a much lower degree of weight loss that is typically difficult to maintain long-term,[5, 6] RYGB patients can expect at least 50% excess weight loss (EWL) that is generally sustained (e.g. at least 2-10 years post-RYGB).[7-11] Weight loss achieved through conventional methods is likely difficult to maintain because the body tightly regulates changes in weight through a compensatory mechanism that increases appetite and decreases energy expenditure. However, for an unknown reason, this adaptive response does not tend to occur following RYGB.

It is clear that RYGB is an effective tool for substantial weight loss, however, the mechanisms behind its success are not completely understood and it is thought that surgery-induced changes in gastrointestinal (GI) hormones, leptin, and/or subjective satiety play a role. Several GI hormones have been identified for their effects on appetite, including glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and ghrelin and the adipose derived hormone leptin. Previous research suggests that the satiety promoting hormones, GLP-1 and PYY increase after RYGB, while the orexigenic GI hormone ghrelin and the adipocytokine leptin decrease. These changes generally occur before weight loss, suggesting that a component of the surgery is responsible. In addition, subjective satiety has also been reported to increase after RYGB, likely because of changes in the GI hormones and due to the reduced stomach capacity after surgery. However, it is currently not well understood what effect different macronutrients have on the GI hormones and satiety in the post-RYGB patient population and what the time course for these changes are. From a clinical perspective, there is a need for understanding how various macronutrients affect these parameters, as this is useful information that might allow for better dietary recommendations after RYGB. It is also important to understand the time course for these changes for optimal nutrition care. This dissertation will address these aims. Chapter 2 presents a literature review that will provide relevant background for these aims. Chapter 3 focuses on the macronutrient-specific changes in the GI hormones in both the early and later postoperative time points. Chapter 4 pertains to macronutrient-specific changes in satiety soon after and up to one-year post-RYGB and evaluates if changes in satiety are related to GI hormone changes. Last, Chapter 5 provides an overall summary with suggestions for future research.

CHAPTER 2: LITERATURE REVIEW*

*Publication Citation (portions of this Literature Review have been published): Beckman LM, Beckman TR, Earthman CP. Changes in gastrointestinal hormones and leptin after the Roux-en-Y gastric bypass procedure: A review. *Journal of the American Dietetic Association*. 2010;110(4):571-584.

ROUX-EN-Y GASTRIC BYPASS

There are several bariatric surgeries currently performed, including vertical banded gastroplasty, gastric banding, sleeve gastrectomy, biliopancreatic diversion, duodenal switch, and the Roux-en-Y gastric bypass (RYGB). The present dissertation research focused on the RYGB, consequently only this bariatric procedure will be discussed.

RGYB is the safest and most efficacious bariatric surgery and thus it is currently the most commonly performed weight loss operation in the US and Canada,[3] comprising about 51% of all bariatric procedures.[3] It can be performed using either open or laparoscopic techniques, with laparoscopic being the preferred method because of its quicker recovery time and decreased post-operative complications.[12, 13] Weight loss is thought to be equivalent between the two methods as the primary difference between open and laparoscopic RYGB is the method of access.[2] While the RYGB was classically described as having both malabsorptive and restrictive components, it is now understood that malabsorption of macronutrients (e.g. calories) is not common after the proximal RYGB[14], the most common form of RYGB performed, and instead is characterized by restrictive and hormonal components.[15] The restrictive component is achieved by creating a gastric pouch which promotes early satiety[4, 12] and thereby decreases intake.[4] During the RYGB surgical procedure, the distal jejunal limb is connected to the new gastric pouch, creating a Roux limb,[2] also known as the alimentary limb which functions to transport nutrients.[4] The gastric pouch is on average 15-30mL in volume,[2, 4, 12] while Roux limbs vary in length, and typically range from 75-150cm for the proximal RYGB and are > 150cm for the distal RYGB.[2, 4] The gastrointestinal (GI) tract before (Figure 2-

1) and after the RYGB procedure (Figure 2-2) is depicted below. The hormonal component of the RYGB procedure will be discussed in the next major section (See: *Gastrointestinal Hormones and Leptin*).

Figure 2-1: The Gastrointestinal Tract Before the Roux-en-Y Gastric Bypass Procedure

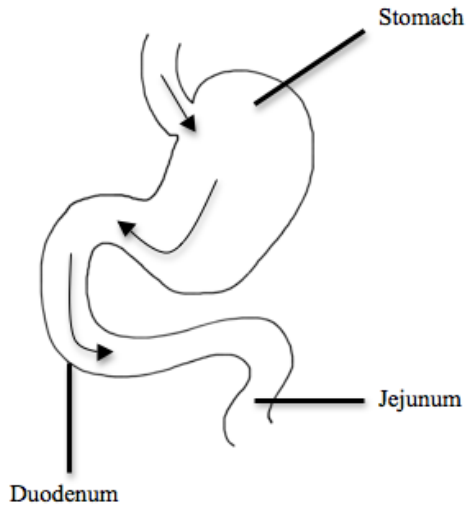
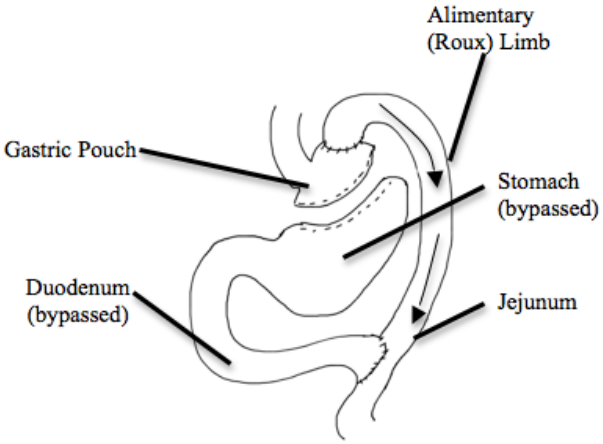


Figure 2-2: The Gastrointestinal Tract After the Roux-en-Y Gastric Bypass Procedure



Weight Loss After Roux-en-Y Gastric Bypass

RYGB imparts substantial weight loss in a short period of time that is generally maintained long-term. Although results are variable, studies have commonly reported 50-80% excess weight loss (EWL[a]) within the first year after RYGB[13, 16-25] and 60-80% long-term (e.g. > one-year post-RYGB).[13, 19, 26, 27]

Surgical success has typically been defined by weight loss (e.g. %EWL, BMI change). One method to determine success is the modified Reinhold classification.[28] An individual is considered to have good or excellent weight loss if their BMI is ≤ 35 kg/m^2 or $\geq 50\%$ EWL, while failure is defined as a BMI > 35 kg/m^2 or %EWL $< 50\%$.[28] According to these criteria, about 70-90% of patients who undergo RYGB are considered successful by one-year post-RYGB[7, 10, 16, 17, 29] and 50-80% long-term (2-10 years post-RYGB).[7-11] Although successful, the mechanisms for weight loss are not completely understood and it is thought that surgery-induced changes in several GI hormones and leptin might play a role.

GASTROINTESTINAL HORMONES AND LEPTIN

Several GI hormones have been identified for their effects on appetite, including glucagon-like peptide-1 (GLP-1), peptide Tyrosine Tyrosine (PYY), and ghrelin and the adipose derived hormone leptin (Table 2-1). While numerous hormones are involved in appetite regulation (e.g. CCK, GIP, oxyntomodulin), changes in the aforementioned hormones have been implicated as possible contributors to the substantial weight loss that occurs with RYGB and consequently are the focus of this dissertation. The following

[a] %EWL = 100*(weight loss)/(initial weight – ideal body weight*)
*Ideal body weight is determined from Metropolitan Life Weight Tables

Table 2-1: Overview of Gastrointestinal Hormones and Leptin

Hormone	Mechanism of Action
Glucagon-Like Peptide-1 (GLP-1)	***Secretion of GLP-1 reduces hunger and imparts satiety by:*** • Slowing gastric emptying[30, 31] • Promoting insulin release[31] • Inhibiting glucagon secretion[31] • Inhibiting gastric acid secretion[30] • Acting on the central nervous system[32-35]
Peptide Tyrosine Tyrosine (PYY)	***Secretion of PYY reduces hunger and imparts satiety by:*** • Delaying gastric emptying[36] • Inhibiting gastric acid secretion[36]
Ghrelin	***Secretion of ghrelin stimulates appetite by:*** • Increasing gastrointestinal motility[37, 38] • Decreasing insulin secretion[37, 38]
Leptin	***Secretion of leptin decreases food intake and increases energy expenditure by:*** • Acting on the hypothalamus[39-41]

discussion will describe GLP-1, PYY, ghrelin, and leptin in normal physiology and how they change after RYGB.

Overview of Glucagon-Like Peptide-1 in Normal Physiology

The incretin GLP-1 is secreted from the L cells in the distal ileum and colon in response to energy intake.[37, 42-44] GLP-1 has two biological forms which are equivalent in potency: GLP-$1_{7\text{-}36amide}$ and GLP-$1_{7\text{-}37}$ with GLP-$1_{7\text{-}36amide}$ being the most prevalent isoform.[32, 42] A majority of the studies presented here measured total GLP-1 (both GLP-$1_{7\text{-}37}$ and GLP-$1_{7\text{-}36amide}$ forms) and consequently unless otherwise noted, reference to total GLP-1 will be notated as GLP-1.

GLP-1 is considered an appetite-regulating hormone because secretion of it reduces hunger and imparts satiety.[45] After enteral intake, GLP-1 is secreted into circulation in two phases: 1) early (within 5 to 15 minutes)[44, 46] and 2) late (30 – 60

minutes later).[44] The mechanism by which GLP-1 promotes satiety is thought to be multifaceted because it slows gastric emptying,[30, 31] promotes insulin release,[31] inhibits glucagon secretion,[31] inhibits gastric acid secretion,[30] and acts on the central nervous system to induce satiety and decrease food intake[32-35] (Table 2-1). Because of these aforementioned effects, it is considered to play an important role in the "ileal brake" mechanism,[32] which regulates the passage of nutrients through the GI tract.[47] The satiety-promoting effects of GLP-1 are evident when it is peripherally administered, as it has been found to reduce appetite and energy intake in healthy weight humans[48] as well as those with obesity;[49] consequently, it has been investigated as a therapy for weight loss. Given the mechanism of action of GLP-1 it would be expected that postprandial plasma levels would be elevated in normal-weight individuals and lower in those with obesity, and there are some data to support this.[50]

Glucagon-Like Peptide-1 Changes After Roux-en-Y Gastric Bypass

Fasted GLP-1 levels are generally unchanged after RYGB[31, 51] and consequently the following section will only focus on postprandial GLP-1 concentrations. The data concerning postprandial GLP-1 changes after RYGB are relatively consistent across studies. In cross-sectional analyses that compared RYGB patients to obese, overweight, and/or normal-weight non-surgical controls, all of the reviewed studies reported significantly higher postprandial GLP-1 levels after RYGB[52-61] (Table 2-2). To control for the effects of weight loss on GLP-1 changes, a majority of the aforementioned studies weight-matched the participants to the pre-RYGB weight (e.g. obese individuals)[52, 53, 55, 57, 58, 61] and/or the post-RYGB weight (e.g. overweight or obese individuals).[53, 54, 56, 59, 60]

Even when weight-matched, RYGB patients had significantly greater postprandial GLP-1 values compared with either obese or overweight individuals. Moreover, one would expect that normal-weight individuals would have higher postprandial GLP-1 values compared with post-RYGB patients who were still overweight or obese. Studies that compared post-RYGB patients to normal-weight individuals did not find this at 6-36 months post-op when participants were still considered overweight/obese,[57, 61] suggesting that a component of the RYGB procedure alters the GLP-1 profile and weight loss is not a major factor involved in the change.

When comparing RYGB to other surgical procedures, all of the reviewed studies found that postprandial GLP-1 levels were significantly higher in the RYGB patients compared with those who underwent gastric banding[14, 54, 60, 62] (Table 2-2). In these studies, the postoperative weight was not statistically different between groups, suggesting weight loss is not a primary regulator of GLP-1. Similarly, other studies have compared the GI hormone profile of post-RYGB patients to that of a conventional weight loss group (e.g. diet)[52, 55, 56, 59] (Table 2-2). Although the mean body weight was not significantly different between the groups, postprandial GLP-1 levels were significantly higher in the post-RYGB group compared with the post-diet induced weight loss group.[52, 55, 59] As previously mentioned, GLP-1 is secreted from the distal ileum in response to nutrient intake. Increased GLP-1 levels post-RYGB have been hypothesized to occur because of the surgical component that promotes a more rapid delivery of nutrients to the distal gut.[64, 65]

In studies that prospectively compared pre- to post-RYGB, increased postprandial GLP-1 levels were observed after surgery[14, 52, 56, 59, 62, 66-72] (Table 2-3). Findings from le

Table 2-2: Postprandial GLP-1 Summary in RYGB Patients - Cross-Sectional Analyses

Study	Study Groups (n)	Time after Surgery or Weight Loss	Stimulus Details	Postprandial GLP-1 (Peak, AUC, and/or Concentration)
Bose et al.[62]	Post-RYGB (11); Post-GB (9)	RYGB: 1mo and 1y; GB: 3mo and 1y	50g OGTT	Post-RYGB > Post-GB
Evans et al.[52]	Post-RYGB (10); Non-surgical BMI-matched (10)	RYGB: 2-3wk; Non-Surgical: after 7d low calorie liquid diet	Liquid meal (262kcal) mixed-nutrient: (32% protein, 47% CHO, 21% fat); high-fat: (22% protein, 33% CHO, 45% fat)	Post-RYGB > Non-surgical
Goldfine et al.[53]	Post-RYGB (21); Overweight (10); Obese (5)	2-4y	Liquid standard mixed meal⌘(250kcal, 240mL)	Post-RYGB > Overweight and Obese
Korner et al.[54]	Post-RYGB (13); Overweight (13); Post-GB (10)	16-34mo	Liquid standard mixed meal⌘(320kcal, 474mL)	Post-RYGB > Overweight and Post-GB
Korner et al.[14]	Post-RYGB (28); Post-GB (15)	2, 12, 26, and 52wk	Liquid standard mixed meal⌘(320kcal, 474mL)	Post-RYGB > Post-GB (26 and 52wk)
Laferrere et al.[55]	Post-RYGB (9); Conventional (10)	RYGB: 1mo; Conventional: after 10kg weight loss	50g OGTT	Post-RYGB > Conventional
Laferrere et al.[56]	Post-RYGB (10); Conventional matched for pre-RYGB weight and BMI (10)	RYGB: 1mo; Conventional: after equivalent weight loss	50g OGTT	Post-RYGB > Conventional
le Roux et al.[57]	Post-RYGB (6); Post-GB (6); Obese matched for pre-surgery BMI (12);	6-36mo	Mixed meal (420kcal; nutrient details not reported)	Post-RYGB > Post-GB, Obese, and Normal-weight

	Normal-weight (15)			
Morinigo et al.[58]	Post-RYGB (9); Obese (6)	6wk	Liquid standard mixed meal⌘(398 kcal, 250mL)	Post-RYGB > Obese
Olivan et al.[59]	Post-RYGB (11); Conventional matched for RYGB weight loss (10)	After 10kg weight loss	50g OGTT	Post-RYGB > Conventional
Pournaras et al.[63]	Pre-RYGB (17); 12mo post-RYGB (6); 18mo post-RYGB (5), 24mo post-RYGB (6)	12-24mo	Mixed meal (400kcal, 10.2% protein, 48.8% CHO, 41% fat)	Post-RYGB (all time points) = Pre-RYGB
Rodieux et al.[60]	Post-RYGB (8); Post-GB (6); Non-surgical matched for post-surgery weight (8)	RYGB: 9-48mo; GB: 25-85mo	Oral U 13C-labeled glucose load (0.5g/kg, 2% enriched with 13C glucose)	Change from fasted: Post-RYGB > Post-GB and Non-surgical
Vidal et al.[61]	Post-RYGB (24); Normal-weight (8); Obese matched to pre-RYGB BMI (8)	9-30mo	Liquid standard mixed meal⌘(398kcal, 250mL)	Post-RYGB > Obese and Normal-weight

AUC: area under the curve; conventional: conventional weight loss (e.g. diet, exercise); CHO: carbohydrate; d: days; GB: gastric band; GLP-1: glucagon-like-peptide-1; h: hours; min: minutes; mo: months; OGTT: oral glucose tolerance test; RYGB: Roux-en-Y gastric bypass; y: years; wk: weeks; ⌘: standard mixed meal = 50% CHO, 35% fat, 15% protein

Table 2-3: Postprandial GLP-1 Summary in RYGB Patients - Prospective Analyses

Study	n	Time Points	Stimulus Details	Postprandial GLP-1 (Peak, AUC, and/or Concentration)
Bose et al.[62]	11	Before and 1mo and 1y post-RYGB	50g OGTT	Post-RYGB > Pre-RYGB
Bose et al.[66]	11	Before and 1, 6, and 12mo post-RYGB	50g OGTT	Post-RYGB > Pre-RYGB
Borg et al.[67]	6	Before and 1, 3, and 6mo post-RYGB	Mixed meal (420kcal; nutrient details not reported)	Post-RYGB (6mo) > Pre-RYGB
Evans et al.[52]	10	Before and 2-3wk post-RYGB	Liquid meal (262kcal) mixed-nutrient: (32% protein, 47% CHO, 21% fat); high-fat: (22% protein, 33% CHO, 45% fat)	Post-RYGB > Pre-RYGB (both stimulus groups)
Falkén et al.[68]	12	Before and 3d, 2mo, and 1y post-RYGB	Liquid meal (300kcal, 11% protein, 89% CHO, 0% fat, 200mL)	Post-RYGB (2mo and 1y) > Pre-RYGB
Hansen et al.[69]	9	Before and 1 and 6wk post-RYGB	Liquid meal (250kcal, 9g protein, 40g CHO, 6g fat, 8oz)	Post-RYGB > Pre-RYGB
Korner et al.[14]	28	Before and 2, 12, 26, and 52wk post-RYGB	Liquid standard mixed meal⌘(320kcal, 474mL)	Post-RYGB (26 and 52wk) > Pre-RYGB
Laferrere et al.[70]	8	Before and 1mo post-RYGB	OGTT	Post-RYGB > Pre-RYGB
Laferrere et al.[56]	10	Before and 1mo post-RYGB	50g OGTT	Post-RYGB > Pre-RYGB
le Roux et al.[71]	16	Before and 2, 4, 7, and 42d post-RYGB	Mixed meal (400kcal; nutrient details not reported)	Post-RYGB > Pre-RYGB
Olivan et al.[59]	11	Before and after 10kg weight loss	50g OGTT	Post-RYGB > Pre-RYGB
Pournaras et al.[63]	6	Before and 18-24mo post-RYGB	Mixed meal (400kcal, 10.2% protein, 48.8% CHO, 41% fat)	Post-RYGB = Pre-RYGB

AUC: area under the curve; CHO: carbohydrate; d: days; GLP-1: glucagon-like-peptide-1; h: hours; min: minutes; mo: months; OGTT: oral glucose tolerance test; RYGB: Roux-en-Y gastric bypass; y: years; wk: weeks

Roux et al.[71] suggest that this change occurs as early as two days post-RYGB, before any weight loss and data from the same group indicates that this effect can be seen as long as 24 months post-RYGB.[72] To summarize, the body of literature suggests that postprandial GLP-1 substantially increases after RYGB and levels are greater in this population compared to those who are normal-weight, overweight, obese, have undergone other bariatric surgeries, or lost weight via conventional methods (Table 2-4).

Table 2-4: Summary of GI Hormones and Leptin Findings in the Post-RYGB Patient Population Compared to Pre-RYGB and Non-RYGB*

	Postprandial GLP-1	Postprandial PYY	Fasting and/or Postprandial Ghrelin	Fasting Leptin
Post-RYGB	↑	↑	↓ or ←→	↓ or ←→

* Non-RYGB patients include those who are normal-weight, overweight, or obese, other bariatric surgery patients, and those who underwent weight loss via conventional methods; GI: Gastrointestinal; RYGB: Roux-en-Y gastric bypass; GLP-1: glucagon-like peptide-1; PYY: peptide YY; ↑: increased; ↓: decreased; ←→: not different

Overview of Peptide YY in Normal Physiology

Similar to GLP-1, PYY is secreted from the L cells of the gut after a meal.[31, 37, 39, 42] It occurs in two forms: PYY_{1-36} and PYY_{3-36} with PYY_{3-36} being the major circulating form.[39] A majority of the studies presented here measured total PYY (both PYY_{1-36} and PYY_{3-36} forms) and consequently unless otherwise noted, reference to total PYY will be notated as PYY.

PYY is also considered an appetite-regulating hormone given that secretion of it reduces hunger and imparts satiety.[45] PYY has a biphasic secretion pattern, similar to GLP-1; it is released into circulation soon after enteral intake (e.g. 15-30 minutes),[73] plateaus after 1-2 hours,[57, 73, 74] and remains elevated for up to six hours.[75] One mechanism by which PYY is thought to promote satiety is through its role in the "ileal

brake".[32] In other words, PYY delays gastric emptying and inhibits gastric acid secretion, as discussed in a review by le Roux and Bloom (Table 2-1).[36] Intravenous PYY infusion has been found to decrease energy intake and reduce hunger in healthy individuals[76] and those with obesity.[77] It has been reported that fasting and postprandial PYY levels are lower in those with obesity and higher in normal-weight individuals,[77, 78] but not all studies have found this to be true.[79, 80] As a result, it has been suggested that reduced PYY release is not likely to be a mechanism involved in the etiology of obesity.[42, 77]

Peptide YY Changes After Roux-en-Y Gastric Bypass

Fasted PYY levels are generally unchanged after RYGB[31, 51] and consequently the following section will only focus on postprandial PYY concentrations. The general consensus of cross-sectional analyses is that postprandial PYY levels are higher in post-RYGB individuals compared with normal-weight,[57, 81-84] overweight,[60, 82] obese,[25, 52, 56-58, 81, 83] other bariatric surgical procedure patients,[14, 25, 57, 60, 62, 82] and/or in those who lost weight via conventional methods (e.g. diet)[25, 52, 56, 59] (Table 2-5). More specifically, a majority of the aforementioned studies determined that compared to the other study populations, the PYY response to a test meal or oral glucose tolerance test (OGTT) was exaggerated, occurred earlier, and/or remained elevated above baseline levels for much of the sampling time period.[14, 25, 52, 56-58, 60, 62, 81-83] Similar to GLP-1, higher PYY levels occurred in post-RYGB study populations, despite equivalent body weight/BMI in the comparison groups.[14, 25, 52, 56, 57, 62, 81-83] In addition, PYY levels were lower in normal-weight subjects compared to post-RYGB subjects who were still classified as

Table 2-5: Postprandial PYY Summary in RYGB Patients - Cross-Sectional Analyses

Study	Study Groups (n)	Time after Surgery or Weight Loss	Stimulus Details	Postprandial PYY (Peak, AUC, and/or Concentration)
Bose et al.[62]	Post-RYGB (11); Post-GB (9)	RYGB: 1mo and 1y; GB: 3mo and 1y	50g OGTT	Post-RYGB > Post-GB
Chan et al.[81]	Post-RYGB (6); Obese (12); Normal-weight (5)	1-2y	75g OGTT	Post-RYGB > Obese and Normal-weight
Evans et al.[52]	Post-RYGB (10); Non-surgical BMI-matched (10)	RYGB: 2-3wk; BMI-matched: after 7d low calorie liquid diet	Liquid meal (262kcal) mixed-nutrient: (32% protein, 47% CHO, 21% fat); high-fat: (22% protein, 33% CHO, 45% fat)	Post-RYGB > Non-surgical (both stimulus groups)
Korner et al.[14]	Post-RYGB (28); post-GB (15)	2, 12, 26, and 52wk	Liquid standard mixed meal⌘ (320kcal, 474mL)	Post-RYGB > Post-GB (26 and 52wk)
Korner et al.[83]	Post-RYGB (12); Normal-weight (8); Non-surgical matched for post-RYGB BMI (12)	30-40mo	Liquid standard mixed meal⌘ (320kcal, 474mL)	Post-RYGB > Normal-weight and Non-surgical
Korner et al.[82]	Post-RYGB (9); Overweight (11); Post-GB (9); Normal-weight (8)	25-28mo	Liquid standard mixed meal⌘ (320kcal, 474mL)	Post-RYGB > Overweight, Post-GB, and Normal-weight
Laferrere et al.[56]	Post-RYGB (10); Conventional matched for pre-RYGB weight and BMI (10)	RYGB: 1mo; conventional: after equivalent RYGB weight loss	50g OGTT	Post-RYGB > Conventional
le Roux et	Post-RYGB (6);	6-36mo	Mixed meal (420kcal;	Post-RYGB > Post-GB, Obese, and

al.[57]	Post-GB (6); Obese (12); Normal-weight (15)		nutrient details not reported)	Normal-weight
Morinigo et al.[58]	Post-RYGB (9); Obese (6)	6wk	Liquid standard mixed meal[⌘] (398kcal, 250mL)	Post-RYGB > Obese
Morinigo et al.[84]	Post-RYGB (25); Normal-weight (6); Obese (10)	6 and 52wk	Liquid standard mixed meal[⌘] (398kcal, 250mL)	Post-RYGB > Normal-weight (no comparison made with Obese)
Olivan et al.[59]	Post-RYGB (11); Conventional matched for RYGB weight loss (10)	After 10kg weight loss	50g OGTT	Post-RYGB > Conventional
Pournaras et al.[63]	Pre-RYGB (17); 12mo post-RYGB (6); 18mo post-RYGB (5), 24mo post-RYGB (6)	12-24mo	Mixed meal (400kcal, 10.2% protein, 48.8% CHO, 41% fat)	Post-RYGB (all groups) > Pre-RYGB
Rodieux et al.[60]	Post-RYGB (8); Post-GB (6); Non-surgical matched for post-surgery weight (8)	RYGB: 9-48mo; GB: 25-85mo	Oral U 13C-labeled glucose load (0.5g/kg, 2% enriched with 13C glucose)	Change from fasted: Post-RYGB > Post-GB and Non-surgical
Valderas et al.[25]	Post-RYGB (8); Post-SG (8); MED (8); Normal-weight (8)	2mo	Liquid meal (355kcal, 13g protein, 50g CHO, 11g fat, 237mL)	Post-RYGB > Post-SG; Post-RYGB > Normal-weight; Post-RYGB and Post-SG > Post-MED

AUC: area under the curve; CHO: carbohydrate; conventional: conventional weight loss (e.g. diet, exercise); d: days; GB: gastric band; h: hours; min: minutes; MED: medical treatment; mo: months; OGTT: oral glucose tolerance test; PYY: Peptide YY; RYGB: Roux-en-Y gastric bypass; SG: sleeve gastrectomy; y: years; wk: weeks; [⌘]standard mixed meal = 50% CHO, 35% fat, 15% protein

obese.[25, 57, 81-84] Taken together, these findings suggest that the alteration in PYY after RYGB is related to the surgery itself and not to weight loss.

In prospective studies that compared pre-RYGB to post-RYGB status, all found higher postprandial PYY levels after surgery compared with pre-RYGB[25, 52, 56, 59, 62, 63, 67, 71, 72] (Table 2-6). Findings from le Roux et al.[71] suggest that this change occurs as early as two days post-RYGB, before any weight loss and data indicates that this effect can be seen as long as 18-24 months post-RYGB.[63, 72] The finding that elevated levels occur right after surgery provides further evidence that a component of the RYGB is responsible for the change, as if weight loss were the primary mechanism, elevated levels would not be expected to occur until at least one month. In sum, the body of literature suggests that PYY substantially increases soon after RYGB and levels are greater in this population compared to those who are normal-weight, overweight, obese, have undergone other bariatric surgeries, or lost weight via conventional methods (Table 2-4).

Overview of Ghrelin in Normal Physiology

Ghrelin is another appetite regulating GI hormone, although, unlike GLP-1 and PYY it is the only known orexigenic (i.e. appetite stimulating) hormone.[37, 39, 42, 43] Ghrelin circulates in two forms: active (acyl) and inactive (desacyl).[85] A majority of the studies presented here measured total ghrelin (both active and inactive forms) and consequently total ghrelin will be notated as simply ghrelin.

Ghrelin is released both centrally (pituitary) and peripherally (stomach) and its anti-satiating properties may be due to its biological effects to increase GI motility and decrease insulin secretion[37, 38] (Table 2-1). Ghrelin levels increase in the absence of

Table 2-6: Postprandial PYY Summary in RYGB Patients - Prospective Analyses

Study	n	Time Points	Stimulus Details	Postprandial PYY (Peak, AUC, and/or Concentration)
Bose et al.[62]	11	Before and 1mo and 1y post-RYGB	50g OGTT	Post-RYGB > Pre-RYGB
Borg et al.[67]	6	Before and 1, 3, 6mo post-RYGB	Mixed meal (420kcal; nutrient details not reported)	Post-RYGB (3 and 6mo) > Pre-RYGB
Evans et al.[52]	10	Before and 2-3wk post-RYGB	Liquid meal (262kcal) Mixed-nutrient: (32% protein, 47% CHO, 21% fat); High-fat: (22% protein, 33% CHO, 45% fat)	Post-RYGB = Pre-RYGB Post-RYGB: Mixed-nutrient > High-fat
Korner et al.[14]	28	Before and 2, 12, 26, and 52wk post-RYGB	Liquid standard mixed meal[⌘](320kcal, 474mL)	Post-RYGB (26 and 52wk) > Pre-RYGB 52wk Post-RYGB > 26wk Post-RYGB
Laferrere et al.[56]	10	Before and 1mo post-RYGB	50g OGTT	Post-RYGB > Pre-RYGB
le Roux et al.[71]	16	Before and 2, 4, 7, and 42d post-RYGB	Mixed meal (400kcal; nutrient details not reported)	Post-RYGB (2, 4, and 42d) > Pre-RYGB
Olivan et al.[59]	11	Before and after 10kg weight loss	50g OGTT	Post-RYGB > Pre-RYGB
Pournaras et al.[63]	6	Before and 8-24mo post-RYGB	Mixed meal (400kcal, 10.2% protein, 48.8% CHO, 41% fat)	Post-RYGB > Pre-RYGB
Valderas et al.[25]	11	Before and 2mo post-RYGB	Liquid meal (355kcal, 13g protein, 50g CHO, 11g fat, 237mL)	Post-RYGB > Pre-RYGB

AUC: area under the curve; CHO: carbohydrate; d: days; h: hours; min: minutes; mo: months; OGTT: oral glucose tolerance test; PYY: Peptide YY; RYGB: Roux-en-Y gastric bypass; y: years; wk: weeks

enteral intake and decrease right after meal initiation.[86] Circulating ghrelin levels are increased during states of negative energy balance,[87] including diet-induced weight loss in obese individuals[88] and decreased during feeding.[89] This mechanism has been postulated to be a protective response to stimulate energy intake in the underweight and to suppress it in the overweight.[43] However, in obesity, ghrelin is not suppressed with food intake;[90] therefore, this mechanism may be an important factor in the etiology of obesity or it may actually be a consequence of obesity that results from chronic over feeding.

Ghrelin Changes After Roux-en-Y Gastric Bypass

A considerable amount of research has been devoted to ghrelin changes in the RYGB patient population.[14, 21, 26, 56, 57, 59, 60, 62, 67, 68, 71, 81-84, 88, 91-107] Reduced ghrelin levels in response to the RYGB procedure was first described by Cummings et al.[88] in 2002. This finding was received with much curiosity because Cummings et al. also found that ghrelin levels significantly increased when weight loss was achieved by caloric restriction,[88] suggesting that the RYGB procedure had a positive effect on the "hunger hormone" while other methods of weight loss do not. Since that landmark study, others have shown in cross-sectional analyses that the postprandial and/or fasted ghrelin concentration is significantly lower in post-RYGB patients compared with those about to undergo RYGB,[105] normal-weight,[26, 57, 81, 88, 97, 105] overweight,[60, 82] obese,[81, 88, 93, 97, 100, 105] other bariatric surgical procedure patients,[21, 60, 82, 95, 97] and/or those who lost weight via conventional methods (e.g. diet)[21, 59] (Table 2-7). The finding of decreased ghrelin levels post-RYGB offers a partial explanation for the success of the procedure, since low

Table 2-7: Fasted and Postprandial Ghrelin Summary in RYGB Patients - Cross-Sectional Analyses

Study	Study Groups (n)	Time after Surgery/Weight Loss	Stimulus Details	Fasted Ghrelin Concentration	Postprandial Ghrelin (Nadir, AUC, and/or Concentration)
Bose et al.[62]	Post-RYGB (11); Post-GB (9)	RYGB: 1mo and 1y; GB: 3mo and 1y	50g OGTT	Post-RYGB = Post-GB	Post-RYGB = Post-GB
Chan et al.[81]	Post-RYGB (6); Obese (12); Normal-weight (5)	1-2y	75g OGTT (350kcal)	Post-RYGB < Obese and Normal-weight	Post-RYGB < Obese and Normal-weight
Christou et al.[26]	Post-RYGB BMI >35 (16); Post-RYGB BMI <35 (20); Normal-weight (8)	3-5y	Breakfast and lunch (nutrient details not provided)	Post-RYGB groups < Normal-weight	Post-RYGB groups = Normal-weight
Cummings et al.[88]	Post-RYGB (5); Normal-weight (10); Conventional matched for post-RYGB weight (13)	Post-RYGB: 9-31mo; Conventional: after 6mo weight loss program	Breakfast, lunch, dinner (nutrient details not provided)	Post-RYGB < Normal-weight and Conventional (statistical difference not reported)	Post-RYGB < Normal-weight and Conventional; Secretion pattern after RYGB: flat and did not have the meal-related oscillations nor the diurnal rhythm seen in other groups
Engstrom et al.[93]	Middle-aged post-RYGB (10); Middle-aged obese (10); Middle-aged normal	31-54mo	Mixed meal (770kcal, 15-20% protein, 50-55% CHO, 30% fat)	Post-RYGB > Obese	Suppression: Post-RYGB = Young normal-weight; Post-RYGB and Young normal-weight > Obese

	weight (10); Young normal-weight (10)				
Fruhbeck et al.[21]	Post-RYGB (8); Post-GB (8); Conventional matched for pre-surgery BMI (8); Post-TG (6)	6mo	No stimulus	Post-RYGB and Post-TG < Post-GB and Conventional; Post-RYGB = Post-TG	NA
Fruhbeck et al.[95]	Post-RYGB (6); Post-GB (7); Post-BPD (3)	6mo	No stimulus	Post-RYGB < Post-GB and Post-BPD	NA
Holdstock et al.[108]	Post-RYGB (10); non-surgical matched for post-RYGB BMI (10)	12mo	No stimulus	Post-RYGB = Non-surgical	NA
Korner et al.[82]	Post-RYGB (9); Overweight (11); Post-GB (9); Normal-weight (8)	25-28mo	Liquid standard mixed meal⌘(320kcal, 474mL)	Total ghrelin: Post-RYGB = Overweight = Post-GB = Normal-weight; Acyl ghrelin: Post-RYGB, Overweight, and Post-GB < Normal-weight	Total ghrelin suppression: Post-RYGB and Normal-weight > Post-GB and Overweight; Acyl ghrelin suppression: Post-RYGB > Post-GB
Korner et al.[14]	Post-RYGB (28); Post-GB	2, 12, 26, and 52wk	Liquid standard mixed meal⌘(320kcal, 474mL)	Post-RYGB = Post-GB	Post-RYGB = Post-GB

	(15)				
Laferrere et al.[56]	Post-RYGB (10); Conventional matched for pre-RYGB weight and BMI (10)	RYGB: 1mo; conventional: after equivalent RYGB weight loss	No stimulus for ghrelin analysis	Post-RYGB = Conventional	NA
Leonetti et al.[97]	Post-RYGB (11); Post-GB (10); Obese matched for post-surgery BMI (10); Post-TG (8); Normal-weight (8)	9-15mo	Mixed breakfast and lunch with standard Italian nutrient composition (details not reported)	Post-RYGB < Post-GB, Normal-weight, and Obese; Post-TG < Post-RYGB, Post-GB, Normal-weight, and Obese	Change from fasted: NS changes in post-RYGB, post-GB, or post-TG
le Roux et al.[57]	Post-RYGB (6); Post-GB (6); Obese (12); Normal-weight (15)	6-36mo	No stimulus for ghrelin analysis	Post-RYGB = Post-GB = Obese	NA
Martins et al.[100]	Post-RYGB (9); Conventional (8); Pre-bariatric surgery (9)	3y	Liquid meal (400kcal, 20% protein, 45% CHO, 35% fat, 200mL)	Post-RYGB > Conventional	Post-RYGB = Conventional = Pre-bariatric surgery
Olivan et al.[59]	Post-RYGB (11); Conventional	After 10kg weight loss	50g OGTT	Post-RYGB < Conventional	Suppression: Post-RYGB = Conventional

	matched for RYGB weight loss (10)				
Rodieux et al.[60]	Post-RYGB (8); Post-GB (6); Non-surgical matched for post-surgery weight (8)	RYGB: 9-48mo; GB: 25-85mo	Oral U 13C-labeled glucose load (0.5g/kg, 2% enriched with 13C glucose)	Post-RYGB = Post-GB = Non-surgical	Suppression: Post-RYGB > Post-GB and Non-surgical
Stoeckli et al.[103]	Post-RYGB (5); Post-GB (8); Obese (7)	3, 6, 12, and 24mo	No stimulus	NA	Post-RYGB = Obese (difference between RYGB and GB not tested)
Tritos et al.[105]	Post-RYGB (6); Obese pre-RYGB (6); Obese, non-surgical (6); Normal-weight (5)	2y	75g OGTT	Post-RYGB < Normal-weight and Obese pre-RYGB	Post-RYGB < Normal-weight, Obese pre-RYGB, and Obese non-surgical

AUC: area under the curve; BPD: Biliopancreatic diversion; CHO: carbohydrate; conventional: conventional weight loss (e.g. diet, exercise); GB: gastric band; h: hours; min: minutes; mo: months; NA: not applicable; NS: not significant (as reported in study); OGTT: oral glucose tolerance test; RYGB: Roux-en-Y gastric bypass; TG: total gastrectomy; y: years; wk: weeks; ⌘standard mixed meal = 50% CHO, 35% fat, 15% protein

ghrelin levels would not contribute to feelings of hunger. Significantly decreased ghrelin occurs immediately post-op, as Lin et al.[98] evaluated participants 30 minutes post-RYGB and found significantly decreased ghrelin levels compared with pre-RYGB. Studies indicate that this ghrelin effect is maintained for more than one year after RYGB.[26, 57, 60, 81, 82, 88, 93, 97, 105] However, not all research has found lower ghrelin in post-RYGB patients compared to other patient populations (e.g. normal-weight, obese),[14, 56, 62, 93, 103, 108] perhaps due to differing study methodologies. At this time, the role of ghrelin in post-RYGB weight loss remains unclear.

Studies that prospectively compared ghrelin levels between the pre- and post-RYGB state obtained inconsistent results (Table 2-8). For example, a majority of the reviewed studies did not find a difference in fasted[14, 56, 59, 62, 67-69, 91, 92, 96, 99, 103, 106, 108] and/or postprandial[14, 59, 62, 68, 69, 71, 96, 99, 101] ghrelin levels between pre- and 0-14 days,[14, 68, 71], 1-3,[14, 56, 59, 62, 67, 68, 71, 91, 101] 6,[14, 67, 67, 92, 99] 12,[14, 62, 68, 96] or 24 months post-RYGB[103] and several studies found that ghrelin increased during this same time period.[62, 104, 106, 106, 108] Morinigo et al.[84] found that at six weeks post-RYGB fasted ghrelin levels had significantly decreased compared with pre-RYGB, however at 52 weeks, there was a significant increase in the hormone compared with the six week measurement and levels were comparable to that of baseline. On the other hand, several studies did find a significant decrease in fasted ghrelin levels 30 minutes to 2 years after RYGB.[21, 98, 101, 102] It is unclear why there are inconsistent findings between these prospective studies. Sample size may be an issue; Couce et al.[92] initially found a significant decline in ghrelin from pre- to both two hours and 10 days post-RYGB when the N was 49 and 18, respectively. However, at six months post-op, the study N had decreased to 11 and the

Table 2-8: Fasted and Postprandial Ghrelin Summary in RYGB Patients - Prospective Analyses

Study	n	Time Points	Stimulus Details	Fasted Ghrelin	Postprandial Ghrelin (Nadir, AUC, and/or Concentration)
Breitman et al.[91]	30	Before and 2 and 8wk post-RYGB	No stimulus	Post-RYGB (2wk) < Pre-RYGB; Post-RYGB (8wk) = Pre-RYGB	NA
Bose et al.[62]	11	Before and 1mo and 1y post-RYGB	50g OGTT	Post-RYGB = Pre-RYGB	Nadir at 1mo and 1y: Post-RYGB = Pre-RYGB**;** AUC at 1mo: Post-RYGB = Pre-RYGB; AUC at 1y: Post-RYGB > Pre-RYGB
Borg et al.[67]	6	Before and 1, 3, and 6mo post-RYGB	No stimulus for ghrelin analysis	Post-RYGB = Pre-RYGB	NA
Couce et al.[92]	49	1h before and 2h, 10d, and 6mo post-RYGB	No stimulus	Post-RYGB (2h and 10d) < Pre-RYGB; Post-RYGB (6mo) = Pre-RYGB	NA
Falkén et al.[68]	12	Before and 3d, 2mo, and 1y post-RYGB	Liquid meal (300kcal, 11% protein, 89% CHO, 0% fat, 200mL)	Post-RYGB = Pre-RYGB	Post-RYGB (3d) < Pre-RYGB; Post-RYGB (2mo and 1y) = Pre-RYGB
Fruhbeck et al.[21]	8	Before and 6mo post-RYGB	No stimulus	Post-RYGB < Pre-RYGB	NA
Hansen et al.[69]	9	Before and 1 and 6wk post-RYGB	Liquid meal (250kcal, 9g protein, 40g CHO, 6g fat, 8oz)	Total ghrelin: Post-RYGB = Pre-RYGB; Acyl ghrelin: Post-RYGB < Pre-RYGB	Total ghrelin: Post-RYGB = Pre-RYGB; Acyl ghrelin: Post-RYGB < Pre-RYGB

Holdstock et al.[108]	66	Before and 6 and 12mo post-RYGB	No stimulus	Post-RYGB > Pre-RYGB	NA
Karamanakos et al.[96]	16	Before and 1, 3, 6, and 12mo post-RYGB	Mixed meal (420kcal, 16% protein, 55% CHO, 29% fat) (n=6), otherwise no stimulus (n=10)	Post-RYGB = Pre-RYGB	Change from fasted: NS (all time points)
Korner et al.[14]	28	Before and 2, 12, 26, and 52wk post-RYGB	Liquid standard mixed meal⌘ (320kcal, 474mL)	Post-RYGB = Pre-RYGB	Post-RYGB (26 and 52wk) > Pre-RYGB
Laferrere et al.[56]	10	Before and 1mo post-RYGB	No stimulus for ghrelin analysis	Post-RYGB = Pre-RYGB	NA
le Roux et al.[71]	16	Before and 2, 4, 7, and 42d post-RYGB	Mixed meal (400kcal; nutrient details not provided)	Not reported	Post-RYGB = Pre-RYGB
Lin et al.[98]	34	30min before RYGB, 10min after transecting the jejunum, 10min after completely dividing the stomach to form the gastric pouch, and 30min post-RYGB	No stimulus	After dividing the stomach < Pre-RYGB; 30min post-RYGB < Pre-RYGB	NA
Mancini et al.[99]	10	Before and 6mo post-RYGB	4 meals/day with fixed calorie level	Post-RYGB = Pre-RYGB	Post-RYGB = Pre-RYGB
Morinigo et al.[101]	8	Before and 6wk post-RYGB	Liquid mixed meal (398kcal, 14.5% protein, 50% CHO, 35% fat, 250mL)	Post-RYGB < Pre-RYGB	Post-RYGB = Pre-RYGB
Morinigo et al.[84]	25	Before and 6 and 52wk, and 31-34mo post-RYGB	No stimulus for ghrelin analysis	Post-RYGB (6wk) < Pre-RYGB; Post-RYGB (52wk) = Pre-RYGB	NA
Olivan et al.[59]	11	Before and after 10kg weight loss	50g OGTT	Post-RYGB = Pre-RYGB	Suppression: Post-RYGB = Pre-RYGB
Roth et	18	Before and 2y post-	No stimulus	Post-RYGB < Pre-RYGB	NA

al.[102]		RYGB			
Stoeckli et al.[103]	5	Before and 3, 6, 12, and 24mo post-RYGB	No stimulus	Post-RYGB = Pre-RYGB	NA
Sundbom et al.[104]	15	Before and 1, 2, 4, and 6d and 1, 6, and 12mo post-RYGB	No stimulus	Post-RYGB (1-6d) < Pre-RYGB; Post-RYGB (1, 6, and 12mo) > Pre-RYGB (significance not reported)	NA
Vendrell et al.[106]	34	Before and 24wk post-RYGB	No stimulus	Post-RYGB > Pre-RYGB	NA

AUC: area under the curve; CHO: carbohydrate; d: days; h: hours; min: minutes; mo: months; NA: not applicable; NS: not significant (as reported in study); OGTT: oral glucose tolerance test; RYGB: Roux-en-Y gastric bypass; y: years; wk: weeks; ⌘standard mixed meal = 50% CHO, 35% fat, 15% protein

finding was no longer statistically significant.[92] However, this cannot be the primary reason for inconsistent results because with the exception of Borg et al.[67] who did not report attrition, the remainder of the studies reported 100% follow-up rates from pre- to post-RYGB. In sum, it appears that compared to other patient populations (e.g. normal-weight, obese), ghrelin is generally lower after RYGB, but strong conclusions cannot be made when evaluating the prospective studies, as ghrelin levels were either decreased or unchanged (Table 2-4).

Overview of Leptin in Normal Physiology

Since the discovery of leptin in 1994, a plethora of research has been conducted to establish its role in the pathogenesis of obesity. Leptin is a product of the obesity gene (*ob* gene) and is understood to be involved in long-term energy balance.[40] It is secreted by adipocytes and influences energy intake primarily by acting on the hypothalamus[39-41] to decrease food intake and increase energy expenditure (Table 2-1).[41] Plasma leptin concentrations are not known to be regulated by macronutrients, but rather by adipose tissue mass. Leptin circulates in proportion to whole-body adipose tissue mass[40] and strong evidence exists that leptin decreases with reduction in adipose tissue.[59, 109-111] Increased body fat results in increased leptin, which ultimately stimulates reduced food intake and the converse is also true with decreased body fat.[40] However, increased leptin levels do not prevent obesity;[39] therefore, it has been suggested that the progression of obesity is not a result of leptin deficiency but instead there is some degree of leptin resistance.[41]

Leptin Changes After Roux-en-Y Gastric Bypass

A majority of the leptin results in the RYGB patient population were as would be expected. For example, significantly lower fasting leptin concentrations were found in the post-RYGB patient population compared with normal-weight,[112] overweight,[82] and obese individuals[103, 113] and in those who underwent other bariatric procedures[56, 62] (Table 2-9). However, others did not find a statistically significant difference between study populations[56, 59, 83, 95, 114, 115] and this was likely due to the fact that body weight/BMI were similar between groups and as previously mentioned, leptin is positively related to body weight.[14, 83, 103] On the other hand, Korner et al.[83] found similar leptin concentrations between normal-weight and post-RYGB patients and these two groups had significantly lower levels than the obese controls who were weight-matched to the post-RYGB group. This is an interesting finding, as the post-RYGB patients were still considered overweight or obese and it would be expected that the lean individuals would have significantly lower leptin compared with the other groups who were either overweight or obese.

In prospective analyses that evaluated leptin before and after surgery, all found post-RYGB levels to be significantly decreased compared with pre-RYGB participants[14, 56, 59, 62, 67-69, 91, 101, 103, 106, 114-125] (Table 2-10). These findings are as expected, considering that leptin is released in proportion to fat mass and after RYGB a considerable amount of fat mass is lost. The change in leptin was found by two weeks post-RYGB[91] and maintained for at least two years.[103] In contrast to GLP-1 and PYY, it is unlikely that a component of the surgery is responsible for increased leptin levels, as Falkén et al.[68] evaluated the hormone three days after surgery and did not find altered levels. Overall, the data strongly suggest that leptin decreases post-RYGB and is related to weight loss

Table 2-9: Fasted Leptin Summary in RYGB Patients - Cross-Sectional Analyses

Study	Study Groups (n)	Time after Surgery/Weight Loss	Fasted Leptin Concentration
Bose et al.[62]	Post-RYGB (11); Post-GB (9)	1mo and 1y	Post-RYGB < Post-GB (1y)
Faraj et al.[112]	Post-RYGB (8); Normal-weight (8)	2-3y	Post-RYGB < Normal-weight
Fruhbeck et al.[95]	Post-RYGB (6); Post-GB (7); Post-BPD (3)	6mo	Post-RYGB = Post-GB = Post-BPD
Hickey et al.[113]	Post-RYGB (6); Obese (6)	24-30mo	Post-RYGB < Obese
Korner et al.[82]	Post-RYGB (9); Overweight (11); Post-GB (9); Normal-weight (8)	25-28mo	Post-RYGB < Overweight
Korner et al.[83]	Post-RYGB (12); Normal-weight (8); non-surgical BMI matched to post-RYGB (12)	30-40mo	Post-RYGB = Normal-weight; Post-RYGB and Normal-weight < Non-surgical
Korner et al.[14]	Post-RYGB (28); Post-GB (15)	2, 12, 26, and 52wk	Post-RYGB < Post-GB (12, 26, and 52wk)
Laferrere et al.[56]	Post-RYGB (10); Conventional matched for pre-RYGB weight and BMI (10)	RYGB: 1mo; Conventional: after equivalent weight loss	Post-RYGB = Conventional
Olivan et al.[59]	Post-RYGB (11); Conventional matched for RYGB weight loss (10)	After 10kg weight loss	Post-RYGB = Conventional
Riedl et al.[114]	Post-RYGB (30); Post-GB (10)	1y	Post-RYGB = Post-GB
Stoeckli et al.[103]	Post-RYGB (5); Post-GB (8); Obese (7)	3, 6, 12, and 24mo	Post-RYGB and Post-GB < Obese (24mo)
Woelnerhanssen et al.[115]	Post-RYGB (12); Post-SG (11)	1wk and 3 and 12mo	Post-RYGB = Post-SG

BPD: Biliopancreatic diversion; conventional: conventional weight loss (e.g. diet, exercise); mo: months; GB: gastric band; RYGB: Roux-en-Y gastric bypass; SG: sleeve gastrectomy; y: years

Table 2-10: Fasted Leptin Summary in RYGB Patients - Prospective Analyses

Study	n	Time Points	Fasted Leptin Concentration
Bobbioni-Harsch et al.[125]	20	Before and 3, 6, and 12mo post-RYGB	Post-RYGB < Pre-RYGB
Borg et al.[67]	6	Before and 1, 3, 6mo post-RYGB	Post-RYGB (3 and 6mo) < Pre-RYGB
Bose et al.[62]	11	Before and 1mo and 1y post-RYGB	Post-RYGB < Pre-RYGB
Breitman et al.[91]	30	Before and 2 and 8wk post-RYGB	Post-RYGB < Pre-RYGB
Czupryniak et al.[116]	68	Before and every 1-2mo during first year post-RYGB and every 3mo thereafter for 2y	Post-RYGB < Pre-RYGB (at time of maximum weight loss ~15mo)
Das et al.[123]	30	Before and 1y post-RYGB	Post-RYGB < Pre-RYGB
Falkén et al.[68]	12	Before and 3d, 2mo, and 1y post-RYGB	Post-RYGB (2mo and 1y) < Pre-RYGB
Hansen et al.[69]	9	Before and 1 and 6wk post-RYGB	Post-RYGB < Pre-RYGB
Korner et al.[14]	28	Before and 2, 12, 26, and 52wk after RYGB	% Change from Pre-RYGB: Significant
Laferrere et al.[56]	10	Before and 1mo post-RYGB	Post-RYGB < Pre-RYGB
Meier et al.[117]	15	Before and 6mo post-RYGB	Post-RYGB < Pre-RYGB
Miller et al.[118]	15	Before and 3wk and 3 and 6mo post-RYGB	Post-RYGB < Pre-RYGB
Molina et al.[119]	29	Before and 1, 3, and 6mo post-RYGB	Post-RYGB < Pre-RYGB
Morinigo et al.[101]	8	Before and 6wk post-RYGB	Post-RYGB < Pre-RYGB
Olivan et al.[59]	11	Before and after 10kg weight loss	Post-RYGB < Pre-RYGB
Ramos et al.[120]	20	Before and 1 and 3mo post-RYGB	Post-RYGB < Pre-RYGB
Riedl et al.[114]	30	Before and 1y post-RYGB	Post-RYGB < Pre-RYGB
Rubino et al.[121]	10	Before and 3wk post-RYGB	Post-RYGB < Pre-RYGB
Stoeckli et al.[103]	5	Before and 3, 6, 12, and 24mo post-RYGB	Post-RYGB < Pre-RYGB
Swarbrick et al.[122]	19	Before and 1, 3, 6, and 12mo post-RYGB	Post-RYGB < Pre-RYGB
Vendrell et al.[106]	34	Before and 24wk post-RYGB	Post-RYGB < Pre-RYGB
Whitson et al.[124]	10	Before and 6mo post-RYGB	Post-RYGB < Pre-RYGB
Woelnerhanssen et al.[115]	12	Before and 1wk and 3 and 12mo post-RYGB	Post-RYGB < Pre-RYGB

d: days; mo: months; RYGB: Roux-en-Y gastric bypass; y: years; wk: weeks

(Table 2-4).

GASTROINTESTINAL HORMONE ALTERATIONS IN RESPONSE TO MACRONUTRIENTS

The previous sections described what is known about the postprandial behavior of the GI hormones in post-RYGB patients compared to the pre-RYGB physiological state and other patient populations (e.g. normal-weight, other bariatric surgery). Taking this one step forward, it is of interest to evaluate if these hormones are released differently after consumption of various macronutrients. There are no standard dietary recommendations in terms of macronutrient proportions following bariatric surgery and what is currently used by many bariatric centers is based on expert opinion or observational studies. Understanding how various macronutrients affect GLP-1, PYY, and ghrelin could lead to better nutrition care recommendations after RYGB because favorable alterations in the hormones would likely increase satiety and therefore result in decreased food intake. There has been limited study of the GI hormone response to different macronutrients post-RYGB. The following sections will focus primarily on what is known about the GI hormone response to various macronutrients in non-RYGB subjects and animal models, and where available, in post-RYGB subjects.

Macronutrients and Glucagon-Like Peptide-1 Secretion

GLP-1 is secreted in response to ingested macronutrients.[31] More specifically, there are limited data suggesting that GLP-1 is stimulated by fat intake in a dose dependent manner in rats,[126] normal-weight adults,[127, 128] and obese boys.[129] Other data

suggests that GLP-1 is stimulated by protein more than carbohydrate intake.[130, 131] To date, only one study has looked at the effect of different macronutrients on GLP-1 in the post-RYGB patient population. Evans et al.[52] evaluated the effect of two liquid meals that were both 262kcal: 1) mixed-nutrient (32% protein, 47% CHO, 21% fat) and 2) high-fat (22% protein, 33% CHO, 45% fat) before and 2-3 weeks after RYGB. Post-RYGB GLP-1 concentrations significantly increased after both meals, however the duration of increased levels above fasting was longer for the mixed-nutrient group compared to the high-fat meal group. Despite this prolonged duration, concentrations, peak, and AUC values were not different between treatment groups. Because of the lack of studies investigating the macronutrient effect on GLP-1 after RYGB, it is not possible currently to make conclusions about this relationship.

Macronutrients and Peptide YY Secretion

PYY is released into circulation in proportion to the number of calories ingested.[73] Similar to GLP-1, fat[73, 132-135] and protein[132, 135, 136] have been found to be important regulators of PYY secretion in obese[132, 135, 136] and normal-weight individuals.[133, 134, 136] In terms of the post-RYGB patient population, only one study has looked at the effect of different macronutrients on PYY in the post-RYGB patient population. Evans et al.[52] evaluated the effect of a high-fat meal versus control (mixed-meal) on GI hormones and subjective satiety (details of liquid meals provided above) before and 2-3 weeks after RYGB. Post-RYGB PYY concentrations significantly increased after both meals, however the duration of increased levels above fasting was longer for the high-fat meal group compared to the mixed-nutrient group. Despite this

prolonged duration, the mixed-nutrient group had a greater AUC value than the high-fat meal group (P=0.04). At this time, due to limited data, it is not possible to make conclusions about the macronutrient effect on PYY levels after RYGB.

Macronutrients and Ghrelin Secretion

The macronutrient that induces the greatest ghrelin suppression is not well defined. For instance, some research suggests that carbohydrate is suppressive[137] and one study found that it induces greater suppression compared to either a high-fat or high-protein meal.[138] Alternatively, others have found protein to more potent in terms of prolonged suppression compared to carbohydrate[130, 131, 138-141] and/or fat.[138] Erdmann et al.[137] also found that fat was a potent suppressor, especially in the late postprandial state (i.e. 60-180 minutes) with similar findings by Tannous dit El Khoury et al.[138] In terms of the post-RYGB patient population, no studies to date have evaluated the macronutrient effect on ghrelin.

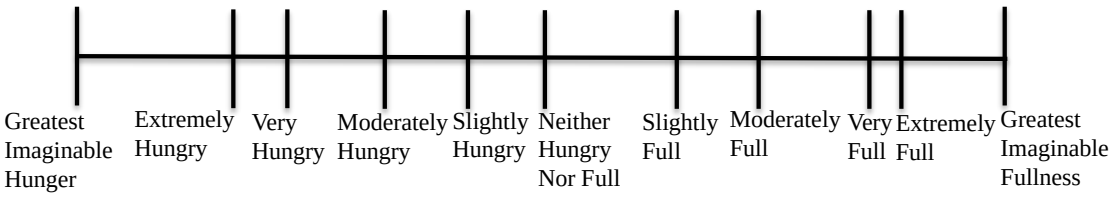
SATIETY

Satiety is commonly defined as the absence of hunger during the inter-meal period.[139] The following sections will describe the common methodology used to measure satiety, satiety changes after RYGB, the relationship between satiety and the GI hormones, and the current dietary recommendations after RYGB.

Subjective Satiety Assessment

Satiety can be measured either subjectively or objectively. In terms of subjective measurements, line scales are often used to measure satiety. Study participants mark on a straight line how hungry or full they feel. The line is commonly anchored on each side by opposing statements, similar to the following range: "*not at all hungry*" to "*very hungry*". Researchers then measure the distance from one end of the extreme to the point on the line marked by the participant to determine their satiety. While quick and easy to administer, the aforementioned line scale has several limitations, including a lack of multiple scale labels, which limits its reliability. Consequently, Cardello et al.[142] developed the Satiety Labeled Intensity Magnitude (SLIM) scale (Figure 2-3). It improves upon the traditional line scale by including numerous verbal labels within the scale that are unequally spaced. The labels themselves and the spacing have been experimentally determined.[142] The verbal labels are easy to understand and they provide a rough meaning to the subject. When evaluated against other scales, the SLIM scale was shown to have greater reliability and sensitivity compared to traditional line scales.[142] While the SLIM scale appears to be a dependable tool for the measurement of subjective satiety, it has not previously been used in the RYGB patient population.

Figure 2-3: Satiety Labeled Intensity Magnitude Scale



In contrast, measurement of GI hormone concentrations is an objective measure of satiety. Most researchers collect fasted blood samples and then continue sampling for a set time period after enteral intake to determine postprandial GI hormone responses. Ideally, researchers would collect subjective satiety data at the same time point of blood sampling. Overall, when collected and analyzed simultaneously, subjective satiety data and GI hormone concentrations have the potential to provide a robust measure of satiety.

Subjective Satiety Changes After Roux-en-Y Gastric Bypass

The evaluation of subjective satiety changes in the RYGB patient population has not been commonly measured compared with the GI hormones. Overall, similar results were evident between these studies and suggest that RYGB positively impacts satiety and has an immediate effect when individuals are still obese. le Roux et al.[71] found significant changes in hunger and fullness scores within days after surgery. For example, at 2, 4, 7, and 42 days post-RYGB, the hunger score was reduced by ~50% while the fullness score doubled compared with the pre-RYGB state.[71] Other investigators have also found significant increases in postprandial satiety at three days[68], one month,[67] six weeks,[58] and two months[25, 68] post-RYGB, when participants were still considered markedly obese, compared with the pre-RYGB time point. The increased satiety observed soon after RYGB is quite reasonable considering that the stomach capacity post-surgery is drastically reduced. The real question that is of importance to long-term weight loss and/or maintenance is whether or not this favorable alteration is maintained months to years post-surgery.

Evidence exists that the increased subjective satiety found soon after RYGB is also present even after significant weight loss. Borg et al.[67] reported that in addition to a reduction in BMI ($P < 0.001$) at three and six months post-RYGB, there was also a significant decrease in postprandial hunger score and increase in postprandial fullness score compared with pre-RYGB. In a cross-sectional analysis Pournaras et al.[63] evaluated postprandial satiety scores in four groups of patients: 1) pre-RYGB; 2) 12 months post-RYGB; 3) 18 months post-RYGB; and 4) 24 months post-RYGB. They also evaluated another group of patients prospectively (before RYGB and 18-24 months after). When subjective satiety data from both the cross-sectional and prospective analyses were combined, satiety significantly increased at all time points after surgery compared with pre-RYGB levels.[63] The Borg et al.[67] and Pournaras et al.[63] studies demonstrated that appetite did not return after significant weight loss, which is contradictory to what typically occurs after conventional weight loss methods. Nonetheless, more studies with longer follow-up times similar to that of Pournaras et al.[63] are necessary to ascertain the longevity of this effect. Clinically, these results are of particular interest as changes in subjective satiety occurred when individuals were still considered morbidly obese and were maintained after substantial weight loss, suggesting that body weight loss is partially explained by an immediate improvement in satiety perception and this alteration is sustainable.

The pharmacological inhibition of gut hormone release and its effect on subjective satiety has also been evaluated. le Roux et al.[71] studied individuals who had had either undergone RYGB or laparoscopic adjustable gastric banding (LAGB). In a double-blind randomized fashion, participants received either saline or octreotide

subcutaneously. Octreotide inhibits the secretion of GI hormones and these investigators were interested in evaluating appetite and food intake after its administration, to determine if there was a difference in effect between the surgeries. Blood was collected before administration of either saline or octreotide and at several time points after for three hours. Participants were provided with an *ad libitum* semi-liquid meal at one hour post-injection in 50mL doses every five minutes to control the rate of consumption. Participants completed subjective satiety assessments pre-meal and every five minutes thereafter. Results indicated that octreotide inhibited the release of GI hormones in both groups while saline allowed an early and exaggerated response of GLP-1 and PYY only in the RYGB group. There was a significant decrease in the fullness score and nearly double the food intake in the RYGB group after octreotide compared with saline with no significant findings in the LAGB group. This is of clinical relevance because these results suggest that RYGB alters the satiety response more so than LAGB, a finding first reported by Korner et al.[82] Taken together, perhaps amelioration of GI hormones and subjective satiety are major factors in the long-term success of RYGB compared with other surgical techniques. Considering the aforementioned findings concerning satiety it is also important to determine if subjective satiety is related to objective measures of satiety (e.g. GI hormones).

Relationship Between Subjective Satiety and Glucagon-Like Peptide-1 After Roux-en-Y Gastric Bypass

Limited data are available concerning the relationship between GLP-1 and subjective satiety in the RYGB patient population, as few studies have actually conducted

correlation analyses. Evans et al.[52] found a significant correlation between GLP-1 and hunger score, while Morinigo et al.[58] failed to find a significant relationship. Considering that GLP-1 is an appetite-regulating GI hormone, one would expect that it would be related to subjective satiety, but it is clear from the lack of studies that more research is needed before conclusions can be made.

Relationship Between Subjective Satiety and Peptide YY After Roux-en-Y Gastric Bypass

It is unclear if postprandial PYY levels are related to subjective satiety after RYGB, as only a few investigators have analyzed the relationship. Most did not find an association between hunger and/or fullness and PYY.[52, 58, 83] Considering that PYY secretion reduces hunger and imparts satiety[45] it would be reasonable to expect that postprandial PYY would be inversely correlated with hunger and positively correlated with fullness score. Lack of correlations between the two variables may be due to the large inter-individual variability inherent of subjective satiety data as suggested by Korner et al.[83] and larger sample sizes may be necessary to see such an effect.

Relationship Between Subjective Satiety and Ghrelin After Roux-en-Y Gastric Bypass

A few of the reviewed studies that measured ghrelin in the RYGB patient population also analyzed subjective satiety.[26, 67, 71, 82, 83, 96] Of these studies, only one study conducted a correlation analysis between ghrelin concentration and satiety scores. Christou et al.[26] did not find a correlation between any of the satiety scores at any time

point and their respective ghrelin level. Lower ghrelin levels would have been expected to correlate with subjective satiety scores and it is unclear why the results were not more robust, however, the unexpected findings might be partially explained by the high inter-individual variation inherent to subjective satiety analyses.[83]

Macronutrients and Subjective Satiety

Protein has been suggested to be the most satiating of the three macronutrients.[143, 144] In fact, decreased appetite scores have been reported after protein preloads compared with glucose[139] and others have found higher satiety scores after a high protein (25% energy) meal compared with an "appropriate/normal" protein (10% energy) meal.[145, 146] To date, we are aware of only one study that has evaluated the macronutrient effect on subjective satiety after RYGB. As described above, Evans et al.[52] compared a high-fat liquid meal to that of a control group (equicaloric mixed-nutrient meal) before and 2-3 weeks post-RYGB and found that feelings of hunger decreased after surgery in only the mixed nutrient group, suggesting that fat is not an important factor in subjective satiety in this patient population. However, because of the dearth of data, the macronutrient effect on satiety after RYGB remains unknown. From a clinical perspective, this information is important for optimal diet management after the surgery.

Dietary Recommendations After Roux-en-Y Gastric Bypass

Life-long diet modifications are required for long-term weight management success after RYGB. Key components of the diet after RYGB include caloric restriction while also consuming adequate amounts of the macro- and micronutrients. Currently,

dietary recommendations after RYGB simply focus on how and when to progress from the various food consistencies (e.g. clear liquids, soft foods) (see Kulick et al. reference[147] for an example of dietary advancement after bariatric surgery). However, it is not well defined as to how much or what percentage of each macronutrient should be consumed in the early or later postoperative time period to achieve optimal results. As previously described, fat and/or protein have been shown to favorably alter the GI hormones and subjective satiety in non-RYGB subjects and this could also be possible after RYGB. Continued research on post-RYGB macronutrient recommendations is warranted with the ultimate goal of developing evidence-based dietary guidelines for this increasing population.

SUMMARY

RYGB is an effective weight loss treatment option for those in which traditional therapies have failed. It offers long-term weight loss that may be the result of the physiological and GI hormone changes associated with the procedure. This literature review has summarized the current evidence showing that alterations in GLP-1, PYY, ghrelin, and leptin, do occur postoperatively and generally do so in a favorable direction that is supportive of ongoing weight loss. In the majority of studies, post-RYGB GLP-1 and PYY concentrations were usually found to be higher, while ghrelin and leptin levels were typically lower compared to the concentrations of these hormones in individuals undergoing other surgical procedures, individuals who were normal and overweight, and those who lost weight by conventional methods.

There are notable gaps in the literature. First, follow-up times varied substantially between studies, and in some instances only one time point was measured (i.e. cross-sectional studies), therefore it was difficult to make comparisons between studies. Studies that evaluated the GI hormone, leptin, and subjective satiety profile in both the early and later postoperative time periods are also lacking. In addition, while many studies have evaluated the GI hormone response following RYGB, few have evaluated the relationship between the GI hormones and subjective satiety. Considering that the GI hormones are appetite-regulating, it is of interest to better understand if these measures are related to subjective feelings of satiety in those who have undergone RYGB. Furthermore, while others have evaluated the GI hormone and subjective satiety response to various macronutrients in non-RYGB subjects, it is not well understood if these variables are related to protein, carbohydrate, or fat ingestion in the post-RYGB patient population. These evaluations are needed to better define post-RYGB nutrition recommendations.

THE CURRENT STUDY

Substantial weight loss occurs after RYGB and it is thought that changes in GI hormones/leptin and satiety after RYGB play a large role in the success of the surgery. However, it is not well understood if the GI hormones and subjective satiety are altered differently by various macronutrients after RYGB and if time after surgery has an impact on these relationships. Investigating the role of diet in GI hormone and subjective satiety changes is of relevance to the ever-growing bariatric surgery patient population. Currently, the dietary recommendations after RYGB are vague guidelines that are not

based on extensive clinical research. Continued research in this area that attempts to better understand dietary components that could ultimately lead to successful weight loss/maintenance is needed for proper nutrition care. Understanding the time course for the GI hormone and subjective satiety changes and how these variables are impacted by different macronutrients could lead to refined nutrition care recommendations that have the potential to aid with weight loss and/or maintenance in the post-RYGB patient population. In this dissertation, the GI hormone and subjective satiety response to either protein or fat in both the early and later postoperative time period was evaluated. Chapter 3 focuses on the macronutrient-specific changes in the GI hormones in both the early and later postoperative time points. Chapter 4 pertains to macronutrient-specific changes in subjective satiety soon after and up to one-year post-RYGB and evaluates if changes in satiety are related to GI hormone changes. Chapter 5 presents overall conclusions and outlines directions for future research.

RESEARCH QUESTIONS, HYPOTHESES, AND STATISTICS

Chapter 3: Changes in Gastrointestinal Hormones and Leptin After Roux-en-Y Gastric Bypass Surgery

Primary Research Questions

- At what time point post-RYGB do changes in the GI hormone and leptin profile occur? *(Hypotheses 1 and 2)*
- Is there a difference in GI hormone responses after short-term administration of either protein or fat? *(Hypotheses 3, 4, and 5)*

Hypothesis #1 – Postprandial PYY and GLP-1 will increase and ghrelin will decrease at all visits after RYGB compared to the Pre-RYGB visit.

- These changes will occur as early as two weeks post-RYGB and will remain elevated/decreased for the duration of the study.

Statistics: Mixed-effects linear model

Hypothesis #2 – Fasted leptin will reach its lowest level at one-year after RYGB.

Statistics: Mixed-effects linear model

Hypothesis #3 – GLP-1 and PYY will be greater after protein consumption compared to fat at all visits.

Statistics: Mixed-effects linear model

Hypothesis #4 – Ghrelin will be lower after protein consumption compared with fat at all visits.

Statistics: Mixed-effects linear model

Hypothesis #5 – Fasted levels of GLP-1, PYY, ghrelin, and leptin will not differ between treatment groups at any visit.

Statistics: Mixed-effects linear model

Chapter 4: Subjective Satiety Before and Up to One Year After Roux-en-Y Gastric Bypass

Primary Research Questions

- Is there a difference between fat and protein in terms of subjective satiety? *(Hypothesis 1)*
- Does subjective satiety change after RYGB? If subjective satiety changes after RYGB, at what time point does this occur? Is this change maintained long-term (e.g. 1 year)? *(Hypothesis 2)*
- Are changes in subjective feelings of satiety associated with changes in objective measures of satiety? *(Hypothesis 3)*
- Is weight loss associated with changes in GI hormones and/or subjective satiety? *(Hypotheses 4 and 5)*

Hypothesis #1 – Greater satiety will be reported after protein consumption compared to fat at all visits.

Statistics: Mixed-effects linear model

Hypothesis #2 – Subjective satiety will increase at all visits after RYGB compared to the Pre-RYGB visit.

Statistics: Mixed-effects linear model

Hypothesis #3 – Subjective satiety will be associated with the GI hormones after RYGB.

- Positive relationship between GLP-1/PYY and subjective satiety
- Inverse relationship between ghrelin and subjective satiety

Statistics: Mixed-effects linear model

Hypothesis #4 – Changes in GLP-1, PYY, and subjective satiety will be positively associated with weight loss after RYGB.

Statistics: Mixed-effects linear model

Hypothesis #5 – Change in ghrelin will be inversely associated with weight loss after RYGB.

Statistics: Mixed-effects linear model

CHAPTER 3: CHANGES IN GASTROINTESTINAL HORMONES AND LEPTIN AFTER ROUX-EN-Y GASTRIC BYPASS SURGERY*

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CHAPTER SYNOPSIS

Background: Roux-en-Y gastric bypass (RYGB) imparts long-term weight loss, the mechanisms for which are not well understood. Changes in leptin and gastrointestinal (GI) hormones, including glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and ghrelin, may contribute to the relative success of RYGB compared with conventional weight loss methods. This study evaluated changes in GI hormones and leptin post-RYGB. The study also evaluated whether GI hormones differed after a short-term dose of protein or fat. **Methods:** GLP-1, PYY, ghrelin, and leptin were assessed in 16 women before and up to one year after RYGB. Plasma was collected before and at several times after a short-term equicaloric dose of protein or fat. **Results:** GLP-1 AUC increased at week 6 and 52 weeks in the fat beverage (FAT-BEV) group compared with Baseline. PYY AUC remained elevated at 52 weeks in the FAT-BEV group. Ghrelin AUC decreased at weeks 2, 6, and 52 in the protein beverage (PRO-BEV) group compared with Baseline. Ghrelin AUC was lower in the PRO-BEV group compared with the FAT-BEV group at 6 weeks. Fasted leptin decreased at all visits in both groups and was lower in the FAT-BEV compared with the PRO-BEV group at 52 weeks. **Conclusions:** Changes from Baseline were evident for all GI hormones and leptin; some differences were evident soon after surgery (ghrelin, leptin) while others were maintained long-term (GLP-1, PYY, ghrelin, leptin). In response to a short-term stimulus, protein suppressed ghrelin and fat potently stimulated GLP-1 and PYY. Future work in this area is warranted.

INTRODUCTION

Bariatric surgery is the most effective treatment for morbid obesity in terms of achieving major, long-term weight loss.[148] Consequently, the prevalence of such surgery is increasing worldwide. The Roux-en-Y gastric bypass (RYGB) is currently the most common bariatric procedure performed, comprising about 70-75% of all bariatric procedures.[2] The RYGB procedure imparts substantial long-term weight loss; however, the mechanisms by which this occurs are not well understood. Restriction and bypass components characterize this procedure.[149] The restrictive component is achieved by creating a gastric pouch (~20-30mL) which promotes early satiety[4, 12] and thereby decreases intake[4] which is considered to be the primary reason for weight loss after RYGB.[150] In addition, the distal stomach, duodenum, and proximal jejunum are bypassed.[12] Malabsorption of energy yielding macronutrients is not common after RYGB but in some cases malabsorption of micronutrients (e.g. iron, vitamin B12) does occur.[14, 24] In addition to these physiological modifications, changes in leptin and several gastrointestinal (GI) hormones, including glucagon-like peptide 1 (GLP-1), peptide YY (PYY), and ghrelin, may explain the relative success of the RYGB procedure.[67, 151]

GLP-1 and PYY are considered appetite-regulating hormones given that secretion of each reduces hunger and imparts satiety.[57] They are secreted from the L cells of the distal ileum and colon in response to enteral nutrient intake.[31, 37, 39, 42-44, 152] The mechanism by which these hormones promote satiety is thought to be multifaceted because they slow gastric emptying,[30, 31, 36] promote insulin release,[31, 45] and inhibit gastric acid secretion.[30, 36] Because of these aforementioned effects, they are considered to play an important role in the "ileal brake" mechanism,[32] which regulates the passage of

nutrients through the GI tract.[47] GLP-1 and PYY are secreted within 15 to 30 minutes after food intake.[46, 73, 153] More specifically, data suggest that GLP-1 is stimulated by fat intake in rats[126] and humans,[127, 129] and other data support that it is stimulated by protein more than carbohydrate intake.[130, 131] Similarly, fat[132-134] and protein[132, 135, 136] have been found to be important regulators of PYY secretion in obese[132, 135, 136] and normal-weight individuals.[133, 134, 136] Some data support that fasting and postprandial GLP-1 and PYY secretion are higher in normal-weight individuals compared with those who are obese or overweight.[50, 77, 78, 154] Higher GLP-1 and PYY levels have been reported in post-RYGB patients compared with pre-RYGB,[57, 67, 71, 107, 155] normal-weight,[57, 81-84] overweight,[53, 54, 60, 60, 82] and obese individuals,[57, 58, 81, 83] and in those who underwent other weight loss surgeries.[54, 57, 60, 60, 82]

Ghrelin is the only GI hormone known to stimulate appetite and therefore it is considered to be orexigenic.[37, 39, 42, 43] It is released both centrally (hypothalamus) and peripherally (stomach) and its anti-satiating properties may be due to its biological effects to increase GI motility and decrease insulin secretion.[37, 38] Ghrelin levels increase in the absence of enteral intake and decrease right after meal initiation[86] and the macronutrient that induces the greatest suppression is not well defined. For instance, some research suggests that carbohydrate is suppressive[137, 138] whereas others have found protein to be more potent in terms of prolonged suppression.[130, 131, 138-141] Erdmann et al.[137] also found that fat was a potent suppressor, especially in the late postprandial state (i.e. 60-180 minutes) with similar findings by Tannous dit El Khoury et al.[138] In addition, increased ghrelin levels have been reported in individuals undergoing diet-induced weight loss, which might explain the difficulty in maintaining weight loss achieved with conventional

methods.[156] However, reduced ghrelin has actually been reported after RYGB weight loss compared with pre-RYGB,[92, 98, 101, 105] normal-weight,[26, 57, 81, 97, 101, 105, 156] overweight,[60, 82] and obese[81, 93, 97, 105, 156] people and/or those who undergo other restrictive bariatric surgical procedures.[60, 82, 97] However, not all research has found this to be the case,[14, 67, 67, 71, 83, 93, 96, 99, 103, 104, 108, 157, 158] therefore, the role of ghrelin in post-RYGB weight loss remains unclear.

The adipocytokine, leptin, is a product of the obesity gene (*ob* gene) and is understood to be involved in long-term energy balance.[40] It is secreted mainly by adipocytes and, to a lesser degree, gastric chief cells[159] and influences energy intake, primarily by acting on the hypothalamus[39-41] to decrease food intake and increase energy expenditure.[41] Plasma leptin concentrations are not known to be regulated by macronutrients, but rather by adipose tissue mass. Leptin circulates in proportion to whole-body adipose tissue mass[40] and strong evidence exists that leptin decreases with reduction in adipose tissue.[59, 109-111] As would be expected, decreased leptin has been reported post-RYGB compared with pre-RYGB,[59, 67, 95, 101, 114, 116, 117, 119-122, 124, 125, 158, 160] normal-weight,[112] overweight,[82] and obese[53, 103, 113] patients.

The objective of this study was to evaluate the changes in GLP-1, PYY, ghrelin, and leptin post-RYGB. In addition, we sought to determine whether there was a difference in GI hormone levels after a short-term dose of either protein or fat.

METHODS

Women with Class III obesity (i.e. body mass index (BMI) $\geq 40 kg/m^2$) who planned to undergo the laparoscopic RYGB procedure were recruited from the Weight

Loss Surgery Center at the University of Minnesota Medical Center – Fairview. Participants for this longitudinal analysis were also enrolled in a broader longitudinal study investigating body composition and metabolic changes after RYGB surgery. This study included five visits that were 24 hours in duration: 30-70 days pre-RYGB (Baseline); two weeks post-RYGB (Week 2); six weeks post-RYGB (Week 6); 26 weeks post-RYGB (Week 26); and 52 weeks post-RYGB (Week 52). The timing of the study visits was established in order to evaluate the clinical outcomes in both the early (i.e. Weeks 2 and 6) and late (i.e. Weeks 26 and 52) postoperative stages. GI hormones and leptin were analyzed in 16 participants. Exclusion criteria included the following: use of corticosteroids, testosterone, or anabolic agents; internally placed biomedical device (e.g. pacemaker); liver, renal, or heart failure; pulmonary hypertension; thyroid disease (included if treated and within normal limits); neoplastic disease; Type 1 or uncontrolled Type 2 diabetes mellitus (defined by HbA1c > 7%); pregnancy; or previous weight loss surgery. The study protocol was reviewed and approved by the Institutional Review Board and the General Clinical Research Center (GCRC) at the University of Minnesota, and participants provided written, informed consent before enrollment in the study.

The day before admission to the GCRC, participants were instructed to avoid caffeine, alcohol, and vigorous exercise for 24 hours before testing. Participants were also asked to be in the fasted state for at least two hours before GCRC admission. Participants were randomized to one of two groups: 1) PRO-BEV group: those receiving a sugar-free, high-protein beverage (20g protein, 0g fat, 2g carbohydrate, 90 kilocalories, 8oz) (protein: whey protein isolate, supplied by Davisco Foods International, Inc., Eden Prairie, MN) and 2) FAT-BEV group: those receiving a sugar-free high-fat beverage (0g

protein, 9g fat, 3g carbohydrate, 90 kilocalories, 8oz). At all visits, a baseline four hour fasted plasma sample was collected for the GI hormone and leptin analyses and 15 minutes later, particpants consumed the assigned beverage over a period of not more or less than 30 minutes (60 minutes at Week 2 to accommodate the reduced stomach capacity). Blood draws were taken at specific time points after initiation of beverage consumption (Time 0). All plasma samples were stored at -70°C before analysis of GLP-1, PYY, ghrelin, and leptin concentrations. Tables 3-1 and 3-2 depict when the hormones were sampled at each visit.

Table 3-1: GI Hormone and Leptin Analysis Time Points for Baseline, Week 6, Week 26, and Week 52

Visit	Time 0 minutes	+30 minutes	+60 minutes	+90 minutes
Baseline, Week 6, Week 26, and Week 52	Leptin			
	GLP-1	GLP-1	GLP-1	
	PYY		PYY	PYY
	Ghrelin		Ghrelin	Ghrelin

GI: gastrointestinal; GLP-1: glucagon-like peptide 1; PYY: peptide YY

Table 3-2: GI Hormone and Leptin Analysis Time Points for Week 2

Visit	Time 0 minutes	+60 minutes	+90 minutes	+120 minutes
Week 2	Leptin			
	GLP-1	GLP-1	GLP-1	
	PYY		PYY	PYY
	Ghrelin		Ghrelin	Ghrelin

GI: gastrointestinal; GLP-1: glucagon-like peptide 1; PYY: peptide YY

The longitudinal study from which these data were analyzed was originally aimed to investigate clinical outcomes including body composition, energy metabolism, and nutritional status changes after RYGB. One of the components of the longitudinal study was a double-blinded randomized six-week supplementation period immediately after

surgery to determine if patients consuming more protein in the early post-operative period would lose less lean tissue. The participants consumed the randomly assigned beverage at each testing visit as described above and were also instructed to consume two of the assigned beverages daily throughout the initial six-week postoperative period. For the purposes of this analysis, only the short-term response data from the beverage consumed at the testing visits are discussed.

Dietary Intake Assessment

Information regarding dietary intake was obtained through the use of diet records. During the week before each testing visit, participants recorded their food intake on two assigned weekdays and one weekend day. During the six-week supplementation period (visits at Weeks 2 and 6), participants were instructed to record study beverage intake. Recording errors were minimized by providing the participants with detailed instructions on how to record their intake, including estimation of portion size. Diet records were reviewed with the participants at each testing visit and were analyzed later for nutrient content using the Food Processor SQL software (version 10.4 ESHA Research, Salem, OR). To obtain an estimation of the participants' intake during the week before the visit, an average of the recorded macronutrients and calories was calculated.

Gastrointestinal Hormone and Leptin Assays

All samples were assayed in duplicate. PYY-like immunoreactivity was measured with a specific and sensitive radioimmunoassay that measured both the full length (PYY_{1-36}) and the fragment (PYY_{3-36}).[77, 161] Plasma active GLP-1 and total ghrelin

were measured by established in-house radioimmunoassay.[162-164] Plasma leptin was measured with a Linco Research assay kit.

Anthropometric Measurements

Height and weight were measured using standardized procedures. Height was measured to the nearest 0.1cm by a wall-mounted stadiometer (model S100; AYRTON Corporation, Prior Lake, MN) at Baseline only. At all testing visits, weight was measured to the nearest 0.1kg on a digital scale (model 5002, SCALE-TRONIX, White Plains, NY). BMI was calculated as the patient's weight in kilograms divided by her height in meters squared (kg/m^2).

Surgical Technique

All RYGB procedures were performed laparoscopically. A small gastric pouch (approximately 15mL) was created and the duodenum and proximal portion of the jejunum were bypassed to create a Roux limb. In most cases, the length of the Roux limb was 150cm, according to Weight Loss Surgery Center at the University of Minnesota Medical Center-Fairview protocol.

Statistical Analysis

Study endpoints were defined as change from the Baseline visit in body weight, fasted GI hormones, and fasted leptin and change from Baseline in area under the curve (AUC) for GLP-1, PYY, and ghrelin. AUC for the Week 2 visit was rescaled to the same length of time as the other visits for comparability. Supplement groups were compared in

mixed-effects linear models with a random effect to model the correlation of repeated measurements within each participant. SAS 9.2 software (SAS Institute, Inc., Cary, NC) was used for statistical analyses. Appendix C contains example SAS codes for this analysis.

RESULTS

Twenty-nine women participated in the study. GI hormones and leptin were analyzed in only the 16 women who completed all 5 study visits and who had a sufficient amount of plasma for analyses. All participants were Caucasian, with a mean age of 49 ± 9 years. At Baseline, there were no statistically significant differences between the groups in terms of age, weight, height, or BMI (Table 3-3). In both groups, BMI and absolute body weight significantly decreased at Weeks 26 and 52 compared with Baseline. At Week 26, weight decreased by a mean 34kg (27% weight loss) and at Week 52, weight decreased by a mean 43kg (34% weight loss).

Table 3-3: Baseline Characteristics of Study Participants

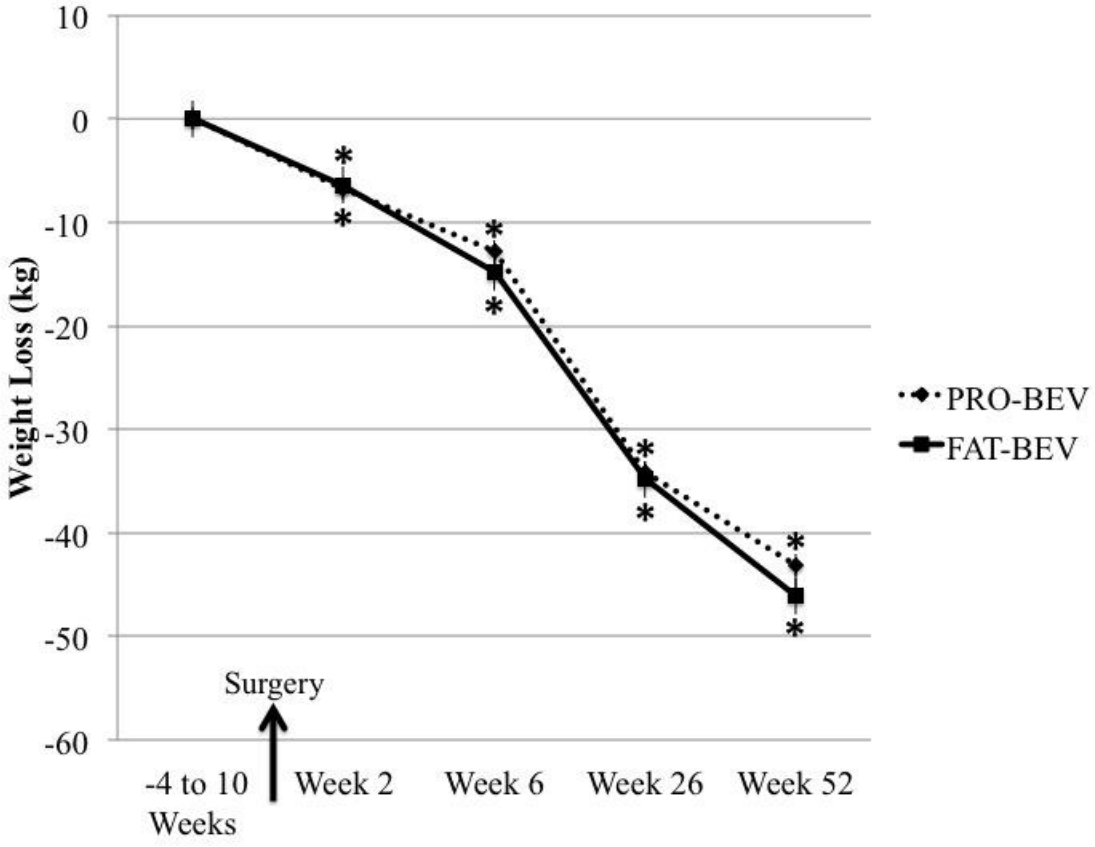
	PRO-BEV (N=8)	FAT-BEV (N=8)
Age (years)	51 ± 7	47 ± 11
Weight (kg)	124 ± 13	128 ± 15
Height (cm)	166 ± 5	166 ± 4
BMI (kg/m^2)	45 ± 5	47 ± 6

Means ± standard deviation; no significant differences between treatments; BMI: body mass index; FAT-BEV: fat beverage group; PRO-BEV: protein beverage group

Body Weight Change from Baseline Visit

Change in body weight for all 29 participants are reported in Figure 3-1. Weight loss was significant at all visits in both treatment groups, with no differences between treatment groups.

Figure 3-1: Body Weight Change from Baseline Visit



* Significantly different from Baseline, $P < 0.05$; Mean ± SE.

Dietary Intake Assessment

At the Baseline visit, the FAT-BEV group was consuming significantly more calories than the PRO-BEV group (2305 ± 456kcal vs. 1696 ± 457kcal, respectively; $P = 0.02$). For the remainder of the study, no differences in caloric intake were evident

between the two treatment groups. At Week 2, participants reported consuming on average 385 kcal (range: 101-963kcal) per day. By Week 6, caloric levels increased to an average 692 kcal (range: 397-1150kcal) per day. At Week 26, participants were consuming about 1120 kcal (range: 645-1565kcal) a day, and by the Week 52 follow-up visit, participants reported consuming about 1350 kcal (range: 755-1774kcal) per day.

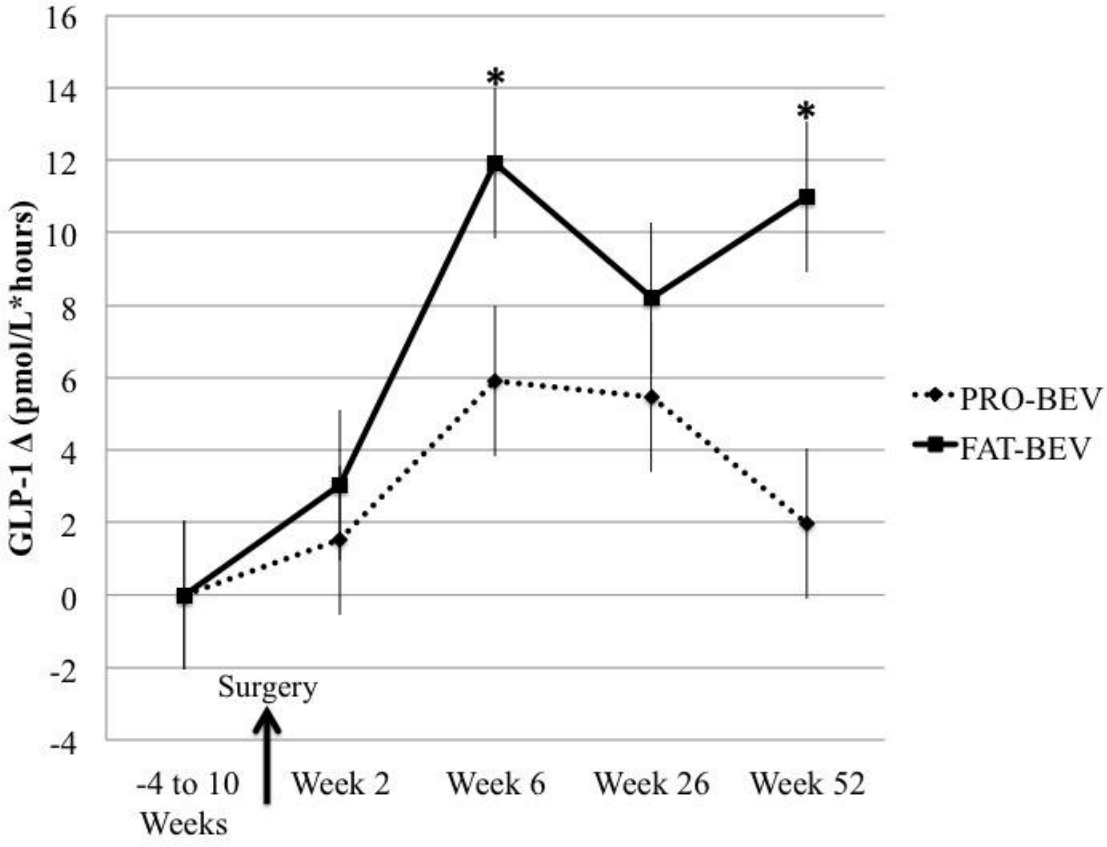
At the Baseline visit, the FAT-BEV group was consuming significantly more protein than the PRO-BEV group (105 ± 27g vs. 76 ± 26g, respectively; P = 0.05). However, by Week 2, the PRO-BEV group was consuming significantly more protein than the FAT-BEV group (41 ± 24g vs. 11 ± 15g; P = 0.01, respectively) and this difference remained significant at Week 6 (PRO-BEV: 57 ± 9g vs. FAT-BEV: 36 ± 25g, P = 0.04). This discrepancy in protein intake between the two groups at Week 2 and 6 is due to the fact that that we asked the participants in both groups to consume the assigned beverage for 6 weeks (immediately after surgery until the Week 6 visit). Participants were instructed to drink the assigned beverage twice a day in addition to consuming their regular meals and snacks. Therefore, the PRO-BEV group was potentially receiving an additional 40 grams of protein per day. Protein consumption was no longer different between the groups at Weeks 26 (59g/day) and 52 (65g/day).

There were no differences in fat consumption between the groups at any visit. At Baseline, participants reported consuming an average 82g of fat per day. At Week 2, fat consumption decreased and was about 12g per day. At Week 6 fat consumption increased, and participants reported an average intake of 25g per day. Fat consumption continued to increase, and average reported intakes were 44g at Week 26 and 53g at Week 52.

GLP-1 AUC Change from Baseline Visit

In the FAT-BEV group, GLP-1 AUC significantly increased at Weeks 6 and 52 compared with Baseline (Figure 3-2).

Figure 3-2: Glucagon-Like Peptide-1 (GLP-1) Area Under the Curve Change from Baseline Visit

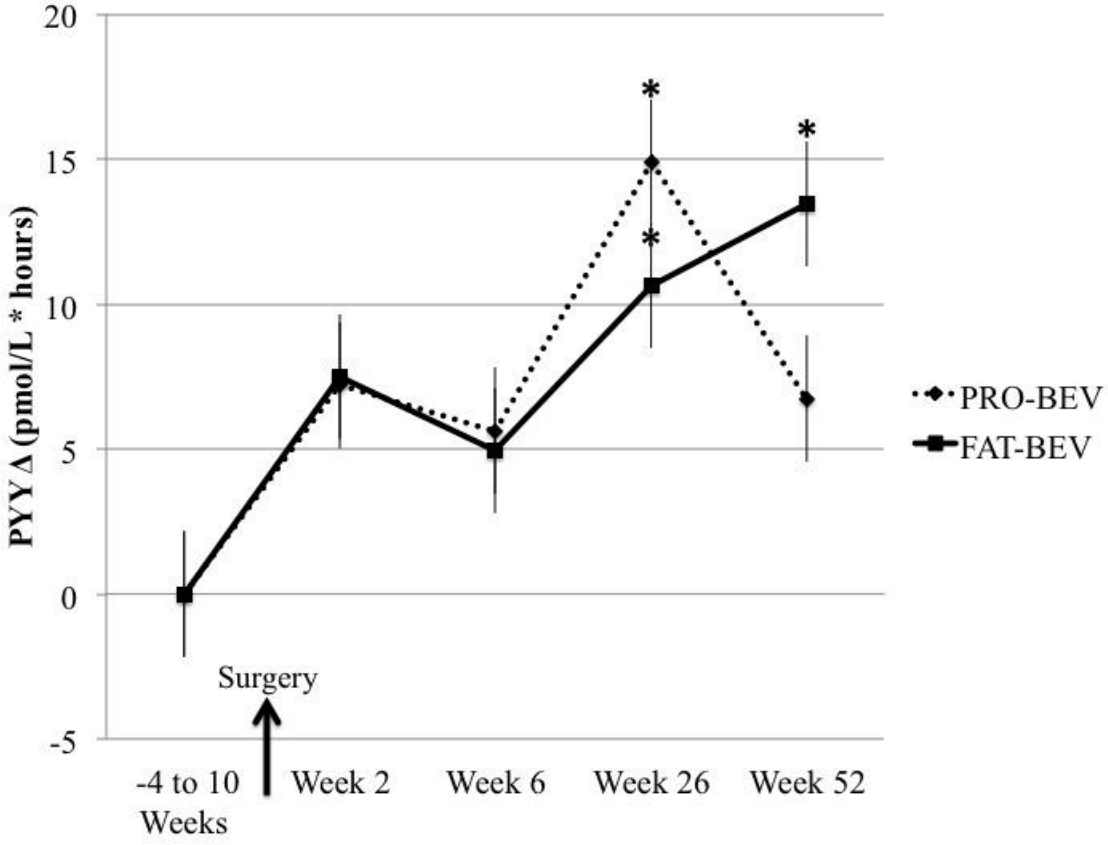


* Significantly different from Baseline, $P < 0.05$; Mean ± SE.

PYY AUC Change from Baseline Visit

In both the PRO-BEV and FAT-BEV groups, PYY AUC significantly increased at Week 26 compared with Baseline (Figure 3-3). In addition, PYY AUC remained higher compared with Baseline at Week 52 Post-RYGB in the FAT-BEV group.

Figure 3-3: Peptide YY (PYY) Area Under the Curve Change from Baseline Visit

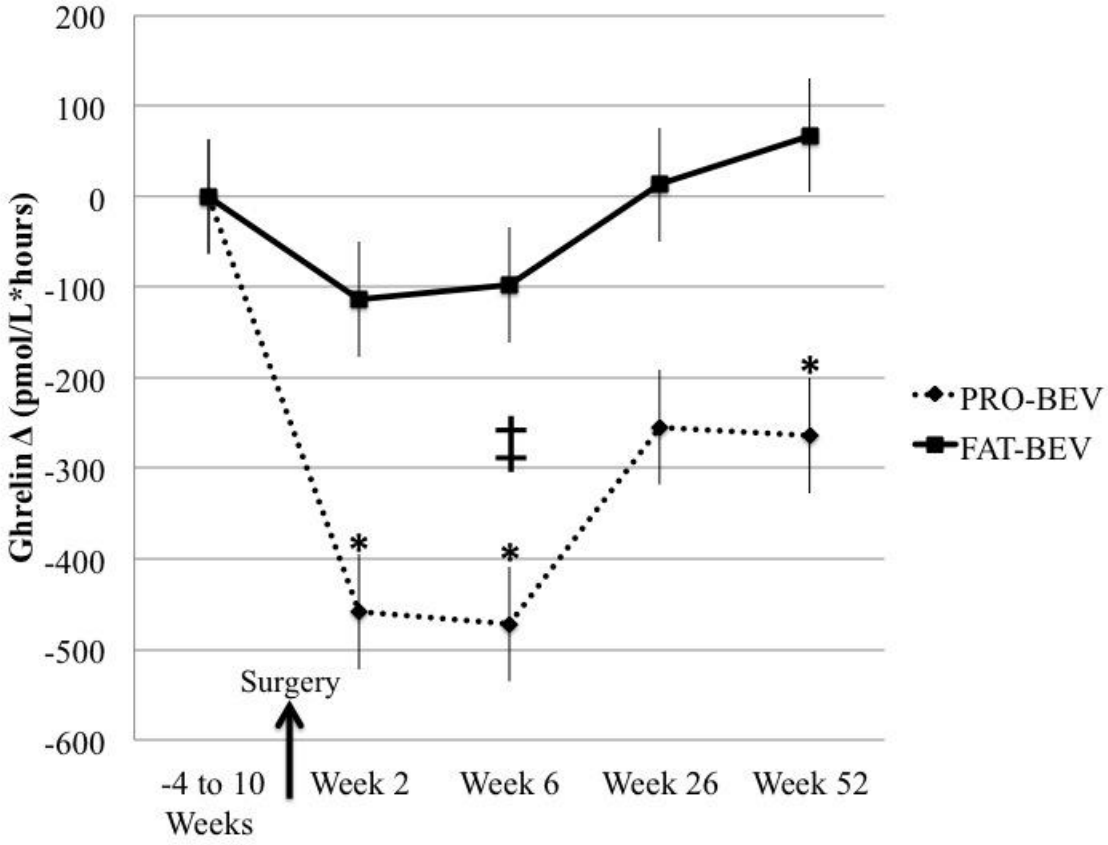


* Significantly different from Baseline, $P < 0.05$; Mean ± SE.

Ghrelin AUC Change from Baseline Visit

Ghrelin AUC was significantly lower at Week 2 compared with Baseline in the PRO-BEV group (Figure 3-4). Moreover, this alteration remained significant at Weeks 6 and 52. Ghrelin AUC levels were significantly different between the PRO-BEV and FAT-BEV group at Week 6, with lower levels in the PRO-BEV group.

Figure 3-4: Ghrelin Area Under the Curve Change from Baseline Visit

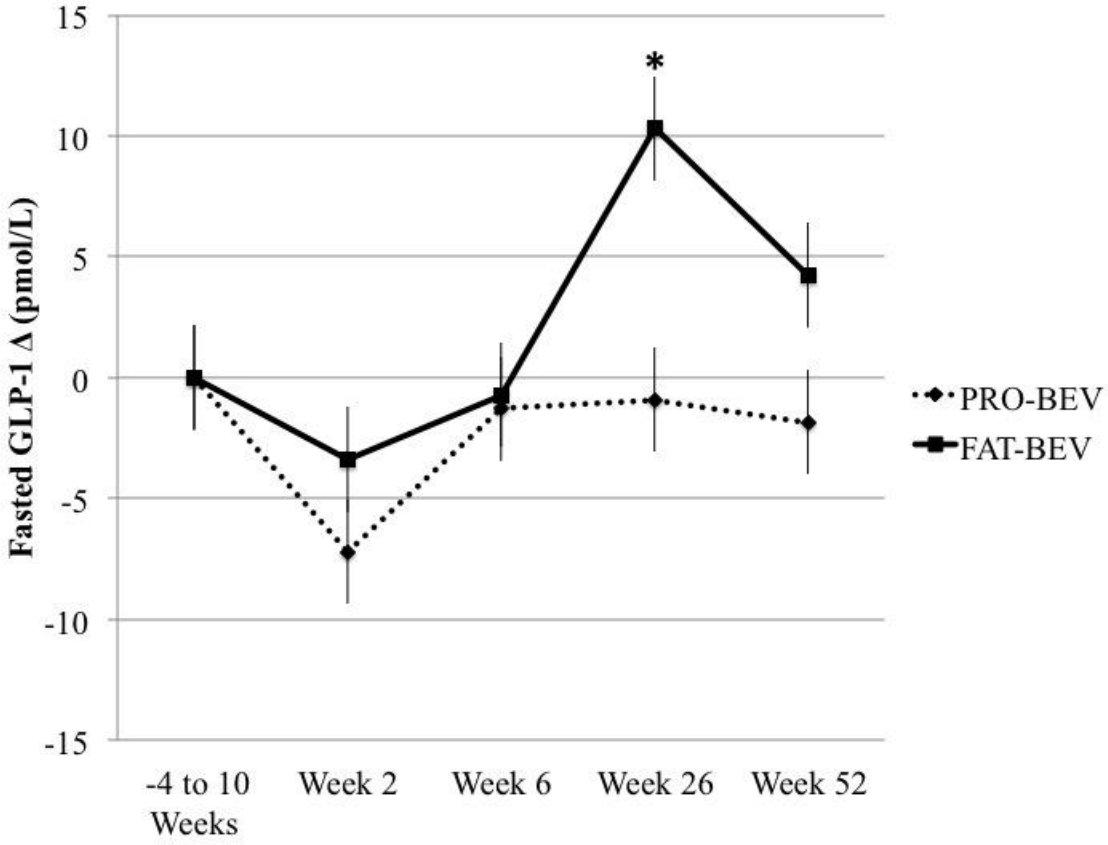


* Significantly different from Baseline, P < 0.05; ‡ Significantly different between groups, P < 0.05; Mean ± SE.

Fasted GLP-1 Change from Baseline Visit

At Week 26, fasted GLP-1 increased significantly compared with Baseline in the FAT-BEV group (Figure 3-5). No differences in fasted GLP-1 levels between treatments were evident at any visit.

Figure 3-5: Fasted Glucagon-Like Peptide-1 (GLP-1) Change from Baseline Visit



* Significantly different from Baseline, $P < 0.05$; Mean ± SE.

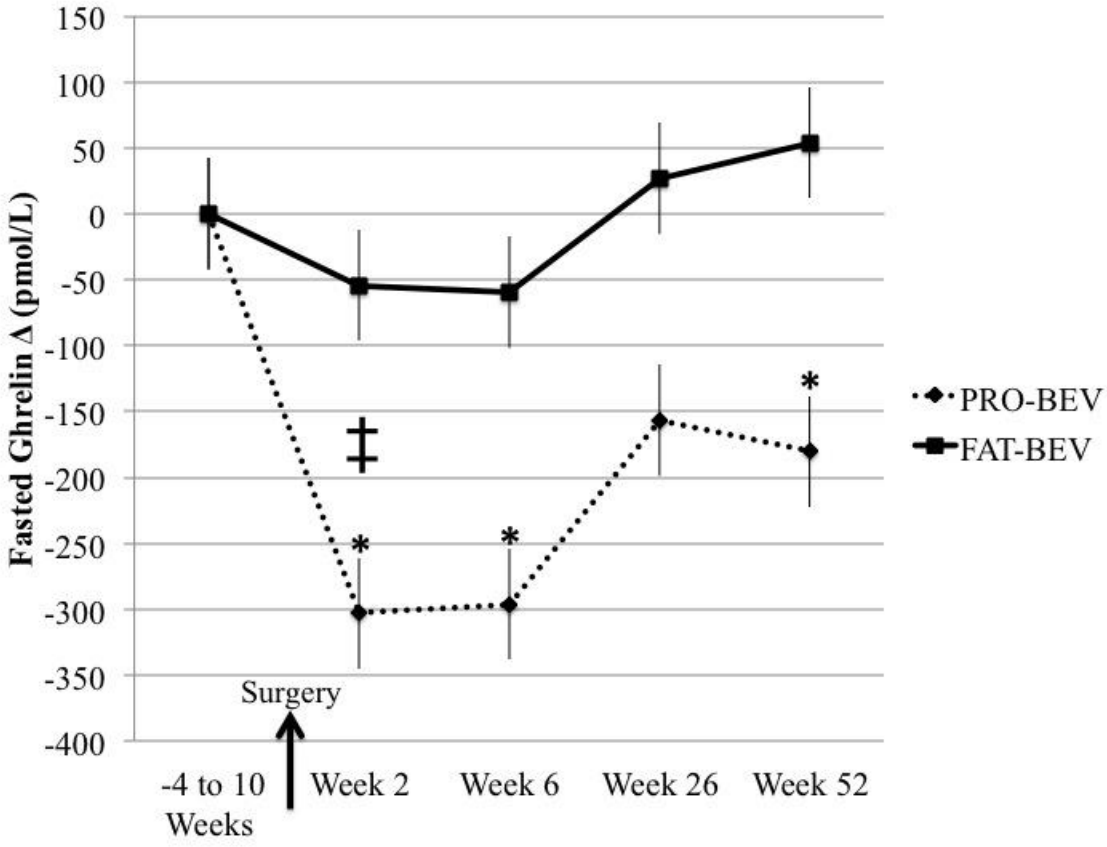
Fasted PYY Change from Baseline Visit

No significant differences in fasted PYY levels were evident between visits (data not presented). There was no difference in fasted PYY levels between treatment groups at any visit.

Fasted Ghrelin Change from Baseline Visit

Fasted ghrelin was significantly lower at Weeks 2, 6, and 52 compared with the Baseline visit in the PRO-BEV group (Figure 3-6). At Week 2, fasted ghrelin was significantly different between treatments, with lower levels in the PRO-BEV group.

Figure 3-6: Fasted Ghrelin Change from Baseline Visit

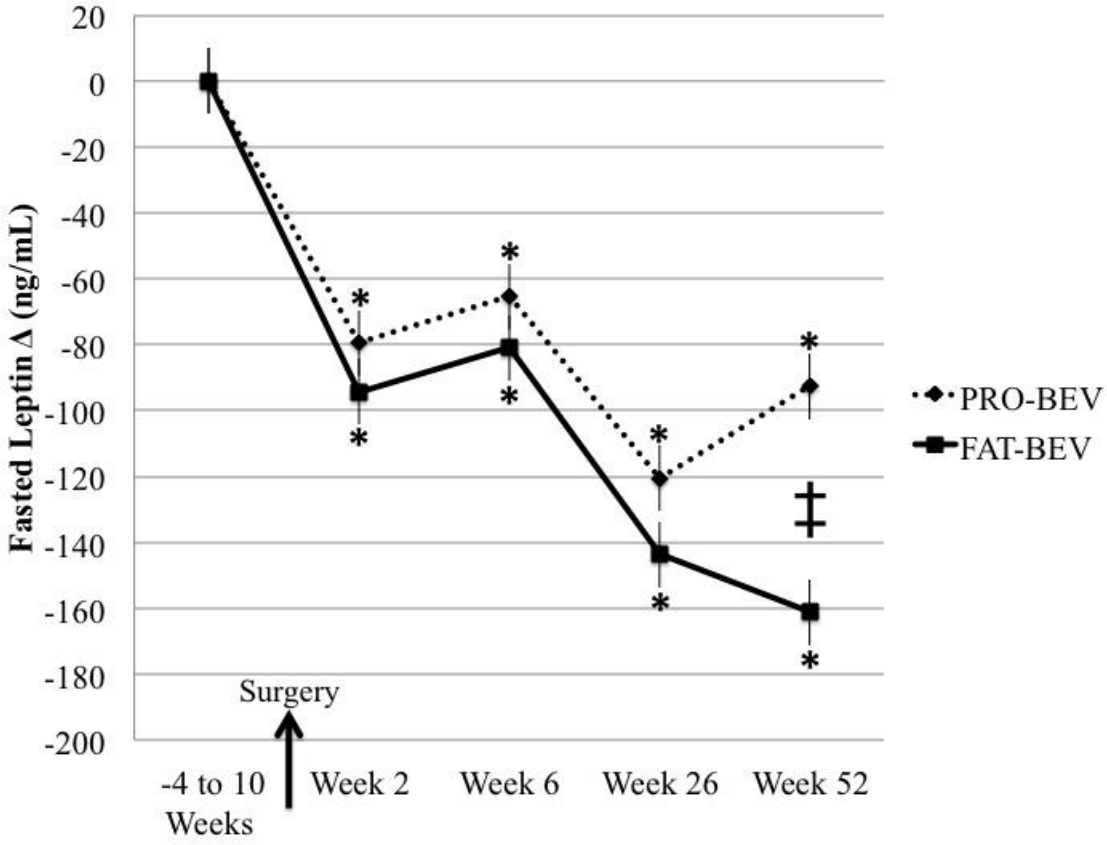


* Significantly different from Baseline, $P < 0.05$; ‡ Significantly different between groups, $P < 0.05$; Mean ± SE.

Fasted Leptin Change from Baseline Visit

In both the PRO-BEV and FAT-BEV groups, leptin significantly decreased compared with Baseline at all time points after RYGB (Figure 3-7). Leptin levels were significantly different between the PRO-BEV and FAT-BEV groups at Week 52, with lower leptin in the FAT-BEV group.

Figure 3-7: Fasted Leptin Change from Baseline Visit



* Significantly different from Baseline, $P < 0.05$; ‡ Significantly different between groups, $P < 0.05$; Mean ± SE.

DISCUSSION

This study investigated the effect of the RYGB procedure on body weight and levels of GLP-1, PYY, ghrelin, and leptin. We also evaluated the effect of short-term administration of protein and fat on GI hormone levels. In patients with morbid obesity, RYGB resulted in significant weight loss, which was maintained for at least one year after surgery. In addition to the favorable change in weight, alterations in GI hormones and leptin also occurred and in some cases these changes were macronutrient specific.

General Findings for Both Beverage Groups

Similarities between treatment groups were evident for fasted leptin at all visits and PYY AUC at Week 26. In regard to fasted leptin, the adipocytokine decreased from Baseline at all visits. This is an expected result and similar to what has been previously reported. Decreased leptin has consistently been found after a reduction in adipose tissue mass after weight loss, either by conventional methods[59, 110, 111] or via RYGB.[53, 59, 67, 82, 95, 101, 103, 112-114, 116, 117, 119-122, 124, 125, 158, 160] Of interest however, we observed that fasted leptin was significantly different as soon as two weeks post-RYGB, before significant changes in absolute body weight were observed. This immediate change was also found by Rubino et al.[121] at three weeks post-RYGB, before significant weight changes and might be a result of the RYGB procedure itself.[121] Leptin is secreted in proportion to adipose tissue mass,[40] and it would be expected that this change would not occur until a later follow-up visit (e.g. Week 26). In addition to adipocytes, leptin is also secreted from the stomach,[159] which is substantially altered after RYGB. This manipulation might explain the decreased fasted leptin levels we found soon after surgery.

PYY AUC was higher in both groups at Week 26 Post-RYGB compared with Baseline. Our results are similar to those who have evaluated PYY at 26 weeks post-RYGB.[14, 67] However, we are not aware of any study that has looked at the effect of different macronutrients on PYY in the post-RYGB state. Given what has been reported in overweight and normal-weight individuals, we would expect PYY to increase after a short-term dose of either protein[132, 135, 136] or fat,[132-134] which is in line with our results.

Specific Findings - Protein Beverage Group

In the PRO-BEV group, a short-term dose significantly lowered ghrelin AUC at Weeks 2, 6, and 52 compared with Baseline levels. In terms of the ghrelin AUC results at two weeks, this early change is congruent with what has been found in previous research 30 minutes to 10 days after surgery[92, 98] and, similar to leptin, occurred before a significant change in absolute body weight occurred, suggesting that a component of the RYGB procedure is involved in this result. Through repeated measurements before, during, and after the RYGB procedure, Lin et al.[98] found that the greatest decline in ghrelin occurs after complete division of the stomach, offering an explanation as to why this favorable change is seen so soon after surgery. In terms of the macronutrient effect on ghrelin AUC, the fact that we found an effect in the PRO-BEV group after RYGB is a novel result. To our knowledge no study has compared the effect of macronutrients on ghrelin in the post-RYGB state. We expected that protein would suppress ghrelin AUC in our patient population at two weeks post-RYGB, because research done in overweight and obese individuals indicates that ghrelin is lower after protein intake compared with the respective baseline levels,[138, 140] carbohydrate intake,[130, 131, 138, 139] and/or fat intake.[138] However, more research is needed in this area to clarify if the ghrelin response post-RYGB is macronutrient specific. Such research has the potential to better define the diet recommendations for patients post-RYGB.

Ghrelin AUC was also significantly reduced at six weeks post-RYGB compared with both Baseline and the FAT-BEV group. By this time point, participants had a significant change in body weight, yet were still considered to be obese. Limited research has evaluated ghrelin at six weeks post-RYGB, and contrary findings are

evident. At four weeks post-RYGB, Sundbom et al.[104] reported an increase in ghrelin to levels comparable with pre-RYGB, whereas two studies[59, 101] did not find a significant ghrelin suppression compared with pre-RYGB levels. In addition, le Roux et al.[71] found an insignificant increase in ghrelin at six weeks compared with baseline. Discrepancies might be related to differences in study protocol. For example, Morinigo et al.[101] measured ghrelin after a mixed 398-kcal liquid meal with 14.5% protein, and le Roux et al.[71] measured ghrelin after a mixed 400 kcal meal (protein amount not specified) whereas Sundbom et al.[104] measured only fasted ghrelin. Olivan et al.[59] measured ghrelin after a 50g oral glucose tolerance test. We measured ghrelin after a 90kcal protein beverage (88.9% protein), and our significant finding might be due to the higher proportion of protein. Studies that evaluated patients of similar weight status (obese and/or overweight) have observed protein to potently suppress ghrelin.[130, 131, 139, 140] Although fat has been found to suppress ghrelin,[137, 138] we did not find this at Week 6 and instead observed significantly lower ghrelin in the PRO-BEV group compared with the FAT-BEV group. Perhaps the best explanation is that protein may be more suppressive of the GI hormone than fat,[138, 140] especially in post-RYGB patients. Satiety research suggests that protein is the most and fat the least satiating,[144] which may account for our findings.

The suppressed ghrelin AUC levels observed at Week 2 and 6 were maintained long-term in this study. Ghrelin AUC was significantly suppressed at one-year Post-RYGB compared with Baseline levels. At this time point, almost half of the participants were considered to be overweight (n = 7) with the remainder still classified as obese (n = 9). Reduced ghrelin levels have been reported in post-RYGB patients 1-2 years post-

surgery, compared with other surgical procedure patients,[97] obese,[81, 97] and normal-weight individuals.[81, 97] This finding of suppressed ghrelin levels at one-year post-RYGB is encouraging, and might offer a long-term explanation for the relative success of the procedure. Future work should compare weight loss outcomes with ghrelin levels to better establish ghrelin's role in weight loss after RYGB.

Fasted ghrelin was significantly lower at Weeks 2, 6, and 52 compared with Baseline in only the PRO-BEV group and was significantly lower than in the FAT-BEV group at Week 2. Treatment specific alterations in the fasted ghrelin levels were unexpected and prompted us to investigate our diet data for possible explanations. As described in the Methods, this analysis was part of a larger study evaluating body composition changes and the impact of protein intake on those changes in the RYGB patient population and was not initially designed to evaluate GI hormones and leptin. Consequently, participants in this study only fasted for four hours before the Baseline blood draw. At Weeks 2 and 6, analysis of the meal eaten 4-6 hours before the Baseline blood draw indicated that the percent of calories from protein was significantly greater in the PRO-BEV group compared with the FAT-BEV group (Week 2: PRO-BEV: 42%, FAT-BEV: 20%, P = 0.05; Week 6: PRO-BEV: 31%, FAT-BEV: 14%, P = 0.04). Ghrelin, being orexigenic, increases in the absence of enteral intake and, in this study was higher in the FAT-BEV group. Protein has been suggested to be the most satiating of the three macronutrients,[143] and perhaps a sustained reduced ghrelin level between meals is a mechanism for this. Because the study participants fasted for between 4-6 hours before the study, it might be more appropriate to consider them to be in a postprandial rather than fasting state. It is certainly possible that our levels of Baseline fasting ghrelin were

susceptible to some previous meal effects.

Although we observed an apparent difference between the treatment groups in fasting ghrelin concentrations at some of the postoperative time points, we hesitate to make general recommendations regarding the clinical significance and implication of these findings, for several reasons. First, we had a small sample size (n = 8 in each group) and might not have had enough power to detect real differences. Second, because this study was not originally designed to evaluate GI hormones, the fasting time period might not have been long enough to ensure an adequate preprandial state. Overall, we cannot be certain whether the difference in fasted ghrelin concentrations between groups was true or spurious, and thus further work is needed to elucidate the GI hormone profile in response to different macronutrients in the post-RYGB patient population.

Specific Findings - Fat Beverage Group

GLP-1 AUC was greater at six weeks and one-year Post-RYGB in the FAT-BEV group compared with Baseline levels. Other research has investigated GLP-1 four[55, 59, 67, 70] to six[58, 59, 71, 155, 165] weeks post-RYGB and overall the findings were similar to ours. Most researchers have found significantly increased levels at this time point compared with baseline,[55, 58, 70, 71, 155] although two studies did not find such an effect.[67, 165] Clements et al.[165] may not have found increased GLP-1 at six weeks post-RYGB because they only measured the GI hormone in the fasted state, and GLP-1 is released in response to nutrient intake. The changes that we and others observed occurred when participants were still considered obese; therefore, the weight loss is not driving the changes in GLP-

1. Instead, increased GLP-1 levels post-RYGB are thought to occur because of a surgical component that promotes more rapid delivery of nutrients to the distal gut.[64, 65]

The increased GLP-1 AUC levels found at Week 6 were maintained long term in our study (i.e. one year Post-RYGB). Few other studies have evaluated GLP-1 changes at this specific time point post-RYGB. Korner et al.[14] found GLP-1 increased significantly at one year post-RYGB compared with Baseline, whereas Moringigo et al.[155] did not find this. Increased GLP-1 levels have been found at post-RYGB in studies of longer duration (e.g. > one year) compared with those who underwent other weight loss procedures[54, 57, 60] and non-surgical controls[53, 54, 57, 61, 151] and this alteration has been hypothesized to play a role in the success of the procedure. le Roux et al.[71] evaluated those who had "good" and "poor" weight loss outcomes, defined as >30% and <25% weight loss after RYGB, respectively (RYGB was performed 25.3 ± 1.7 months before the study). Patients considered to have poor weight loss had significantly lower GLP-1 compared with those who were considered to have good weight loss.[71]

In contrast to the lack of significant change in GLP-1 AUC levels in the PRO-BEV group from Baseline to all time points following surgery, we found that GLP-1 AUC levels were increased in response to an short-term administration of fat at Weeks 6 and 52. This was a novel finding, as no other study to our knowledge has looked at GLP-1 changes in response to different macronutrients before and after RYGB. We found no significant differences at any of the time points between the groups in GLP-1 AUC. Graphic representation of the data suggests that the short-term response to protein was not stimulatory of GLP-1, and there may have been a difference between groups that we were unable to detect because of an insufficient sample size. Although it has been

reported that both protein and fat can potently stimulate GLP-1 release, fat has been reported to stimulate GLP-1 more potently than protein in normal-weight individuals[127] and obese boys.[129] Lipids are thought to be the most potent stimulus for GLP-1 secretion, and ingestion of fats leads to sustained secretion.[152] From our data in the RYGB patient population, fat ingestion more potently stimulated GLP-1 compared with protein. More work is needed in this area to establish the most effective post-RYGB diet recommendations.

Fasted GLP-1 levels were significantly greater at Week 26 compared with Baseline in only the FAT-BEV group. This result was unexpected, and we evaluated the macronutrient content of the meal eaten 4-6 hours before the Baseline blood draw in order to better understand the mechanism involved. Compared with either protein or carbohydrate, fat most potently slows GI transit.[166] In addition, GLP-1 secretion peaks after meal termination and remains elevated for several hours afterward.[53-55, 57-61, 70] Although both protein[130, 131] and fat[127, 129] stimulate GLP-1 secretion, it is possible that the FAT-BEV group had an elevated GLP-1 level secondary to the meal eaten before the visit, which was higher in the percentage of calories from fat compared with the PRO-BEV group and might not have been completely digested and absorbed because of the slower transit time of fat (FAT-BEV: 46% vs PRO-BEV: 33%, P = NS).

At one-year Post-RYGB, PYY AUC increased significantly compared with Baseline levels. Studies that have specifically looked at this time point[14, 84] have reported similar findings. Likewise, in cross-sectional studies that compared PYY levels in pre-RYGB patients,[63] other weight loss surgical patients,[57, 60] and nonsurgical controls[57, 60, 81, 83, 151] with those of patients who were at least one year post-RYGB found higher PYY in

the post-RYGB group. It is thought that increased PYY levels, similar to GLP-1, mediate the long-term weight loss/maintenance found in RYGB patients. In the le Roux et al.[71] study that classified post-RYGB patients according to their weight loss outcome, those who were considered to have good weight loss had significantly higher PYY compared with the poor weight loss group.

This is the first study we know of that evaluated the PYY response after different macronutrients in the Pre- and Post-RYGB patient population. We found that at one-year Post-RYGB, PYY AUC only increased in response to the short-term administration dose of fat. Prior work evaluating the PYY response to different macronutrients in normal-weight individuals found that PYY is secreted in response to fat[133, 134] and protein[136] and we are not sure why we did not see such an effect at Week 52 for protein in our study. PYY AUC in the PRO-BEV group reached its peak at Week 26 and by Week 52 decreased to a value similar to what was seen at Week 2. Based on these results, we suggest that PYY is more sensitive to fat in the Post-RYGB patient population, but more research in this area is clearly needed before a definitive conclusion can be made.

LIMITATIONS

Several limitations need to be considered when evaluating our results. First, we had a small sample size (n = 8 in each treatment group) and may not have had enough power to detect real differences. Second, our study sample included only Caucasian women, and therefore are not generalizeable to the population. Third, because of cost and time constraints, participants were not able to serve as their own control for the two treatments and instead they received only one beverage type for all five visits. Fourth,

this analysis was part of a larger study evaluating body composition changes in the RYGB patient population and was not initially designed to evaluate leptin and the GI hormones. Consequently, participants in this study were only fasted for four hours before the Baseline blood draw for GI hormones and leptin. The possibility of altered results secondary to the meal eaten four hours before the analyses cannot be ruled out.

CONCLUSIONS

We have shown that in addition to substantial weight loss, multiple changes in GI hormones and leptin occur after RYGB. Some differences were evident quite soon after surgery (two weeks: ghrelin, leptin) whereas others were maintained long-term (one year: GLP-1, PYY, ghrelin, and leptin). Overall, the success of the procedure is likely attributable to the synergistic effect of these changes. To our knowledge, this is the first study to evaluate the effect of different macronutrients on GI hormones in the post-RYGB patient population. Significant differences between macronutrient groups in terms of GI hormones and leptin were evident for ghrelin and leptin. In addition, differences were observed within treatment groups. Notably, we found that protein suppressed ghrelin while fat was a potent stimulator of GLP-1 and PYY. This line of research has particular relevance to nutrition support practitioners because it may facilitate improvements in the nutritional care of post-RYGB patients. Future work in this area has the potential to better define post-RYGB macronutrient recommendations to impart greater satiety and thereby promote more effective weight loss and/or weight maintenance in this growing patient population.

FUNDING SOURCES

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CHAPTER 4: SUBJECTIVE SATIETY BEFORE AND UP TO ONE YEAR AFTER ROUX-EN-Y GASTRIC BYPASS*

*Paper under review

CHAPTER SYNOPSIS

Background: Favorable alterations in several appetite-regulating gastrointestinal (GI) hormones occur after Roux-en-Y gastric bypass (RYGB), and may positively affect satiety and contribute to weight loss after surgery. We aimed: to evaluate changes in subjective satiety before and after RYGB; to determine if subjective satiety is different after a protein or fat dose; and to investigate if subjective satiety, the gastrointestinal (GI) hormones, and/or weight loss are related post-RYGB. **Methods:** Twenty women (Age: 48±2 years; BMI before RYGB: 48±1 kg/m^2, mean±SEM) were studied before and 2, 6, 26, and 52 weeks after RYGB. Subjective satiety and GI hormones were measured before and after consumption of a protein- or fat-containing beverage. **Results:** Subjective satiety was not different between treatments. Satiety increased at Week 2 ($p=0.009$) compared to before surgery. Satiety scores returned to pre-RYGB levels after Week 6. Subjective satiety was unrelated to the GI hormones. In a separate analysis, weight loss was unrelated to changes in subjective satiety and the GI hormones. **Conclusions:** This study was novel in that macronutrient effects on satiety post-RYGB were evaluated. Although no strong macronutrient effects were observed, further work is warranted to better define post-RYGB nutrition recommendations. Subjective satiety increased in the early postoperative time period, but the improvement was not maintained at Week 52. Further research on the role of diet in GI hormone and satiety changes after RYGB could ultimately lead to refinements in evidence-based nutrition care for more effective support of weight loss/maintenance in this patient population.

INTRODUCTION

Following Roux-en-Y gastric bypass (RYGB), a myriad of changes occur that are thought to contribute to the substantial weight loss seen after the procedure. Of interest, favorable alterations in several appetite-regulating gastrointestinal (GI) hormones occur, including glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and ghrelin. Generally, GLP-1[71] and PYY[71] increase and ghrelin[68, 92, 98, 104] decreases within days after RYGB, suggesting that weight loss is not the primary mechanism behind these changes, but instead anatomical surgical changes directly promote the favorable hormonal changes in some way. These hormonal changes ultimately have a positive impact on subjective feelings of satiety, or the absence of hunger during the intermeal period,[139] and decrease caloric intake, which results in weight loss.

Previous studies that have evaluated subjective satiety in the RYGB patient population suggest that the surgery positively impacts satiety and has an immediate effect when individuals are still obese. le Roux et al.[71] found significant changes in hunger and fullness scores within days after surgery. For example, at 2, 4, 7, and 42 days post-RYGB, the hunger score was reduced by ~50% while the fullness score doubled compared with the pre-RYGB state.[71] Other investigators have also found significant increases in postprandial satiety at three days,[68] one month,[67] six weeks,[58] and two months[25, 68] post-RYGB, when participants were still considered markedly obese, compared with the pre-RYGB time point. The increased satiety observed soon after RYGB is quite reasonable considering that the stomach capacity post-surgery is drastically reduced. The real question that is of importance to long-term weight loss

and/or maintenance is whether or not this favorable alteration is maintained months to years after RYGB.

Limited evidence exists that the increased subjective satiety found soon after RYGB is also present even after significant weight loss. Borg et al.[67] reported that in addition to a reduction in BMI ($P < 0.001$) at three and six months post-RYGB, there was also a significant decrease in postprandial hunger score and increase in postprandial fullness score compared with pre-RYGB. Similarly, in a cross-sectional analysis Pournaras et al.[63] evaluated postprandial satiety scores in four groups of patients: 1) pre-RYGB; 2) 12 months post-RYGB; 3) 18 months post-RYGB; and 4) 24 months post-RYGB. They also evaluated another group of patients prospectively (before RYGB and 18-24 months after). When subjective satiety data from both the cross-sectional and prospective analyses were combined, satiety significantly increased at all time points after surgery compared with pre-RYGB levels.[63] The Borg et al.[67] and Pournaras et al.[63] studies demonstrated that appetite did not return after significant weight loss, which is contradictory to what typically occurs after conventional weight loss methods (e.g. diet and exercise). Clinically, these studies are of particular interest as changes in subjective satiety occurred when individuals were still considered morbidly obese and were maintained after substantial weight loss, suggesting that body weight loss is partially explained by an immediate improvement in satiety perception and this alteration is sustainable.

Considering that GLP-1, PYY, and ghrelin are appetite-regulating GI hormones, it is of interest to understand if these objective measures of satiety are related to subjective satiety measurements. When collected and analyzed simultaneously, GI hormone

concentrations and subjective satiety data have the potential to provide a robust measure of satiety. However, in the post-RYGB patient population, few evaluations of this relationship have been conducted.[25, 26, 52, 58, 83] Understanding if these variables are related has clinical utility because measuring postprandial GI hormone responses is labor intensive, expensive, and requires strict assay protocols, therefore, in clinical settings where GI hormone measurements are not feasible, subjective satiety might be an appropriate surrogate measure.

Protein has been suggested to be the most satiating of the three macronutrients.[143, 144] In fact, decreased appetite scores have been reported after protein preloads compared with glucose[139] and others have found higher satiety scores after a high protein (25% energy) meal compared with an "appropriate/normal" protein (10% energy) meal.[145, 146] To date, we are aware of only one study that has evaluated the macronutrient effect on subjective satiety after RYGB. Evans et al.[52] compared a high-fat liquid meal (262kcal, 13g fat, 14g protein) to that of a control group (equicaloric mixed-nutrient meal, 6g fat, 20g protein) before and 2-3 weeks post-RYGB and found that feelings of hunger decreased after surgery in only the mixed-nutrient group, suggesting that fat is not an important factor in subjective satiety in this patient population. However, because of the dearth of data, the macronutrient effect on satiety after RYGB remains unknown, especially in the long-term. From a clinical prospective, this information is important for optimal diet management after the surgery.

The primary objective of this analysis was to evaluate subjective satiety both in the early and later postoperative period and to determine if subjective satiety, the GI hormones, and/or weight loss are related. An additional objective was to determine if

perceived satiety is different after a dose of either protein or fat. We hypothesized the following: 1) those who were randomized to the protein beverage would report significantly greater satiety compared with the fat beverage at all time points after consumption for all visits; 2) subjective satiety would significantly increase at all visits after RYGB compared to the Pre-RYGB visit; 3) subjective satiety would be significantly associated with the GI hormones after RYGB; and 4) the GI hormones and subjective satiety would be related to weight loss after RYGB.

METHODS

Subjects

Women who planned to undergo the laparoscopic RYGB procedure were recruited from the Weight Loss Surgery Center at the University of Minnesota Medical Center – Fairview. Subjects for this longitudinal analysis participated in a broader longitudinal study investigating body composition and metabolic changes after RYGB surgery. This study included five visits: 30-70 days before RYGB (Pre-RYGB); two weeks post-RYGB (Week 2); six weeks post-RYGB (Week 6); six months post-RYGB (Week 24); and one year post-RYGB (Week 52). The timing of the study visits was established in order to evaluate the clinical outcomes in both the early (e.g. Weeks 2 and 6) and late (e.g. Weeks 24 and 52) post-operative stages. Exclusion criteria included the following: use of corticosteroids, testosterone, or anabolic agents; internally placed biomedical device (e.g. pacemaker); liver, renal, or heart failure; pulmonary hypertension; thyroid disease (included if treated and within normal limits); neoplastic disease; Type 1 or uncontrolled Type 2 diabetes mellitus (defined by HbA1c > 7%);

pregnancy; and/or history of previous weight loss surgery. The study protocol was reviewed and approved by the Institutional Review Board and the General Clinical Research Center (GCRC) at the University of Minnesota, and subjects provided written, informed consent before participating in the study.

Study Visits

Participants were instructed the day before admission to the GCRC to avoid caffeine, alcohol, and vigorous exercise for 24 hours before testing. Subjects were also asked to be in the fasted state for at least two hours prior to GCRC admission. Subjects were randomized to one of two groups: 1) a sugar-free high protein beverage (20g protein, 0g fat, 2g carbohydrate, 90kcal, 8oz; PRO-BEV) (protein: whey protein isolate, supplied by Davisco Foods International, Inc., Eden Prairie, MN) or 2) a sugar-free high fat equicaloric beverage (0g protein, 9g fat, 3g carbohydrate, 90kcals, 8oz; FAT-BEV). At all visits, a baseline four hour fasted plasma sample was collected for the GI hormone analyses before subjects consumed the assigned study beverage (Time 0). Subjects consumed the assigned beverage over a period of not more or less than 30 minutes (60 minutes at Week 2 to accommodate the reduced stomach capacity). Blood draws were taken at specific time points after initiation of beverage consumption. All plasma samples were stored at -70°C before analysis of GLP-1, PYY, and ghrelin. Table 4-1 depicts when the hormones were sampled at each visit.

Table 4-1: GI Hormone and Subjective Satiety Analysis Time Points

Visit								
Pre-RYGB, Week 6, 26, and 52	Time 0	+30	+45	+60	+75	+90	+120	+150
Week 2	Time 0	+60	+75	+90	+105	+120	+150	+180
Variable								
GLP-1	X	X		X				
PYY	X			X		X		
Ghrelin	X			X		X		
Satiety	X	X	X	X	X	X	X	X

GI: Gastrointestinal; GLP-1: glucagon-like peptide-1; PYY: peptide YY
Notes: Sampling time points are different at Week 2 to accommodate for the reduced stomach capacity after surgery; Times are in minutes.

Gastrointestinal Hormone Assays

All samples were assayed in duplicate. PYY-like immunoreactivity was measured with a specific and sensitive radioimmunoassay, which measures both the full length (PYY_{1-36}) and the fragment (PYY_{3-36}).[77, 161] Plasma active GLP-1 and total ghrelin were measured by established in-house radioimmunoassay.[162-164]

Subjective Satiety Measurement

Subjective satiety was measured using the Satiety Labeled Intensity Magnitude (SLIM) scale[142] before and at several time points after beverage consumption at each testing visit. Subjects were asked to mark on a 150mm straight line how hungry or full they felt. The scales were anchored with *greatest imaginable hunger* at 0mm, *extremely hungry* at 25mm, *very hungry* at 34mm, *moderately hungry* at 47mm, *slightly hungry* at 61mm, *neither hungry nor full* at 75mm, *slightly full* at 98mm, *moderately full* at 111mm, *very full* at 128mm, *extremely full* at 132mm, and *greatest imaginable fullness* at 150mm. Table 4-1 depicts when subjective satiety was measured at each visit. Subjects used paper and pen at the study visits to record subjective satiety and then study staff

transposed the scores to the software program SIMS 2000 (Version 6.0; Sensory Information Management System, Morristown, NJ).

Anthropometric Measurements

Height and weight were measured using standardized procedures. Height was measured to the nearest 0.1cm by a wall-mounted stadiometer (model S100; AYRTON Corporation, Prior Lake, MN) at the Pre-RYGB visit only. At all testing visits, weight was measured to the nearest 0.1kg on a digital scale (model 5002, SCALE-TRONIX, White Plains, NY). Body mass index (BMI) was calculated as body weight/height^2 (kg/m^2).

Surgical Technique

All RYGB procedures were performed laparoscopically. A small gastric pouch (approximately 15mL) was created and the duodenum and proximal portion of the jejunum were bypassed to create a Roux-limb. In most cases, the length of the Roux-limb was 150cm, according to the University of Minnesota Weight Loss Surgery Center protocol.

Statistical Analysis

All longitudinal comparisons against baseline and between treatments were performed using a mixed-effects linear model (SAS Proc Mixed) with treatment and visit number and adjustors as fixed effects, and random intercept for each subject to model the correlation between repeated measurements from that subject. Associations between

change in the GI hormones and change in SLIM scale score used this model, with GI hormones as the response and adjustors age and the caloric content of the meal eaten 4-6 hours prior to the Time 0 blood draw. Two additional relationships were similarly evaluated, while adjusting for Pre-RYGB weight, age, and the kcal content of the meal eaten before the study visit: 1) weight loss and the change in the GI hormones over time and 2) weight loss and the change in the SLIM scale score over time.

Area under the curve (AUC) for the satiety score was calculated using the trapezoidal rule. A P value < 0.05 was considered significant. Unless otherwise noted, data are presented as mean ± standard error. The software SAS (version 9.2; SAS Institute, Inc., Cary, NC) was utilized for statistical analyses. Appendix C contains example SAS codes for this analysis.

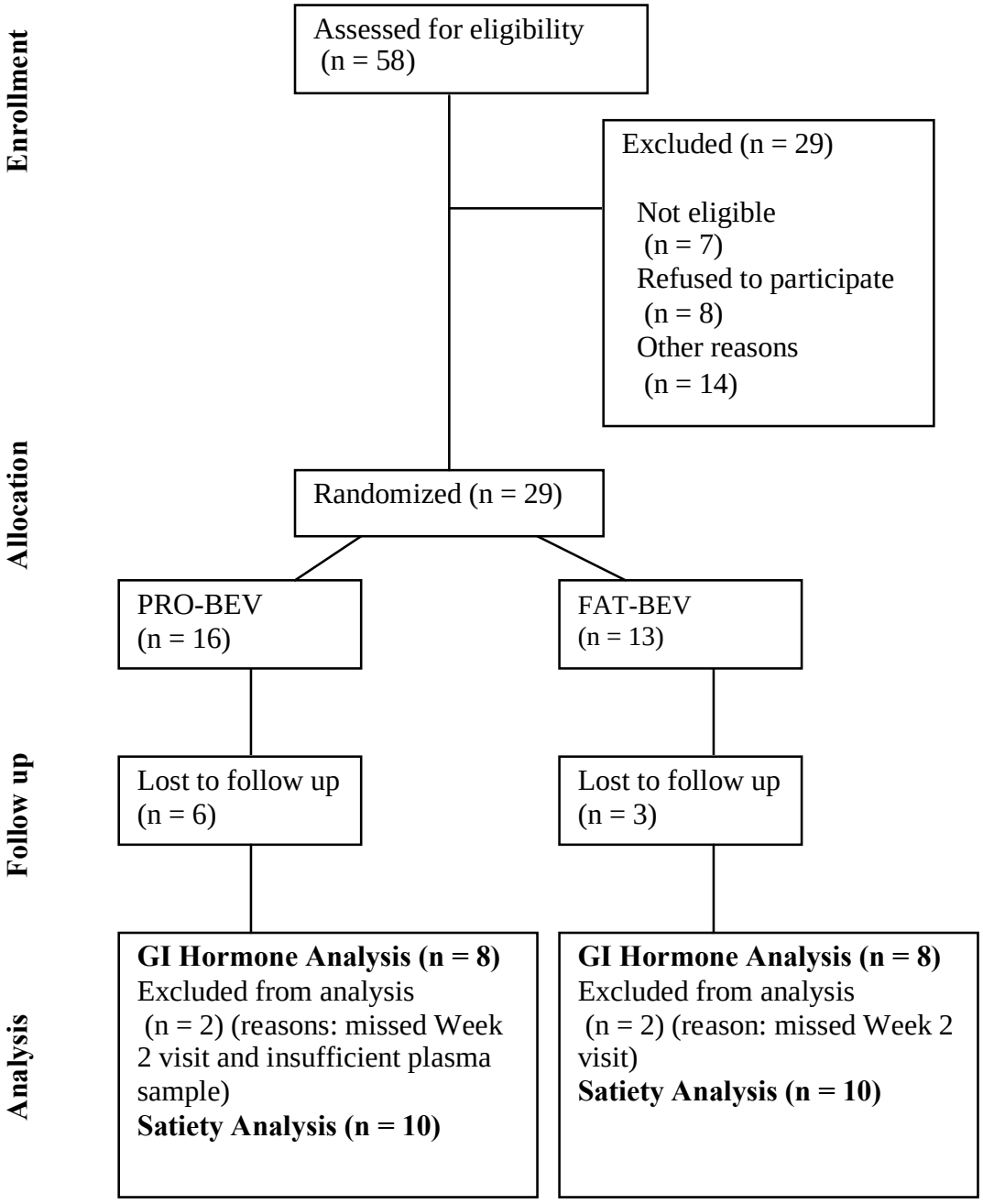
RESULTS

Subject Characteristics

Twenty-nine women participated in this study and 20 completed at least four study visits (PRO-BEV n=10; FAT-BEV n=10). We report satiety results from these 20 women, and GI hormone results from the 16 women who completed all five visits and who had a sufficient amount of plasma for analyses (PRO-BEV n=8; FAT-BEV n=8) (Figure 4-1). Results of the GI hormone response to the study beverage in this study population were previously reported.[167]

All 20 participants were Caucasian with a mean age of 48 years (Table 4-2). Baseline BMI was significantly greater in those who completed the study (n=20) compared to those who did not (n=9). Age, body weight, BMI were not different

Figure 4-1: Study Flow Diagram



FAT-BEV: fat beverage group; GI: gastrointestinal; PRO-BEV: protein beverage group

Table 4-2: Subject Characteristics

	Pre-RYGB (n=20)	Week 2 (n=17)	Week 6 (n=20)	Week 26 (n=20)	Week 52 (n=20)
Age (years)	48 ± 2				
Body Weight (kg)	132 ± 4 A	125 ± 4 B	118 ± C	98 ± 4 D	88 ± 4 E
BMI (kg/m^2)	48 ± 1 A	45 ± 1 B	43 ± 1 C	35 ± 1 D	32 ± 1 E

Mean ± SEM; RYGB: Roux-en-Y gastric bypass; Different letters within the same row indicate significant difference at $P < 0.05$.

between treatment groups.[167] In addition, subjective satiety was not different between treatments, therefore data were pooled and randomized treatment ignored in the analysis.

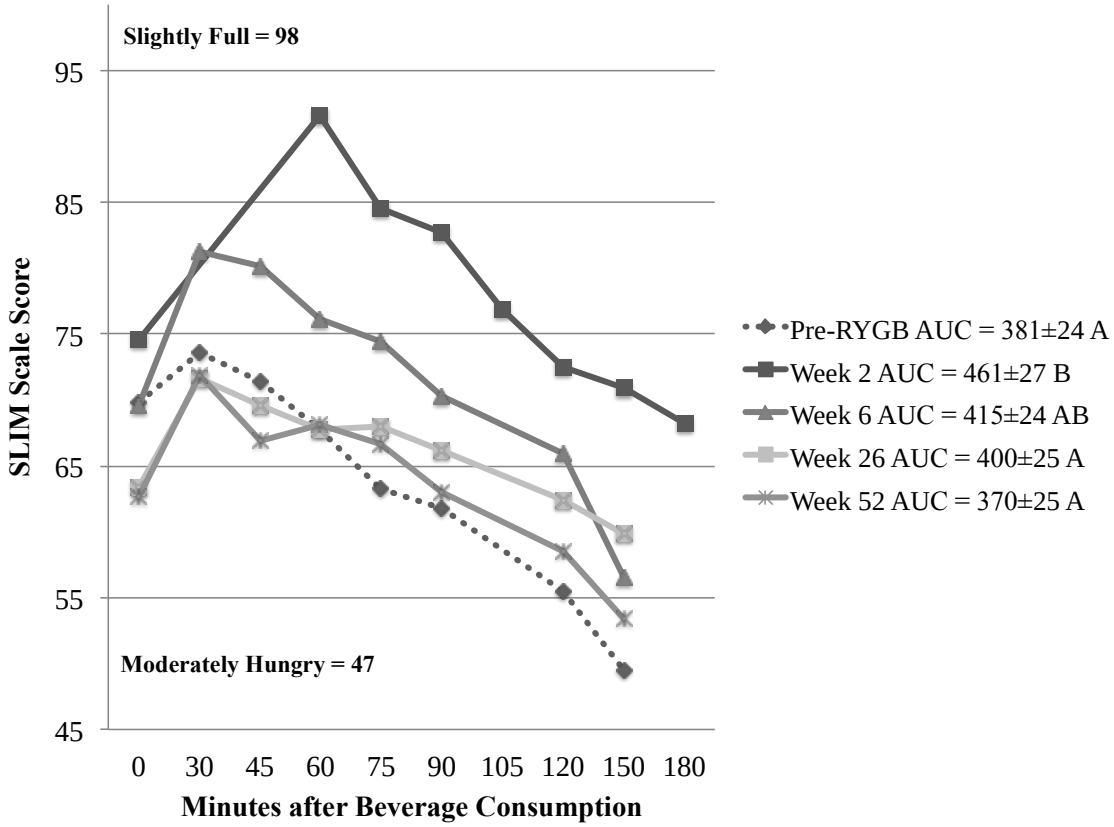
Subjective Satiety Assessment

The SLIM scale AUC generally increased from Pre-RYGB to Weeks 2 and 6, indicating greater satiety during the 2-3 hours after consuming the test beverage (Figure 4-2). SLIM scale AUC was significantly greater at Week 2 compared to Pre-RYGB ($p=0.009$). SLIM scores had generally returned to Pre-RYGB levels after the Week 6 visit (Figure 4-2).

Relationship Between Gastrointestinal Hormones and Subjective Satiety

AUC for GI hormones showed no association with the SLIM scale score AUC, both unadjusted and after adjusting for potential influencing factors on GI hormone concentrations and subjective satiety, including age and the kcal value of the meal eaten prior to the visit (data not shown).

Figure 4-2: Subjective Satiety Scores Before and After Beverage Consumption



In the legend, the numbers after each visit represent AUC score ± SEM. The visits with different letters have significantly different AUCs; SLIM scale scores were collected at the following time points for Pre-RYGB, Week 6, Week 26, and Week 52: 0, 30, 45, 60, 75, 90, 120, and 150 minutes; SLIM scale scores were collected at the following time points for Week 2: 0, 60, 75, 90, 105, 120, 150, and 180 minutes; RYGB: Roux-en-Y gastric bypass; SLIM: Satiety Labeled Intensity Magnitude

Relationship Between Weight Loss and Changes in Gastrointestinal Hormones and Subjective Satiety

In two separate models, the relationships between weight loss and change in the GI hormones and SLIM scale score were evaluated. Weight loss was unrelated to change in the GI hormone AUC's ($P > 0.05$) and SLIM scale score AUC ($P > 0.05$) (data not

shown). Further adjustment for age, kcal value of the meal eaten 4-5 hours prior to the study visit, and weight at Pre-RYGB visit did not change the results.

DISCUSSION

We previously reported on the GI hormone changes after RYGB.[167] Briefly, changes from the Pre-RYGB visit were evident for all of the GI hormones and in some cases there was a macronutrient effect. Differences from the Pre-RYGB visit were evident in the early postoperative period (e.g. Weeks 2 and 6) only for ghrelin, while changes in GLP-1, PYY, and ghrelin were present long-term (e.g. Weeks 26 and 52). In response to the study beverage, protein suppressed ghrelin (significant difference at Week 6), while fat tended to stimulate GLP-1 and PYY though the difference between groups did not reach statistical significance. Considering that GLP-1, PYY, and ghrelin are understood to be appetite-regulating hormones, the next step for us was to evaluate the relationship between the GI hormones and feelings of satiety after RYGB. We also wanted to better understand the macronutrient effect on subjective satiety.

Subjective Satiety Assessment

Subjective satiety is often measured using line scales. Study participants mark on a straight line how hungry or full they feel. The common line scale used in satiety research is anchored on each side by opposing statements, similar to the following range: "*not at all hungry*" to "*very hungry*". Researchers then measure the distance from one end of the extreme to the point on the line marked by the participant to determine their satiety. While quick and easy to administer, the aforementioned line scale has several

limitations, including a lack of multiple scale labels, which limits its reliability. Consequently, Cardello et al.[142] developed the SLIM scale. It improves upon the traditional line scale by including numerous verbal labels within the scale that are unequally spaced. The labels themselves and the spacing have been experimentally determined.[142] The verbal labels are easy to understand and they provide a rough meaning to the subject. When evaluated against other scales, the SLIM scale was shown to have greater reliability and sensitivity compared to a traditional line scale.[142] While the SLIM scale appears to be a dependable tool for the measurement of subjective satiety, it has not been used in the RYGB patient population, as previous research has used traditional line scales. Because of the strengths of the SLIM scale and that expected differences in satiety were reflected in the scale, we feel that we successfully characterized feelings of satiety in this study.

Macronutrient Effect on Subjective Satiety

Despite the understanding that protein is the most satiating of the macronutrients, subjective satiety was not different between the PRO-BEV and FAT-BEV after RYGB in this study. It is possible that the study beverage did not contain enough protein to elicit a response. Prior studies evaluating macronutrient effects on subjective satiety in overweight individuals have used larger doses (e.g. ~50g)[139] and it is possible that we would have found an effect had the beverage contained more protein. However, it should be noted that during the planning phases of this study, it was determined that 20g was the highest amount of protein that could be included in one 240mL beverage at the desired 90kcal level and still be palatable. Due to the limited stomach capacity of RYGB

patients, it was deemed appropriately necessary to limit the beverage volume to a maximum of 240mL. The kcal content of the supplement was also smaller than what is commonly used as a stimulus in GI hormone and subjective satiety studies. Assessment of the GI hormones and subjective satiety at the testing visits was an added component and this study was not initially designed to evaluate these parameters. The beverage was originally included in the study protocol to be consumed twice a day for six weeks after surgery to assess lean tissue changes in response to protein supplementation. Because the beverage was intended to be consumed in addition to the subject's meal plan, the kcal content could not be increased, due to dietary restrictions after surgery.

Continued work on the macronutrient effect on subjective satiety is warranted in this patient population. Currently, dietary recommendations after RYGB are simply suggestions as how and when to progress from the various food consistencies (e.g. clear liquids, soft foods) (see Kulick et al. reference[147] for an example of dietary advancement after bariatric surgery). Many bariatric centers recommend consuming 60-80g protein per day, but the basis for the recommendation is unclear and currently there are no guidelines for how much of the other macronutrients should be consumed in the early or later postoperative time period. If the patients in our study at Week 52 followed the 60-80g protein per day suggestion, they would be consuming on average 0.68-0.91g/kg protein per day (60-80g/88kg). While 0.91g/kg is in accordance with the dietary reference intake, it might not be enough to elicit a satiety response. Protein has been shown to favorably alter subjective satiety in non-RYGB subjects and this could also be possible after RYGB. Continued research on post-RYGB macronutrient

recommendations is warranted with the ultimate goal of developing evidence-based dietary guidelines for this increasing population.

Subjective Satiety Changes after RYGB

Subjective satiety increased in the early postoperative period after surgery compared to the Pre-RYGB visit. This is not surprising given that the stomach capacity after surgery is greatly reduced and because others have also found significant improvements soon after RYGB.[25, 58, 67, 68, 71] However, the finding that subjective satiety returned to Pre-RYGB levels at Week 52 was not expected. Pournaras et al.[63] found that the improved satiety response was maintained in the long-term (e.g. 18-24 months) and we hypothesized that we too would find this. The Pournaras et al. study used a stimulus that was 400kcal and perhaps in the later postoperative time period, more kcals are needed to elicit a satiety response, considering that at one year post-RYGB, patients are consuming greater amounts of kcals, with reports of about 1400kcal per day,[167, 168] compared to 400-700kcal typical of the earlier time periods after surgery.[167] However, because the study beverage was calorically equivalent to a commonly recommended post-RYGB snack item, one cup of skim milk, it is interesting to note that intake at this kcal level did not alter satiety at Week 52. It is possible that in the later postoperative time period, patients become desensitized to satiety signals and therefore may not "hear" these important cues at lower kcal doses. Weight maintenance or regain tends to occur after about 1-2 years after surgery.[169, 170] Could a return to pre-surgery satiety levels be a factor in this reduced weight loss trajectory? While Pournaras et al. found increased subjective satiety in the later postoperative time period, this is the only study to our

knowledge that has evaluated this variable in the long-term and we cannot say with certainty what happens to subjective satiety at one year or more after RYGB. Clearly more research that evaluates subjective satiety at later time points after surgery (≥ one year) is needed to better understand these possibilities.

Relationship between Subjective Satiety and the GI Hormones

The GI hormones GLP-1, PYY, and ghrelin are appetite regulating hormones. GLP-1 and PYY promote satiety,[45] while ghrelin is orexigenic (i.e. appetite stimulating).[42, 43] In this study, none of the GI hormones were related to subjective satiety, and this is consistent with other studies in this patient population.[26, 58, 83] While GLP-1 and PYY increased and ghrelin decreased after surgery (GLP-1: Weeks 6 and 52; PYY: Weeks 26 and 52; ghrelin: Weeks 2, 6, and 52),[167] subjective satiety was only improved in the early postoperative period (e.g. Weeks 2). Lack of correlations between the GI hormones and the SLIM scale score may be due to the large inter-individual variability inherent of subjective satiety data as suggested by Korner et al.[83] and larger sample sizes may be necessary to see such an effect. Alternatively, these patients may have become desensitized to their satiety cues, thereby reverting to their pre-surgical eating habits, and they may be used to eating for other reasons, an issue not effectively dealt with in the interim, so satiety is not a cue they normally use for meal termination. More work is needed to understand the eating behaviors in this population. While the GI hormones were significantly and favorably altered in response to the study beverage at most visits after RYGB,[167] suggesting satiation, these individuals (18 of which were overweight or obese at all visits after RYGB) might not be able to decipher the

physiological cues that indicate fullness, as suggested by the lack of significant change in the SLIM scale score in the later postoperative period. Evidence for the possibility of this inability to "hear" the satiety cues is provided by a limited number of studies in normal-weight and overweight/obese individuals. In these studies, satiety scores were positively associated with GLP-1[171] and PYY[172] in normal-weight subjects, while no relationship was found in those who were overweight or obese.[129, 173] In light of this possibility, further assessments of subjective satiety and eating behaviors in this patient population are needed, and should include both diagnostic and interventional counseling components, taking into account the fact that many overweight and obese people are often times not eating because of metabolic need or hunger, but instead, are eating for a whole host of other, often hedonic, reasons.[174] With proper diagnostic approaches and counseling tailored to the disordered eating patterns that often plague this patient population, there may be better long term clinical outcomes. Also, with education and counseling about satiety signals and bodily cues of fullness, individuals who are undergoing weight loss (either by traditional methods or bariatric) might have a better chance for long-term success, if they can learn to appropriately respond to these physiologic cues and terminate eating. However, if they start eating for non-metabolic reasons, then they are not likely to stop eating because of common physiologic satiety signals. More research in this area of satiety, eating behaviors, meal initiation, meal termination, and the role of GI hormones is critical to improving clinical outcomes in this patient population.

Relationship between Weight Loss and Changes in GI Hormones and Subjective Satiety

From a physiological standpoint, it is understood that certain GI hormones have anorexigenic effects on the brain that can promote satiety; these effects could play an important role in the substantial weight loss observed after RYGB. Consequently, we evaluated if weight loss was related to either changes in the GI hormones or subjective satiety in two separate models. We did not find that changes in GLP-1, PYY, ghrelin, or SLIM scale score were related to weight loss. We are not certain as to why we did not find a relationship between these variables, considering that others have suggested that changes in the GI hormones and subjective satiety are likely important factors in the weight loss seen after surgery.[175, 176] We acknowledge that GI hormones and subjective satiety are not the only variables involved in weight loss and it is likely a synergistic effect between changes in GI hormones, subjective satiety, long-term dietary habits, physical activity, and psychological factors. In addition, GLP-1, PYY, and ghrelin are not the only GI hormones involved in appetite and other hormones such as CCK, GIP, and oxyntomodulin also deserve continued research. In sum, further evaluations of these relationships are needed in larger-scale studies of longer duration (e.g. > one year).

Limitations and Strengths

Several limitations need to be considered when evaluating our results. First, we had a small sample size that consisted entirely of Caucasian women. Small sample sizes are not uncommon in bariatric research, considering the difficulty in recruitment, and in our study, the five visits were 24 hours in duration, making it even more difficult to

acquire patients because of schedule conflicts. Second, because of cost and time constraints, subjects were not able to serve as their own control for the two study beverages. Third, this analysis was part of a broader study evaluating body composition changes after RYGB and was not initially designed to evaluate the hormones or subjective satiety. Consequently, subjects were only fasted for 4 hours before the Time 0 blood draw and subjective satiety assessment. Although we attempted to control for the kcal value of the meal eaten 4 hours before the study visits, we cannot rule out the possibility of altered results secondary to the short fasting duration. In addition, the protein and kcal content of the study beverage was smaller than what is commonly used as a stimulus for GI hormone and subjective satiety assessments and additional studies with greater protein doses are needed to further evaluate the macronutrient effect. Similarly, the stimulus used in this study was in liquid form and prior work evaluating high-protein meals in liquid versus solid food suggests that liquids are less satiating.[177] While frequent consumption of protein-rich foods as liquids (e.g. skim milk) is not uncommon in this patient population, it is also important to characterize satiety after solid foods to better describe real-life conditions. Consequently additional studies with solid food are warranted. Last, the time points for subjective satiety assessment might not have been appropriate to capture the immediate effects of the beverage. It is possible that the peak feeling of satiety occurred before the 30-minute measurement and additional studies that measure 10-15 minutes after beverage consumption are warranted.

The strengths of this study include its prospective study design with data collection before and at several time points for one year after RYGB. We also used the SLIM scale to measure subjective satiety, which has been shown to be more reliable and

sensitive than a traditional line scale. In addition, evaluation of the macronutrient effect on subjective satiety is a unique aspect of this study, considering only one other study to our knowledge has conducted such an analysis in this patient population.[52] While Evans et al. evaluated the effect of both a mixed- and high-fat meal on subjective satiety, we build upon that work by including a protein treatment. Continued efforts in this line of research have possible implications for better nutritional care after RYGB in terms of dietary recommendations that promote satiety and consequently weight loss and/or maintenance. We also assessed if the GI hormones are related to changes in subjective satiety. While the GI hormones are well researched after RYGB, subjective satiety is not routinely evaluated, with even less focus on the relationship between the variables. Considering the cost constraints of GI hormone analyses that likely limit its use in the clinical setting, assessing subjective satiety could be an appropriate surrogate; but at this time, we are unable to make such a suggestion and recommend further exploration of this relationship. Last, we assessed subjective satiety in both the early postoperative time period and up to one year after RYGB. Few studies have evaluated this variable in the long-term. Given our finding that satiety-inducing hormones remain elevated one year after RYGB, yet satiety scores revert back to pre-surgical levels over the long-term, research involving neurobehavioral physiology in these subjects deserves further attention to better understand and possibly develop strategies to prevent the recidivism for weight gain in the long-term.

CONCLUSIONS

We evaluated the macronutrient effect on subjective satiety using the SLIM scale. We also assessed if either subjective satiety or weight loss are related to changes in several GI hormones. Although we did not find a difference in subjective satiety between beverage groups, assessment of the macronutrient effect on satiety is a novel analysis in this patient population, and further work is needed to better define the current post-RYGB nutrition recommendations. We did find that subjective satiety increased in the early postoperative time period, but the improvement was not maintained at Week 52, and this may be due to the low kcal content of the beverage. Few studies have evaluated the long-term subjective satiety changes after RYGB and continued work on this topic is needed. In conclusion, investigating the role of diet in GI hormone and subjective satiety changes is of relevance to the ever-growing bariatric surgery patient population. Currently, the dietary recommendations after RYGB are vague guidelines that are not based on extensive clinical research. Continued research in this area that attempts to better understand dietary components that could ultimately lead to successful weight loss/maintenance is needed for proper nutrition care after RYGB.

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CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

CONCLUSIONS

The number of individuals seeking bariatric surgery will likely continue to increase as a result of the burgeoning obesity population. The nutritional needs of this population are complex and dynamic, yet they are not well defined. The success of the RYGB relies heavily on proper dietary management, consequently, continued research on this matter is paramount.

Previous investigators have reported favorable alterations in GLP-1, PYY, ghrelin, leptin, and subjective satiety after RYGB. However, the macronutrient effect on these variables is not well researched in the RYGB patient population. These evaluations are needed to better define post-RYGB nutrition recommendations. In addition, few have evaluated the relationship between the GI hormones and subjective satiety. Considering that the GI hormones are appetite-regulating, it is of interest to better understand if these measures are related to subjective feelings of satiety in those who have undergone RYGB.

One purpose of this dissertation research was to investigate the macronutrient effect on the GI hormones and subjective satiety and the time course for changes in these parameters. GI hormones were modestly impacted by protein and fat in this study in both the early and later postoperative time periods. Specifically, greater ghrelin suppression occurred after the PRO-BEV and GLP-1 and PYY levels were only increased above Pre-RYGB levels in the FAT-BEV group. While these findings do suggest a macronutrient effect and that a combination of protein and fat might be needed to elicit favorable alterations in the GI hormones, we are cautious regarding recommendations in light of our small sample size and the fact that subjects were only fasted for 4-6 hours prior to the

study visit. We found that subjective satiety increased only in the early postoperative time period and was not different between treatment groups. It is likely that the macronutrient dose was not enough to elicit a response. Additional studies with a larger stimulus are needed to better understand how these variables are impacted by fat, protein, and carbohydrate. Alternatively, even though the GI hormones were favorably altered after RYGB, patients might not be able to "hear " the satiety cues, especially in the later postoperative period. The disconnect between the objective (e.g. GI hormones) and subjective (e.g. SLIM scale score) measures of satiety is a topic that should be explored further in this patient population. If these patients are indeed desensitized to satiety signals, counseling on what it means to be "hungry" and/or "full" might be of great use for weight management after RYGB.

In conclusion, the overall aim of this dissertation research was to better understand the role of various macronutrients in GI hormone and subjective satiety responses after RYGB. We found that protein and fat had a modest impact on the GI hormones, but not subjective satiety. Further investigation of these questions is warranted, and could lead to a better understanding of dietary components that might be important for successful weight loss/maintenance, and ultimately facilitate development of evidence-based nutrition guidelines for this ever-growing patient population.

FUTURE DIRECTIONS

This dissertation research provides a foundation from which additional questions concerning GI hormones and subjective satiety in the RYGB patient population can be further explored. In addition to addressing the previously described limitations of this

research, other investigations are warranted. The following sections describe possible future directions for this research.

Validation of Subjective Satiety Tools in the Bariatric Patient Population

Assessment of subjective satiety in the bariatric surgical patient population has traditionally been done using simple line scales. These line scales are commonly anchored on each side by opposing statements, similar to the following range: "*not at all hungry*" to "*very hungry*". While an acceptable tool, the reliability and sensitivity has been shown to be better with the SLIM scale, because of its experimentally determined scale design. This study was the first to use the SLIM scale in those who had undergone RYGB and while that is a unique component of the study it is also a limitation in that we are not certain if it is a valid tool in this population. Consequently, there is a need for validation studies in this patient population so that subjective satiety can be accurately assessed.

GI Hormone and Subjective Satiety Assessment in "Successful Weight Loss Responders"

This study and the results of others have generally reported favorable changes in GI hormones and subjective satiety after RYGB and it has been suggested that these changes are partly responsible for the substantial weight loss seen after the procedure. However, limited work has been done evaluating differences in these variables between those who are considered "successful" and those deemed not (commonly accepted definitions of success were described in the Literature Review). While the surgery is largely successful in many, some do not achieve optimal weight loss and it is not clearly

understood why. If it is found that GI hormone and/or subjective satiety differences are part of the problem, continued research on potential treatments for this deficit would be needed.

Assessment of Subjective Satiety and Eating Behaviors After Interventional Counseling Component

Results of this dissertation research indicate that subjective satiety increases in the early postoperative period but is not maintained in the long-term, despite favorably altered GI hormones. In addition, GI hormones were not related to subjective feelings of satiety in this study, despite reports in normal-weight individuals that these variables are associated. These results suggest that there is a disconnect between the GI hormone response and feelings of satiety and these patients might not be able to "hear" the satiety signals. If patients are counseled about what it means to feel "hungry" and/or "full" they might be better equipped to decipher these feelings and either eat because they are hungry or stop because they are full. In addition, these patients are likely eating for reasons besides physiological need and hunger, including psychological stress and hedonics. With counseling and education, patients can learn to identify these altered eating patterns and employ techniques to overcome the desire to eat for reasons beyond hunger. Overall, an interventional study of this kind has the potential to provide bariatric patients with a better chance for long-term success and has far-reaching implications to overweight and obese individuals undergoing conventional weight loss treatments.

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APPENDIX A: CONSENT FORM

The University of Minnesota, Twin Cities Campus
Department of Food Science and Nutrition
CONSENT TO BE A RESEARCH SUBJECT

Study Title: Clinical Implications of Gastric Bypass Surgery

You are invited to participate in a research study on the effects of gastric bypass surgery on health and well-being. You were selected as a possible participant because you have decided to undergo a laparoscopic Roux-en-Y gastric bypass operation. If you do not undergo a Roux-en-Y gastric bypass operation, your study participation will end after the collection of fat tissue at the time of your surgery. To be a subject in this study, you must currently not be pregnant or become pregnant during the study. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

This study is being conducted by Carrie Earthman, PhD, RD of the Department of Food Science and Nutrition, Sayeed Ikramuddin, MD and Todd Kellogg, MD of the Department of Surgery, Shalamar Sibley, MD, MPH and Tiffany Beckman, MD, MPH of the Department of Medicine, Endocrine Division, at the University of Minnesota.

Study Purpose
The purpose of the study is to look at changes in the amount of lean and fat tissue, and water in the body (this is also called body composition), after gastric bypass surgery, and to explore factors that might affect these changes, including dietary supplementation. We are also interested in exploring how changes in body composition after surgery might affect how you feel about your life.

Study Procedures
If you agree to participate in this study, we would ask you to do the following:

General Study Overview:
Sixty subjects will be enrolled to participate in this study. Study participants will be randomly assigned to one of three groups: 1) a high-protein supplemental beverage group; 2) a low-protein supplemental beverage group; or 3) a no supplemental beverage group. The decision as to which group you will be assigned will be made randomly. This method is similar to the toss of a coin and is determined by chance.

<u>Supplemental beverage groups:</u>
Twenty study participants will receive a sugar-free, high-protein beverage ("high protein") and twenty will receive a sugar-free, low-protein beverage ("low protein") for six weeks following gastric bypass surgery. The high protein and low protein beverages are made of natural products commonly found in commercially available foods, and contain the same amount of calories. You will not know whether you will receive the high protein or low protein beverage.
Instructions for the supplemental beverage:

Beginning as soon as possible (preferably within 1 – 5 days) after your gastric bypass surgery procedure, you will consume the assigned beverage (if assigned to either the low or high protein beverage) twice a day for approximately 6 weeks, until the day that you come in for the 6-week post-operative testing visit (Visit 3). The time of day you consume the beverage is between meals. You will not consume any other supplemental protein beverages during the six-week period between your surgery and Visit 3. You will be provided a container with small packets of powdered beverage mix, along with a booklet for recording when you drink the beverage and how you feel before and after drinking it. Before you drink the beverage, you will be asked to record how hungry you are and how you feel physically. After you drink the beverage, you will record your response to those same questions, including how well you liked the beverage. You will also save the empty packages so that we can collect these from you at a later time.

Unsupplemented group

Twenty subjects will be assigned to the unsupplemented group. If you are in this group, you will receive the standard care you would have received had you not been in the study. Subjects in this group will go through the same testing procedures at each of the 5 testing visits to the GCRC as all the other subjects.

Orientation: Once you have provided consent, you will be shown how to keep a record of what you eat, and how to collect a 24-hour urine sample. These instructions will be given to you at a brief meeting that will take place at the General Clinical Research Center (GCRC) at the University of Minnesota, or at another mutually acceptable location, and will take approximately 1 hour of your time.

Testing Visits:

You will return to the GCRC on 5 separate occasions for testing: Time 1 (Baseline, approximately 30 – 70 days prior to your gastric bypass surgery), Time 2 (approximately 6 – 15 days after your surgery), Time 3 (approximately 6 weeks after your surgery), Time 4 (approximately 6 months after your surgery), and Time 5 (approximately 1 year after your surgery). You will write down everything you eat and drink in a dietary record on 3 assigned days during the week before you are scheduled for each of these testing visits to the GCRC. One of these days will be the day before testing, and you will continue recording your intake after you arrive at the GCRC. You will collect your urine for 24 hours (the entire day) before testing. You will continue your urine collection by bringing your container with you when you come to the GCRC.

On the day of your admission before testing, you will be asked not to eat or drink anything but water after 12:00 noon. You will report to the GCRC at 2:00 PM that afternoon, and be admitted for an overnight stay. You will then have an intravenous (IV) line inserted into an arm vein. You will be asked to provide a urine sample. Pregnant women should not participate in this study, due to the potential risk to a developing fetus of dual-energy x-ray absorptiometry (DXA) scans and fasting. Therefore, at each testing visit we will conduct a pregnancy test on your urine sample. If you suspect you are pregnant, please notify the study staff. Pregnancy at any point during this study will cause you to be withdrawn from the study. Several standard laboratory tests will be

conducted on your urine sample, so that we can evaluate your fluid and nutritional status. After the urine sample has been collected, you will receive IV fluids over an eight-hour period.

At 4:00 PM, you will have a small amount of blood drawn through the IV. Immediately following, if you are in one of the supplemental beverage groups, you will drink a single dose of your assigned supplement. Similarly, if you are in the unsupplemented group, you will be randomly assigned to drink a single dose of either the "high protein" or "low protein" beverage. This will be followed by blood sampling, again through the IV. During this time, you will also fill out questionnaires on your hunger. After this test, you will be provided an evening meal. You will fast (meaning nothing to eat or drink except water) overnight (after 8 PM). You will continue to fast until testing is completed, by approximately 1 PM the next day. At that point, you will be offered a lunch meal, and you will be free to leave the GCRC.

Specific Information Regarding Study Procedures:

Medical History

During your admission to the GCRC, you will have a brief physical examination by a study physician. Your medical records will be reviewed to learn about your current and previous medical conditions, current medications, recent hospitalizations, and other medical information. In addition, your medical records will be reviewed to learn about your smoking history, alcohol use, exercise and other health habits, and demographic information. Lastly, you may be asked questions so that we can learn more about your medical and health history. You will be asked to complete a physical activity questionnaire at each testing visit.

Quality of Life

You will be asked to complete a questionnaire on your feelings about yourself, your work, your sexual life, and other aspects of life.

Dietary Intake:

Information about your dietary intake will be gathered in several different ways. Before you undergo baseline (Time 1) testing visit, you will be told how to accurately record amounts of foods and liquids consumed and how to complete the 3-day food records. You will record everything you eat and drink, including dietary supplements, in a food record on 3 assigned days during the week before each testing visit to the GCRC. One of those 3 days will be the day before testing, and you will bring your record with you when you report to the GCRC so you can continue recording through that evening meal.

Dietary records will be reviewed at each testing visit. You will be asked to answer questions about your *usual* food preferences and eating habits; this is called a food frequency questionnaire. Beginning one week after Time 1 testing, and then throughout the study on a weekly basis, you will be contacted by telephone by study personnel, and you will be asked what you ate and drank over the previous day; this is called a 24-hour dietary recall. During this phone call, you will also be asked about: symptoms you might be experiencing, such as nausea, vomiting, or diarrhea; changes in your ability to tolerate

specific foods; your current usual food intake; your current weight (if you have a home scale); current activity level; and when you may have started adding more solid foods back to your diet.

Body Measurements

You will be asked to remove your outer clothing, so that you are dressed only in underwear, socks, and a hospital gown during measurements. Your height and body weight will be measured at Time 1, and your body weight will be measured again at each visit. At each testing visit, your waist and hips will be measured using a simple tape measure.

Body Composition by Multiple Dilution

A method called "multiple dilution" using deuterium and bromide will be used to measure the total amount of water you have in your body, and how much water is inside and outside your cells. This information can be used to tell us how your body is changing after your surgery. For example, the information about the amount of water inside your cells tells us how much lean tissue you have in your body (termed "body cell mass"). For this method, you will need to have 2 teaspoons of blood taken through the IV line immediately after you have had your weight measured. An additional 2 teaspoons of blood will be taken not through the IV line, but from another site (e.g. the arm not containing the IV), 4 hours after the infusion is completed. After the initial blood draw, a solution of sodium bromide will be given to you through the IV line over a period of approximately one hour. You will also be given, through the IV line, a small measured dose of "heavy water" or deuterium oxide. Both deuterium oxide and sodium bromide are harmless in the amounts given and will pass through your urine. Once the sodium bromide and deuterium oxide have been completely infused, the IV line will be removed. You will be asked to urinate the morning of the test and every hour up to 4 hours after you were given the deuterium oxide. During this time, your urine will be collected so that the total volume can be measured, and samples will be stored for analysis. You will not be allowed to eat or drink anything after the test starts, and until the second blood draw that occurs 4 hours after completion of the sodium bromide infusion. During this 4-hour period, you will undergo other procedures, including bioimpedance spectroscopy, dual-energy x-ray absorptiometry, and indirect calorimetry, all explained below.

Body Composition by Dual-Energy X-Ray Absorptiometry

Your body composition will be evaluated by a dual-energy x-ray absorptiometry (DXA) system. This procedure involves lying on a padded table for 10 - 20 minutes as a moving arm on a machine is passed over your whole body. Although you will need to lie very still during the measurement, you will feel no discomfort. You will be dressed in a hospital gown, and will be asked to remove all jewelry, watches, keys, and any other sources of metal from your person, as well as your bra. At Visit 1 only, you will be scanned three times. After being scanned without your bra, you will also be scanned with a thin piece of acrylic placed under your arm, which will serve to keep your breast from falling over your right arm during the scan. A third scan will be performed after you have had your chest wrapped with soft material.

Body Composition by Bioimpedance Spectroscopy

Your body water will be measured by bioimpedance spectroscopy. Four sticky electrodes will be placed on your right hand and foot after cleaning with alcohol, and then a small electrical current that you will not be able to detect will be sent through wires attached to the electrodes. Several measurements, each taking approximately 45 seconds, will be made at 5, 10, and 30 minutes after you lie down.

Indirect Calorimetry

This procedure measures how many calories you are burning at rest. This information can be used to tell us how your body is changing after your surgery. Before the measurement begins, we will measure your blood pressure and body temperature, and then you will lie quietly on your back for 30 minutes. A large, clear plastic hood will be placed over your head and shoulders, and a 20-minute testing period will begin. During the 20 minutes, you will continue to lie quietly on your back, and you will breathe room air normally. Your breath will be detected by a machine that will measure how much oxygen you breathe in, and how much carbon dioxide you breathe out every minute. This is a painless procedure; however, people who are uncomfortable in confined spaces may find this test stressful. Another indirect calorimetry instrument will also be used to measure your resting energy expenditure. This hand-held device will require you to breathe through a mouthpiece attached to it for ~10 minutes, while a nose clip prevents you from breathing through your nose. This is also a painless procedure; however, you may experience some mild discomfort by having your nose held closed by the nose clip. Both procedures will be repeated several times.

Other Laboratory Tests on your Blood and Urine

You will be admitted to the GCRC by 2:00 PM on the afternoon before testing, and we will ask you not to eat or drink anything but water after 12:00 noon that day. At approximately 4:00 PM, you will have a blood sample taken from your IV line in order to evaluate several hormones and other substances in your blood related to obesity and weight loss. You will be asked to complete a brief set of questions about your hunger level, and then consume the supplemental beverage you were assigned. You will be asked to complete the same set of questions after you have finished drinking the beverage, and additional blood will be taken. The amount of blood drawn for this test will be less than 8 tablespoons.

The next day, a small sample of urine will be collected and an additional 6 teaspoons of blood will be taken through your IV line so that several standard laboratory tests can be obtained. These laboratory tests will be used to evaluate your fluid and nutritional status, and to test for hormones and other substances related to blood pressure, body fluids, blood vessel injury and obesity. In addition, your urine sample will be tested for albumin (a blood protein normally excreted in small amounts in the urine). Proteins that are related to fluid status, blood pressure, blood vessel injury, and obesity will also be measured in your urine.

Hand Muscle Strength

You will be asked to complete a simple test of your muscle strength by using what is called a hand dynamometer. A hand dynamometer looks like a pair of pliers, and requires you to squeeze as hard as you can without causing yourself discomfort. You will do this as each visit to the GCRC (Times 1 – 5).

Sampling of Fat Tissue During Gastric Bypass Surgery

During your gastric bypass surgery, a small sample (about 10 grams, which is equivalent to two pats of butter) of your fat tissue will be taken by your surgeon, from two places: just under your skin, and close to your stomach. These two samples will be tested for proteins that are related to fluid status, blood pressure, blood vessel injury, and obesity. The procedure will take only a small amount of time and poses no additional risks to you.

24-Hour Urine Collection

You will be given a large container and will be expected to collect your urine starting by 7:15 AM the day you are scheduled to report to the GCRC. You will bring the container with you when you check in at the GCRC the afternoon before testing, and you will continue to collect your urine throughout the evening and night before testing, stopping the collection by 7:15 AM the morning of testing.

Risks of Study Participation

The study has the following risks.

Blood Draws

The risks of drawing blood may include temporary discomfort from the needle stick, bruising, and rarely, infection. You will have your blood drawn through an IV line and through a needle stick. A temporary black and blue mark results from placement of an IV. Placement of an IV can be painful, but it carries no significant risks. Infections from IVs rarely occur from the relatively brief (less than 24 hours) period required for testing. However, if any signs of infection appear, the IV will be removed and the area will be treated. During each testing visit (Times 1 - 5), a maximum of 160 ml of blood (less than 11 tablespoons, or about 2/3 of a cup) will be drawn.

Hospitalization

During your overnight hospitalization, you will be confined to the GCRC, an inpatient study unit of the Fairview-University Medical Center. On the first day of your admission, you will need to fast from 12:00 noon until the evening dinner meal. You will also need to fast overnight for 12 hours prior to testing, which means nothing to eat or drink except water after 8 PM on the first day of your admission. On the following morning, you will continue to not eat and you will not be allowed to drink anything starting from approximately 7 AM and throughout the testing period until approximately 1:00 PM, when you will be offered a meal. You will not eat for as long as 17 hours, and this may cause you some discomfort. As a result of fasting this long, you might feel hungry, lightheaded or dizzy, weak, tired, and there is a slight possibility that you might pass out. Although pregnancy would prevent you from participating in this study, you should know that fasting is associated with some possible risk to a developing fetus, by reducing the amount of nutrients that would be available. Although you will be occupied

with several procedures during the morning of testing, confinement to the GCRC may be boring. You will be discharged from the GCRC at the end of testing.

DXA Scans

As part of this study you will be exposed to ionizing radiation through a DXA x-ray performed once. The DXA is a test to measure body composition that is estimated to provide less than 0.03% of that received from natural sources of radiation by a Minnesota resident in one year. Seven DXA scans will be performed over the course of the year-long study. Although pregnancy would prevent you from participating in this study, you should know that it is not recommended for pregnant women to undergo DXA scans. Although the risk is considered minimal, there is a slight possibility that harm could result for the developing fetus. For this reason, we will conduct a pregnancy test on your urine at each testing visit admission to the GCRC. If you have participated in any other research study involving ionizing radiation exposure in the past 12 months, discuss this with the Investigator to determine if you are eligible to participate in this study.

Administration of Deuterium Oxide and Sodium Bromide

Deuterium oxide (heavy water) is colorless and odorless. Deuterium is a natural molecule that is present in all drinking water. Deuterium is not a radioactive substance, and has no recognized harmful effects. Sodium bromide is colorless and odorless, found normally in nature, and is not toxic in the amount given.

Bioimpedance Spectroscopy

Although bioimpedance spectroscopy presents no risk to most individuals, it is generally not recommended for individuals with pacemakers or other similar internally placed biomedical devices; therefore, if you have such an implant, you will be excluded from participation in this study.

Benefits of Study Participation

There are no benefits to you for participating in the test. However, an understanding of how dietary intake affects body composition and health after gastric bypass surgery may lead to improved clinical and nutritional management of gastric bypass surgery patients.

Alternatives to Study Participation

You may choose not to participate in this study.

Study Costs and Subject Compensation

You will not be charged for any of the procedures or materials used in this study. In return for your time, effort, and inconvenience associated with participation in this study, you will be reimbursed a total of $300 over the 5 testing visits. After completion of each of the first 4 testing visits, you will receive $50, and after completion of the final testing visit (Time 5), you will receive $100. It will take 4 – 6 weeks before you receive reimbursement after each of the tests. Payment will be made to you in the form of a check issued by a University Department.

Research Related Injury

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner to you or your insurance company. If you think that you have suffered a research related injury, let the study investigators know right away.

Confidentiality
The records of this study will be kept private. All records will be coded and kept in a locked file. In any publications or presentations, we will not include any information that will make it possible to identify you as a subject. Your record for the study may, however, be reviewed by departments at the University with appropriate regulatory oversight. If you have not been seen before at Fairview-University Medical Center, a permanent medical record will be created at the hospital under your name. Your study data will be added to your medical record. While confidentiality cannot be guaranteed, it will be protected to the fullest extent possible.

Protected Health Information (PHI)
Your PHI created or received for the purposes of this study is protected under the federal regulation known as HIPAA. Refer to the attached HIPAA authorization for details concerning the use of this information.

Voluntary Nature of the Study
Participation in this study is voluntary. Your decision whether or not to participate in this study will not affect your current or future relations with the University of Minnesota or the Fairview University Obesity Surgery Center. If you decide to participate, you are free to withdraw at any time without affecting those relationships.

Contacts and Questions
The researchers conducting this study are Carrie Earthman, PhD, RD, Sayeed Ikramuddin, MD, Todd Kellogg, MD, Shalamar Sibley, MD, MPH, and Tiffany Beckman, MD, MPH. You may ask any questions you have now, or if you have questions later, **you are encouraged to** contact Dr. Earthman at 612-624-9278. If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), **you are encouraged to** contact the Fairview Research Helpline at telephone number 612-672-7692 or toll free at 866-508-6961. You may also contact this office in writing or in person at University of Minnesota Medical Center, Fairview Riverside Campus, 2200 Riverside Avenue, Minneapolis, MN 55454.

You will be given a copy of this form to keep for your records.

Statement of Consent
I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

Subject's signature	Date	Person obtaining consent	Date

APPENDIX B: SUPPLEMENT NUTRIENT COMPOSITION AND PREPARATION METHOD

Nutrition Facts	
*Protein Beverage (PRO-BEV)**	
Serving Size 24g	
Amount Per Serving	
Calories 90	
Calories from Fat 0	
	% Daily Value
Total Fat 0g	0%
Saturated Fat 0g	0%
Trans Fat 0g	0%
Cholesterol 5mg	1%
Sodium 135mg	6%
Total Carbohydrate 2g	1%
Dietary Fiber 0g	0%
Sugars 0g	
Protein 20g	

Nutrition Facts	
*Fat Beverage (FAT-BEV)**	
Serving Size 13g	
Amount Per Serving	
Calories 90	
Calories from Fat 80	
	% Daily Value
Total Fat 9g	13%
Saturated Fat 0g	0%
Trans Fat 0g	0%
Cholesterol 0mg	0%
Sodium 0mg	0%
Total Carbohydrate 3g	1%
Dietary Fiber 0g	0%
Sugars 0g	
Protein 0g	

Note: Nutrition facts are the same for all three flavors (strawberry, orange, and grape)

*Beverages developed and supplied by Davisco Foods International, Inc, Eden Prairie, MN).

Preparation Method:

1. Fill small blender cup with ~7oz tepid tap water, up to the top of the maximum fill line. Do not use cold water, as it won't mix well.
2. Empty 1 supplement packet into blender cup.
3. Blend on "liquefy" for ~2 minutes.
4. Pour into cup and chill 15-30 minutes and then stir well to re-blend it before drinking.

APPENDIX C: EXAMPLE SAS CODES

Chapter 3: Changes in Gastrointestinal Hormones and Leptin After Roux-en-Y Gastric Bypass Surgery

SAS Code for Body Weight Changes from Baseline (Figure 3.1)

```
proc glimmix;
class id treatment visit ;
model WeightChangeFromPreRYGB = treatment visit treatment*visit /
ddfm=kenwardroger;
random intercept / subject=id type=un v vcorr;
lsmeans treatment*visit / slice = visit  alpha=.333
plots=(meanplot(cl join sliceby=treatment plotby=treatment));
run;
```

*SAS Code for GI Hormone and Leptin Changes from Baseline (Figures 3.2-3.7)**

```
proc glimmix;
class id treatment visit;
model FastedGLP = treatment visit treatment*visit / ddfm=kenwardroger;
random intercept / subject=id type=un v vcorr;
lsmeans treatment*visit / slice = visit  alpha=.333
plots=(meanplot(cl join sliceby=treatment plotby=treatment));
where visit > 1;
run;
```

*Same code used for fasted PYY, fasted ghrelin, fasted leptin, GLP-1 AUC, PYY AUC, and ghrelin AUC

Chapter 4: Subjective Satiety Before and Up to One Year After Roux-en-Y Gastric Bypass

*SAS Code for Difference in Subject Characteristics Between Treatment Groups**

```
Proc Mixed;
Class id visit;
Model Weight = Treatment visit treatment*visit /Solution;
random int / subject = id;
Run;
```

*Same code used for age and BMI

*SAS Code for Subject Characteristics (Table 4.2)**

```
Proc Mixed;
Class id visit;
Model Weight = visit /Solution;
LSMEANS visit/diff;
random int / subject = id;
Run;
```

*Same code used for age and BMI

SAS Code for Assessing Difference in Subjective Satiety Between Beverage Groups

```
Proc Mixed;
Class id visit treatment;
Model SatietyAUC = Treatment visit treatment*visit /Solution;
random int / subject = id;
Run;
```

SAS Code for Subjective Satiety Before and After Roux-en-Y Gastric Bypass (Figure 4.2)

```
Proc Mixed;
Class id visit;
Model SatietyAUC = visit /Solution;
LSMEANS visit/diff;
random int / subject = id;
Run;
```

SAS Code for Relationship Between Subjective Satiety and the GI Hormones

```
Proc Mixed;
Class id visit;
Model SatietyAUC = GLPAUC PYYAUC GhrelinAUC visit age TestDayCalories
/Solution;
LSMEANS visit/diff;
random int / subject = id;
Run;
```

SAS Code for Relationship Between Weight Loss and Change in GI Hormones

```
Proc Mixed;
Class id visit;
Model WeightLoss = dAUC_GLP dAUC_PYY dAUC_Ghrelin visit age
TestDayCalories PreRYGBWeight/Solution;
LSMEANS visit/diff;
random int / subject = id;
Run;
```

SAS Code for Relationship Between Weight Loss and Change in Subjective Satiety

```
Proc Mixed;
Class id visit;
Model WeightLoss = dAUC_HungerFull visit age TestDayCalories
PreRYGBWeight /Solution;
LSMEANS visit/diff;
random int / subject = id;
Run;
```