APPETITE HORMONES FOLLOWING ROUX-EN-Y GASTRIC BYPASS: WHAT IS THE MAGNITUDE OF CHANGE WITH TIME?

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GUT PEPTIDES BEFORE AND FOLLOWING ROUX-EN-Y GASTRIC BYPASS:
A SYSTEMATIC REVIEW AND META-ANALYSIS

Abstract

Background. Roux-en-Y gastric bypass (RYGB) is an effective treatment for obesity, where gut peptides such as ghrelin, glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) play an instrumental role in reduced appetite after RYGB. This systematic review and meta-analysis aimed to establish the magnitude of change of ghrelin, GLP-1, PYY and appetite sensation following RYGB. **Methods.** A systematic search was conducted in Medline Ovid, Embase, Scopus, and Cochrane Central Register of Controlled Trials up until March 2021. Two independent reviewers screened articles for studies that evaluated ghrelin, GLP-1, PYY or appetite sensation via visual analogue scales (VAS) before and after RYGB in adults. Risk of bias was assessed with the quality assessment tool for before-after studies with no control group from the National Heart, Lung and Blood Institute (NHLBI). A multilevel model with random effects for study and follow-up time points nested in study was fit to the data. The model included kilocalorie consumption as a covariate and time points as moderators. **Results.** Among the 2,559 articles identified, 47 met the inclusion criteria, among which k=19 evaluated ghrelin, k=40 GLP-1, k=22 PYY and k=8 appetite sensation via VAS. Our results indicate that fasting ghrelin levels are decreased 2 weeks post-RYGB ($p = .005$) but do not differ from baseline from 6 weeks to 1-year post-RYGB. Postprandial ghrelin levels at 6 months and 1-year post-RYGB were not different from pre-surgical values ($p = .51$). Fasting GLP-1 levels were not different from pre-surgical levels up to 2 years post-RYGB. Postprandial levels of GLP-1 increased significantly from 1 week ($p < .001$) to 2 years post-RYGB ($p < .01$) compared to before surgery. Compared to pre-RYGB levels, fasting PYY increased at 6 months ($p = .034$) and 1 year ($p = .0299$) post-surgery and postprandial levels were increased up to 1 year ($p < .01$). Heterogeneity was significant in most analyses. Insufficient data on appetite sensation was available to be meta-analyzed. **Conclusion.** Our analyses illustrate the magnitude of change of ghrelin, GLP-1 and PYY before and after RYGB surgery. Importantly, between study

heterogeneity within the current literature warrants more standardized protocols and studies with longer follow-up periods for better comprehension of changes in gut peptides following RYGB surgery.

Keywords: Roux-en-Y gastric bypass; Ghrelin; Glucagon-like peptide-1; Peptide YY; Appetite; Meta-analysis; Systematic review

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List of Abbreviations:

AUC: Area under the curve

BA: Before and after study design

BBB: Blood-brain barrier

BMI: Body mass index

CI: Confidence interval

EBW: Excess body weight

ECLIA: Electrochemiluminescence-based immunoassays

EE: Energy expenditure

EI: Energy intake

ELISA: Enzyme-linked immunosorbent assay

GI: Gastrointestinal

GLP-1: Glucagon-like peptide-1

Kcal: kilocalorie

NGT: Normal glucose tolerance

NR: Not Reported

PYY: Peptide YY

QE: Quasi-experimental study design

RCT: Randomized control trial

RIA: Radioimmunoassay

RYGB: Roux-en-Y gastric bypass

SD: Standard deviation

T2D: Type 2 diabetes

USA: United States of America

VAS: Visual analogue scale

Chapter 1: Introduction

Living with obesity has become increasingly common across the world as over 650 million individuals were reported living with obesity worldwide in 2016 according to the World Health Organization (WHO), making this chronic illness a major health concern. This trend is similarly observed in Canada, where over half of adult Canadians (63%, in 2018) are classified as living with overweight or obesity (*Obesity in Canada: Causes, Consequences and the Way Forward - BR2015-12.Pdf*, 2015.). What is more, the prevalence of obesity is continuously rising and has been doing so for the last decades and with weight gain being exacerbated since the recent Covid-19 pandemic (Seal et al., 2022). The World Obesity Federation estimates the global prevalence of obesity to be 16.1% of adults in 2025 and 17.5% by 2030 (Lobstein et al., 2022). Living with increased adiposity often leads to comorbidities such as type 2 diabetes (T2D), hypertension, and dyslipidemia, in addition to putting the individual at a higher risk for major health risks such as heart disease and stroke, which are the leading causes of death worldwide (WHO, 2021).

Body mass index (BMI) is a measure used to determine the weight category of individuals which consists of dividing their mass (kg) by the scale value of their height (m). A measure of BMI <18.5 kg/m^2 is classified as underweight; 18.5 to 24.9 kg/m^2 is normal weight; and 25 to 29.9 kg/m^2 is overweight. Measures of 30 to 34.9 kg/m^2 are classified as obesity class I; 35 to 39.9 kg/m^2 as obesity class II, and ≥40 kg/m^2 as obesity class III (obesity class II and III are often referred to as severe obesity) (Weir & Jan, 2022). BMI is a good tool to standardize weight or weight loss given the height of participants and is still frequently used in the literature despite the criticism it receives due to the metric not taking into consideration body composition or other factors to categorize individuals. Of the 84 risk factors evaluated by the Global Burden of Disease Study in 2017, BMI was among the top 5 risk factors for death and disability-adjusted life years

(GBD 2017 Risk Factor Collaborators, 2018). Obesity has and continues to burden health globally and greatly impacts mental health, quality of life and the health care system. It is clear that individuals need support more than ever to reduce the burden of obesity on overall health.

Interventions for weight loss vary widely from diets, physical activity, or pharmaceuticals, to surgical approaches, and often combinations of the aforementioned interventions. One of the axioms of obesity treatment is that there cannot be meaningful weight loss without a prolonged energy deficit. Dietary modifications and increased physical activity participation are very common weight loss interventions, whereby energy balance is targeted by either reducing energy intake and/or increasing energy expenditure. A two-year intervention trial in participants living with obesity class II and III using a low-energy diet and behavioural group treatment showed a peak weight loss of 18.9% at 6 months. However, even with continued support, at two years the participants demonstrated an average weight loss of 7.2% compared to baseline (Karlsson et al., 2021). While this type of intervention can aid in weight loss in the short-term, long-term effects are typically minimal as weight loss is often regained in individuals living with severe obesity.

Although currently approved pharmaceutical approaches do provide interesting long-term results for body weight control (O'Neil et al., 2018), the success is limited (Rubino et al., 2022), and weight relapse occurs when the treatment is terminated (Khera et al., 2016; Rubino et al., 2021). As such, individuals living with severe obesity often require more intensive interventions in order to improve obesity-related comorbidities and health-related quality of life. Accordingly, the first documented surgical intervention for weight loss was performed in 1954 and involved a resection of the small intestine, results from which were reported in 1966 by Mason and Ito (Mason & Ito, 1967; Wiggins et al., 2020). Since then, the number of bariatric surgeries performed around the world has increased exponentially. In Canada alone, the number of surgeries increased from

1,578 in 2006 to 6,525 in 2013 (*Bariatric Surgery in Canada - Report*, 2014). According to The International Federation for the Surgery of Obesity and Metabolic Disorders (IFSO) global registry, between 2015 and 2018, 84.3% of bariatric surgeries in Canada were a Roux-en-Y gastric bypass (RYGB) (Ramos et al., 2019). The RYGB procedure consists of drastically decreasing the size of the stomach and bypassing the proximal part of the small intestine, otherwise known as the duodenum. The anatomical changes produced by the surgery have a resulting major effect on appetite hormones (**Figure 1**). Notably, it procures satiety to the individual very early postprandially, thereby helping them create and sustain the energy deficit needed for larger weight loss. The ingested food that bypasses the duodenum then comes in contact with the cells that produce hunger suppressing hormones earlier than prior to surgery, thus causing an augmented secretion, satiety, and cessation of food intake (Steinert et al., 2017). These appetite-suppressing hormones are primarily GLP-1 and PYY. Contrastingly, the appetite inducing hormone, ghrelin, is lowered following the surgery since its secretion cells are located in the area of the stomach that is resected. Collectively, RYGB patients do not feel as hungry before meals, and satiation is accelerated as a result of this surgical intervention (Holst et al., 2018). These hormones thus represent key factors for the success of the RYGB surgery. Reduced hunger and increased satiety, even with a small energy intake, will translate into a sustained negative energy balance and lead to weight loss that far surpasses that of non-surgical approaches (Steinert et al., 2017).

Chapter 2: Literature Review

Obesity is a chronic disease where an increasing percentage of adults and children are affected every year, worldwide (*Obesity and Overweight*, 2021). Traditional weight loss strategies such as diets, exercise, and pharmaceuticals generally result in reductions in weight, yet with time weight regain occurs (Buchwald et al., 2004). To this day, there are no known weight loss interventions or strategies that are completely effective in the long-term, meaning the weight lost is often regained and individuals are back to their initial or even heavier weight (Fothergill et al., 2016; MacLean et al., 2017; Wing & Phelan, 2005). A meta-analysis pooled traditional weight loss strategy outcomes to a minimum of 12 months post-intervention, where weight loss typically plateaued at 5 to 9% at 6 months and weight regain was observed up to 48 months post-intervention (Franz et al., 2007). To achieve weight loss, an energy deficit or a negative energy balance needs to be created (Müller et al., 2016). Energy balance has two components: energy intake (EI) and energy expenditure (EE). To predict the long-term results of an intervention, it is important to assess not only the energy deficit created during, but also its effects on EI and EE after the intervention. While traditional weight loss induces an energy deficit, the compliance to the deficit, compensation, and the effects of the intervention on EI and EE following the deficit generally lead the individual to regain the initial weight loss (Doucet et al., 2018). Unfortunately, maintaining weight loss has been shown to be difficult and the factors that drive weight regain have yet to be clearly understood because a multitude of factors can affect EI and EE in a weight-reduced state (MacLean et al., 2017).

Diet, exercise, and a combination of both interventions can help individuals living with obesity to lose their excess body weight (EBW). However, as mentioned previously, individuals living with obesity that achieve a diet-induced weight loss, regain the lost weight 80–97% of the

time (Melby et al., 2017; Riou et al., 2015). In addition to the current obesogenic environment favouring overeating and sedentary behaviour, appetite-related hormonal changes also promote weight regain up to one year following diet-induced weight loss (Sumithran et al., 2011). Both sides of the energy balance are affected during and after non-surgical interventions. A decrease in total EE and an increase in appetite leading to a higher EI thereby translates to weight regain (Doucet et al., 2018; Dulloo et al., 1997). For people living with severe obesity, health risks are high and after multiple failed attempts to lose weight with traditional strategies (*Bariatric Surgery in Canada - Report*, 2014), surgical approaches are often considered.

To this day bariatric surgery is the most effective weight-loss strategy, leading to major weight loss and weight loss maintenance over time (Buchwald et al., 2004). Worldwide and in Canada, RYGB is one of the most commonly performed bariatric surgery (Angrisani et al., 2015; *Bariatric Surgery in Canada - Report*, 2014). In 2014, 48% of bariatric surgeries in Canada were RYGB (Anvari et al., 2018). RYGB is deemed successful when the weight loss is equivalent to ≥50% of EBW (Angrisani et al., 2015), where EBW represents the surplus of weight from what is considered a healthy body mass index (BMI) (25 kg/m^2). This means that if 100% EBW loss is achieved, the individual will have reached a BMI of 25 kg/m^2. Weight nadir occurs around two years post-surgery at 74% EBW loss and then slowly increases to about 52% EBW loss at 10-year post-surgery (Mehaffey et al., 2016). In a longitudinal study, at 10-years post-surgery the mean EBW loss was 56%, representing a mean weight loss of 41.3 kg, while nonsurgical matches lost only 7.7% of EBW (Maciejewski et al., 2016). In the same review, RYGB weight loss results were compared to other common types of bariatric surgery such as the sleeve gastrectomy (SG) or the adjustable gastric band surgery (AGB). At one-year post-surgery, RYGB patients lost 7.6% more weight than the SG patients and 17.9% more than the AGB patients. This trend was shown to

continue up to 10 years post-surgery (Maciejewski et al., 2016). RYGB also leads to remission or significant amelioration of comorbidities associated with obesity (Buchwald et al., 2004). In the first two years post-surgery, 80% of RYGB patients no longer require medication for type 2 diabetes (T2D) (Amundsen et al., 2017). In a different study, the remission rate of T2D was 54% at 12 years post-RYGB (Guimarães et al., 2021). The long-term relapse rate of T2D in RYGB patients is lower compared to SG patients (Yu et al., 2021). However, in some cases, similar to other weight-loss strategies, RYGB is followed by weight regain. A recent study observed weight loss failure prevalence of 29.9%, defined as losing less than 20% of initial weight at 10-year post-RYGB (Guimarães et al., 2021). At the 10-year mark, about 5% of the RYGB group regained within 5% of initial body weight, compared to 56% of nonsurgical matches (Maciejewski et al., 2016). Weight regain following RYGB increases health risks, reoccurrence of comorbidities, and cost (Coen & Goodpaster, 2016; Sudan et al., 2015). Prior to attempting prevention of weight regain following RYGB, it is crucial to have a better understanding of the main mechanisms involved for the success of RYGB and the changes throughout time after the surgery.

RYGB Surgery

Patients who undergo RYGB have permanent anatomical changes to their digestive system, affecting gastric emptying, absorption of nutrients, hormone secretion and other appetites-related signals (Steinert et al., 2017). The RYGB surgery consists of separating most of the stomach, which typically ranges from 15 to 30 ml in volume to create a much smaller pouch that is approximately 10% of its original size. The pouch is then reattached mid-jejunum, typically called the roux limb (100-150 cm). The remaining part of the stomach and the proximal part of the small intestine is then reattached further to the jejunum, bypassing most of the stomach and the

duodenum, and forming the biliopancreatic limb (50-75 cm). This is depicted by the Y shape in **Figure 1** (19, 20).

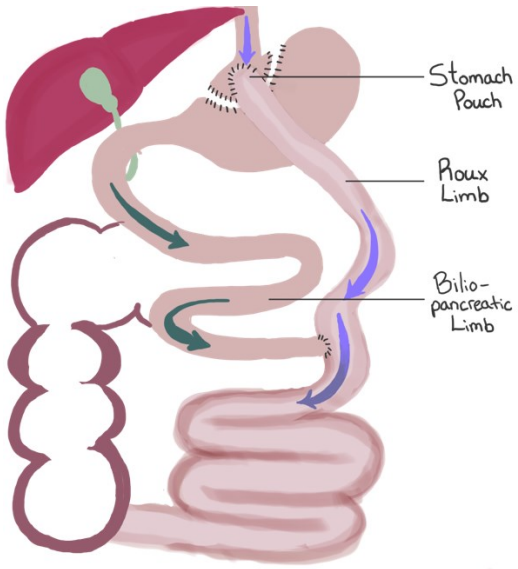

Figure 1: The Digestive System Following the Roux-en-Y Gastric Bypass Surgery.

These changes instigate the significant weight loss that follows RYGB. Initially, EI drastically decreases by 67% compared to pre-operative values (Giusti et al., 2016). Instinctively, it may seem that the restrictive nature of the intervention is the chief reason for the lower EI, however, changes in appetite regulating hormones are thought to play an instrumental role in the decrease and maintenance of reduced EI (Holst et al., 2018).

Appetite Hormones

Appetite is regulated by a multitude of factors and signals. Appetite hormones have a strong influence, along with external stimuli, as illustrated in Figure 2, such as visual cues, olfactory signals, taste, learned behaviour, time, physical and social environment, stress levels and restraint (MacLean et al., 2017).

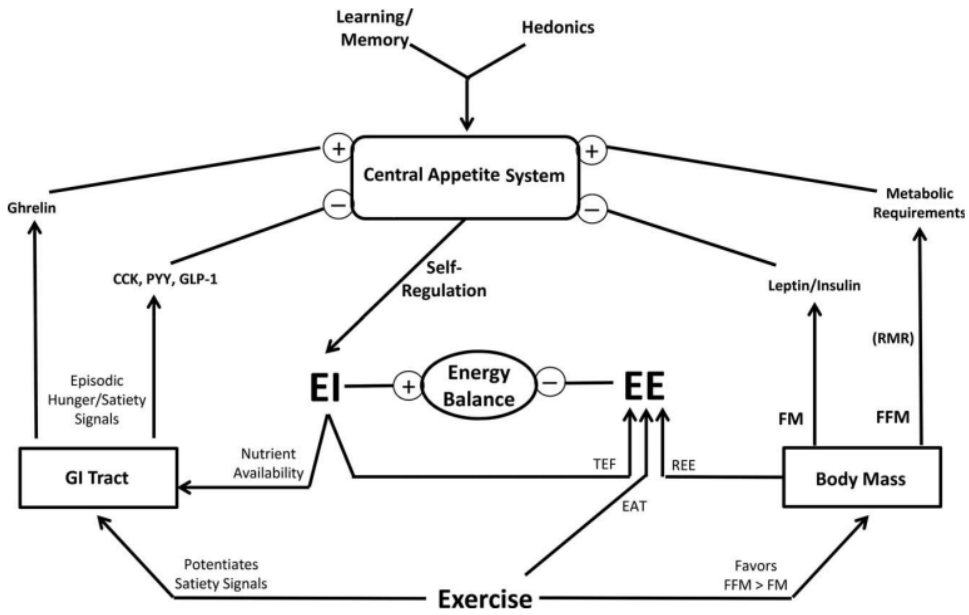

Figure 2: Biological Inputs of Appetite Regulation (MacLean et al., 2017)

Although it is important to keep these factors in mind, this review will focus on direct measurements of key appetite hormones. Surgical, diet, and exercise weight loss interventions all have effects on appetite regulating hormones. Appetite is, in part regulated by both episodic and tonic signals (Blundell et al., 2015). Episodic secretion of hormones such as ghrelin, glucagon-like peptide-1 (GLP-1), and peptide YY (PYY) directly influences satiety and have an impact on food intake with every meal; More precisely, it affects meal initiation, gastric emptying and meal termination (Schubert et al., 2014). A tonic hormone, such as leptin, regulates EI and fluctuates in response to changes in the energy reserves (Liu & Kanoski, 2018). Specifically, leptin procures feedback from the adipose tissues and fluctuates directly with fat mass changes (Strohacker et al., 2014). Although both episodic and tonic appetite regulating signals, impact food consumption post-RYGB, this review will focus on the response of episodic hormones. Ghrelin, GLP-1 and PYY and their influence, fluctuations with food consumption, and the RYGB surgery will be discussed in more detail below.

Ghrelin

Ghrelin is mostly secreted by the P/D1 cells in the oxyntic glands within the fundus of the stomach. Its concentration peaks before meals and mainly influences meal initiation and hunger sensation via its activation of the NPY/AgRP neurones in the arcuate nucleus (Steinert et al., 2017). Ghrelin levels drop in lean individuals with food consumption, however this suppression is lower in individuals living with obesity (Melby et al., 2017). In adults and adolescents living with obesity, weight loss leads to an elevation of ghrelin levels, which is linked to augmented hunger (Melby et al., 2017). For RYGB patients, fasting ghrelin levels are drastically reduced post-surgery. Meal-to-meal oscillations are no longer apparent, and this change has been associated with the reduction of hunger and EI post-surgery (Steinert et al., 2017). Though, this effect appears to be temporary since normal oscillation of ghrelin levels with meals have been observed one-year post-RYGB in some studies. The changes in ghrelin levels post-RYGB in the long term remain to be controversial (Perakakis et al., 2019; Steinert et al., 2017). It is hypothesized that the diminished effect of RYGB surgery on ghrelin levels are caused by the weight loss or adaptation of the gastrointestinal tract (Steinert et al., 2017). This implies that ghrelin might be more important for initial weight loss than a long-term weight maintenance mechanism following RYGB (Steinert et al., 2017). The concentrations and magnitude of change post-surgery need to be clarified as the timeline could potentially link these changes with other events, like weight stabilization. The hunger hormone ghrelin is thought to contribute to the success of the procedure; however, the appetite-suppressing hormones seem to have a crucial role for weight loss and weight loss maintenance.

GLP-1

GLP-1 is an appetite regulating hormone produced by the L-cells of the intestine. Normally secreted 15 to 30 minutes after a meal, GLP-1 inhibits food intake, regulates glycemia, and stimulates insulin production (Lim & Brubaker, 2006). Meal-stimulated secretion of GLP-1 is attenuated in some studies with people living with obesity (Steinert et al., 2017) and is lower than baseline after weight stabilization following a dietary restriction (Lean & Malkova, 2016). GLP-1 receptors are found in a multitude of regions in the brain and its action for appetite inhibition comes primarily from the indirect activation of POMC neurones (Liu & Kanoski, 2018). Ghrelin and GLP-1 have opposite effects and bind to different receptors in the arcuate nucleus in the brain to stimulate or inhibit food intake and food motivation (Liu & Kanoski, 2018). GLP-1 also acts on the reward-related region of the brain, known as the ventral tegmental area, and inhibits food palatability and motivation for highly palatable foods (Liu & Kanoski, 2018). Due to the bypass of the duodenum and proximal part of the jejunum in RYGB patients, the accelerated passage of undigested nutrients to the distal part of the intestine, where L-cells are present, stimulates an exaggerated production of GLP-1 (Steinert et al., 2017). The augmented secretion of this incretin hormone is also thought to be partially responsible for the rapid resolution of type 2 diabetes in RYGB patients (Holst et al., 2018; Steinert et al., 2017). This effect occurs a few days after the intervention, before any noticeable weight loss is observed (Holst et al., 2018) and appears to be sustained over time (Steinert et al., 2017). It is, however, unclear if the levels of GLP-1 secretion remain consistent throughout the years following the procedure. This variation in GLP-1 production is thought to be one of the most important outcomes for the success of weight loss in RYGB patients along with PYY.

PYY

L-cells secreting PYY are located in the distal small intestine and colon. PYY levels begin to increase 15 to 30 minutes postprandial and reach a plateau 60 to 90 minutes after the meal (Steinert et al., 2017). This appetite regulating hormone promotes satiety and gastric emptying and acts both centrally and peripherally. PYY secretion is affected by meal size as well as the type of macronutrient, as lipids specifically result in a higher rate of secretion (Liu & Kanoski, 2018). Similar to GLP-1, the accelerated passage of food to the digestive system densely populated with endocrine L-cells also triggers an exaggerated secretion of PYY following RYGB. When administered together, GLP-1 and PYY have been shown to have a supplementary additive inhibition of food intake (Holst et al., 2018). A counter sur-production of PYY has been observed when a GLP-1 antagonist was introduced, making it difficult to isolate the individual effects of each hormone (Holst et al., 2018). Interestingly, when inhibitors of GLP-1 and PYY were administered to RYGB patients, food intake increased (Holst et al., 2018). The link between obesity and PYY is unclear since results across studies vary for fasting and postprandial levels of total PYY (Steinert et al., 2017). GLP-1 along with PYY work rapidly to signal satiety and their effect appears to last long-term post-RYGB. These hormones could be a key factor to the long-term success of the surgery. The longevity of the augmented GLP-1 and PYY response, however, remains unclear.

Appetite

It is important to also consider perceived appetite while investigating appetite regulating hormones. It has been shown that appetite sensation measurement with visual analogue scales is highly correlated with food intake and suitable in a laboratory setting (Dimitriadis et al., 2017; Holliday et al., 2021). The numerous appetite modulators previously presented collectively impact

appetite sensation, which ultimately provides guidance to feeding. Appetite is not only the result of variations in peripheral peptide signalling, but it is also influenced by external and perceived information by the individual, as previously mentioned and shown in **Figure 2**. Measuring this by instructing participants to answer questions such as "how hungry do you feel?" or "how full do you feel?" on a visual scale is highly accessible, can be accurately reproduced, and can provide additional information on perception as well as more objectively measured biomarkers (Flint et al., 2000). The VAS is frequently used to gather subjective information on different terms surrounding the appetite sensation of the participants, such as hunger, fullness, satiety, and prospective food consumption before and following EI. These terms are referring to the drive to eat, feeling of being full and cessation of the urge to eat, and how much more food could still potentially be eaten respectively (Blundell et al., 2010). During energy restriction, people living with obesity have been shown to display an augmented fasting appetite (Doucet et al., 2000; Hintze et al., 2017). This subjective hunger has been shown to vary in successful vs. non-successful weight loss interventions, where the successful group (group that lost more weight) had a hunger sensation favouring a lower EI (King et al., 2008). When comparing a group living with obesity versus a RYGB group with matched diets that both lost 10 kg, the RYGB group showed lower hunger and higher satiety ratings compared to pre-surgery whereas the diet group showed higher hunger and lower satiety ratings compared to pre-surgery (Halliday et al., 2019). VAS for appetite measurement does not accurately predict EI in most studies according to a recent systematic review (Holt et al., 2017); thus results should be interpreted as complementary information about the individual current state following a meal (Doucet et al., 2003). This additional insight supplements the information provided by gut peptides and provides a more comprehensive picture of appetite control following RYGB.

Leptin

Leptin is an appetite regulating hormone secreted mostly by white adipocytes (Zhang et al., 1994). This tonic appetite hormone is transported through the blood-brain barrier (BBB) to its receptors in the arcuate nucleus of the hypothalamus, leading to inhibition of appetite (Gruzdeva et al., 2019). When fat mass increases, leptin levels also increase and lead to a reduction in EI in lean individuals. However, this response is not observed in people living with obesity (de Git et al., 2018; Gruzdeva et al., 2019; Zhao et al., 2020). The high volume of fat mass loss following RYGB is expected to also lead to proportional decreases in leptin levels with time (Beckman et al., 2010). Although leptin has an important impact on appetite through a tonic response and was originally considered as a secondary analysis during our analyses, it will not be considered a main outcome in this meta-analysis. The RYGB surgery does not have a direct impact on leptin secretion, other than through the increased reduction of adiposity compared to non-surgical approaches. Similar effects of different bariatric surgeries on leptin have been observed (Salman et al., 2020).

Summary of Available Evidence

The RYGB surgery changes the postprandial response of gastrointestinal hormones; most notably increasing GLP-1 and PYY, and lowering ghrelin levels, altogether associated with the decreased appetite seen post-surgery (Giusti et al., 2016). The change of concentration of these hormones are hypothesized to have an important role in food intake control and decreasing motivation to eat in RYGB patients (Beckman et al., 2010; Holst et al., 2018). The changes in the secretion of ghrelin early after the surgery are substantial, with lower levels of ghrelin following a weight loss intervention being unique to the bariatric surgery. Though, this change seems to be temporary and not described in any review addressing hormonal changes following bariatric

surgeries. Regarding ghrelin, the magnitude and duration of the change is unclear. Since weight loss typically stabilizes 12 to 18 months after the RYGB surgery, it would be interesting to examine a possible link between weight stabilization and ghrelin level changes post-RYGB. Potential matching of the time of weight stabilization in patients and ghrelin levels could refine our understanding of weight loss mechanisms following the surgery. Furthermore, though increased levels of GLP-1 and PYY seem to be more durable, a more precise picture of their variation with time is needed. Reviews investigating the changes of appetite hormones only include information about the direction of the changes in these hormones (Beckman et al., 2010; Dimitriadis et al., 2017). The magnitude of change of concentrations throughout time post-surgery and the length of these changes would be key to identifying the importance of the role of these hormones in weight loss maintenance in RYGB patients. It would be relevant to clarify the expected concentrations of these hormones at the different stages following the surgical intervention, since they are thought to be important for the weight loss and maintenance post-RYGB. This type of information would provide, not only the expected ranges, but could also be a way to identify outliers before untimely weight changes occur and potentially provide these individuals additional support before weight regain occurs.

The aim of this review is to provide detailed information on the expected ranges and change over time of key appetite-related hormones (GLP-1, PYY, ghrelin) as well as hunger sensation following the RYGB surgery. Having a clearer view of the hormonal changes throughout time following the surgical intervention could help identify the importance of the role of these appetite signals at different stages following the procedure and potentially link the changes to weight nadir or weight regain.

Research Problem

The RYGB has shown incredible success for weight loss and weight maintenance as well as remission of comorbidities in people living with obesity, yet its effects on the key mechanisms of weight loss are still unclear. Ghrelin, GLP-1 and PYY have a demonstrated effect on food intake (Konturek et al., 2004), although the current literature lacks in describing their magnitude of change throughout time following the RYGB surgery. Appetite hormones such as ghrelin, GLP-1 and PYY have a well-established role in appetite control (Zanchi et al., 2017). As such, it would be important to also know how they fluctuate with time following weight loss induced by RYGB. For the most part, current available literature on appetite hormone following RYGB only provide the direction of the change without giving details on the magnitude of change or whether these changes are sustained over time. Therefore, performing a systematic review focused on illustrating the changes of these hormones would be valuable to further our understanding of these key factors for success following the RYGB surgery and the years succeeding the procedure. This information could lead to providing added support to patients in hopes of reducing the likelihood of weight regain and reoccurrence of comorbidities.

Objectives and Hypotheses

Primary objective

To establish the magnitude of the meal induced response of appetite regulating peptides (ghrelin, GLP-1, and PYY) and appetite sensation via VAS from before compared to post-RYGB surgery.

Secondary objective

To establish the magnitude of change of fasting levels of appetite regulating peptides (ghrelin, GLP-1, and PYY) from before to post-RYGB surgery.

Hypotheses

1. Postprandial ghrelin levels will decrease early post-surgery and increase back to pre-surgical concentrations within the first year post-RYGB.
2. Postprandial GLP-1 and PYY hormones concentrations will be much higher early post-surgery compared to before RYGB and remain high even with weight stabilization.
3. VAS scores will be associated with hunger hormone levels, promoting lower appetite and higher satiety.
4. Fasting levels of ghrelin will be higher following the RYGB surgery and fasting levels of GLP-1 and PYY will remain unchanged after RYGB surgery.

Chapter 3 & 4: Methodology & Results

To avoid redundancy, the methodology and results sections are presented in the form of a paper, entitled: “Gut Peptides Before and Following Roux-en-Y Gastric Bypass: A Systematic Review and Meta-Analysis.” The paper will be submitted for publication.

Gut Peptides Before and Following Roux-En-Y Gastric Bypass: A Systematic Review and Meta-Analysis

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Abstract

Background. Roux-en-Y gastric bypass (RYGB) is an effective treatment for obesity, where gut peptides such as ghrelin, glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) play an instrumental role in reduced appetite after RYGB. This systematic review and meta-analysis aimed to establish the magnitude of change of ghrelin, GLP-1, PYY and appetite sensation following RYGB. **Methods.** A systematic search was conducted in Medline Ovid, Embase, Scopus, and Cochrane Central Register of Controlled Trials up until March 2021. Two independent reviewers screened articles for studies that evaluated ghrelin, GLP-1, PYY or appetite sensation via visual analogue scales (VAS) before and after RYGB in adults. Risk of bias was assessed with the quality assessment tool for before-after studies with no control group from the National Heart, Lung and Blood Institute (NHLBI). A multilevel model with random effects for study and follow-up time points nested in study was fit to the data. The model included kilocalorie consumption as a covariate and time points as moderators. **Results.** Among the 2,559 articles identified, 47 met the inclusion criteria, among which k=19 evaluated ghrelin, k=40 GLP-1, k=22 PYY and k=8 appetite sensation via VAS. Our results indicate that fasting ghrelin levels are decreased 2 weeks post-RYGB ($p = .005$) but do not differ from baseline from 6 weeks to 1-year post-RYGB. Postprandial ghrelin levels at 6 months and 1-year post-RYGB were not different from pre-surgical values ($p = .51$). Fasting GLP-1 levels were not different from pre-surgical levels up to 2 years post-RYGB. Postprandial levels of GLP-1 increased significantly from 1 week ($p < .001$) to 2 years post-RYGB ($p < .01$) compared to before surgery. Compared to pre-RYGB levels, fasting PYY increased at 6 months ($p = .034$) and 1 year ($p = .0299$) post-surgery and postprandial levels were increased up to 1 year ($p < .01$). Heterogeneity was significant in most analyses. Insufficient data on appetite sensation was available to be meta-analyzed. **Conclusion.** Our analyses illustrate the magnitude of change of ghrelin, GLP-1 and PYY before and after RYGB surgery. Importantly, between study

heterogeneity within the current literature warrants more standardized protocols and studies with longer follow-up periods for better comprehension of changes in gut peptides following RYGB surgery.

Keywords: Roux-en-Y gastric bypass; Ghrelin; Glucagon-like peptide-1; Peptide YY; Appetite; Meta-analysis; Systematic review

Introduction

Obesity prevalence rose globally from 11% in 2010 to 15% in 2020 and is projected to reach 18% by 2030 (1). Health risks are high for people living with severe obesity, and after multiple failed attempts to lose weight with traditional strategies, surgical approaches are currently the most robust solution for long-term weight loss (2,3). Roux-en-Y gastric bypass (RYGB) is one of the most performed types of bariatric surgery (3,4). From 2014 to 2018, 38% of bariatric surgeries worldwide were a RYGB surgery, totalling 72,645 RYGB surgeries reported in the registry (5). RYGB benefits are numerous, including critical weight loss, reduced medication use, remission of type 2 diabetes (T2D), dyslipidemia and hypertension, lowered risk of coronary heart disease, reduced obstructive sleep apnea, and improved quality of life (2,6–11). For example, the CROSSROADS randomized controlled trial results showed a 25.8% body weight loss and 60% T2D remission in participants at 1-year post-op RYGB compared to only 6.4% and 5.9% for the intensive lifestyle and medical interventions, respectively (12). When compared to the sleeve gastrectomy, which is the most performed bariatric intervention, RYGB has also shown to procure stronger results for weight loss, both short and long term, as well as for comorbidity remission rates (5,10,13). However, inadequate weight loss which is classified as weight loss ≤50% of excess body weight (4), can occur in patients following bariatric surgery. A recent study identified that at 5 years post-RYGB, 23% of 5,936 participants met at least 1 of the 3 common definitions of unsuccessful weight loss (14). Thus, it is crucial to have a better understanding of the contribution of the main mechanisms involved in successful weight loss following RYGB. Patients who undergo RYGB have permanent anatomical changes to their digestive system, creating a much smaller pouch and bypassing the duodenum (15,16). Intuitively, it may be assumed that the restrictive nature of the intervention is the chief reason for the subsequent lower energy intake, however, changes in appetite regulating hormones are thought to play an instrumental role in the

decrease and maintenance of reduced energy intake (17). It is also important to consider perceived appetite when investigating gut peptides (18,19). This review will focus on key episodic appetite hormones, namely ghrelin, GLP-1 and PYY, that have been demonstrated to influence appetite and food intake (20).

RYGB surgery substantially amplifies the postprandial response of gastrointestinal (GI) hormones. An increase in GLP-1 and PYY as well as a decrease in ghrelin levels are all associated with the reduced appetite demonstrated post-surgery (21). The fluctuation of these hormones are thought to have an important role in food intake control and decreasing motivation to eat for RYGB patients (17,22). The changes in ghrelin levels noted early after RYGB are substantial, and are a common feature of RYGB surgery (15). Although this change has been reported to be only transient (23), it has not been present in any review addressing hormonal changes following RYGB to date. The variation in GLP-1 and PYY levels is thought to be one of the most important outcomes leading to successful weight loss in RYGB patients. Bypassing the duodenum and proximal jejunum results in the accelerated passage of undigested nutrients to the distal part of the intestine, which is more densely populated with L-cells (15). This results in an exaggerated GLP-1 and PYY response to food intake in RYGB patients (15). Increased levels of GLP-1 and PYY appear to be longer lasting, although a clearer depiction of their variation with time is needed. The magnitude of changes of concentrations throughout time post-surgery and the length could more clearly layout normal responses of these hormones in successful RYGB patients.

The aim of this systematic review and meta-analysis is to provide detailed information on the expected ranges and change over time of key appetite-related hormones (ghrelin, GLP-1, PYY) as well as appetite sensation following RYGB.

Methodology

This systematic review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (24), and is registered in the International Prospective Register of Systematic Reviews (PROSPERO) database (registration number CRD42021253743), thereby following the gold standard steps for systematic reviews and meta-analysis to ensure quality of reported results.

Eligibility criteria

Studies were eligible if they (I) included adults who underwent RYGB (II) evaluated participants before and after RYGB surgery, (III) evaluated the meal induced response of ghrelin, GLP-1, PYY and/or appetite sensation with a visual analogue scale (VAS) and (IV) published their results in a peer reviewed journal in English or French. Animal studies, case studies, and cross-sectional studies were excluded. Studies were also excluded if they did not provide the macronutrient composition of meals, did not require a minimum of an 8-hour of fast before the evaluation, collected or reported only fasted appetite-related hormones data, reported postprandial data collection limited to less than 120 minutes postprandial, or did not provide a minimum of 3 time points to calculate the area under the curve (AUC). Appetite sensation consists of measures of hunger, fullness, satiety, prospective food consumption and/or satisfaction. If multiple articles from the same study were initially included, only the article with the longest follow-up period was included.

Information Sources and Search Strategy

Four electronic databases were searched in March 2021: Medline Ovid, Embase, Scopus, and Cochrane Central Register of Controlled Trials with no restriction for the date of publication.

Databases were searched using a combination of MeSH terms and keywords. A search strategy was elaborated based on our research question, population, intervention and outcomes of interest with the support of a university librarian. Search terms included variations of: Gastric bypass, Ghrelin, GLP-1, Peptide YY, Hunger, and Appetite. The details of the search strategy can be found in the supplementary material (page 1). For the databases Medline and Embase, animal studies were excluded within the search strategy. The search strategy was validated by confirming it identified key articles recognized prior as being eligible (25,26). An identical electronic search strategy was performed in March 2022 with a restriction for the date of publication from 2021 to 2022. The search strategy was set to retrieve articles with leptin as one of our outcomes of interest. Subsequently, articles that measured only leptin were excluded. The reasoning for this is that a large number of studies solely evaluating leptin were retrieved and could, by themselves, be studied in a future meta-analysis. Since the RYGB surgery does not have direct effect on leptin concentrations (22,27,28), members of the research group decided not to have leptin as a main outcome in this review. If data from multiple reports corresponded to the same study, only the study with the most information of the outcomes of interest was included.

Article Selection

Articles identified with the search strategy from all 4 databases were imported into Covidence (29). Covidence is an online platform to manage systematic reviews, and was used to track, manage progress and identify conflicts during the progress of screening articles by the authors. Once imported, the duplicates were automatically removed by the platform and manually verified by one author (MS). Two authors independently screened all records by titles and abstracts for potentially eligible studies (MS, EB). They then independently evaluated the full text of

relevant studies to identify eligible articles. A third author resolved any conflicts that arose from each step of the screening process (AB).

Data Extraction

The data was manually extracted onto Microsoft Excel by a single reviewer (MS) and verified by a second reviewer (EB). Fasting and AUC values of ghrelin, GLP-1, PYY and VAS appetite sensation were extracted from included studies. If key information such as total caloric intake of the meal, macronutrient content of the meal, AUC or incremental AUC for Ghrelin, GLP-1, PYY or VAS appetite sensation scores were not presented in the article, the authors were contacted by email on up to two occasions. For each outcome of interest, fasting, AUC or incremental-AUC values were extracted with their standard deviation (SD) at baseline and at each time point evaluated.

Additional data extracted regarding the study details were: the article title, journal, year of publication, country of publication, and design. Regarding the study population, the following data was extracted: number of participants, average and/or age range, ethnicity, number and/or percentage of women included, number of women menopaused, number of participants initially with type 2 diabetes, and comorbidities. The following data regarding the study intervention was extracted: description of any pre-surgery mandatory weight loss, surgery details such as pouch size, biliopancreatic and alimentary limb length, fasting duration, consistency of meal (liquid or solid with description when solid or semi-solid), quantity of test meal (ml), duration of test meal, total caloric intake (kcal), and macronutrient composition (%). Weight and/or BMI and weight loss and/or BMI loss before and after the surgery was also extracted. The following information regarding the outcome measurements were extracted: type of hormone analysis (total/active), type of kit and company for each hormone measures and the description of time points evaluated

postprandially. For studies with a RCT design, the control group data was not extracted. Some assumptions were made when information in the included studies was unclear, such as the test meal was entirely consumed by participants at all time points evaluated and that overnight fasting was assumed to be of a minimum of 8 hours. When not mentioned, AUC were assumed to be calculated with the trapezoid method and to be total AUC unless authors indicated that they calculated incremental AUC.

Risk of Bias Assessment

The quality of included articles was assessed with a standardized tool for before after (pre-post) studies with no control group developed by the National Heart, Lung and Blood Institute (NHLBI, USA) (30) The tool is composed of 12 items designed to evaluate the internal validity of the studies. Three of the 12 criteria were defined as "fatal flaws" when not met or identified: (I) participants representative of clinical population of interest, (II) outcome measures clearly described, valid and reliable (duplicate essays for hormone analysis), and (III) follow-up rate of ≥ 80%. Study quality was defined as good, fair and poor when 0, 1, and ≥2 fatal flaws were identified, respectively. The remaining criteria targeted the study question, eligibility criteria, study population, enrollment of eligible participants, sample size, clear intervention description blinding of outcome assessors, statistical analysis, multiple outcome measures, and group-level interventions and individual level outcome efforts. Two authors independently assessed study quality of included studies (MS, EB) and a third author resolved any conflicts that arose through discussion (AB).

Statistical Analysis

The effect measure used for ghrelin, GLP-1 and PYY was mean AUC and fasting mean. Mean weight was meta-analysed to determine if the weight of the population of included studies was decreasing following the RYGB surgery. Multilevel models with cluster robust variance estimation were performed.

Forest plots were used to visually display results of individual studies and syntheses. When a study contributed multiple arms, each arm was treated as an independent study within the meta-analysis. Forest plots present studies ordered based on the size of their outcome estimate within each time point to facilitate appraisal of outcome distributions. Analyses were performed using R version 4.2.1. Metafor, 3.4.0 (31). For each measure of interest, outcomes were weighted by inverse-variance and analyzed in a multilevel model with random effects for a) study, and b) time points nested within studies (32). The model adjusted for kcal consumption of the test meal. Dependency from repeated measurement was addressed by calculating cluster robust standard errors. Cluster-robust standard errors were calculated when there was sufficient data, otherwise regular standard errors were reported. When cluster robust methods were incorporated, the moderator was tested with an F-test. When standard multi-level methods were applied, the moderator was tested with a Qm test. Since the included studies did not randomly sample participants and did not randomly assign treatment, inferences in this meta-analysis are restricted to the population of participants included in these studies. Statistical heterogeneity was evaluated with Cochrane's Q and was separated into between-study and between-time point components. Heterogeneity variance was calculated as sigma-squared for each component. Prediction intervals were calculated based on the between-subject heterogeneity and sampling error. The 95% prediction intervals in forest plots illustrate the range of mean values expected from a random

study from the same population as our sample. Between study heterogeneity was estimated with Restricted Maximum Likelihood (REML).

There were syntheses that could not be conducted due to insufficient data. Only time points with at least two data points were aggregated in each meta-analysis. If a study presented data with a type of outcome (active, total), time AUC (incremental, total, 120-300 minutes postprandial), or time point post-RYGB that no other included studies presented, the datapoint could not be analyzed in the meta-analysis. There were no statistical methods used to explore causes of heterogeneity because of insufficient power. When large heterogeneity was observed, methodological differences between studies and possible errors in data extraction or original articles was explored. Sensitivity analyses were not conducted.

Results

Study selection

The database search initially identified 4,437 articles that were imported into Covidence. Covidence automatically removed 1,878 duplicates (17 more duplicates were excluded manually). Of the 2,559 articles remaining, 2,211 were deemed irrelevant after titles and abstract screening. The full text was retrieved for 228 studies and of those, 181 studies were excluded. Out of the excluded full texts, 13% (k=23) were the wrong study design, 12% (k=22) did not have a meal test, 11% (k=20) did not measure the postprandial response up to 120 minutes and 40% (k=72) only measured leptin (**Figure 1**). Eight studies would have met the inclusion criteria but were excluded because key information (i.e., meal test kcal, measure of outcomes of interest fasting and AUC) were not presented in the article and the missing information could not be obtained from corresponding authors (33–40). Therefore, a total of 47 studies were included, of which 31

presented sufficient information to be included in the meta-analysis for at least one outcome of interest, and 27 were meta-analyzed for weight (**Figure 1**).

Study characteristics

Study characteristics and main findings are presented in **Table 1**. Included studies were published between 2004 and 2021, with 40% (k = 19) published between 2016 and 2021 (25,26,41–57). The median (range) total sample size was n = 12 (5-60) with a total of n = 904. The median of women evaluated was 75% (24-100%) across included studies and15% (k =7) of studies evaluated women only (41,44,54,58–61). The median (range) follow-up time post-RYGB was 6 months (1 week-48 months), where 51% (k= 24) of studies evaluated their participants up to at least 12 months post-RYGB (25,26,41–43,45,47,48,50,56,62–73). A pre-surgery weight loss or diet was mandatory in 23% (k = 11) of studies (46,49,52,57,63,67,68,71,74–76) For 13% (k = 6) of these studies, a 8% weight loss was mandatory (57,67,69,75–77). In these studies, all pre-RYGB weight, BMI and hormone analyses were done after the pre-surgical weight loss. Most studies did not report weight stabilization before the surgery. Initial BMI was reported by all except 3 studies (45,53,75), and in 94% (k = 43) of studies, the median BMI was 44.8 kg/m^2 (32.1-53.3) kg/m^2. All surgery details were reported by 62% (k = 29) of the studies. Pouch size averaged 26 ml (15-40 ml), biliopancreatic limb length averaged 73 cm (25-150 cm), and alimentary limb length averaged 124 cm (100-150 cm) (presented in supplementary material Table 1). Most studies (85%, k= 40) reported giving a liquid meal tests to their participants (25,26,41,43–45,47–52,57–64,66–83), while 11% (k = 5) of studies reported semi-solid or solid meal tests (44,51–53,82). A further 2% (k = 1) of the studies provided both liquid and solid meal tests on different days (56) (presented in **Supplementary Table 1**). Meal duration was standardized across participants in 57% (k = 27) of studies (25,26,41,44,48,49,51,54–57,61,63–65,67–69,72–79,85), ranging from 3 to 30 minutes.

The median caloric intake (range) of the meal test was 304 kcal (75-600 kcal). Caloric composition of test meals can be found in the supplementary materials. Regarding the outcomes of interest, 40% (k=19) of studies evaluated ghrelin (25,26,41,44,49,52,58,61,62,64,68,73,75,79,81,82,84,85), 85% (k = 40) GLP-1 (26,41–50,52–56,58,60–70,72,74–78,80,82–85), 47% (k=22) PYY (25,26,41,44,47,49,51,52,54,57–61,64,68,69,71,75,80,82,84), and a VAS was used to measure appetite sensation AUC in 17% (k=8) of studies (44,52,54,57,59,61,62,78,80).

Risk of bias

The NHLBI quality assessment tool for pre-post studies with no control group revealed a total of 13% (k=6) of studies with an overall good quality rating, 62% (k=29) of studies with a fair rating, and 26% (k=12) of studies with a poor rating (**Table 1**). The complete summary of risk of bias assessment can be found in the **Supplementary Table 2**. The majority of fatal flaws recorded arose from the same flaw, which related to the quality of the study outcome measures, more precisely in this case, reliability by verifying if assays were duplicated for measurement of Ghrelin, GLP-1 and PYY. All 12 of the poor-quality studies and a total of 41 out of the 47 studies did not assay the peptides in duplicate.

Ghrelin

Pre-RYGB, k=10 eligible studies (11 arms) reported fasting ghrelin (n= 194). The estimated fasting ghrelin concentration was 554 pg/ml (95% CI: 437-670). At 2 weeks post-RYGB, 2 studies (3 arms) (n= 39) showed an estimated fasting ghrelin level of 432 pg/ml (95% CI: 301-562). At 6 weeks post-RYGB, 2 studies (n= 17) showed an estimated fasting ghrelin level of 448 pg/ml (95% CI: 290-605). At 26 weeks post-RYGB, 4 studies (n= 118), showed an estimated fasting ghrelin

level of 541 pg/ml (95% CI: 421-661). Lastly, at 52 weeks post-RYGB, 5 studies (n= 132) revealed an estimated fasting ghrelin level of 553 pg/ml (95% CI: 434-673) for fasting ghrelin (**Figure 2**). These analyses demonstrated that fasting ghrelin two weeks post-surgery was significantly lower compared to pre-surgery levels, (Q_m [df = 4] = 14.82, p = .005). Followed by an increase similar to pre-surgical levels at 6 weeks post-RYGB, at which time values were no longer significantly different from pre-RYGB values. This was sustained from 6 to 52 weeks post-RYGB. The between-study heterogeneity was σ^2 = 35,956, QE(df = 20) = 293.530, p < .001.

Pre-RYGB, and at 26 and 52 weeks post-RYGB, 3 eligible studies reported AUC for ghrelin (n= 90). The estimates were 51,875 pg/ml (95% CI: 37471-66279), 51,389 pg/ml (95% CI: 36,968-65,810) and 51,517 pg/ml (95% CI: 37,003-66,030) respectively (**Figure 3**). No significant difference between follow up measurements of postprandial secretion of ghrelin was found, (Q_m [df = 3] = 2.3, p = .51). The between-study heterogeneity was σ^2 = 151,924,752.4, QE(df = 5) = 55.283, p < .001.

GLP-1

Total GLP-1 fasting concentrations were analyzed pre-RYGB with 11 studies (15 arms; n= 301) and revealed an estimated 9 pmol/L (95% CI: 6-13) pre-RYGB. At 1-week post-*RYGB,* amongst 2 studies (5 arms; n= 55), the fasting GLP-1 concentration was 9 pmol/L (95% CI: 5-13). At 2 weeks post-RYGB, for 2 studies (n= 35), the estimated fasting concentration was 10 pmol/L (95% CI: 4-16). The concentration whereas it was 9 pmol/L (95% CI: 5-13) at 12 weeks post-RYGB for the 4 studies that were analyzed (7 arms; n= 92). At 52 weeks post-RYGB, 5 studies were analyzed (6 arms; n= 174) and the resulting estimated fasting total GLP-1 was 10 pmol/L (95% CI: 6-13). Finally, at 104 weeks, 2 studies were analyzed (3 arms; n= 100) revealing an estimated 8 pmol/L (95% CI: 6-13) (**Figure 4**). However, there was no difference between any

time point in population estimates. The heterogeneity was significant (σ^2 = 24.4, QE([df = 32] = 359.94, $p < .001$).

The estimated active GLP-1 fasting concentration pre-RYGB across 7 studies (10 arms; n= 122) was 5 pmol/L (95% CI: 2-9), 5 pmol/L (95% CI: 1-8) at 2 weeks post-RYGB for 1 study (2 arms; n= 31), 5 pmol/L (95% CI: 2-9) at 12 weeks post-RYGB for 3 studies (n= 33), and 6 pmol/L (95% CI: 2-10) at 52 weeks post-RYGB for 2 studies (n= 24). Forest plots for active fasting GLP-1 can be found in the supplemental materials. However, there was no significant difference among time points estimated for GLP-1 in the population. The heterogeneity was significant (σ^2 = 11.3, QE[df = 13] = 130.7990, $p < .001$).

For the analysis of total GLP-1 Incremental-AUC (180 minutes postprandial) 2 studies were analyzed pre-RYGB (4 arms; n= 35) and other time points 1 study (3 arms; n= 30). Pre-RYGB the estimate was 694 pmol/L (95% CI: 300-1089), at 1 and 12 weeks post-RYGB the estimate was 5360 pmol/L (95% CI: 4530-6190) and at 12 weeks 4366 pmol/L (95% CI: 3740-4993) respectively (**Figure 5**). Postprandial levels of GLP-1 increased significantly at 1-week and 12-weeks compared to pre-RYGB, QM(df = 3) = 274.77, p < .001. The between-study heterogeneity was σ^2 = 106560.87, but the residual heterogeneity was not significant, QE(df = 6) = 6.922, p = 0.328.

The analysis of GLP-1 Incremental-AUC (240 minutes postprandial) pre-RYGB included 3 studies (4 arms; n=43) and the estimate was 1,385pmol/L (95% CI: 144-2,625). At 1-week post-RYGB 2 studies were analyzed (3 arms; n= 34) and the estimate was 6427 pmol/L (95% CI: 4,627-8,227), at 12 weeks values of 3 studies were analyzed (4 arms; n=43) the estimate was 5,398 pmol/L (95% CI:3871-6926). At 52 weeks post-RYGB 1 study were analyzed (2 arms; n= 25) with an estimate of 7,802 pmol/L (95% CI: 5,060-10,543) (**Figure 6**). Differences in postprandial

GLP-1 levels among all time points post-RYGB are significantly higher compared to pre-RYGB, QM(df = 4) = 35.69, p < .01. There was significant residual heterogeneity, QE(df = 8) = 33.27, p-val < .01. Profile analysis showed the model was unable to estimate σ^2 between-studies.

For the analysis of GLP-1 AUC over 240 minutes postprandially, 2 studies were analyzed at baseline (3 arms; n= 74) and 1 study (2 arms) at 26 weeks and 104 weeks post-RYGB (n= 62). The estimates are 1,872 pmol/L (95% CI: 1,740-2,003), 4,805 pmol/L (95% CI:4,406-5,204) and 4,449 pmol/L (95% CI: 4,088-4,810), respectively (**Figure 7**). Again, the postprandial response of GLP-1 was significantly higher at 26 and 104 weeks post-RYGB compared to pre-RYGB (QM (df = 3) = 366.09, p < .01, when compared to pre-RYGB estimates. Residual heterogeneity was not significant, QE(df = 3) = 0.57, p = 0.90. The model could not estimate sigma squared.

PYY

Pre-RYGB, fasting levels of total PYY values were presented by 8 studies (9 arms; n= 187) and the analysis revealed an estimate of 96 pg/ml (95% CI: 80-112). At 2-weeks post-RYGB, 1 study (2 arms) were analyzed (n= 20) with an estimate of 98 pg/ml (95% CI: 64-133), whereas at 12 weeks (2 studies; n=33) the estimate was 120 pg/ml (95% CI: 102-137). For analyses at 26 and 52 weeks post-RYGB (4 studies; n= 89) the estimate were 116 pg/ml (95% CI: 107-125) and 123 pg/ml (95% CI: 104-142) respectively (**Figure 8**). Fasting levels of PYY increased significantly compared to pre-RYGB at 26 and 52 weeks post-RYGB, p = .034 and .0299 respectively. Nevertheless, the omnibus test of the time point moderator failed to reach significance, F(1, 4) = 4.829, p = .0782. There was no significant residual heterogeneity QE(df = 16) = 8.84, p = .92, σ^2 = .001.

Pre-RYGB, total PYY AUC values over 120 minutes in response to the test meal were presented by 4 studies (5 arms; n= 81) and the estimate was 18,314 pg/ml (95% CI: 10,725-25,903) At 2-weeks post-RYGB, 1 study (2 arms) were analyzed (n= 20) and the estimate was 23,362 pg/ml (95% CI: 12,785-33,939). At 26 and 52 weeks post-RYGB, 3 studies were analyzed (n= 61) and the estimate were 36,647 pg/ml (95% CI: 27,982-45,313) and 37,453 pg/ml (95% CI: 28,795-46,112), respectively (**Figure 9**). Post-prandial levels of PYY were significantly increased at 26 and 52 weeks post-RYGB compared to pre-RYGB values (QM(df = 4) = 45.23, $p < .01$). There was significant heterogeneity, QE(df = 8) = 52.45, $p < .01$, $\sigma^2 = 54348043.35$.

Appetite sensation with VAS

Appetite sensation AUC was reported in a low number of studies and could not be meta-analyzed (data available in **Supplementary Table 3**). Hunger AUC was reported in 11% (k = 5) of studies (44,54,57,59,61) (n=50) at pre-RYGB and up to 12 weeks post-RYGB. Although AUC varied pre-RYGB and was not reported in all studies that measured hunger, data available of post-RYGB hunger values were lower than pre-surgical values but not significantly different in 4 studies (44,57,62,80) and significantly lower in 3 studies (54,59,78). One study found significant lower hunger score at 6 weeks post-RYGB but values were no longer different from pre-RYGB at 3 months post-surgery (61). Fullness AUC was reported in 4% of studies (k=2; n=27) (54,61) pre-RYGB and up to 12 weeks post-RYGB, when compared to pre-surgical values fullness increased after RYGB in both studies but only significantly in one (54). Bryant et al. reported increased fullness at 3 days and 2 months post-RYGB but no significant differences at 1-year (62). Satiety was reported by 6% of studies (k=3; n=23) (44,57,59) at baseline and increased post-RYGB in all studies, increase was significant in 2 of the studies (44,59). The prospective food consumption was reported in 4% of studies (k=2; n=33) (44,54) pre-RYGB and up to 12 weeks post-RYGB and

when compared to pre-surgical values of prospective food consumption was significantly lower post-RYGB in both studies. Satisfaction of the test meal was reported in only 4% of studies (k=2; n=23) (54) and was significantly higher 1-month post-RYGB compared to pre-RYGB.

Weight

Pre-RYGB, 24 eligible studies (31 arms) reported mean weight after an overnight fast (n= 446) and the calculated estimate was 124 kg (95% CI: 118-129). The estimates were 119 kg (95% CI: 21-39) at 1-week (6 studies; 11 arms; n= 199), 115 kg (95% CI: 112-119) at 2 weeks (2 studies; 3 arms; n= 54), 113 kg (95% CI: 105-122) at 4 weeks (4 studies; n= 33), 101 kg (95% CI: 95-107) at 12 weeks (7 studies; 12 arms; n=154), 94 kg (95% CI: 91-97) at 26 weeks (5 studies; n= 143), 87 kg (95% CI: 83-92) at 52 weeks (14 studies; 17 arms; n=290) and 90 kg (95% CI: 83-96) at 104 weeks (4 studies; n=93) (**Figure 10**). Mean weight was significantly lower at all timepoints after RYGB compared to pre-RYGB weight, $F(df1 = 7, df2 = 23) = 198.76$, $p < .0001$. Significant heterogeneity, $QE(df = 79) = 4619.62$ $p < .001$, $\sigma^2 = 78.36$.

Discussion

Summary of evidence

To our knowledge, this is the first meta-analysis to investigate and determine the magnitude of change of gut peptides following RYGB. A meta-analysis was conducted for fasting and postprandial response of ghrelin, GLP-1 and PYY, as well as for weight before and following the RYGB surgery.

Our results show that after an initial decrease in fasting ghrelin levels at 2 weeks post-RYGB, levels at other time points analyzed up to 12 months post-RYGB were not significantly different from pre-surgical values (**Figure 2**). In light of the observed heterogeneity, it is unclear if the decrease in fasting ghrelin at 2-week post-RYGB is robust. The postprandial levels of ghrelin

following RYGB were not different from pre-surgical levels at 6 months and 1-year post-surgery (mean pre-RYGB: 51,875 pg/ml, 6 months post: 51,389 pg/ml and 1 year post: 51517 pg/ml). Although available data is limited, **Figure 3** illustrates that following RYGB postprandial levels of ghrelin in the included studies did not change. Our results are in accordance with another previously published meta-analysis that investigated standard mean differences of fasting levels of ghrelin only before and after RYGB, where results were divided into short-term (<3 months) and long-term (>3 months) change post-RYGB (23). They found a significant decrease in fasting ghrelin levels in the short-term and a contrasting increase in the long-term (23). Our analysis also points to a short-term slight but significant decrease in fasting ghrelin levels at 2 weeks post-RYGB, yet levels appear to return to pre-RYGB levels from 6 weeks to 1 year as opposed to the increase described by Xu et al. (23). Potential sources for the different findings could include the lower number of participants post-RYGB in our study since we performed a multilevel analysis while they combined all data >3 months post-RYGB together. A recent non-systematic review described that more studies found no changes in fasting and postprandial ghrelin levels after RYGB, while still a few reported a decrease or increase (86).

Although fasting levels of total and active GLP-1 could not be combined for the analyses, the outcome for the individual analyses were congruent. Indeed, fasting GLP-1 was not different between pre-RYGB and up to 2 years post-surgery for total GLP-1 and up to 1-year post-RYGB for active GLP-1 (**Figure 4**). For both active and total fasting GLP-1, heterogeneity was significant (p< .001). The wide variability in pre-RYGB datapoints indicates a confounder most likely influenced the sample of included studies before the intervention. The source of this confounder could be derived from participant or measurement variability, though the exact cause cannot be identified with the given information in the included studies. Fasting GLP-1 levels are higher

compared to other similar studies at all time points measured, including at baseline in the Nannipieri study (84). Notably, this study did not provide the number of women evaluated or average age of the participants. They also provided a semi-solid test meal whereas some studies in our analysis offered a liquid meal test. These characteristics along with assay kit variability could be potential confounders, however the provided information was not sufficient to consider the study an outlier.

Postprandial responses of GLP-1 were computed in 3 different analyses. There were methodological differences in blood sampling times and appraisal of both incremental and total data in the included studies, thus preventing synthesis of all postprandial GLP-1 in one analysis. Although only a small number of studies were included in each separate analysis, all 3 trended towards similar results. GLP-1 incremental-AUC at 180 minutes increased significantly at 1 week post-RYGB, as well as 3 months post-RYGB, compared to pre-surgical values. The 3-month estimate represents approximately a 6.3-fold increase from pre-RYGB. Results were similar for incremental-AUC at 240 minutes with a significant increase at 1-year post-RYGB. The 1-year estimate represents a 5.6-fold increase compared to pre-RYGB. The third analysis, total GLP-1 at 240 minutes, combined data at time points of 6 months and 2 years post-RYGB where differences compared to pre-RYGB were significantly higher. In this analysis at 6 months and 2-years post-RYGB, the estimate represents a 2.6-fold and 2.4-fold increase, respectively, compared to the pre-RYGB mean. **Figures 5-7** together illustrate the clear increase in GLP-1 levels starting at 1 week extending up to 2 years post-RYGB in our population. Since heterogeneity could not be estimated due to the small study sample size, the results should be interpreted with caution and limited to our population. Nevertheless, consistent with our results are in agreement with the central role of GLP-1 as a modulator of appetite in humans. The results are also in agreement with earlier studies

showing an appetite-suppressing role of GLP-1 (87), as well as more recent trials using GLP-1 agonists for weight loss (88). In 2018, Jirapinyo et al. published a meta-analysis on fasting and post-prandial GLP-1 changes after RYGB (89). Consistent with our findings, they also concluded that fasting GLP-1 was unchanged after RYGB and that postprandial GLP-1 increased following RYGB (89). While direction of changes is consistent with the previous meta-analysis, our analysis provides additional information on the extent of these changes at different time points post-RYGB and expected ranges of GLP-1 in our study population.

Fasting PYY levels were significantly higher than pre-surgical values at both 6 months and 1 year post-RYGB. The 1-year estimate represents approximately a 1.3-fold increase from pre-RYGB (**Figure 8**). However, since the omnibus test of the time point moderator did not reach significance, the fasting PYY results cannot be considered robust. Postprandial values of PYY increased significantly at 6 months (2-fold increase) and 1-year post-surgery (2-fold increase) compared to pre-RYGB. Only a very small number of studies could be analyzed for AUC of PYY (k= 4), the model points towards no between study heterogeneity but rather random sampling error, it is unclear if the analysis results are robust. Our results are in agreement with the appetite-suppressing role of PYY, similarly to GLP-1 as they both exert similar effects on the central nervous system (90,91). Fasting PYY remaining unchanged post-RYGB and higher levels of PYY post-surgery were also reported in a review published in 2021 where no statistical synthesis was performed (86).

Results regarding appetite sensation remain unclear because the data presented in the included studies could not be compared to each other due to the presence of subcategories of appetite sensations (e.g., hunger and satiety) and time points post-RYGB and/or time of AUC that did not match between studies, thus hindering further analyses. In addition, values of AUC over 3

months post-RYGB were unavailable, giving a very small window in time to compare values to pre-surgical scores or further even compare appetite sensation scores to appetite-related hormone changes over time.

Evaluating the changes of weight following RYGB was not a main objective of this review. However, it shows that the RYGB intervention led to significant weight loss in the studies that were included in the systematic review/meta-analysis. As such, these results should not be taken as a reflection of the effects of RYGB on weight loss, but rather to show that the peptide profiles analyzed and presented herein were drawn from a population of individuals with obesity who underwent significant weight losses. As can be expected, participants of the included studies did experience a significant weight loss and reached weight nadir at or after 12 months post-RYGB.

Strength and Limitations

This systematic review and meta-analysis is the first of its kind as it includes multilevel models which allowed for the illustration of the magnitude of change of ghrelin, GLP-1 and PYY at distinct time points post-RYGB compared to pre-surgical values. However, this study is not without significant limitations. Firstly, only articles available in English or French were assessed. Moreover, the included studies show high levels of heterogeneity. Plausible explanations for this could be the variability in type of assay kit, providers, time span between included studies, lack of duplicate assays and even inter-study lot-to-lot variability in longitudinal studies. Assay kit variability cannot be neglected as a source of variability, as pre-RYGB values of main outcomes in this meta-analysis vary greatly, even when corrected for meal test caloric intake. Only a very limited number of included studies (k=6) performed duplicate assays of main outcomes, which is necessary because it leads to higher reliability of results and facilitates the identification of outliers within and between studies (92).

Lastly, only a small number of studies could be compared in the analyses. Variability in protocol of main outcome measurements was high, which further added to the complexity for study comparison. Sub-analyses were required and multiple data points could not be included in the meta-analysis when comparable data was not available. This combined with high heterogeneity between studies at baseline limits our interpretation of results. Although the conclusions should be interpreted with caution, this study sheds light on the current state of the literature. Higher quality measurements of appetite hormones and standardized protocols of post-prandial response measurements could provide more consistent results for future meta-analyses for these outcomes. Since bariatric surgery and appetite hormone antagonists are gaining popularity and availability due to the growing population in need of effective long-term weight loss interventions, pooling data of more studies to solidify knowledge and comprehension of these mechanisms of weight loss following RYGB is required.

Implication of findings and future research

This review provided new information regarding the magnitude of change of appetite-related hormones following RYGB surgery. Although significant changes compared to pre-surgical levels of gut peptides were found, methodological inconsistencies and large heterogeneity between studies prevent strong conclusions regarding the effect of the RYGB surgery on appetite-related hormones. Future research should consider evaluating longer term effects of RYGB on appetite-related hormones and investigate whether fluctuations are present with weight regain over time. Additional studies measuring appetite sensation are required and should be considered when assessing gut peptides. Measuring appetite sensation via a VAS provides additional information on appetite perception of the participants and is highly accessible and reproducible (93). Other considerations for prospective studies include thorough descriptions of participant characteristics,

assay kits, surgery details and meal duration. These additions would improve transparency and potentially lower between-study heterogeneity. Standardization of postprandial response measurements, including units and time of the AUC for each outcome (i.e., ghrelin, GLP-1 and PYY) would be beneficial for future synthesis of results. Additionally, disclosure of mean fasting and AUC levels of peptides in all future published studies is prompted for effective analysis of the literature.

Conclusion

In conclusion, this meta-analysis provides insight on the current state of the literature regarding changes in fasting and postprandial gut peptides before and following RYGB surgery. While heterogeneity between studies limits our findings to the population of included studies, our data shows that following RYGB, fasting ghrelin levels were lower than pre-RYGB only at 2 weeks post surgery. While postprandial levels were unchanged. GLP-1 fasting levels were unchanged after RYGB, although for the postprandial response of GLP-1, an important increase was present early after surgery and up to 2 years post-RYGB. PYY levels were increased at both the fasted state and postprandially after the surgery. More standardized research with longer follow-up periods is needed to confidently understand changes in gut peptides following RYGB surgery.

List of Abbreviations:

AUC: Area under the curve

BA: Before and after study design

CI: Confidence interval

ECLIA: Electrochemiluminescence-based immunoassays

ELISA: Enzyme-linked immunosorbent assay

GLP-1: Glucagon-like peptide-1

Kcal: kilocalorie

NGT: Normal glucose tolerance

NR: Not Reported

PYY: Peptide YY

QE: Quasi-experimental study design

RCT: Randomized control trial

RIA: Radioimmunoassay

RYGB: Roux-en-Y gastric bypass

SD: Standard deviation

T2D: Type 2 diabetes

USA: United States of America

VAS: Visual analogue scale

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Table 1. Summary of study characteristics, main findings and quality assessment rating of included studies.

Author, year of publication (Country) *Design*	n (% W)	Average age±SD (range) [25th, 75th percentile]	Initial BMI Kg/m²±SD (range)	Test meal (kcal)	Post-prandial AUC time (minutes)	Type of assay (Provider)	Time post-RYGB of outcome measures					Outcomes of interest	Main findings	Quality Assessment rating
							Total (m)	0-3 m.	4-6 m.	7-12 m.	12+ m.			
Alamuddin et al., 2017 (41) (USA) *QE*	23 (100)	35.5±2	44.6±0.9	240	180	ELISA (Millipore)	18		✓		✓	Ghrelin GLP-1 PYY	F and AUC Ghrelin: = 6 m, = 12 m (control) F GLP-1: = 6 m, = 12 m AUC GLP-1: ↓ 6m, = 12m F and AUC active PYY: ↓ 6 m, ↓ 12 m	2
Arakawa et al., 2020 (25) (USA) *BA*	40 (80)	43.6±1.6 (22-64)	46.4±0.9 [w]	320	120	RIA (Millipore)	12		✓	✓		Ghrelin [f, a] PYY [f, a]	F and AUC Ghrelin: = 6m, = 12m F PY Y: ↑ 6m, = 12m AUC PYY: ↑ 6m, ↑ 12m	3
Bryant et al., 2013 (62) (England) *BA*	12 (75)	36±2	30.3±1.8	300	180	RIA (NR)	12	✓		✓		Ghrelin GLP-1 VAS	AUC Ghrelin: = 3 days, = 2m, = 12m AUC GLP-1: = 3 days, ↑ 2m, ↑ 12m AUC VAS hunger: = 3 days, = 2m, = 12m AUC VAS Fullness: ↑ 3 days, ↑ 2m, = 12m	1
Campos et al., 2010 (63) (USA) *QE*	12 (75)	47.4±8.7	48.4±6.8	300	180	ELISA (Millipore)	6	✓	✓			GLP-1	AUC GLP-1: ↑ 6m	2
Cazzo et al., 2017 (42) (Brazil) *BA*	11 (55)	36.7±8.2	46.3±3.1 [w]	515	180	ELISA (Millipore)	12			✓		GLP-1 VAS	AUC GLP-1: = 12m °VAS hunger: ↑ 12m °VAS fullness: ↑ 12m °VAS satisfaction: ↑ 12m °VAS PFC: ↓ 12m °VAS one point post-prandial only	1
Chronaiou et al., 2012 (64) (Greece) *RCT*	12 (67)	NR	46.1±2.2 [w]	300	120	RIA (Millipore)	12	✓	✓	✓		Ghrelin [f, a] GLP-1 PYY [f, a]	F ghrelin: ↓ 3m, = 6m, ↑12m F GLP-1: = 3m, = 6m, = 12m F PYY: = 3m, ↑ 6m, ↑ 12m P values for AUC NR	3
Evans et al., 2012 (78) (USA) *QE*	20 (70)	49.6±11.2	45.6±7.6	262	120	Multiplex immunoassay (Millipore)	0.5	✓				GLP-1 PYY [f, a] VAS	F GLP-1: = 2 weeks AUC GLP-1: ↑ 2 weeks F PYY: = 2 weeks AUC PYY: = 2 weeks AUC VAS hunger: ↓ 2 weeks	2
Fellici et al., 2015 (65) (Brazil) *BA*	36 (47)	47.4±8.4 (31-54)	32.1±0.3 [w]	515	180	ELISA (Linco)	12	✓	✓	✓		GLP-1	AUC GLP-1: ↑ 3m. ↑ 6m, ↑ 12m	1

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Author, year of publication (Country) *Design*	n (% W)	Average age±SD (range) [25th, 75th percentile]	Initial BMI Kg/m²±SD (range)	Test meal (kcal)	Post-prandial AUC time (minutes)	Type of assay (Provider)	Time post-RYGB of outcome measures					Outcomes of interest	Main findings	Quality Assessment rating
							Total (m)	0-3 m.	4-6 m.	7-12 m.	12+ m.			
Foschi et al., 2008 (79) (Italy) *BA*	10 (90)	43.1±4.1	32.1±0.3	504	180	RIA (DRG diagnostics)	6		✓			Ghrelin	P values for comparison pre-RYGB NR	2
Griffo et al., 2016 (43) (Italy) *BA*	9 (56)	46±7	42±6 [w]	304	180	ELISA (Millipore)	24				✓	GLP-1	AUC GLP-1: ↑ 24m	1
Halliday et al., 2019 (44) (USA) *QE*	6 (100)	37.6±4.6	41.4±1.4	200	180	ELISA (Alpco diagnostics)	1	✓				Ghrelin [f] GLP-1 [f] PYY [f] VAS	F ghrelin: ↓ 1m F GLP-1: = 1m F PYY: = 1m F VAS hunger, satiety and PFC: = 1m AUC ghrelin: ↓ 1m AUC GLP-1: = 1m AUC PYY: = 1m AUC VAS hunger: ↓ 1m AUC VAS satiety and PFC: ↑ 1m	2
Hansen et al., 2011 (85) (USA) *BA*	9 (67)	41±11	51.9±6 [w]	250	240	RIA (Millipore)	1.5	✓				Ghrelin [f] GLP-1	P values for comparison to pre-RYGB NR	2
Ilesanmi et al., 2021 (45) (United Kingdom) *BA*	10 (80)	50.2±13.2	NR [w]	330	180	Milliplex magnetic bead-based multi-analyte (Millipore)	12	✓		✓		GLP-1 [f]	F GLP-1: = 1m, ↑ 12m AUC GLP-1: ↑ 1m, ↑ 12m	1
Jørgensen et al., 2013 (74) (Denmark) *BA*	9 (33)	50±3	39.5±2.5	300	240	RIA (NR)	3	✓				GLP-1 [a]	F GLP-1: ↓ 1 week, ↓ 3m	2
Jacobsen et al., 2012 (75) (Denmark) *BA*	8 (75)	35.5±8.8 (26-55)	*NR [w]	300	210	RIA (Linco)	0.5	✓				Ghrelin [f] GLP-1 [f] PYY	F and AUC Ghrelin: ↓ 2 weeks F GLP-1: = 2 weeks AUC GLP-1: ↓ 2 weeks F and AUC PYY: ↓ 2 weeks	1
Jacobsen et al., 2013 (76) (Denmark) *BA*	12 (75)	NR	*39.2±1.8	300	240	RIA (NR)	3	✓				GLP-1 [f]	F and AUC GLP-1: ↑ 3m	2
Jiménez et al., 2012 (66) (Spain) *BA*	6 (NR)	43±13	44.8±4.6	398	120	RIA (Millipore)	47				✓	GLP-1	P values for comparison to pre-RYGB NR	2

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Author, year of publication (Country) *Design*	n (% W)	Average age±SD (range) [25th, 75th percentile]	Initial BMI Kg/m²±SD (range)	Test meal (kcal)	Post-prandial AUC time (minutes)	Type of assay (Provider)	Time post-RYGB of outcome measures					Outcomes of interest	Main findings	Quality Assessment rating
							Total (m)	0-3 m.	4-6 m.	7-12 m.	12+ m.			
Jørgensen et al., 2012 (67) (Denmark) *BA*	25 (75)	52±9	NGT (a) *41.5±4.8 [w] T2D (b) *43.1±5.1 [w]	300	240	RIA (NR)	12	✓		✓		GLP-1 [f, a]	F GLP-1 NGT: = 1 week, = 3m, =12m F GLP-1 T2D: = 1 week, = 3m, ↑12m AUC GLP-1 NGT and T2D: ↑ 1week, ↑ 3m, ↑ 12m	2
Korner et al., 2009 (68) (USA) *BA*	28(86)	45±2	48±1	320	120	RIA (Linco)	12		✓	✓		Ghrelin [f] GLP-1 [f] PYY [f]	F Ghrelin: = 2 weeks, = 3m, = 6m, = 12m F GLP-1: = 6m, ↑ 12m F PYY: = 2 weeks, = 3m, = 6m, ↑ 12m	3
Lindegaard et al., 2015 (69) (Denmark) *BA*	25 (NR)	NR	NGT (a) *41.5±4.8 [w] T2D (b) *43.2±5.3 [w]	300	240	RIA (NR)	12	✓		✓		GLP-1 [a]	AUC GLP-1: ↑ 1week, ↑ 3m, ↑12m	2
Lips et al., 2014 (58) (The Netherlands) *QE*	31 (100)	NGT (a) 18.6±1.6 T2D (b) 51.3±1.9	NGT (a) 44.2±0.8 [w] T2D (b) 43.5±1.1 [w]	400	180	ELISA (Millipore)	0.75	✓				Ghrelin [f] GLP-1 [f] PYY	F and AUC Ghrelin NGT and T2D: ↓ 3 weeks F GLP-1 NGT and T2D: = 3weeks AUC GLP-1 NGT and T2D: ↑ 3weeks F PYY NGT: = 3 weeks F PYY T2D: ↓ 3 weeks AUC PYY NGT and T2D: ↑ 3 weeks	2
Magro et al., 2018 (46) (Brazil) *BA*	11 (55)	36.7±8.2	46.3±3.1 [w]	515	180	ELISA (Millipore)	12			✓		GLP-1	AUC GLP-1: = 12m	2
Martinussen et al., 2015 (77) (Denmark) *BA*	30 (67)	NGT (a) *41±2.8 IGT (b) *42±3.3 T2D (c) *46±2.8	NTG (a) *43.2±1.3 [w] IGT (b) *40.4±1.4 [w] T2D (c) *41.2±1.3 [w]	300	180	RIA (NR)	3	✓				GLP-1 [f, a]	F GLP-1 NGT, IGT and T2D: = 1 week, = 3m AUC GLP-1 NGT, IGT and T2D: ↑ 1 week. ↑ 3m	2
Miras et al., 2021 (47) (United Kingdom) *Mechanistic study*	53 (59)	49±10 Long (b) 48±9	42±6 Long (b) 43±8	300	120	Multiplexed Magpix immunoassay (NR)	12	✓	✓	✓		GLP-1 PYY	AUC GLP-1: = 2 weeks, other time points NR AUC PYY: = 2 weeks, other time points NR Compared to pre-RYGB	2
Morínigo et al., 2004 (81) (Spain) *QE*	8 (63)	46±3.1	49.5±1.8	398	120	RIA (Linco)	1.5	✓				Ghrelin [f]	F Ghrelin: ↓ 6 weeks AUC Ghrelin: = 6 weeks	2

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Author, year of publication (Country) *Design*	n (% W)	Average age±SD (range) [25th, 75th percentile]	Initial BMI Kg/m²±SD (range)	Test meal (kcal)	Post-prandial AUC time (minutes)	Type of assay (Provider)	Time post-RYGB of outcome measures					Outcomes of interest	Main findings	Quality Assessment rating
							Total (m)	0-3 m.	4-6 m.	7-12 m.	12+ m.			
Morínigo et al., 2006 (80) (Spain) *QE*	9 (78)	39.4±10.7	47.4±6.1	398	120	RIA (Linco)	1.5	✓				GLP-1 PYY VAS	AUC GLP-1: ↑ 6 weeks AUC PYY: ↑ 6 weeks AUC VAS hunger: = 6weeks AUC VAS satiety: ↑ 6 weeks	2
Morínigo et al., 2006 (70) (Spain) *BA*	NGT (a) 12 (75) IGT (b) 12 (75) T2D (c) 10 (50)	NGT (a) 41.8±2.7 IGT (b) 46.8±3.2 T2D (c) 50.05±3	NGT (a) 49.7±1.7 IGT (b) 48.6±1.8 T2D (c) 49±2.1	398	120	RIA (Linco)	12	✓		✓		GLP-1 [f]	F GLP-1 NGT, IGT and T2D: = 6 weeks AUC GLP-1 NGT, IGT: ↑ 6 weeks, ↑ 12m AUC GLP-1 T2DL = 6 weeks, ↑ 12m	1
Morínigo et al., 2008 (71) (Spain) *QE*	25 (24)	43.6±2.1	48.8±1.2	398	120	RIA (Linco)	12	✓		✓		PYY	F PYY NGT and T2D: = 6 weeks, = 12m AUC PYY NGT and T2D: ↑ 6 weeks, ↑ 12m	2
Nannipieri et al., 2013 (84) (Italy) *BA*	23 (NR)	NR	41.8±5.4 [w]	350	300	ELISA (Millipore)	12	✓		✓		Ghrelin GLP-1 [f] PYY	F and AUC Ghrelin: = 2 weeks, ↓ 12m F GLP-1: ↑ 2 weeks, ↓ 12m AUC GLP-1: ↑ 2 weeks, = 12m F and AUC PYY: = 2 weeks, ↑ 12m	2
Nguyen et al., 2015 (72) (USA) *RCT*	60 (63)	49±9	35.8±0.5	250	120	ELISA (Millipore)	12			✓		GLP-1 [f]	F GLP-1 : ↓ 12m	1
Nosso et al., 2016 (48) (Italy) *BA*	14 (57)	49±7	42±6 [w]	304	180	ELISA Merck-(Millipore)	12			✓		Ghrelin GLP-1 [f]	F ghrelin: = 12m F GLP-1: = 12m AUC GLP-1: ↑ 12m	2
Peterli et al., 2009 (82) (Switzerland) *Randomized parallel group study*	13 (NR)	41.8±10.4 (23.6-53.9)	47±6.4 [w]	400	180	ELISA (Linco)	3	✓				Ghrelin GLP-1 [f] PYY	AUC Ghrelin: ↑ 1 week, ↑ 3m AUC GLP-1: ↑ 1 week, ↑ 3m AUC PYY: ↑ 1 week, ↑ 3m F P values NR	2
Pop et al., 2018 (94) (USA) *BA*	10 (NR)	53.2 (48-58.4)	51.14 (45.96-56.32)	360	360	ECLIA (Meso Scale Discovery)	0.3	✓				Ghrelin GLP-1 PYY	F and AUC Acyl ghrelin: ↓ 10 days F and AUC GLP-1: ↑ 10 days F PYY: = 10 days AUC PYY: ↑ 10 days	2

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Author, year of publication (Country) *Design*	n (% W)	Average age±SD (range) [25th, 75th percentile]	Initial BMI Kg/m²±SD (range)	Test meal (kcal)	Post-prandial AUC time (minutes)	Type of assay (Provider)	Time post-RYGB of outcome measures					Outcomes of interest	Main findings	Quality Assessment rating
							Total (m)	0-3 m.	4-6 m.	7-12 m.	12+ m.			
Purnell et al., 2018 (50) (USA) *BA*	62 (77)	NGT (a) 52 [45, 54] T2D (b) 52 [47, 57]	NGT (a) 45.7 [43.6, 50.7] T2D (b) 47.9 [43.1, 54.8]	360	240	Single-plex immunoassays (Meso Scale Discovery)	24		✓		✓	GLP-1 [f, a]	F GLP-1 NGT and T2D: ↓ 6m, ↓ 24m AUC GLP-1 NGT and T2D: ↑ 6m, ↑ 24m	2
Rao et al., 2017 (51) (USA) *QE*	21 (81)	43.1±2.4	47.8±1.7 [w]	320	120	Single-plex immunoassays (Meso Scale Discovery)	6		✓			PYY [f]	F PPY: = 6m AUC PYY: ↑ 6m	3
Schmidt et al., 2016 (52) (Denmark) *QE*	14 (71)	37.7±10.4	45.4±5.1	310	180	Milliplex (Linco)	1	✓				Ghrelin GLP-1 PYY VAS	P values for comparison to pre-RYGB NR	2
Shah et al., 2019 (53) (USA) *BA*	5 (80)	51±6	NR [w]	220	180	C-terminal assay (Millipore)	1	✓				GLP-1 [a]	F GLP-1: = 1m	1
Shankar et al., 2017 (54) (USA) *QE*	17 (100)	45±10	53.3±3.5	389	120	RIA (Millipore)	1	✓				GLP-1 PYY VAS	AUC GLP-1: ↑ 2 weeks, ↑1m AUC PYY: ↑ 2 weeks, ↑1m AUC VAS hunger: ↓ 2 weeks, ↓ 1m AUC VAS Fullness, Meal satisfaction and PFC: ↑ 2 weeks, ↑ 1m	2
Stano et al., 2017 (56) (USA) *BA*	21 (92)	L-group (a) 35.3±10.5 S-group (b) 34.8±9.2	L-group (a) 47.4±6.62 [w] S-group (b) 48.02±9.28	600	360	RIA (Millipore)	12			✓		GLP-1	L-group AUC GLP-1: ↑ 12m S-group AUC GLP-1: = 12m	1
Steven et al., 2016 (55) (United Kingdom) *QE*	9 (NR)	NR	43±1.1	100	120	ELISA (AlpcoDiagnositics)	0.25	✓				GLP-1	AUC GLP-1: ↑ 1 week	2
Svane et al., 2016 (57) (Denmark) *BA*	9 (33)	50±3	*39.2±2.5	301	240	RIA (Millipore)	3	✓				GLP-1 [f, a] PYY VAS	F GLP-1: = 3m AUC GLP-1: ↑ 3m F PYY: = 3m AUC PYY: ↑ 3m AUC VAS hunger and satiety: = 3m	2

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Author, year of publication (Country) *Design*	n (% W)	Average age±SD (range) [25th, 75th percentile]	Initial BMI Kg/m²±SD (range)	Test meal (kcal)	Post-prandial AUC time (minutes)	Type of assay (Provider)	Time post-RYGB of outcome measures					Outcomes of interest	Main findings	Quality Assessment rating
							Total (m)	0-3 m.	4-6 m.	7-12 m.	12+ m.			
Tsouristakis et al., 2019 (95) (USA) *BA*	38 (NR)	43.8±2.5	47.2±0.7 [w]	320	120	RIA (Millipore)	48		✓	✓	✓	Ghrelin [f, a] GLP-1 PYY [f, a]	F and AUC Ghrelin: = 6m, = 12m, ↑ 24m, ↑ 36m, ↑ 48m F GLP-1: = 6m, = 12m, = 24m, = 36m, = 48m AUC GLP-1: ↑ 6m, ↑ 12m, ↑ 24m, ↑ 36m, ↑ 48m F and AUC PYY: ↑ 6m, ↑ 12m, ↑ 24m, ↑ 36m, ↑ 48m	3
Umeda et al., 2011 (83) (Brazil) *BA*	10 (NR)	NR	39.7±1.9 [w]	353	120	ELISA (Linco)	3	✓				GLP-1 [f]	F GLP-1: = 1 week, ↓ 1m, = 3m AUC GLP-1: = 1 week, ↑ 1m, ↑ 3m	1
Valderas et al., 2010 (59) (Chile) *QE*	8 (100)	38.7±4.5	37.8±0.8	355	180	RIA (Linco)	2	✓				PYY [f] VAS	F PYY: = 2m AUC PYY: ↑ 2m AUC VAS hunger: ↓ 2m AUC VAS satiety: ↑ 2m	2
Yan et al., 2014 (60) (USA) *QE*	20 (100)	NGT (a) 36.7±2.4 T2D (b) 49.1±2.0	NGT (a) 44.3±1.1 [w] T2D (b) 46.6±2.3 [w]	75, 150 and 300	120	ELISA (Millipore)	3	✓				GLP-1 PYY	75 kcal NGT and T2D AUC GLP-1: = 1 week, = 3m 150 kcal NGT AUC GLP-1: ↑ 1 week, ↑ 3m 150 kcal T2D AUC GLP-1: ↑ 1 week, = 3m 300 kcal NGT and T2D AUC GLP-1: ↑ 1 week, ↑ 3m 75 kcal NGT and T2D AUC PYY: = 1 week, = 3m 150 kcal NGT and T2D AUC PYY: ↑ 1 week, ↑ 3m 300 kcal NGT and T2D AUC PYY: ↑ 1 week, ↑ 3m	1
Yang et al., 2014 (73) (China) *BA*	16 (56)	35.2±11.8 (20-55)	38.6±6.4 [w]	300	120	ELISA (Novatein)	12			✓		Ghrelin	F ghrelin: ↑ 12m	3
Yousseif et al., 2014 (61) (United Kingdom) *BA*	10 (100)	46.8±1.5	45±1.5	500	180	ELISA (Millipore)	3	✓				Ghrelin GLP-1 [f] PYY VAS	F and AUC ghrelin: = 6 weeks, = 3m F GLP-1: = 6 weeks, = 3m AUC GLP-1: ↑ 6 weeks, ↑ 3m F PYY: = 6 weeks, = 3m AUC PYY: ↑ 6 weeks, ↑ 3m F VAS hunger: ↓ 6 weeks, ↓ 3m AUC VAS hunger: ↓ 6 weeks, = 3m F VAS fullness: = 6 weeks, = 3m AUC VAS fullness: ↓ 6 weeks, ↓ 3m	1

^{f} = fasting data was meta-analyzed; ^{a} = AUC data was meta-analyzed; ^{w} = weight was meta-analyzed; ***** Initial BMI pre-RYGB after 8% mandatory weight loss; ELISA = Enzyme-linked immunosorbent assay; ECLIA = Electrochemiluminescence-based immunoassays; RIA = Radioimmunoassay; m = months; AUC = area under the curve; F = fasting, PFC = prospective food consumption. All significant ($p < 0.05$) differences compared to pre-RYGB (unless stated otherwise) are represented by arrows in the direction of the change (↓ or ↑), non-significant change is represented by (=); NR = not reported

Figure Legends:

Figure 1. PRISMA 2020 Flow Chart for identification of studies.

Figure 2. Fasting ghrelin levels and estimates (pg/ml) of included studies before and 2, 6, 26 and 52 weeks post-RYGB.

Figure 3. Postprandial response (120 minutes; total AUC) of ghrelin levels and estimates (pg/ml) of included studies before RYGB, 26 and 52 weeks post-RYGB.

Figure 4. Fasting GLP-1 levels and estimates (pmol/L) of included studies before and 1, 2, 12, 52 and 104 weeks post-RYGB.

Figure 5. Postprandial response (180 minutes; incremental-AUC) of GLP-1 levels and estimates (pmol/L) of included studies before RYGB, 1,12 and 52 weeks post-RYGB.

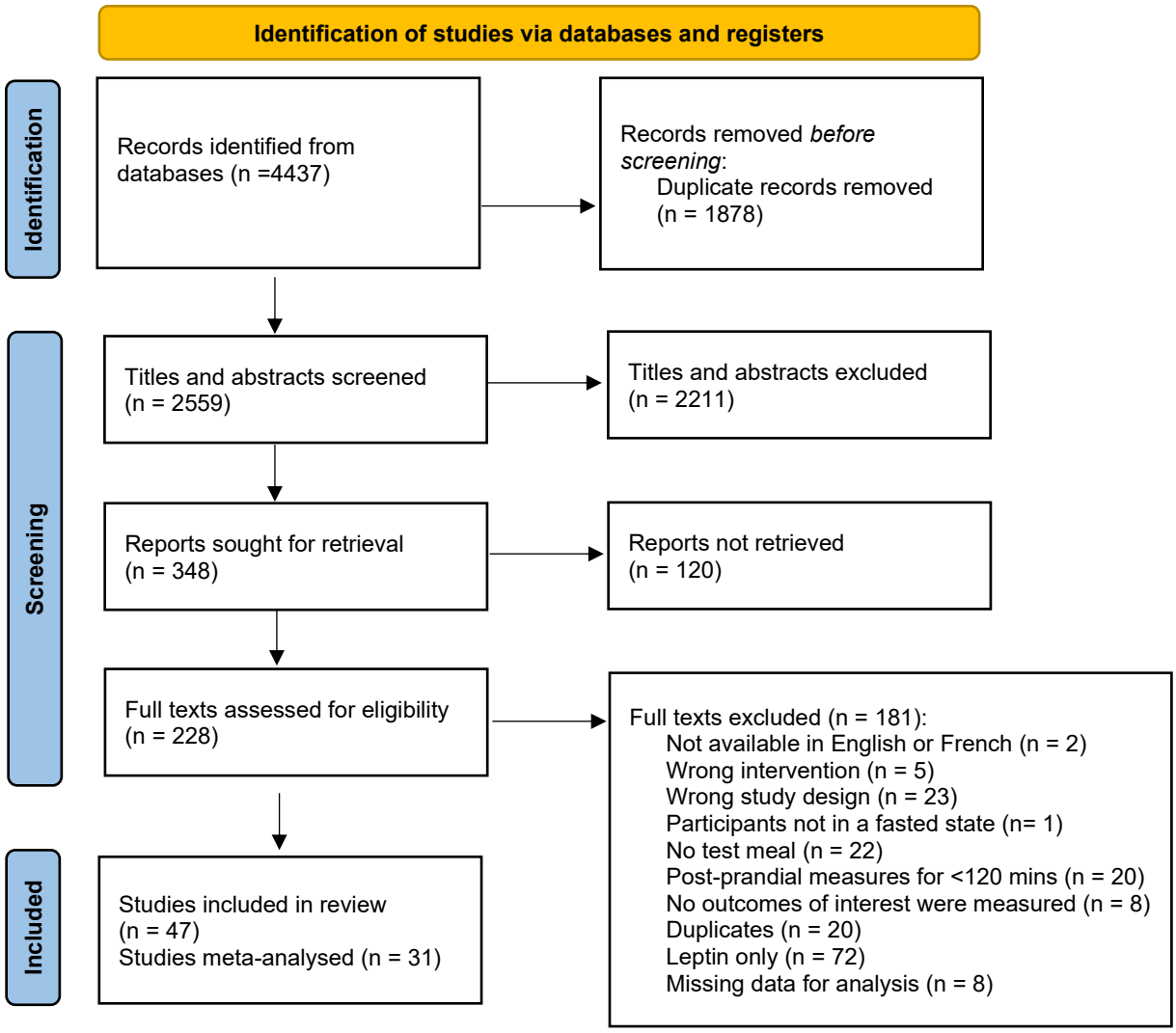
Figure 6. Postprandial response (240 minutes; incremental-AUC) of GLP-1 levels and estimates (pmol/L) of included studies before RYGB, 1, 12 and 52 weeks post-RYGB.

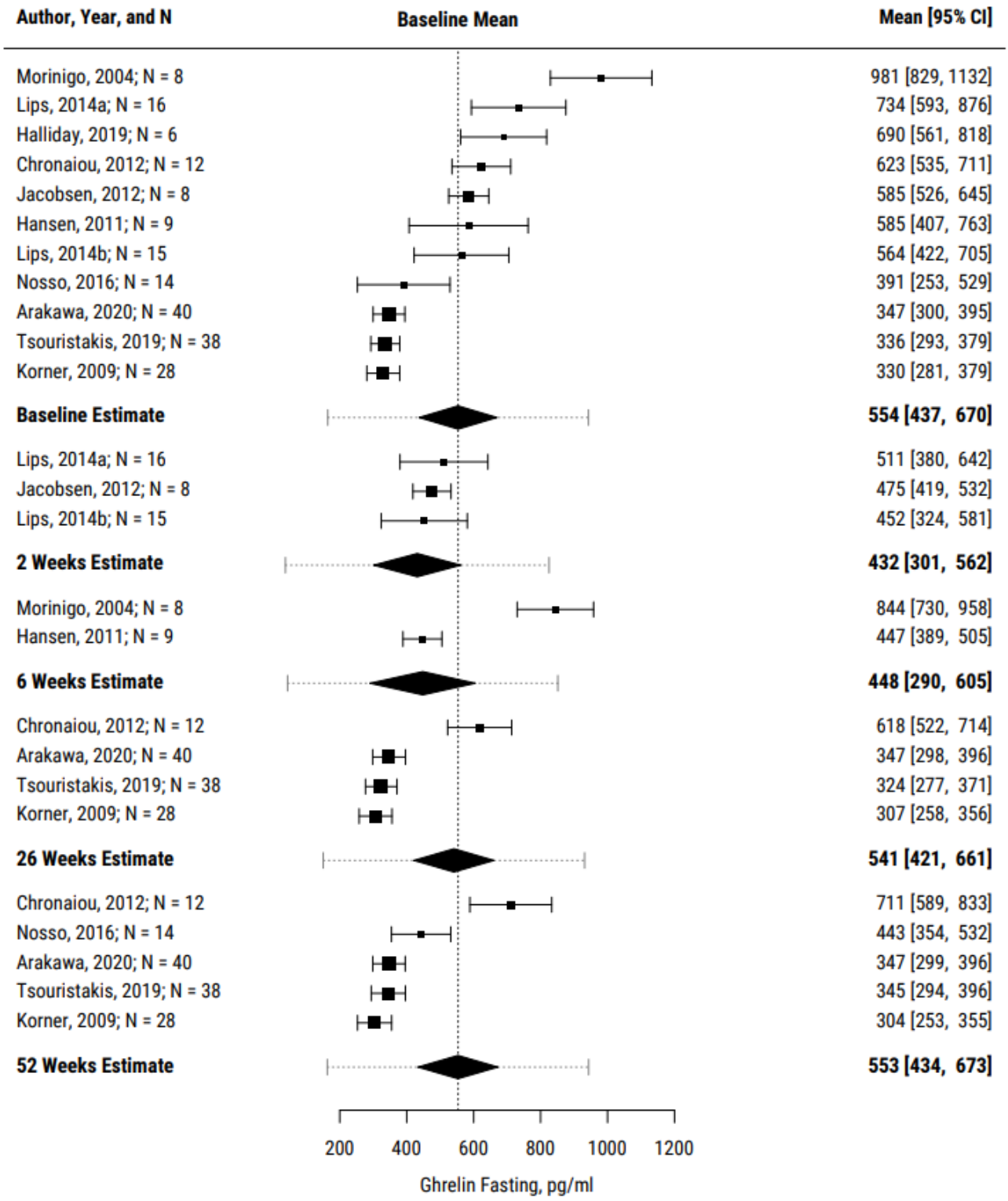
Figure 7. Postprandial response (240 minutes; total AUC) of GLP-1 levels and estimates (pmol/L) of included studies before RYGB, 26 and 104 weeks post-RYGB.

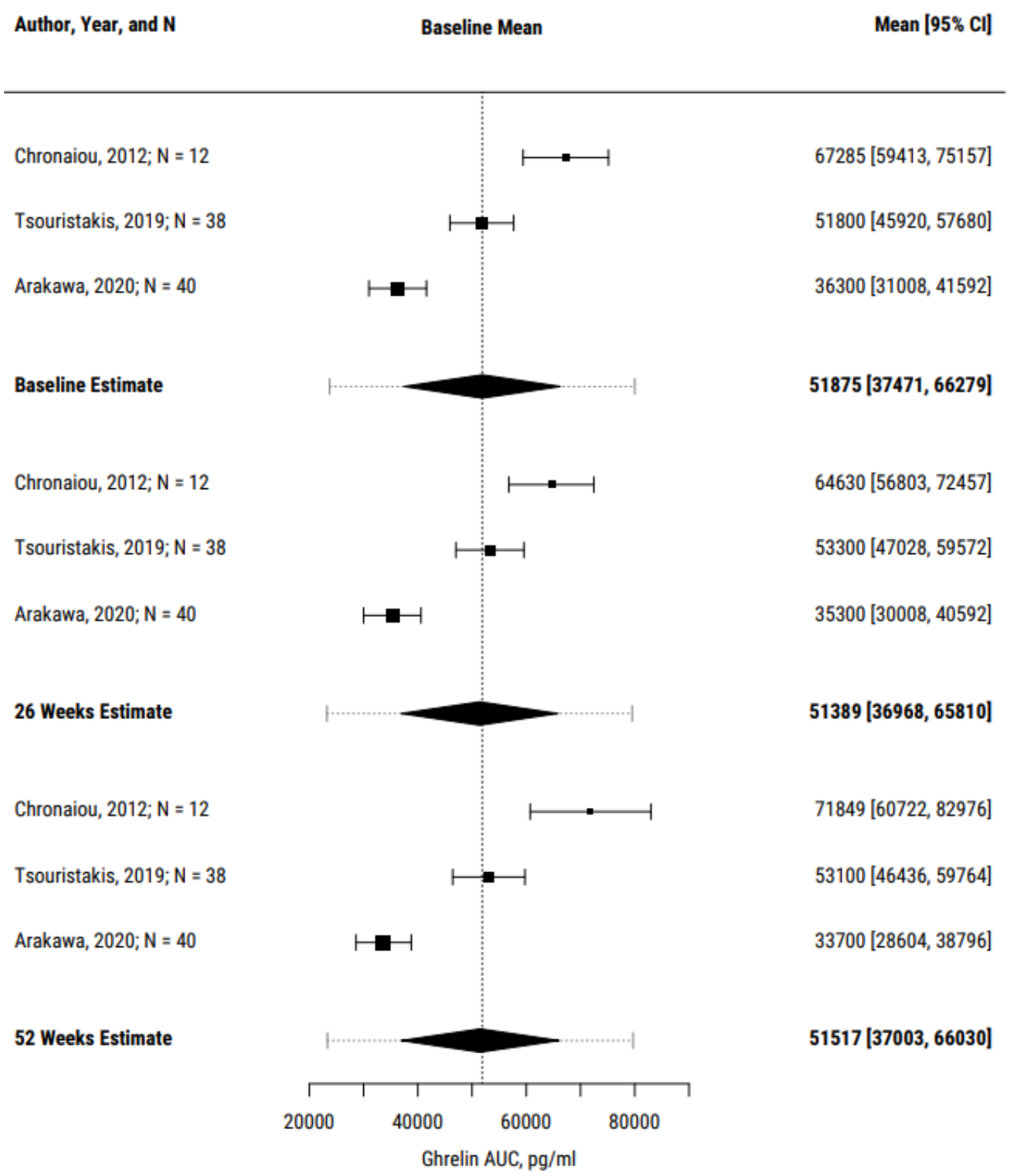
Figure 8. Fasting PYY levels and estimates (pg/ml) of included studies before and 2, 12, 26 and 52 weeks post-RYGB.

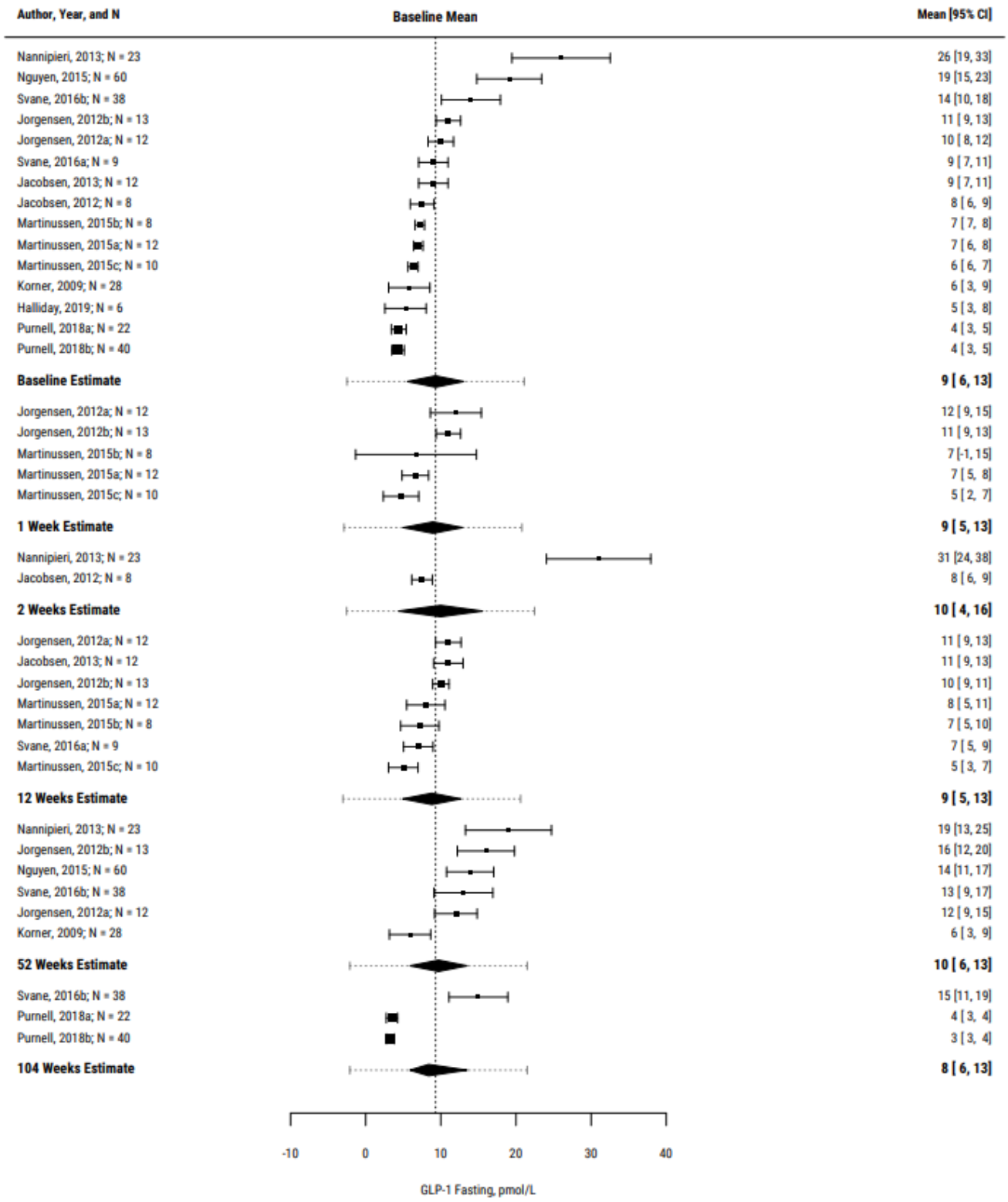
Figure 9. Postprandial response (120 minutes; total AUC) of PYY levels and estimates (pg/ml) of included studies before RYGB, 2, 26 and 52 weeks post-RYGB.

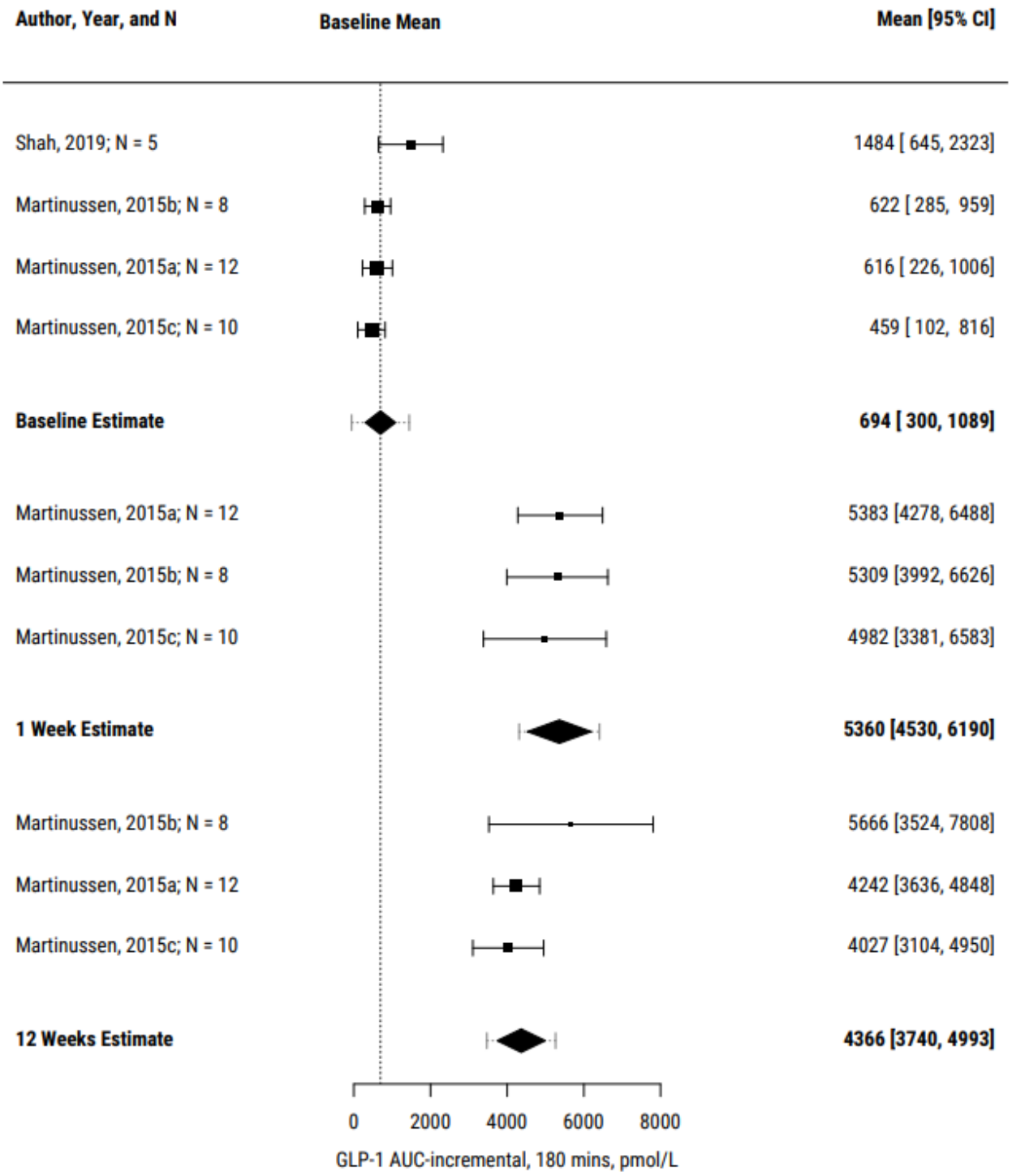
Figure 10. Mean weight (kg) of included studies before RYGB and 1, 2, 4, 12, 26, 52 and 104 weeks post-RYGB.

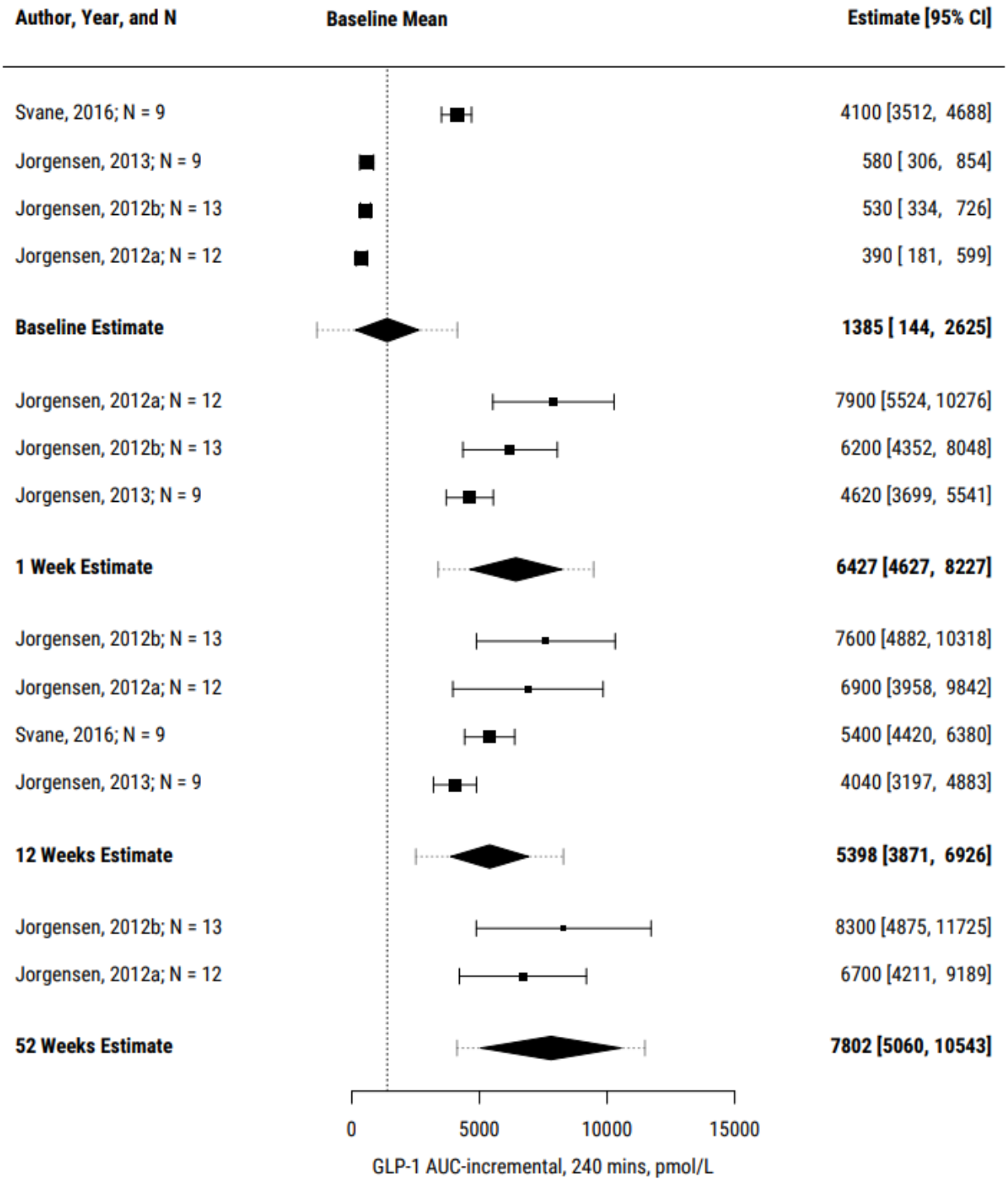

Identification of studies via databases and registers
Identification
Records identified from databases (n =4437)
Records removed *before screening*:
Duplicate records removed (n = 1878)
Screening
Titles and abstracts screened (n = 2559)
Titles and abstracts excluded (n = 2211)
Reports sought for retrieval (n = 348)
Reports not retrieved (n = 120)
Full texts assessed for eligibility (n = 228)
Full texts excluded (n = 181):
Not available in English or French (n = 2)
Wrong intervention (n = 5)
Wrong study design (n = 23)
Participants not in a fasted state (n= 1)
No test meal (n = 22)
Post-prandial measures for <120 mins (n = 20)
No outcomes of interest were measured (n = 8)
Duplicates (n = 20)
Leptin only (n = 72)
Missing data for analysis (n = 8)
Included
Studies included in review (n = 47)
Studies meta-analysed (n = 31)

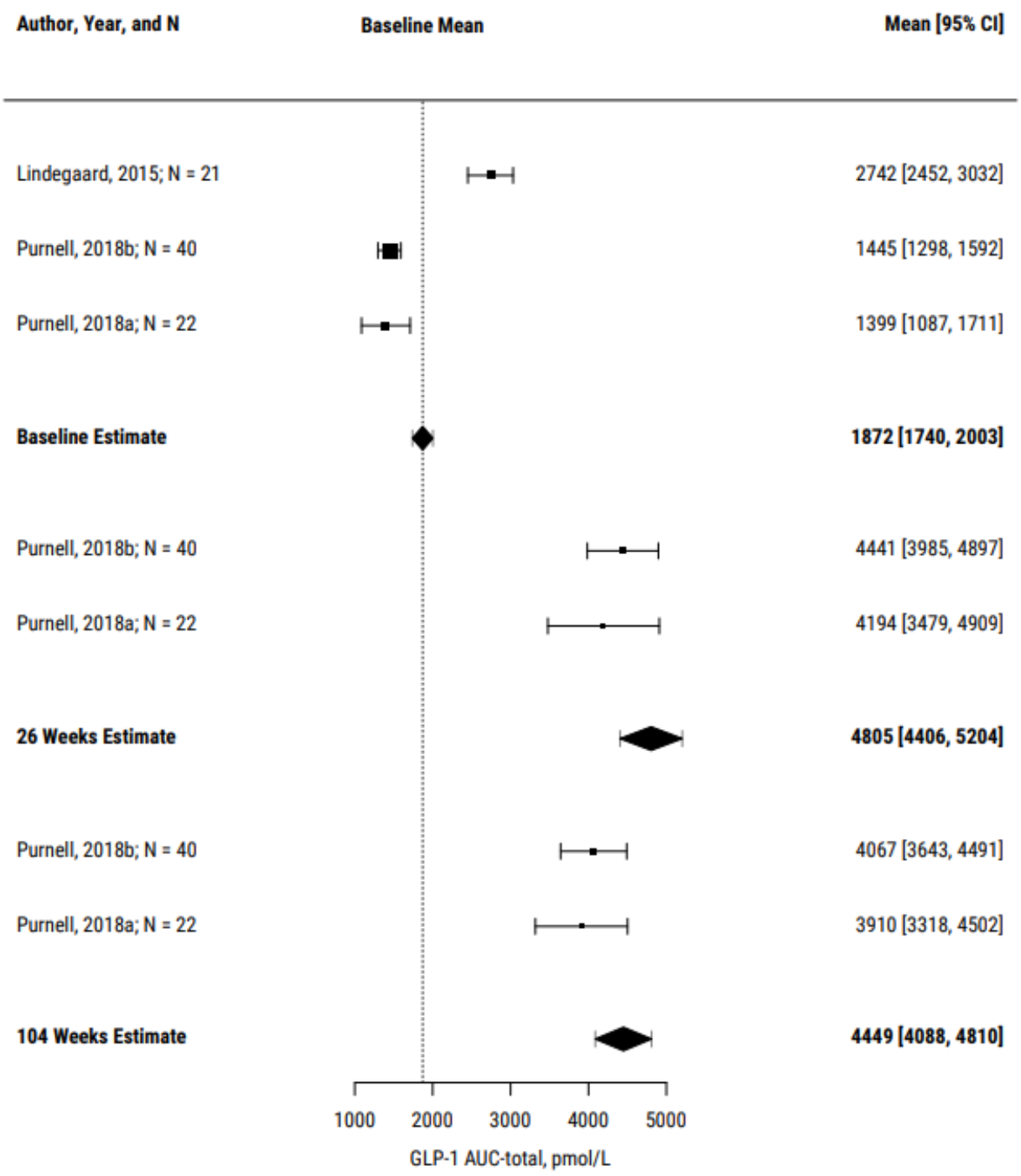

Author, Year, and N
Baseline Mean
Mean [95% CI]
Morinigo, 2004; N = 8
981 [829, 1132]
Lips, 2014a; N = 16
734 [593, 876]
Halliday, 2019; N = 6
690 [561, 818]
Chronaiou, 2012; N = 12
623 [535, 711]
Jacobsen, 2012; N = 8
585 [526, 645]
Hansen, 2011; N = 9
585 [407, 763]
Lips, 2014b; N = 15
564 [422, 705]
Nosso, 2016; N = 14
391 [253, 529]
Arakawa, 2020; N = 40
347 [300, 395]
Tsouristakis, 2019; N = 38
336 [293, 379]
Korner, 2009; N = 28
330 [281, 379]
Baseline Estimate
554 [437, 670]
Lips, 2014a; N = 16
511 [380, 642]
Jacobsen, 2012; N = 8
475 [419, 532]
Lips, 2014b; N = 15
452 [324, 581]
2 Weeks Estimate
432 [301, 562]
Morinigo, 2004; N = 8
844 [730, 958]
Hansen, 2011; N = 9
447 [389, 505]
6 Weeks Estimate
448 [290, 605]
Chronaiou, 2012; N = 12
618 [522, 714]
Arakawa, 2020; N = 40
347 [298, 396]
Tsouristakis, 2019; N = 38
324 [277, 371]
Korner, 2009; N = 28
307 [258, 356]
26 Weeks Estimate
541 [421, 661]
Chronaiou, 2012; N = 12
711 [589, 833]
Nosso, 2016; N = 14
443 [354, 532]
Arakawa, 2020; N = 40
347 [299, 396]
Tsouristakis, 2019; N = 38
345 [294, 396]
Korner, 2009; N = 28
304 [253, 355]
52 Weeks Estimate
553 [434, 673]
200
400
600
800
1000
1200
Ghrelin Fasting, pg/ml

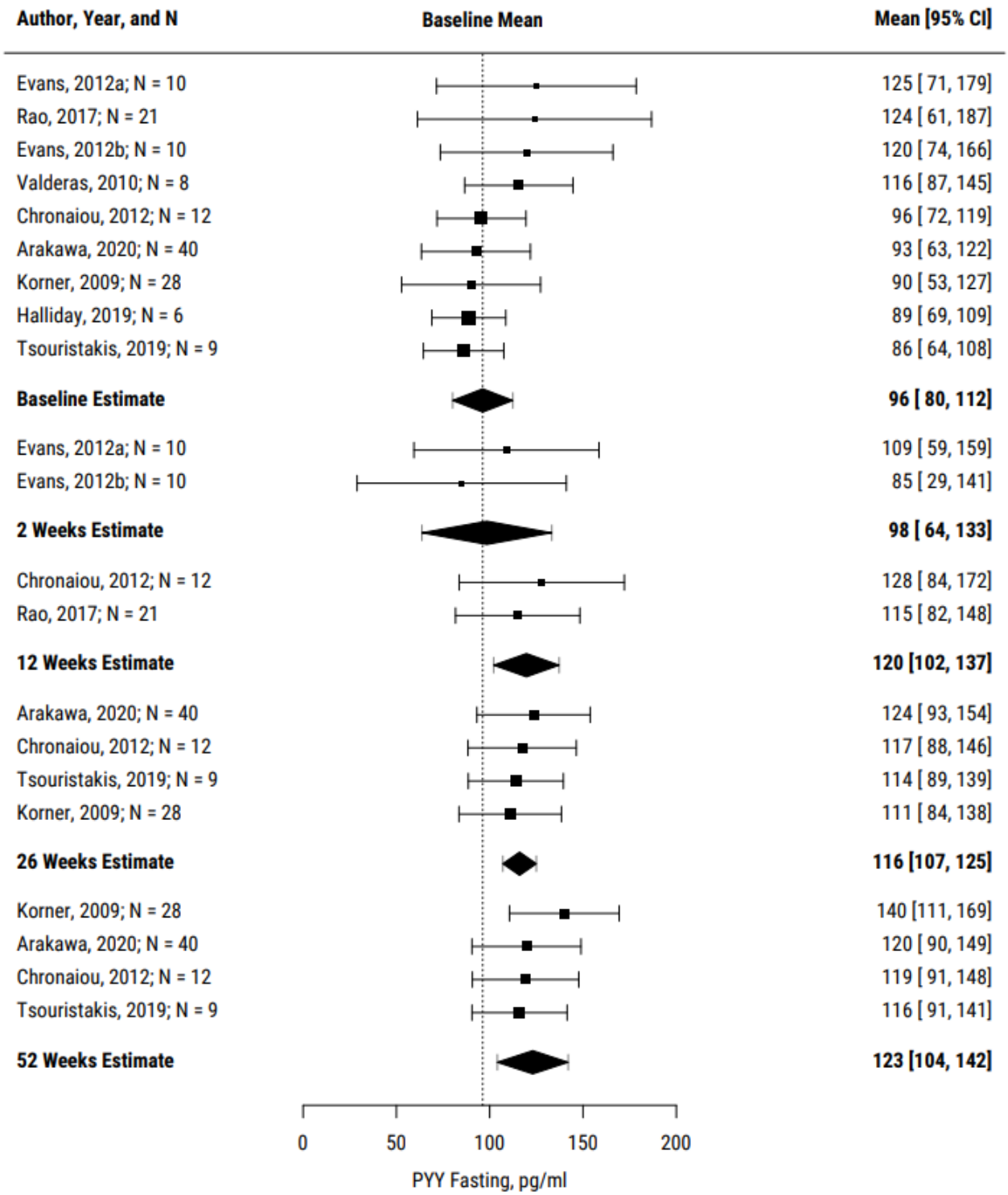

Author, Year, and N
Baseline Mean
Mean [95% CI]
Chronaiou, 2012; N = 12
67285 [59413, 75157]
Tsouristakis, 2019; N = 38
51800 [45920, 57680]
Arakawa, 2020; N = 40
36300 [31008, 41592]
Baseline Estimate
51875 [37471, 66279]
Chronaiou, 2012; N = 12
64630 [56803, 72457]
Tsouristakis, 2019; N = 38
53300 [47028, 59572]
Arakawa, 2020; N = 40
35300 [30008, 40592]
26 Weeks Estimate
51389 [36968, 65810]
Chronaiou, 2012; N = 12
71849 [60722, 82976]
Tsouristakis, 2019; N = 38
53100 [46436, 59764]
Arakawa, 2020; N = 40
33700 [28604, 38796]
52 Weeks Estimate
51517 [37003, 66030]
20000
40000
60000
80000
Ghrelin AUC, pg/ml

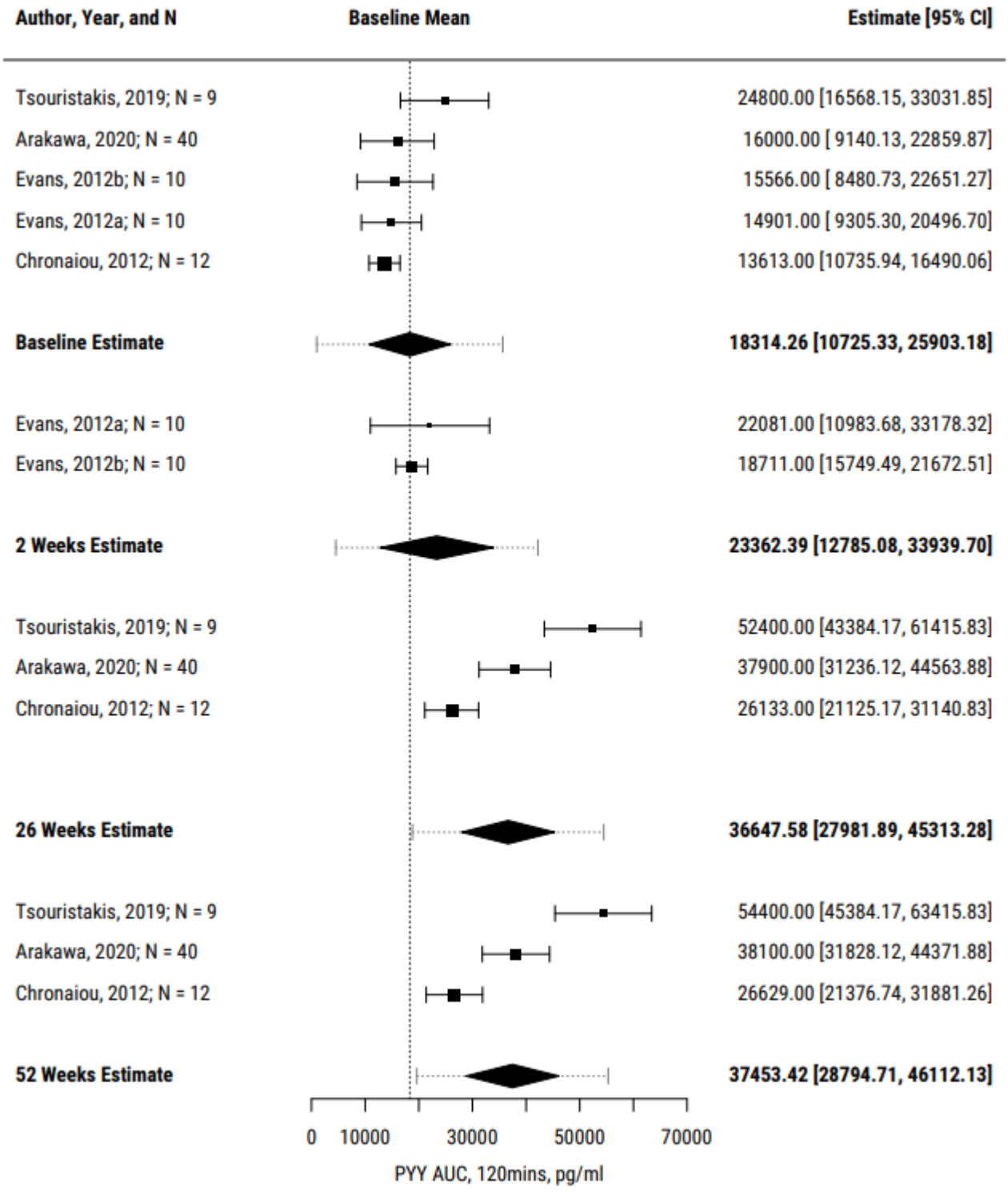

Author, Year, and N
Baseline Mean
Mean [95% CI]
Nannipieri, 2013; N = 23 26 [19, 33]
Nguyen, 2015; N = 60 19 [15, 23]
Svane, 2016b; N = 38 14 [10, 18]
Jorgensen, 2012b; N = 13 11 [9, 13]
Jorgensen, 2012a; N = 12 10 [8, 12]
Svane, 2016a; N = 9 9 [7, 11]
Jacobsen, 2013; N = 12 9 [7, 11]
Jacobsen, 2012; N = 8 8 [6, 9]
Martinussen, 2015b; N = 8 7 [7, 8]
Martinussen, 2015a; N = 12 7 [6, 8]
Martinussen, 2015c; N = 10 6 [6, 7]
Korner, 2009; N = 28 6 [3, 9]
Halliday, 2019; N = 6 5 [3, 8]
Purnell, 2018a; N = 22 4 [3, 5]
Purnell, 2018b; N = 40 4 [3, 5]
Baseline Estimate 9 [6, 13]
Jorgensen, 2012a; N = 12 12 [9, 15]
Jorgensen, 2012b; N = 13 11 [9, 13]
Martinussen, 2015b; N = 8 7 [-1, 15]
Martinussen, 2015a; N = 12 7 [5, 8]
Martinussen, 2015c; N = 10 5 [2, 7]
1 Week Estimate 9 [5, 13]
Nannipieri, 2013; N = 23 31 [24, 38]
Jacobsen, 2012; N = 8 8 [6, 9]
2 Weeks Estimate 10 [4, 16]
Jorgensen, 2012a; N = 12 11 [9, 13]
Jacobsen, 2013; N = 12 11 [9, 13]
Jorgensen, 2012b; N = 13 10 [9, 11]
Martinussen, 2015a; N = 12 8 [5, 11]
Martinussen, 2015b; N = 8 7 [5, 10]
Svane, 2016a; N = 9 7 [5, 9]
Martinussen, 2015c; N = 10 5 [3, 7]
12 Weeks Estimate 9 [5, 13]
Nannipieri, 2013; N = 23 19 [13, 25]
Jorgensen, 2012b; N = 13 16 [12, 20]
Nguyen, 2015; N = 60 14 [11, 17]
Svane, 2016b; N = 38 13 [9, 17]
Jorgensen, 2012a; N = 12 12 [9, 15]
Korner, 2009; N = 28 6 [3, 9]
52 Weeks Estimate 10 [6, 13]
Svane, 2016b; N = 38 15 [11, 19]
Purnell, 2018a; N = 22 4 [3, 4]
Purnell, 2018b; N = 40 3 [3, 4]
104 Weeks Estimate 8 [6, 13]
-10
0
10
20
30
40
GLP-1 Fasting, pmol/L

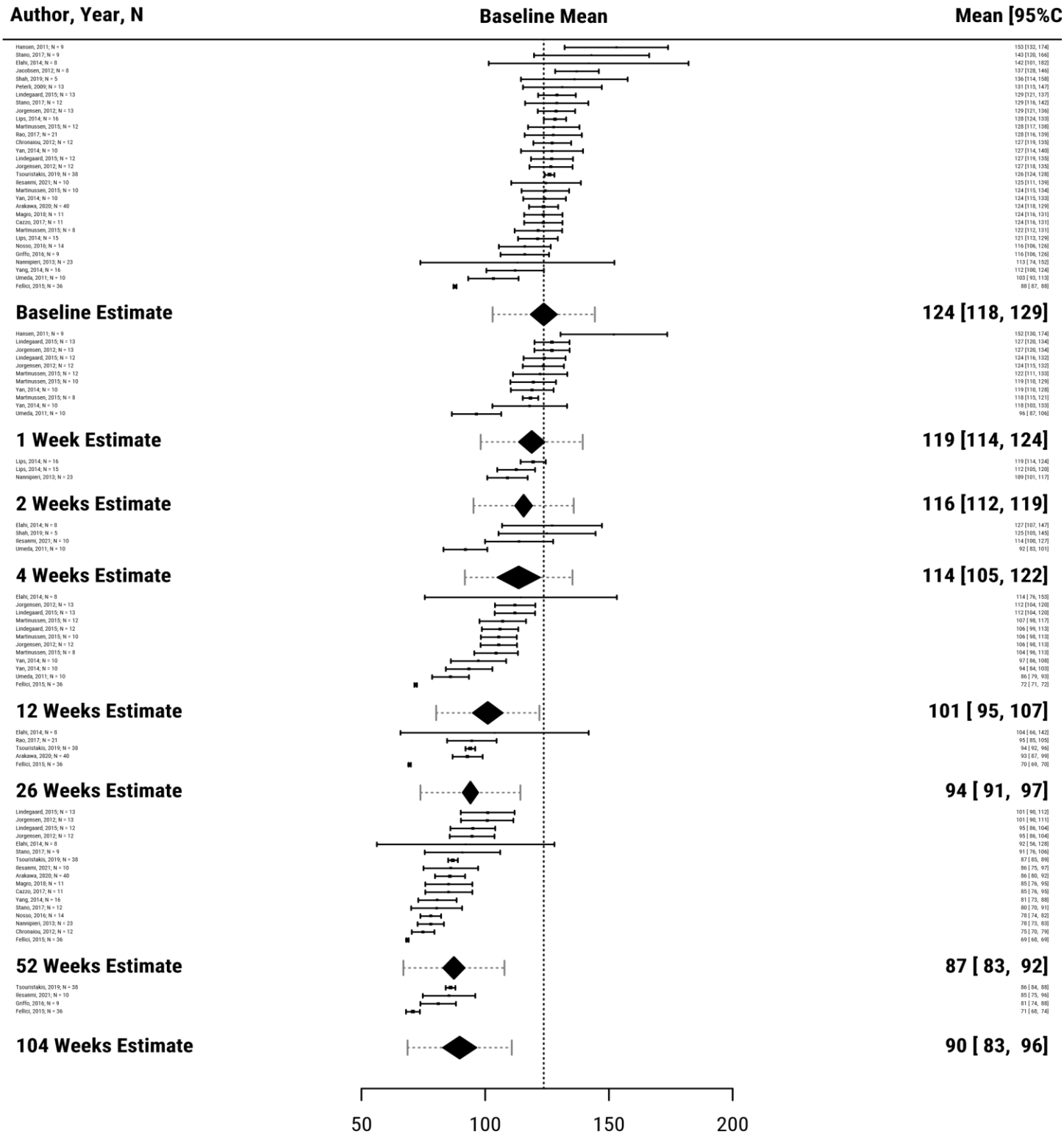

Author, Year, and N
Baseline Mean
Mean [95% CI]
Shah, 2019; N = 5
1484 [645, 2323]
Martinussen, 2015b; N = 8
622 [285, 959]
Martinussen, 2015a; N = 12
616 [226, 1006]
Martinussen, 2015c; N = 10
459 [102, 816]
Baseline Estimate
694 [300, 1089]
Martinussen, 2015a; N = 12
5383 [4278, 6488]
Martinussen, 2015b; N = 8
5309 [3992, 6626]
Martinussen, 2015c; N = 10
4982 [3381, 6583]
1 Week Estimate
5360 [4530, 6190]
Martinussen, 2015b; N = 8
5666 [3524, 7808]
Martinussen, 2015a; N = 12
4242 [3636, 4848]
Martinussen, 2015c; N = 10
4027 [3104, 4950]
12 Weeks Estimate
4366 [3740, 4993]
0
2000
4000
6000
8000
GLP-1 AUC-incremental, 180 mins, pmol/L


Author, Year, and N
Baseline Mean
Estimate [95% CI]
Svane, 2016; N = 9
4100 [3512, 4688]
Jorgensen, 2013; N = 9
580 [306, 854]
Jorgensen, 2012b; N = 13
530 [334, 726]
Jorgensen, 2012a; N = 12
390 [181, 599]
Baseline Estimate
1385 [144, 2625]
Jorgensen, 2012a; N = 12
7900 [5524, 10276]
Jorgensen, 2012b; N = 13
6200 [4352, 8048]
Jorgensen, 2013; N = 9
4620 [3699, 5541]
1 Week Estimate
6427 [4627, 8227]
Jorgensen, 2012b; N = 13
7600 [4882, 10318]
Jorgensen, 2012a; N = 12
6900 [3958, 9842]
Svane, 2016; N = 9
5400 [4420, 6380]
Jorgensen, 2013; N = 9
4040 [3197, 4883]
12 Weeks Estimate
5398 [3871, 6926]
Jorgensen, 2012b; N = 13
8300 [4875, 11725]
Jorgensen, 2012a; N = 12
6700 [4211, 9189]
52 Weeks Estimate
7802 [5060, 10543]
0
5000
10000
15000
GLP-1 AUC-incremental, 240 mins, pmol/L


Author, Year, and N
Baseline Mean
Mean [95% CI]
Lindegaard, 2015; N = 21
2742 [2452, 3032]
Purnell, 2018b; N = 40
1445 [1298, 1592]
Purnell, 2018a; N = 22
1399 [1087, 1711]
Baseline Estimate
1872 [1740, 2003]
Purnell, 2018b; N = 40
4441 [3985, 4897]
Purnell, 2018a; N = 22
4194 [3479, 4909]
26 Weeks Estimate
4805 [4406, 5204]
Purnell, 2018b; N = 40
4067 [3643, 4491]
Purnell, 2018a; N = 22
3910 [3318, 4502]
104 Weeks Estimate
4449 [4088, 4810]
1000
2000
3000
4000
5000
GLP-1 AUC-total, pmol/L


Author, Year, and N
Baseline Mean
Mean [95% CI]
Evans, 2012a; N = 10
125 [71, 179]
Rao, 2017; N = 21
124 [61, 187]
Evans, 2012b; N = 10
120 [74, 166]
Valderas, 2010; N = 8
116 [87, 145]
Chronaiou, 2012; N = 12
96 [72, 119]
Arakawa, 2020; N = 40
93 [63, 122]
Korner, 2009; N = 28
90 [53, 127]
Halliday, 2019; N = 6
89 [69, 109]
Tsouristakis, 2019; N = 9
86 [64, 108]
Baseline Estimate
96 [80, 112]
Evans, 2012a; N = 10
109 [59, 159]
Evans, 2012b; N = 10
85 [29, 141]
2 Weeks Estimate
98 [64, 133]
Chronaiou, 2012; N = 12
128 [84, 172]
Rao, 2017; N = 21
115 [82, 148]
12 Weeks Estimate
120 [102, 137]
Arakawa, 2020; N = 40
124 [93, 154]
Chronaiou, 2012; N = 12
117 [88, 146]
Tsouristakis, 2019; N = 9
114 [89, 139]
Korner, 2009; N = 28
111 [84, 138]
26 Weeks Estimate
116 [107, 125]
Korner, 2009; N = 28
140 [111, 169]
Arakawa, 2020; N = 40
120 [90, 149]
Chronaiou, 2012; N = 12
119 [91, 148]
Tsouristakis, 2019; N = 9
116 [91, 141]
52 Weeks Estimate
123 [104, 142]
0
50
100
150
200
PYY Fasting, pg/ml


Author, Year, and N
Baseline Mean
Estimate [95% CI]
Tsouristakis, 2019; N = 9
24800.00 [16568.15, 33031.85]
Arakawa, 2020; N = 40
16000.00 [9140.13, 22859.87]
Evans, 2012b; N = 10
15566.00 [8480.73, 22651.27]
Evans, 2012a; N = 10
14901.00 [9305.30, 20496.70]
Chronaiou, 2012; N = 12
13613.00 [10735.94, 16490.06]
Baseline Estimate
18314.26 [10725.33, 25903.18]
Evans, 2012a; N = 10
22081.00 [10983.68, 33178.32]
Evans, 2012b; N = 10
18711.00 [15749.49, 21672.51]
2 Weeks Estimate
23362.39 [12785.08, 33939.70]
Tsouristakis, 2019; N = 9
52400.00 [43384.17, 61415.83]
Arakawa, 2020; N = 40
37900.00 [31236.12, 44563.88]
Chronaiou, 2012; N = 12
26133.00 [21125.17, 31140.83]
26 Weeks Estimate
36647.58 [27981.89, 45313.28]
Tsouristakis, 2019; N = 9
54400.00 [45384.17, 63415.83]
Arakawa, 2020; N = 40
38100.00 [31828.12, 44371.88]
Chronaiou, 2012; N = 12
26629.00 [21376.74, 31881.26]
52 Weeks Estimate
37453.42 [28794.71, 46112.13]
0
10000
30000
50000
70000
PYY AUC, 120mins, pg/ml


Author, Year, N
Baseline Mean
Mean [95%C
Hansen, 2011; N = 9 153 [132, 174]
Stano, 2017; N = 9 143 [120, 166]
Elahi, 2014; N = 8 142 [101, 182]
Jacobsen, 2012; N = 8 137 [128, 146]
Shah, 2019; N = 5 136 [114, 158]
Peterli, 2009; N = 13 131 [115, 147]
Lindegaard, 2015; N = 13 129 [121, 137]
Stano, 2017; N = 12 129 [116, 142]
Jorgensen, 2012; N = 13 129 [121, 136]
Lips, 2014; N = 16 128 [124, 133]
Martinussen, 2015; N = 12 128 [117, 138]
Rao, 2017; N = 21 128 [116, 139]
Chronaiou, 2012; N = 12 127 [119, 135]
Yan, 2014; N = 10 127 [114, 140]
Lindegaard, 2015; N = 12 127 [119, 135]
Jorgensen, 2012; N = 12 127 [118, 135]
Tsouristakis, 2019; N = 38 126 [124, 128]
Ilesanmi, 2021; N = 10 125 [111, 139]
Martinussen, 2015; N = 10 124 [115, 134]
Yan, 2014; N = 10 124 [115, 133]
Arakawa, 2020; N = 40 124 [118, 129]
Magro, 2018; N = 11 124 [116, 131]
Cazzo, 2017; N = 11 124 [116, 131]
Martinussen, 2015; N = 8 122 [112, 131]
Lips, 2014; N = 15 121 [113, 129]
Nosso, 2016; N = 14 116 [106, 126]
Griffo, 2016; N = 9 116 [106, 126]
Nannipieri, 2013; N = 23 113 [74, 152]
Yang, 2014; N = 16 112 [100, 124]
Umeda, 2011; N = 10 103 [93, 113]
Fellici, 2015; N = 36 88 [87, 88]
Baseline Estimate
124 [118, 129]
Hansen, 2011; N = 9 152 [130, 174]
Lindegaard, 2015; N = 13 127 [120, 134]
Jorgensen, 2012; N = 13 127 [120, 134]
Lindegaard, 2015; N = 12 124 [116, 132]
Jorgensen, 2012; N = 12 124 [115, 132]
Martinussen, 2015; N = 12 122 [111, 133]
Martinussen, 2015; N = 10 119 [110, 129]
Yan, 2014; N = 10 119 [110, 128]
Martinussen, 2015; N = 8 118 [115, 121]
Yan, 2014; N = 10 118 [103, 133]
Umeda, 2011; N = 10 96 [87, 106]
1 Week Estimate
119 [114, 124]
Lips, 2014; N = 16 119 [114, 124]
Lips, 2014; N = 15 112 [105, 120]
Nannipieri, 2013; N = 23 109 [101, 117]
2 Weeks Estimate
116 [112, 119]
Elahi, 2014; N = 8 127 [107, 147]
Shah, 2019; N = 5 125 [105, 145]
Ilesanmi, 2021; N = 10 114 [108, 127]
Umeda, 2011; N = 10 92 [83, 101]
4 Weeks Estimate
114 [105, 122]
Elahi, 2014; N = 8 114 [76, 153]
Jorgensen, 2012; N = 13 112 [104, 120]
Lindegaard, 2015; N = 13 112 [104, 120]
Martinussen, 2015; N = 12 107 [98, 117]
Lindegaard, 2015; N = 12 106 [99, 113]
Martinussen, 2015; N = 10 106 [98, 113]
Jorgensen, 2012; N = 12 106 [98, 113]
Martinussen, 2015; N = 8 104 [96, 113]
Yan, 2014; N = 10 97 [86, 108]
Yan, 2014; N = 10 94 [84, 103]
Umeda, 2011; N = 10 86 [79, 93]
Fellici, 2015; N = 36 72 [71, 72]
12 Weeks Estimate
101 [95, 107]
Elahi, 2014; N = 8 104 [66, 142]
Rao, 2017; N = 21 95 [85, 105]
Tsouristakis, 2019; N = 38 94 [92, 96]
Arakawa, 2020; N = 40 93 [87, 99]
Fellici, 2015; N = 36 70 [69, 70]
26 Weeks Estimate
94 [91, 97]
Lindegaard, 2015; N = 13 101 [90, 112]
Jorgensen, 2012; N = 13 101 [90, 111]
Lindegaard, 2015; N = 12 95 [86, 104]
Jorgensen, 2012; N = 12 95 [86, 104]
Elahi, 2014; N = 8 92 [56, 128]
Stano, 2017; N = 9 91 [76, 106]
Tsouristakis, 2019; N = 38 87 [85, 89]
Ilesanmi, 2021; N = 10 86 [75, 97]
Arakawa, 2020; N = 40 86 [80, 92]
Magro, 2018; N = 11 85 [76, 95]
Cazzo, 2017; N = 11 85 [76, 95]
Yang, 2014; N = 16 81 [73, 88]
Stano, 2017; N = 12 80 [70, 91]
Nosso, 2016; N = 14 78 [74, 82]
Nannipieri, 2013; N = 23 78 [73, 83]
Chronaiou, 2012; N = 12 75 [70, 79]
Fellici, 2015; N = 36 69 [68, 69]
52 Weeks Estimate
87 [83, 92]
Tsouristakis, 2019; N = 38 86 [84, 88]
Ilesanmi, 2021; N = 10 85 [75, 96]
Griffo, 2016; N = 9 81 [74, 88]
Fellici, 2015; N = 36 71 [68, 74]
104 Weeks Estimate
90 [83, 96]
50
100
150
200
Weight (kg)

GUT PEPTIDES BEFORE AND FOLLOWING ROUX-EN-Y GASTRIC BYPASS:
A SYSTEMATIC REVIEW AND META-ANALYSIS

Supplementary materials

Search Strategy

Medline (OVID)

Gastric Bypass/ AND (Roux* OR RY OR RYGB).ti,ab,kf.
Anastomosis, Roux-en-Y/
("Roux-en-Y" or "roux en y" OR "roux-en y" OR "roux en-y" OR "RY" OR "RYGB"). ti,ab,kf.
1 OR 2 OR 3
Ghrelin/
(ghrelin or ppghrelin or (motilin adj2 peptide) or ghrl or obestatin or ppmtlrp or "appetite regulating hormone" or Anamorelin or Ipamorelin or Eganamorelin or Hexarelin or MK-677 or Rikkunshito).ti,ab,kf.
Glucagon-like peptide 1/
("glucagon-like peptide 1" or "glucagon like peptide 1" OR "GLP 1" OR "GLP1" OR "GLP-1" OR "glucagon-like peptide-1").ti,ab,kf.
Peptide YY/
("peptide YY" or "PYY" or "PYY peptide" or peptide-YY).ti,ab,kf.
Leptin/
(leptin).ti,ab,kf.
Hunger/
Appetite/
Satiation/
Satiety response/
(hungry OR hunger OR Appetit* OR fullness OR Crav* OR Satiety OR Satiation OR discomfort OR (food adj2 reward*) OR want* OR liking OR tast* OR palat* OR (desire adj2 eat) OR (Prospective ajd2 consumption)).ti,ab,kf.
5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17
4 AND 18
exp animals/ not humans.sh.
19 not 20

Embase ovid

Gastric Bypass/ AND (Roux* OR RY OR RYGB).ti,ab,kw.
Anastomosis, Roux-en-Y/
("Roux-en-Y" or "roux en y" OR "roux-en y" OR "roux en-y" OR "RY" OR "RYGB"). ti,ab,kw.
1 OR 2 OR 3
Ghrelin/
(ghrelin or ppghrelin or (motilin adj2 peptide) or ghrl or obestatin or ppmtlrp or "appetite regulating hormone" or Anamorelin or Ipamorelin or Eganamorelin or Hexarelin or MK-677 or Rikkunshito).ti,ab,kw.
Glucagon-like peptide 1/
("glucagon-like peptide 1" or "glucagon like peptide 1" OR "GLP 1" OR "GLP1" OR "GLP-1" OR "glucagon-like peptide-1").ti,ab,kw.

Peptide YY/
("peptide YY" or "PYY" or "PYY peptide" or peptide-YY).ti,ab,kw.
Leptin/
(leptin).ti,ab,kw.
Hunger/
Appetite/
Satiation/
Satiety response/
(hungry OR hunger OR Appetit* OR fullness OR Crav* OR Satiety OR Satiation OR discomfort OR (food adj2 reward*) OR want* OR liking OR tast* OR palat* OR (desire adj2 eat) OR (Prospective ajd2 consumption)).ti,ab,kw.
5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17
4. AND 18
exp animal/ not human/
19 not 20

CCTR

Gastric Bypass/ AND (Roux* OR RY OR RYGB).ti,ab.
Anastomosis, Roux-en-Y/
("Roux-en-Y" or "roux en y" OR "roux-en y" OR "roux en-y" OR "RY" OR "RYGB"). ti,ab.
1 OR 2 OR 3
Ghrelin/
(ghrelin or ppghrelin or (motilin adj2 peptide) or ghrl or obestatin or ppmtlrp or "appetite regulating hormone" or Anamorelin or Ipamorelin or Eganamorelin or Hexarelin or MK-677 or Rikkunshito).ti,ab.
Glucagon-like peptide 1/
("glucagon-like peptide 1" or "glucagon like peptide 1" OR "GLP 1" OR "GLP1" OR "GLP-1" OR "glucagon-like peptide-1").ti,ab.
Peptide YY/
("peptide YY" or "PYY" or "PYY peptide" or peptide-YY).ti,ab.
Leptin/
(leptin).ti,ab.
Hunger/
Appetite/
Satiation/
Satiety response/
(hungry OR hunger OR Appetit* OR fullness OR Crav* OR Satiety OR Satiation OR discomfort OR (food adj2 reward*) OR want* OR liking OR tast* OR palat* OR (desire adj2 eat) OR (Prospective ajd2 consumption)).ti,ab.
5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17
4 AND 18

GUT PEPTIDES BEFORE AND FOLLOWING ROUX-EN-Y GASTRIC BYPASS:
A SYSTEMATIC REVIEW AND META-ANALYSIS

Scopus

1	TITLE-ABS-KEY ("Roux-en-Y" OR "roux en y" OR "RYGB" OR "RY" OR "roux-en y" or "roux en-y" OR "Roux* W/5 bypass")
2	TITLE-ABS-KEY (ghrelin or ppghrelin or (motilin W/2 peptide) or ghrl or obestatin or ppmtlrp or "appetite regulating hormone" or Anamorelin or Ipamorelin or Eganamorelin or Hexarelin or MK-677 or Rikkunshito)
3	TITLE-ABS-KEY ("glucagon-like peptide 1" or "glucagon like peptide 1" OR "GLP 1" OR "GLP1" OR "GLP-1" OR "glucagon-like peptide-1")
4	TITLE-ABS-KEY ("peptide YY" or "PYY" or "PYY peptide" or peptide-YY)
5	TITLE-ABS-KEY (leptin)
6	TITLE-ABS-KEY (hungry OR hunger OR Appetit* OR fullness OR Crav* OR discomfort OR Satiety OR Satiation OR "food W/2 reward" OR want* OR liking OR tast* OR palat* OR "desire W/2 eat" OR "Prospective W/2 consumption")
7	#2 OR #3 OR #4 OR #5 OR #6
8	#1 AND #7

TITLE-ABS-KEY ("Roux-en-Y" OR "roux en y" OR "RYGB" OR "RY" OR "roux-en y" OR "roux en-y" OR "Roux* W/5 bypass") AND TITLE-ABS-KEY (ghrelin OR ppghrelin OR (motilin W/2 peptide) OR ghrl OR obestatin OR ppmtlrp OR "appetite regulating hormone" OR anamorelin OR ipamorelin OR eganamorelin OR hexarelin OR mk-677 OR rikkunshito) OR TITLE-ABS-KEY ("glucagon-like peptide 1" OR "glucagon like peptide 1" OR "GLP 1" OR "GLP1" OR "GLP-1" OR "glucagon-like peptide-1") OR TITLE-ABS-KEY ("peptide YY" OR "PYY" OR "PYY peptide" OR peptide-yy) OR TITLE-ABS-KEY (leptin) OR TITLE-ABS-KEY (hungry OR hunger OR appetit* OR fullness OR crav* OR discomfort OR satiety OR sat iation OR "food W/2 reward" OR want* OR liking OR tast* OR palat* OR "desire W/2 eat" OR "Prospective W/2 consumption")

GUT PEPTIDES BEFORE AND FOLLOWING ROUX-EN-Y GASTRIC BYPASS:
A SYSTEMATIC REVIEW AND META-ANALYSIS

Supplementary Table 1. Summary of invention characteristics of included studies.

Study Reference	RYGB Surgery			Meal Test						
								Macronutrient Composition		
	Pouch Size (ml)	Biliopancreatic Limb Length (cm)	Alimentary Limb Length (cm)	Consistency	Quantity (ml)	Meal Duration (min)	Kcal	Glucose %	Lipid %	Protein %
Alamuddin et al., 2017 (41)	30	100	NR	L	240	8	240	55	20	25
Arakawa et al., 2020 (25)	15-30	50-100	100-150	L	474	15	320	50	15	35
Bryant et al., 2013 (62)	NR	100	NR	L	200	NR	300	89	0	11
Campos et al., 2010 (63)	30	50	100	L	100	20	300	50	20	30
Cazzo et al., 2017 (42)	30	100	150	NR	NR	NR	515	40.7	41.8	17.5
Chronaiou et al., 2012 (64)	15-25	50	150	L	NR	10	300	55	27	18
Evans et al., 2012 (78)	30	30	100	L	237	20	262	47 33	21 45	32 22
Fellici et al., 2015 (65)	30	100	150	NR	NR	10	515	40.7	41.8	17.5
Foschi et al., 2008 (79)	10%	100 140 (BMI ≥45)	NR	L	500	5-10	504	52	30.3	17.6
Griffo et al., 2016 (43)	40	NR	100-150	L	NR	NR	304	55	27	18
Halliday et al., 2019 (44)	20	25	100 150 (BMI ≥50)	L	NR	20	200	20	40	40
Hansen et al., 2011 (85)	25	NR		L	237	20	250	64	22	14
Ilesanmi et al., 2021 (45)		NR		L	137.5	NR	330	44	32	16
Jørgensen et al., 2013 (74)		NR		L	200	30	300	50	35	15
Jacobsen et al., 2012 (75)	25	75	100	L	200	30	300	49	35	16

Study Reference	RYGB Surgery			Meal Test						
								Macronutrient Composition		
	Pouch Size (ml)	Biliopancreatic Limb Length (cm)	Alimentary Limb Length (cm)	Consistency	Quantity (ml)	Meal Duration (min)	Kcal	Glucose %	Lipid %	Protein %
Jacobsen et al., 2013 (76)		NR		L	200	30	300	50	35	15
Jiménez et al., 2012 (66)	20	40	150	L	250	NR	398	50	35	15
Jørgensen et al., 2012 (67)	25	75	100	L	200	30	300	50	35	15
Korner et al., 2009 (68)	15-30	50-100	100-150	L	474	15	320	50	15	35
Lindegaard et al., 2015 (69)	25	75	100	L	200	30	300	50	35	15
Lips et al., 2014 (58)	25	NR	100	L	266	NR	400	49	35	16
Magro et al., 2018 (46)	30	100	150	Combination	-	NR	515	40.7	41.8	17.5
Martinussen et al., 2015 (77)	25	75	100	L	200	30	300	49	35	16
Miras et al., 2021 (47)	NR	50 150	100	L	125	NR	300	47.9	35.1	17
Morínigo et al., 2004 (81)	15	50	150	L	250	NR	398	50	35	15
Morínigo et al., 2006 (80)		NR		L	250	NR	398	50	35	15
Morínigo et al., 2006 (70)	15	50	150	L	250	NR	398	50	35	15
Morínigo et al., 2008 (71)	20	40	150	L	250	NR	398	50	35	15
Nannipieri et al., 2013 (84)	30-50	120	150	Semi-solid	-	NR	350	45	28	26
Nguyen et al., 2015 (72)	20	100	150	L	NR	15	250	64	22	14
Nosso et al., 2016 (48)	NR		100-150	L	NR	15	304	54	27	17

Study Reference	RYGB Surgery			Meal Test						
								Macronutrient Composition		
	Pouch Size (ml)	Biliopancreatic Limb Length (cm)	Alimentary Limb Length (cm)	Consistency	Quantity (ml)	Meal Duration (min)	Kcal	Glucose %	Lipid %	Protein %
Peterli et al., 2009 (82)	25	50	150	L	200	NR	400	25	63	15
Pop et al., 2018 (94)		NR	100	L	240	5	360	50	35	16
Purnell et al., 2018 (50)		NR		L	NR	NR	360	50	35	16
Rao et al., 2017 (51)		NR		L	474	15	320	50	15	35
Schmidt et al., 2016 (52)	25	75	100	L	175	NR	310	42	17	41
Shah et al., 2019 (53)		NR		Semi-solid	-	NR	220	56	25	19
Shankar et al., 2017 (54)	30	50	100	Semi-solid	140	15	389	26	20	54
Stano et al., 2017 (56)	30	40	150	L Solid	NR	15 15	600 600	50.7 55.4	30 24.6	19.3 20
Steven et al., 2016 (55)	30-50	50-70	100-150	Semi-solid	-	3	100	57	28	13
Svane et al., 2016 (57)	NR	75	125	L	200	30	301	50	35	15
Tsouristakis et al., 2019 (95)	15-30	50-100	100-150	L	474	15	320	50	15	35
Umeda et al., 2011 (83)	20-30	50	100	L	NR	NR	353	46.8	12.5	32.2
Valderas et al., 2010 (59)	15-30	30	150	L	237	NR	355	56	28	15
Yan et al., 2014 (60)	30	60	100	L	NR	NR	75 150 300	40 40 40	43 43 43	17 17 17
Yang et al., 2014 (73)	15-25	100	100	L	NR	10	300	54	31.8	14.2

Study Reference	RYGB Surgery			Meal Test						
								Macronutrient Composition		
	Pouch Size (ml)	Biliopancreatic Limb Length (cm)	Alimentary Limb Length (cm)	Consistency	Quantity (ml)	Meal Duration (min)	Kcal	Glucose %	Lipid %	Protein %
Yousseif et al., 2014 (61)	"small"	80	120	L	250	15	500	43	39	18

Supplementary Table 2. Complete results of quality assessment of included studies.

Article reference	Criteria number												Total of "yes"	Total of "no"	Total of other	# of fatal flaws	Quality rating
	1	2	**3**	4	5	6	**7**	8	**9**	10	11	12					
Alamuddin et al., 2017 (41)	Yes	Yes	**No**	NR	No	Yes	**Yes**	NR	**Yes**	Yes	No	NA	6	3	3	1	Fair
Arakawa et al., 2020 (25)	Yes	No	**Yes**	NR	Yes	Yes	**Yes**	NR	**Yes**	Yes	No	NA	7	2	3	0	Good
Bryant et al., 2013 (62)	Yes	No	**NR**	NR	NR	No	**No**	NR	**Yes**	Yes	No	NA	3	4	5	2	Poor
Campos et al., 2010 (63)	Yes	Yes	**Yes**	No	NR	Yes	**No**	NR	**Yes**	Yes	No	NA	6	3	3	1	Fair
Cazzo et al., 2017 (42)	Yes	Yes	**NR**	NR	No	Yes	**No**	NR	**Yes**	Yes	No	NA	5	3	4	2	Poor
Chronaiou et al., 2012 (64)	Yes	Yes	**Yes**	NR	No	Yes	**Yes**	No	**Yes**	Yes	No	NA	7	3	2	0	Good
Evans et al., 2012 (78)	Yes	Yes	**Yes**	NR	NR	Yes	**No**	No	**Yes**	Yes	No	NA	6	3	3	1	Fair
Fellici et al., 2015 (65)	No	Yes	**CD**	NR	NR	Yes	**No**	No	**Yes**	Yes	No	NA	4	4	4	2	Poor
Foschi et al., 2008 (79)	Yes	No	**Yes**	NR	NR	No	**No**	No	**Yes**	Yes	No	NA	4	5	3	1	Fair
Griffo et al., 2016 (43)	Yes	Yes	**NR**	NR	Yes	Yes	**No**	No	**Yes**	Yes	No	NA	6	3	3	2	Poor
Halliday et al., 2019 (44)	Yes	Yes	**Yes**	NR	No	Yes	**No**	No	**Yes**	Yes	No	NA	6	4	2	1	Fair
Hansen et al., 2011 (85)	Yes	Yes	**Yes**	NR	NR	Yes	**No**	No	**Yes**	Yes	No	NA	6	3	3	1	Fair
Ilesanmi et al., 2021 (45)	No	No	**NR**	NR	Yes	NR	**No**	No	**Yes**	Yes	No	NA	3	5	4	2	Poor

Article reference	Criteria number												Total of "yes"	Total of "no"	Total of other	# of fatal flaws	Quality rating
	1	2	**3**	4	5	6	**7**	8	**9**	10	11	12					
Jørgensen et al., 2013 (74)	Yes	Yes	**Yes**	NR	No	NR	**No**	No	**Yes**	Yes	No	NA	5	4	3	1	Fair
Jacobsen et al., 2012 (75)	Yes	Yes	**Yes**	NR	No	Yes	**No**	No	**No**	Yes	No	NA	6	4	2	1	Poor
Jacobsen et al., 2013 (76)	Yes	Yes	**Yes**	NR	NR	NR	**No**	No	**Yes**	Yes	No	NA	5	3	4	1	Fair
Jiménez et al., 2012 (66)	Yes	Yes	**Yes**	No	NR	NR	**No**	No	**Yes**	Yes	No	NA	5	4	3	1	Fair
Jørgensen et al., 2012 (67)	Yes	Yes	**Yes**	NR	NR	Yes	**No**	No	**Yes**	Yes	No	NA	6	3	3	1	Fair
Korner et al., 2009 (68)	Yes	Yes	**Yes**	NR	NR	Yes	**Yes**	No	**Yes**	Yes	No	NA	7	2	3	0	Good
Lindegaard et al., 2015 (69)	Yes	Yes	**Yes**	NR	NR	Yes	**No**	No	**Yes**	Yes	No	NA	6	3	3	1	Fair
Lips et al., 2014 (58)	Yes	Yes	**Yes**	NR	NR	Yes	**No**	No	**Yes**	Yes	No	NA	6	3	3	1	Fair
Magro et al., 2018 (46)	Yes	Yes	**Yes**	NR	CD	Yes	**No**	No	**Yes**	Yes	No	NA	6	3	3	1	Fair
Martinussen et al., 2015 (77)	No	Yes	**Yes**	NR	Yes	Yes	**No**	No	**Yes**	Yes	No	NA	6	4	2	1	Fair
Miras et al., 2021 (47)	Yes	Yes	**Yes**	NR	Yes	Yes	**No**	Yes	**Yes**	Yes	No	NA	8	2	2	1	Fair
Morínigo et al., 2004 (81)	Yes	Yes	**Yes**	NR	Yes	Yes	**No**	No	**Yes**	Yes	No	NA	7	3	2	1	Fair
Morínigo et al., 2006 (80)	Yes	No	**Yes**	NR	No	CD	**No**	No	**Yes**	Yes	No	NA	4	5	3	1	Fair

Article reference	Criteria number												Total of "yes"	Total of "no"	Total of other	# of fatal flaws	Quality rating
	1	2	**3**	4	5	6	**7**	8	**9**	10	11	12					
Morínigo et al., 2006 (70)	Yes	Yes	**Yes**	NR	No	NR	**No**	No	**Yes**	Yes	No	NA	5	4	3	1	Fair
Morínigo et al., 2008 (71)	Yes	Yes	**Yes**	NR	CD	Yes	**No**	No	**Yes**	Yes	No	NA	6	3	3	1	Fair
Nannipieri et al., 2013 (84)	No	Yes	**Yes**	NR	NR	Yes	**No**	No	**Yes**	Yes	No	NA	5	4	3	1	Fair
Nguyen et al., 2015 (72)	Yes	Yes	**Yes**	No	NR	Yes	**No**	No	**CD**	Yes	No	NA	5	4	3	2	Poor
Nosso et al., 2016 (48)	Yes	Yes	**Yes**	NR	NR	Yes	**No**	No	**Yes**	Yes	No	NA	6	3	3	1	Fair
Peterli et al., 2009 (82)	Yes	Yes	**Yes**	NR	NR	Yes	**No**	No	**Yes**	Yes	No	NA	6	3	3	1	Fair
Pop et al., 2018 (94)	Yes	Yes	**Yes**	NR	No	Yes	**No**	No	**Yes**	Yes	No	NA	6	4	2	1	Fair
Purnell et al., 2018 (50)	Yes	Yes	**Yes**	NR	Yes	Yes	**No**	No	**Yes**	Yes	No	NA	7	3	2	1	Fair
Rao et al., 2017 (51)	Yes	Yes	**Yes**	No	NR	Yes	**Yes**	No	**Yes**	Yes	No	NA	7	3	2	0	Good
Schmidt et al., 2016 (52)	Yes	Yes	**Yes**	No	Yes	No	**No**	No	**Yes**	Yes	No	NA	6	5	1	1	Fair
Shah et al., 2019 (53)	No	Yes	**Yes**	NR	No	NR	**No**	No	**NR**	Yes	No	NA	3	5	4	2	Poor
Shankar et al., 2017 (54)	Yes	Yes	**Yes**	NR	NR	Yes	**No**	No	**Yes**	Yes	No	NA	6	3	3	1	Fair
Stano et al., 2017 (56)	Yes	Yes	**Yes**	NR	Yes	Yes	**No**	No	**No**	Yes	No	NA	7	3	2	1	Poor
Steven et al., 2016 (55)	Yes	Yes	**Yes**	NR	No	Yes	**No**	No	**Yes**	Yes	No	NA	6	4	2	1	Fair

Article reference	Criteria number												Total of "yes"	Total of "no"	Total of other	# of fatal flaws	Quality rating
	1	2	**3**	4	5	6	**7**	8	**9**	10	11	12					
Svane et al., 2016 (57)	Yes	Yes	**Yes**	NR	No	Yes	**No**	No	**Yes**	Yes	No	NA	6	4	2	1	Fair
Tsouristakis et al., 2019 (95)	No	Yes	**Yes**	No	CD	Yes	**Yes**	No	**Yes**	Yes	No	NA	6	4	2	0	Good
Umeda et al., 2011 (83)	No	Yes	**Yes**	NR	Yes	Yes	**No**	No	**NR**	Yes	No	NA	5	4	3	2	Poor
Valderas et al., 2010 (59)	Yes	Yes	**Yes**	NR	NR	Yes	**No**	No	**Yes**	Yes	No	NA	6	3	3	1	Fair
Yan et al., 2014 (60)	Yes	Yes	**Yes**	NR	NR	Yes	**No**	No	**CD**	Yes	No	NA	5	3	4	2	Poor
Yang et al., 2014 (73)	Yes	Yes	**Yes**	NR	NR	Yes	**Yes**	No	**Yes**	Yes	No	NA	7	2	3	0	Good
Yousseif et al., 2014 (61)	Yes	Yes	**Yes**	NR	No	Yes	**No**	No	**CD**	Yes	No	NA	5	5	2	2	Poor

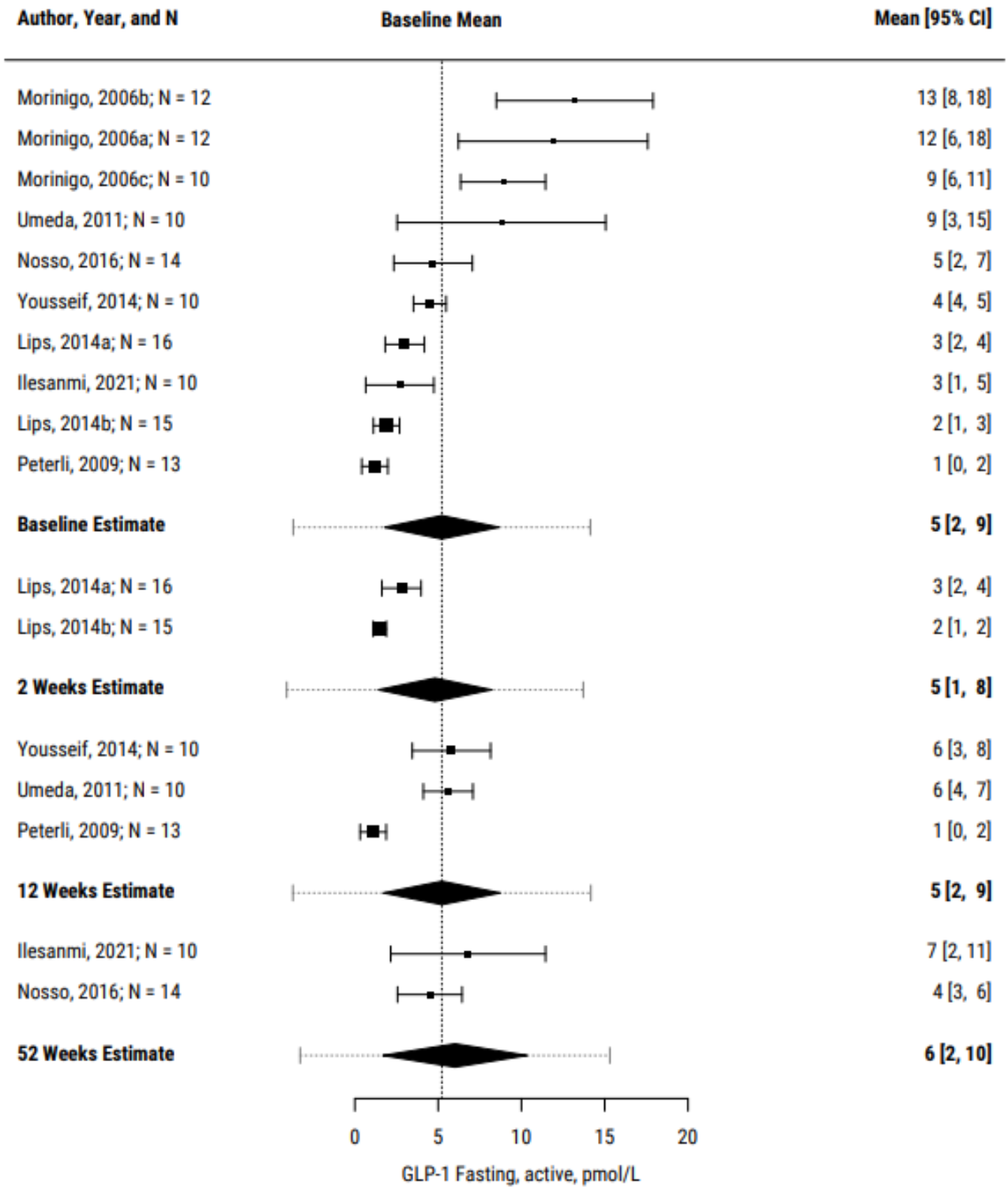
Red flag criteria in bold; NR = not reported; CD = cannot determine; NA = not applicable

Supplementary Table 3. Appetite sensation data from included studies.

Study reference	n	Time of AUC (min)	Pre-RYGB mean (SD)	2 weeks post mean (SD)	4 weeks post mean (SD)	6 weeks post mean (SD)	8 weeks post mean (SD)	12 weeks post mean (SD)
Hunger								
Halliday et al., 2019 (44)	6	180	9855 (867.04)	-	6125 (863.45)	-	-	-
Shankar et al., 2017 (54)	17	120	3231 (144.55)	1474 (113.75)	1293 (68.64)	-	-	-
Svane et al., 2016 (57)	9	240	11160 (543.33)	-	-	-	-	8220 (566.67)
Valderas et al., 2010 (59)	8	180	7357 (463.86)	-	-	-	2981 (331.99)	-
Yousseif et al., 2014 (61)	10	180	5109 (422.16)	-	-	1385 (103.72)	-	2588 (326.66)
Fullness								
Shankar et al., 2017 (54)	17	120	4435 (171.23)	8073 (159.59)	9002 (104.53)	-	-	-
Yousseif et al., 2014 (61)	10	180	6315 (230.53)	-	-	12592 (254.66)	-	1102 (406.73)
Satiety								
Halliday et al., 2019 (44)	6	180	6045 (828.17)	-	10155 (919.74)	-	-	-
Svane et al., 2016 (57)	9	120	10470 (206.67)	-	-	-	-	13550 (486.67)
Valderas et al., 2010 (59)	8	180	8596 (216.73)	-	12992 (508.06)	-	-	-
Prospective food consumption								
Halliday et al., 2019 (44)	6	180	9684 (921.82)	-	4960 (835.81)	-	-	-
Shankar et al., 2017 (54)	27	120	4812 (100.84)	-	2104 (321.20)	-	-	1411 (111.45)
Satisfaction								
Shankar et al., 2017 (54)	27	120	6105 (117.20)	8538 (148.38)	-	-	-	8481 (147.61)

Units are in mm x min

Supplementary Figure 1. Fasting active GLP-1 levels and estimates (pmol/L) of included studies before and 2, 12 and 52 weeks post-RYGB.



Chapter 5: General Discussion and conclusion

We conducted a systematic review and meta-analyses on appetite-related hormones: ghrelin, GLP-1 and PYY following RYGB surgery. This was the first review of its kind attempting to illustrate the magnitude of change of these gut peptides over time after RYGB. Previous reviews only discussed the direction of the changes after surgery (Jirapinyo et al., 2018; Papamargaritis & le Roux, 2021; Xu et al., 2019).

Fasting and postprandial results for ghrelin were not in accordance with our hypothesis. There was a small but statistically significant decrease in fasting ghrelin levels at 2 weeks post-RYGB, however other time points up to 1 year and postprandial response at 6 months and 1 year were not different from pre-surgical values. The small number of studies synthesized and contrastingly large variability across them prompts a cautious interpretation of results. One study that evaluated ghrelin up to 4 years post-RYGB reported no change at 6 months and 1 year, but a significant increase was found in fasting and post-prandial levels of ghrelin at 2, 3, and 4-years post-surgery (**Table 1**) (Tsouristakis et al., 2019). More studies with longer follow-up times are required to understand the full picture of ghrelin fluctuations and to enable more robust conclusions on ghrelin levels following RYGB.

Fasting GLP-1 levels did not change following RYGB compared to pre-surgical values, which was in accordance with our hypothesis. In contrast, fasting PYY concentrations did change, levels increased at 3 months post-RYGB, which was not in accordance with our hypothesis. Heterogeneity could not be assessed for fasting PYY results, which warrants a cautious interpretation of results. More studies are required to consolidate our findings. Postprandial response of GLP-1 and PYY levels were in agreement with our hypotheses as they both increased following RYGB compared to pre-surgical values. Postprandial changes of these appetite-

suppressing hormones are far greater than any changes expected in a healthy population, suggesting that GLP-1 and PYY are key elements in the improvements of appetite control that follows RYGB.

Due to the simplicity of appetite sensation measurements, it was expected that more studies examining appetite hormones in RYGB patients would have also measured appetite sensation. While other factors have an impact on EI, such as restraint, appetite sensations can procure information on the effects of the appetite-related hormones on participants' perceptions of hunger, satiety and fullness postprandially. Furthermore, these would allow to explore if the appetite sensations fluctuate alongside hormone changes over time after RYGB surgery. Unfortunately, we could not proceed as planned since the between-study discrepancies in the number of measurements, timing and follow-ups prevented us from performing meta-analyses on these parameters.

Heterogeneity between the included studies was a challenge that was detected early after data extraction. Before analyses, it was already clear that high levels of heterogeneity were going to be an issue simply by looking at the inter-study variation in the data as well as the different methods used in the studies, which created the need to create multiple subcategories. The different types of assays (active vs. total), times of AUC (varied from 120 to 360 minutes), and studies reporting incremental vs. total AUC prevented us from pooling all studies for analyses, and prompted us to create many subcategories for analyses. Variance in type of assay kits also produced results in different units that could not always be converted. From the very few studies that presented peak peptide values and the knowledge on timing of appetite hormone secretion, protocols for postprandial blood sampling could be narrowed down to shorter times as an effort to standardize and unify the results of future studies. When looking at pre-intervention values of our

outcomes of interest, heterogeneity is already present despite a seemingly similar population. Inclusion criteria of the included studies (not presented) were aligned and this surgical procedure targets a distinct group of people. Kit variability and assay reliability was a challenge that was not anticipated since we expected less methodological variability as well as duplicate assays to be a common practice. While assay reliability and lot-to-lot variability can only be hypothesized to explain some of the heterogeneity, the effects of this barrier could be mostly controlled by transparency of lot-to-lot variation by kit manufacturers and also by implementing duplicate assays within each study (Loh et al., 2022; Plebani & Zaninotto, 2018).

An additional difficulty we faced was the amount of unreported data or methodological details in published articles. Moreover, prevalence of responses from corresponding authors was low. For this project, eight studies could not be included because key information was not reported, additionally some data of the included studies that was measured but not reported could not be included in the analyses, contributing to the low number of studies in each analysis. This barrier was expected as not all studies' main goal was to compare fasting and AUC pre- and post-RYGB. Furthermore, some articles were published as early as 2004 and it is expected that some authors would no longer be available or have access to unpublished data.

Following the process of study selection and initial screening of outcome measures in the included studies, it became evident that the inclusion criteria surrounding leptin needed to be re-evaluated. Originally, there were 72 included studies which only evaluated leptin. After discussions with co-authors, it was decided to exclude studies that only had leptin as one of our outcomes of interest, given that this was a secondary objective. Leptin data was still extracted and analyzed from studies that included at least one other outcome of interest (ghrelin, GLP-1 or PYY). Robust results were produced by the analysis of leptin, but after further thought, results were not

included in the current article because they are not representative of all available data on leptin levels following RYGB. In order to be able to make confident and reliable inferences on the changes in leptin following RYGB, the 72 studies that were excluded would need to be included in the analysis. The large amount of data available on leptin that our search strategy and screening process revealed that a meta-analysis illustrating the changes in leptin levels following RYGB surgery as a main objective could be performed. Leptin results from the population of included studies in the present meta-analysis were aligned with the original hypothesis that with weight loss, leptin levels would subsequently decrease. Since leptin is produced by the adipose tissue and meta-regressions of leptin values and fat mass over time would be interesting, it should be noted that only 2 of the included studies measured fat mass. A possible reason for this could be due to the challenges of performing accurate fat mass measurements in people living with severe obesity. If such a review on leptin were to be conducted, it would be interesting to explore changes after weight nadir and comparison of the years following among participants who regain weight vs. participants who maintained their weight loss.

It is to be noted that even if a meta-analysis on appetite-related hormones was conducted with success, this was my second thesis project attempt. At the beginning of my master's degree journey, I started a project investigating the effects of exercise on appetite hormones, energy consumption and expenditure on RYGB patients who had a weight loss maintenance compared to RYGB patients who had regained weight. In May 2019, I started putting together this research project (ExoABari), with the help of my supervisors, which included multiple questionnaires in French and English, appetite hormone analysis, appetite sensation measures, food preferences assessment, in lab and post-assessment meals, activity trackers and journals, and in lab supervised exercise. This multi-centric project was set to take place in Ottawa, Montreal and Sherbrooke. I

was entirely responsible for the preparation of all the documents necessary for recruitment, including social media ads, initial telephone interviews, email confirmations, oral consent processes to check surgery details in medical files, reminder emails with instructions for pre-visit fasting protocols, and instructions for post-visit protocols (energy intake and expenditure over 3 days). I also prepared the written consent form and questionnaires in French and English. I compiled all the required documents with instructions for all testing days for each participant, which included: a check list of equipment and tasks, the precise timeline from 7 a.m. to 3 p.m. with all protocols to follow at each time point, questionnaires to be filled out, and information to be recorded. Precise bilingual grocery lists with quantities were prepared since participants were to eat breakfast and lunch in the lab and take lunch boxes home for the following 3 days containing food items of their choice with double portions. The documents for testing days included: a questionnaire on depressive symptoms, binge eating and measurement of resting metabolic rate (RMR), personalized food reward questionnaire setup online (French and English with food items preferred by participants) (Leeds food preference questionnaire (LFPQ)), VAS for appetite sensation (bilingual electronic version setup) (7x/test day), anthropometric measurement, body composition (DEXA), exercise with indirect calorimetry, blood sampling (5x/test day) for ghrelin, GLP-1 and PYY, energy intake measurement during test days and the following 3 days, and energy expenditure and activity journals for 3 days. In order to be prepared and competent for the test days, I received training for the RMR, DEXA, and food preparation in the Ottawa lab as well as a day in Sherbrooke training in the wet lab. In March 2020, one week after the Covid-19 pandemic caused shutdowns across the country, we received our last ethics approval for Ottawa. All this to say, unfortunately the project was never able to start in Ottawa because of the pandemic restrictions and since our population of interest was at a higher risk of complications from Covid-19. In

Sherbrooke and Montreal, the project was still able to take place, and I conducted both the participant recruitment using social media ads for targeted RYGB groups and phone interviews to confirm eligibility. Despite not being able to complete the initial project, I still learned tremendously about all of the work that goes into organizing and preparing for experimental research. Successfully completing a systematic review and meta-analysis allowed me to learn all the steps involved in conducting this type of research from the search strategy development to the screening processes and arduous work involved in data extraction and analysis. This project allowed me to sharpen my critical thinking regarding methods, results and appraisal of reported information in study articles.

Conclusion

In conclusion, this meta-analysis on gut peptides before and following RYGB weight loss surgery was able to illustrate the changes of ghrelin, GLP-1 and PYY from pre-surgery to different time points following RYGB. This study reveals large heterogeneity between studies in the current literature on ghrelin, GLP-1 and PYY in RYGB patients. Further investigation will be required to solidify conclusions and to determine if the effects are sustained for years after the weight loss instigated by the RYGB surgery. Longer follow-ups past weight nadir in RYGB patients are also required to understand if changes persist with weight stabilization. Standardization of times of AUC, types of assay kits and implementation of duplicate assays in future studies are suggested as an attempt to limit variability between studies pre-intervention and therefore lead to both stronger conclusions and additional precision on the effects of RYGB on gut peptides.

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