Obesity

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Clinical Trials and Investigations

Glucose-Dependent Insulinotropic Polypeptide and Glucagon After Weight Loss Induced by Diet or Bariatric Surgery

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ABSTRACT

Objective: This study compared the effects of a very low-energy diet (VLED), alone or combined with sleeve gastrectomy (SG) or Roux-en-Y gastric bypass (RYGB), on glucose-dependent insulinotropic polypeptide (GIP) and glucagon concentrations, hormones likely to play a role in weight loss maintenance.

Methods: Participants with severe obesity underwent 10 weeks of VLED alone ($n = 15$) or combined with SG ($n = 15$) or RYGB ($n = 14$). Plasma concentrations of glucagon and GIP (fasting and the first 60 min of a meal), insulin sensitivity, respiratory quotient, and resting energy expenditure (REE) were measured at pre- and post-intervention. Differences in hormone concentrations between groups at follow-up and associations between hormones and metabolic outcomes were evaluated.

Results: Fasting glucagon concentrations were higher, while postprandial GIP concentrations were lower, after RYGB compared to SG. An increase in postprandial glucagon was associated with a decrease in Matsuda index in the RYGB group and with an increase in REE in all groups. An increase in fasting GIP was correlated with an increase in HOMA-IR.

Conclusions: RYGB was associated with lower postprandial GIP and greater glucagon concentrations compared with other groups. These hormonal changes are likely to impact REE, as well as insulin sensitivity, potentially modulating the likelihood of weight loss maintenance.

Trial Registration: ClinicalTrials.gov identifier NCT04051190.

1 | Introduction

Bariatric surgery is one of the most effective treatments for obesity and type 2 diabetes mellitus (T2DM), with long-term safety data that newer anti-obesity medications do not have yet [1]. The two main bariatric surgeries, sleeve gastrectomy (SG) and Roux-en-Y gastric bypass (RYGB), improve glycemic control [2], protect against major adverse cardiovascular events [3], and reduce hepatic steatosis [4]. Some surgical outcomes, such as reduced cardiovascular events or improved insulin sensitivity, are closely linked to the magnitude of weight loss [5, 6]. Other outcomes, such as increased glucose tolerance and hepatic insulin sensitivity, occur before substantial weight loss [7]. Therefore, the changes to the intestinal tract may provide additional

Study Importance

- What is already known?
  - Gastrointestinal and pancreatic hormones are affected by bariatric surgery and may affect the likelihood of long-term weight loss maintenance.
  - Glucose-dependent insulinotropic polypeptide (GIP) and glucagon are associated with insulin resistance and energy expenditure, respectively, making them particularly relevant for people with severe obesity.
- What does this study add?
  - Roux-en-Y gastric bypass (RYGB), compared to sleeve gastrectomy (SG) or diet-induced weight loss, leads to lower postprandial GIP and greater fasting glucagon when weight loss and diet macronutrient composition are similar among treatments.
  - Fasting GIP is correlated with insulin resistance, while postprandial glucagon is associated with resting energy expenditure across the three groups. However, postprandial glucagon is associated with insulin resistance in the RYGB group only.
- How might these results change the direction of research or the focus of clinical practice?
  - Individuals who undergo bariatric surgery or diet-induced weight loss might experience changes in GIP and glucagon that could affect their likelihood of long-term weight loss maintenance.

benefits for glycemic control and weight loss maintenance compared to calorie restriction alone.

Gastrointestinal (GI) hormones are rapidly affected by RYGB and SG, potentially mediating the weight loss-independent effects of bariatric surgery. Glucose-dependent insulinotropic polypeptide (GIP) is one GI hormone that has postprandial effects on lipogenesis and ß-cell function. Additionally, fasting GIP has been correlated with the respiratory quotient (RQ), indicating weight regain risk and reduced fat oxidation [8]. For these reasons, GIP is considered to be an obesogenic hormone, but it is unclear whether GIP is associated with insulin resistance and RQ following bariatric surgeries [9]. Previous research shows increased [10], reduced [11], or no change in postprandial GIP [6] after RYGB. However, there is limited information about GIP concentrations following SG. Therefore, comparing fasting and postprandial GIP after RYGB or SG after similar weight loss is crucial to understanding GIP's role following the two most common bariatric procedures.

Another hormone that could be affected by RYGB and SG is glucagon. Glucagon is a pancreatic hormone secreted during the fasted state, as well as in response to amino acids during a meal. Glucagon promotes glycogenolysis and gluconeogenesis in the liver and is associated with increased energy expenditure (EE), even in animals with little to no brown adipose tissue [12]. Glucagon also may exert incretin-like effects in the fed state by binding to the ß-cell glucagon-like peptide 1 (GLP-1) receptor [12]. Following weight loss, fasting glucagon concentrations appear to decrease [13]. However, research is sparse on postprandial glucagon following different bariatric surgeries [11, 14]. Given glucagon's complex roles in glucose metabolism and EE, researchers and clinicians need to understand whether postprandial glucagon is elevated in one surgery compared to the other and the effects of increased postprandial glucagon in this patient population.

Therefore, this analysis compared GIP and glucagon plasma concentrations across three obesity treatment groups (very low-energy diet [VLED] alone or in combination with SG or RYGB) that lost similar amounts of weight, fat mass (FM), and fat free mass (FFM). To our knowledge, previous research comparing these hormones following different weight loss modalities had significant between-group differences in the magnitude of weight loss and/or in diet macronutrient composition, unlike our study. We hypothesized that postprandial GIP would be lower and glucagon higher following RYGB compared to VLED and SG. Additionally, we explored the association of changes in GIP and glucagon with changes in insulin sensitivity and energy metabolism at follow-up. We were interested in insulin sensitivity and energy metabolism outcomes because they reflect fuel utilization and weight regain risk [8, 12, 15, 16]. We hypothesized that increases in GIP and glucagon would be associated with decreases in insulin sensitivity, that increased glucagon would be associated with increased resting EE (REE), and that increased fasting GIP would be associated with increased RQ.

2 | Methods

2.1 | Study Design

The data used in this analysis were obtained from the parent study, The effect of DIet-induced weight loss versus SG and gastric bypass on appetite (DISGAP). The DISGAP study is a three-armed, prospective, nonrandomized controlled trial that tested whether equivalent weight loss following a VLED alone or in combination with bariatric surgery impacted GI hormones and subjective appetite feelings [17]. Recruitment and data collection took place between September 2019 and January 2022. An outline of the DISGAP study can be seen in Figure 1.

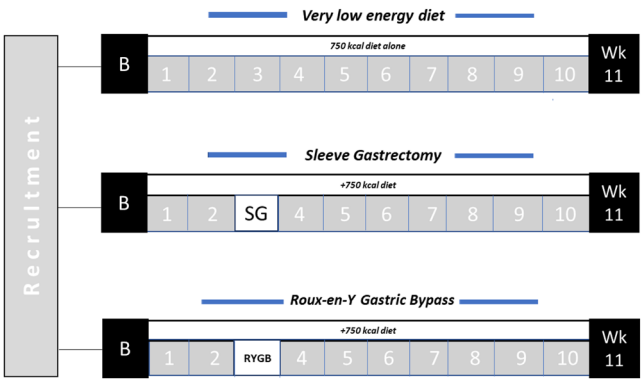

FIGURE 1 | DISGAP study design (nonrandomized study). Assessments were performed at baseline and Week 11. B, baseline; DISGAP, The effect of DIet-induced weight loss versus sleeve gastrectomy and gastric bypass on appetite; RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; Wk, week.

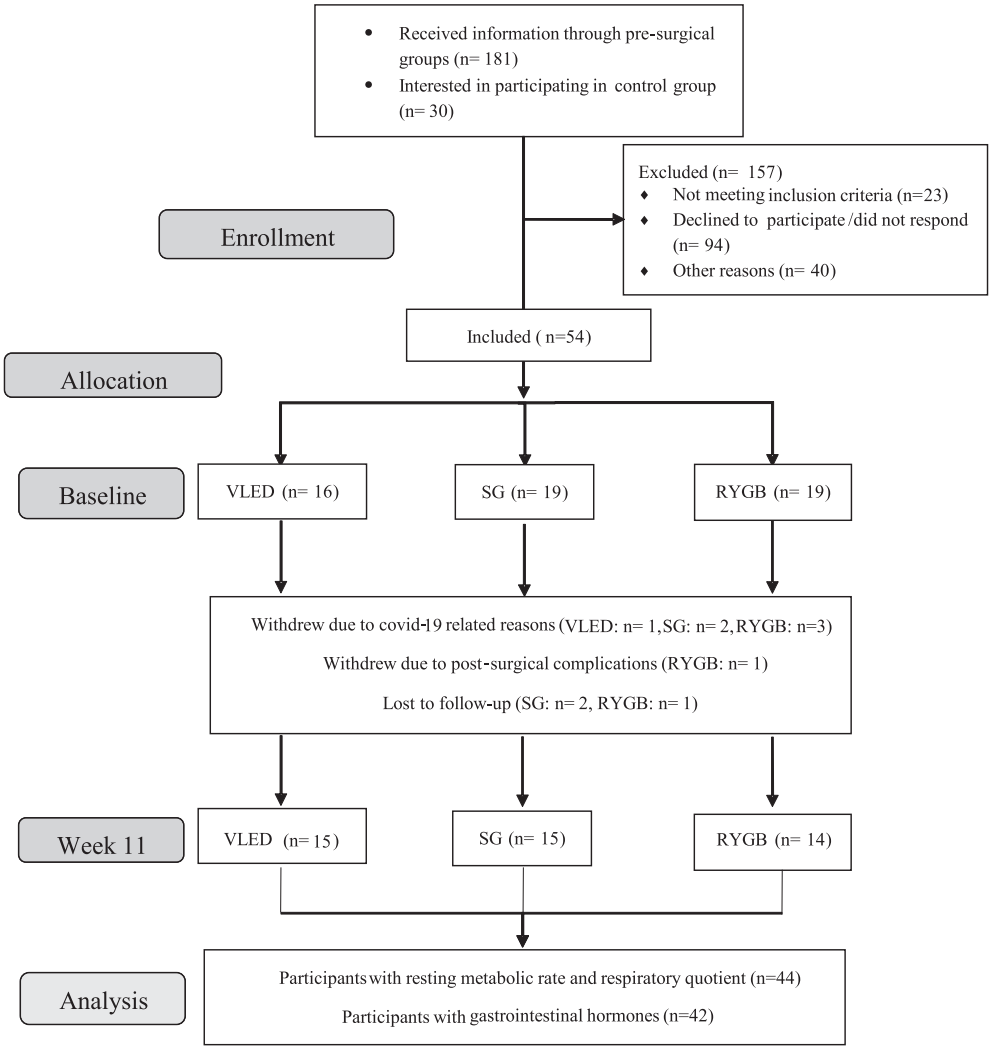

FIGURE 2 | Flow diagram of the study. RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; VLED, very low-energy diet.

The study was approved by the local ethics committee (REK Midt-Norge, Norway; identification number: 2019/252) and was registered in ClinicalTrials.gov (NCT04051190). Informed consent was obtained from all participants in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

2.2 | Participants

DISGAP recruited adults with severe obesity scheduled for RYGB or SG at two local hospitals in the Central Norway Health Region. Patients on a waiting list for bariatric surgery, those who did not meet eligibility criteria for bariatric surgery, and individuals with severe obesity from the local community were recruited for the VLED group. Figure 2 shows the flow diagram for the DISGAP study.

Inclusion criteria were BMI between 35 and 55 kg/m^2, age 18–65, self-reported weight stability (< 2 kg body weight change over the last 3 months), and either a patient in the obesity clinic or one scheduled for bariatric surgery. Exclusion criteria were medications known to affect energy metabolism or appetite, a current cancer diagnosis, or previous bariatric surgery. For this analysis, participants with incomplete or missing data were excluded. Participants with T2DM ($n = 2$, both in the RYGB group) were included in the analysis a priori to maximize statistical power. These participants stopped their medications at least 2 weeks prior to the start of the study after consulting with their physician. More details, including sample size determination, can be found in the parent publication [17].

2.3 | Interventions

2.3.1 | Surgical Procedures

Bariatric surgeries were performed in central Norway at St. Olav University Hospital in Trondheim and the Hospital in Namsos using standard laparoscopic procedures. The SG involved severing the gastrocolic ligament, starting the gastrectomy 4 cm before the pylorus along the greater curvature, and forming the sleeve along the lesser curvature using a 36 French bougie. The RYGB procedure involved forming a small (20–30 mL) proximal

gastric pouch and a stapled gastrojejunostomy. A 75- to 150-cm Roux-Y limb was constructed by transecting the jejunum 60–100 cm distal to the ligament of Treitz and performing a stapled jejunostomy at this site [18].

2.3.2 | Diet Intervention

All participants followed the VLED under the guidance of a registered dietitian for 10 weeks. Participants scheduled for SG and RYGB started the VLED 2 weeks before surgery and continued for another 8 weeks, as part of the surgical protocol in Norway. The VLED consisted of meal replacement food packs and provided 750 kcal per day (LighterLife UK Ltd., Harlow, UK; 26% energy from fat, 36% from carbohydrates, 5% from fiber, and 33% from protein). The meal replacements included soups, shakes, porridge, and bars that were approximately 150 kcal each and similar in macronutrient composition. Participants chose any combination of five products per day. Additionally, participants were encouraged to consume 2.5 L of water per day and up to 100 g of low-starch vegetables. For the first postoperative weeks, surgical patients consumed only liquid meal replacements and gradually introduced more varied food textures into their diet.

All participants were asked to complete a self-reported food diary. At weekly scheduled follow-ups, food diaries were discussed, side effects were recorded, weight was monitored, and acetoacetate was measured in urine with Ketostix (Bayer Ketostix 2880 Urine Reagent Test Strip, Ascensia Diabetes Care, Basel, Switzerland), as a measure of compliance to the VLED. More details on the VLED intervention can be found in prior publications [17, 18].

2.4 | Outcome Variables

After an overnight fast (at least 10 h), participants came to the obesity outpatient clinic at St. Olav University Hospital twice: before the start of the dietary intervention (baseline) and after 10 weeks (Week 11). Body weight and composition, liquid meal tolerance, and energy metabolism were measured at these time points.

2.5 | Body Weight and Composition

Air-displacement plethysmography (BodPod, COSMED, Concord, CA, USA) was used to measure body weight (kg), FM (kg), and FFM (kg).

2.6 | Liquid Meal Tolerance and Hormone Concentrations

Participants consumed a standardized 200-mL commercial low-glycemic drink for the determination of postprandial hormone concentrations (Diben Drink, Fresenius Kabi Norge AS, Fredrikstad, Norway) (300 kcal, 42% fat, 35% carbohydrates, 3% fiber, and 20% protein). This low-glycemic drink was chosen to reduce the risk of dumping syndrome in the operated groups. Blood samples were collected in 4-mL EDTA-coated tubes and drawn at fasting, every 15 min for the first hour, and then at 30-min intervals until 150 min. Participants were asked to consume the drink slowly over a 15-min period to avoid dumping syndrome. Details regarding blood collection and handling were previously published [17].

Plasma insulin was assayed on the Human Metabolic Hormone Magnetic Bead Panel (HMHEMAG-34 K, Merck KGaA) alongside PYY and ghrelin [17]. Plasma glucose was analyzed using a Siemens Atellica CH930 analyzer (Siemens Healthcare Diagnostics). C-peptide was analyzed using a Roche cobas pro e801 analyzer (Roche Diagnostics). The intra- and inter-assay coefficients of variation were $<10\%$ and $<20\%$ for insulin. The analytical variation and intraindividual biological variation were 1.6% and 4.9% for glucose and 1.5% and 16.6% for C-peptide.

Plasma glucagon and active GIP were measured in duplicate using MesoScale Discovery (Rockville, MD) V-PLEX Human Metabolic Panel I kits. The intra- and inter-assay coefficients of variation were $<5\%$ and $<11\%$, respectively, for both GIP and glucagon. The glucagon assay had 30% cross-reactivity with glicentin (1–61), whereas GIP did not have detectable cross-reactivity with selected metabolites and hormones.

For GIP and glucagon, only the first 60 min of the meal challenge was available due to budgetary constraints. The total area under the curve (tAUC) for GIP and glucagon during the meal challenge was estimated using the trapezoidal rule, and incremental AUC (iAUC) was calculated as (tAUC − [fasting value*60 min]).

The Matsuda index [19] and the homeostatic model assessment for insulin resistance (HOMA-IR) [20] were calculated as proxies of insulin sensitivity. For the Matsuda index and for the C-peptide, insulin, and glucose AUCs, data from the full 150-min meal challenge were used.

2.7 | Resting Energy Expenditure and Respiratory Quotient

After resting for 10 min, REE was measured for 15 min with a computerized, open-circuit, ventilated canopy indirect calorimetry system (Vmax Encore 29 N, CareFusion, Baesweiler, Germany). Oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were measured continuously, and values were averaged every minute [21]. The RQ was the average VCO_2/VO_2 during the test.

2.8 | Statistical Analysis

Descriptive statistics were generated for the variables of interest (mean, standard deviation [SD]). Differences in continuous variables between the groups (VLED, SG, RYGB) at baseline were tested using ANOVA. For the ANOVA test, homogeneity of variance was assessed using Levene's test, and skewed variables were log-transformed to meet the normality assumption. We performed the Kruskal–Wallis test when the ANOVA assumptions were violated. Differences in sex distribution between the groups at baseline were tested using a Pearson's chi-square test.

To test whether there were differences between pre- and post-intervention continuous variables within each group, we used paired t-tests or Wilcoxon signed rank tests, as appropriate. To compare the groups in Week 11 on body composition, energy metabolism, insulin sensitivity, and hormones (GIP and glucagon), we used ANCOVA, adjusting for baseline values. We also included age or baseline glucose iAUC as covariates when relevant because these were significantly different between groups at baseline. Post hoc pairwise comparisons of Week 11 outcomes among the three groups were tested using the Bonferroni adjustment.

To identify whether changes in GIP and glucagon were associated with metabolic outcomes of interest (changes in Matsuda index, HOMA-IR, REE, and RQ), we conducted multiple linear regressions with covariates and an interaction term included (hormone*group). Changes were calculated as Week 11 minus baseline. Covariates included age and glucose iAUC for changes in Matsuda index, HOMA-IR, and RQ. Sex was associated with HOMA-IR, so this was included as a covariate for this model as well. For the change in REE, the covariates were age and baseline FFM. The reference group was RYGB for interactions. Residual outliers greater than or less than two SD were removed, and residual plots were visually inspected for normality. Final significant regression models are presented in the results with the model F-statistic (df, n), β coefficient [95% confidence interval], and p values.

All analyses were conducted with and without the participants with T2DM to understand if these participants in the RYGB group affected the results. The results were not substantially different when they were included, especially when we adjusted for baseline glucose concentrations. Therefore, we report the results with the T2DM participants included.

Significance was set at $p < 0.05$. The analyses were performed with SAS statistical software (version 9.4, SAS Institute Inc.). Figures were generated using RStudio (version 4.3.1) and Microsoft Excel for Microsoft 365.

3 | Results

3.1 | Participant Characteristics

Participant characteristics at baseline and Week 11 are shown in Table 1. Forty-four participants completed the intervention ($n = 15$ VLED, $n = 15$ SG, $n = 14$ RYGB) and most participants were female, with no significant sex differences among groups. On average, participants were 43.5 ± 11.3 years of age; however, the RYGB participants were significantly older compared to the SG participants. Besides age, the other baseline characteristic that was different among groups was glucose iAUC. The RYGB group had significantly greater postprandial glucose compared to the VLED group, likely due to the two participants with T2DM in the RYGB group.

Each group showed significant decreases in body weight, FM, and FFM, with an average percent body weight loss of 15.5%. Fasting and postprandial insulin sensitivity improved in all groups over time, and Figure 3A–D shows insulin and glucose concentrations at baseline and Week 11 by group. Despite the general improvements in glycemia, glucose iAUC at Week 11 was greater in the RYGB group compared to the SG group, after adjusting for age and baseline glucose iAUC. There were no other statistically significant between-group differences in body weight or composition, REE, and insulin-related variables at Week 11, after adjusting for baseline values and covariates.

3.2 | GIP and Glucagon Concentrations at Baseline and Week 11

Table 2 and Figure 4A–D show GIP and glucagon concentrations at baseline and Week 11 by group. The results from the pairwise comparisons in the hormone concentrations at Week 11 are shown in Figure 5A–F. At Week 11, there was a main effect of group on fasting glucagon, glucagon tAUC, GIP iAUC, and GIP tAUC. Fasting glucagon and glucagon tAUC were greater following RYGB compared to SG (Figure 5B; $p < 0.05$ and Figure 5F; $p < 0.05$). GIP iAUC and tAUC were lower following RYGB compared to SG (Figure 5C; GIP iAUC; $p < 0.05$ and Figure 5E; GIP tAUC; $p < 0.05$). Fasting GIP and glucagon iAUC were not significantly different among groups at Week 11 (Figure 5A,D).

3.3 | Associations Between Changes in GIP and Glucagon and Changes in Metabolic Outcomes

Figure 6A–C shows the results from the regression models testing the associations between changes in hormones and changes in metabolic outcomes. There was a significant interaction between group and change in glucagon iAUC to explain the change in Matsuda index (Figure 6A; Model $F(7, 32) = 6.78$, $p < 0.001$). An increase in glucagon iAUC was associated with a decrease in Matsuda index for the RYGB group (SG vs. RYGB: $\beta = 0.007$ [0.002, 0.012], $p = 0.009$; VLED vs. RYGB: $\beta = 0.010$ [0.003, 0.017], $p = 0.008$). A different model showed that an increase in glucagon iAUC was also positively correlated with an increase in REE, without a group interaction (Figure 6B; Model $F(5, 34) = 3.62$, $p = 0.012$; $\beta = 0.261$ [0.030, 0.492], $p = 0.028$). Changes in fasting glucagon were not associated with changes in REE ($p > 0.05$).

An increase in fasting GIP was positively associated with an increase in HOMA-IR, without a group interaction (Figure 6C; Model $F(6, 33) = 13.36$, $p < 0.001$; $\beta = 0.118$ [0.026, 0.209], $p = 0.014$). The association between changes in postprandial GIP and changes in Matsuda index was not significant ($p > 0.05$), nor was the change in fasting GIP associated with changes in RQ ($p > 0.05$).

4 | Discussion

The present study investigated the effects of a VLED, alone or combined with RYGB or SG, on GIP and glucagon plasma concentrations. We also explored the associations between changes in GIP and glucagon concentrations with changes in metabolic outcomes. At Week 11, fasting glucagon and glucagon tAUC concentrations were highest in the RYGB group, whereas

TABLE 1 | Participant characteristics at baseline and Week 11, by group.

	VLED ($n=15$)			SG ($n=15$)			RYGB ($n=14$)			
Variables	**Baseline**	**Week 11**	**Baseline vs. Week 11 (p value)**	**Baseline**	**Week 11**	**Baseline vs. Week 11 (p value)**	**Baseline**	**Week 11**	**Baseline vs. Week 11 (p value)**	**Week 11 group (p value)**
Sex (% female)	73.33%	—	—	73.33%	—	—	64.3%	—	—	—
Age (years)	44.5 ± 11.13	—	—	38.0 ± 9.56*	—	—	48.1 ± 11.5*	—	—	—
Weight (kg)	115.1 ± 20.8	97.8 ± 19.6	0.001	120.9 ± 14.7	101.8 ± 12.6	0.001	120.6 ± 13.5	101.8 ± 12.1	0.001	0.292
BMI (kg/m^2)	40.0 ± 3.7	33.7 ± 3.4	0.001	40.5 ± 3.1	34.1 ± 3.0	0.001	41.6 ± 4.9	35.0 ± 5.2	0.001	0.717
FM (kg)	53.9 ± 11.2	40.5 ± 11.2	0.001	58.4 ± 9.0	44.2 ± 8.2	0.001	56.7 ± 11.6	43.8 ± 11.6	0.001	0.407
FFM (kg)	61.1 ± 13.1	57.3 ± 12.2	0.001	62.5 ± 8.9	57.6 ± 7.9	0.001	63.7 ± 10.0	57.9 ± 8.7	0.001	0.069
REE (kcal/day)	2262 ± 544	1935 ± 388	0.001	2219 ± 325	1891 ± 358	0.001	2252 ± 377	1941 ± 351	0.001	0.555
RQ	0.74 ± 0.08	0.69 ± 0.09	0.006	0.76 ± 0.07	0.78 ± 0.13	0.796	0.80 ± 0.07	0.78 ± 0.10	0.459	0.115
Fasting glucose (mg/dL)	88.6 ± 8.2	82.0 ± 4.3	0.006	92.6 ± 10.9	80.6 ± 8.7	0.001	99.8 ± 16.9	86.4 ± 9.1	0.002	0.304
Fasting insulin (μIU/mL)	30.6 ± 26.4	17.6 ± 13.6	0.001	23.7 ± 11.2	14.0 ± 6.3	0.001	24.9 ± 10.1	18.5 ± 14.7	0.024	0.236
Fasting C-peptide (mmol/L)	1.2 ± 0.5	0.7 ± 0.3	0.001	1.0 ± 0.4	0.7 ± 0.2	0.001	1.2 ± 0.4	1.1 ± 0.6	0.001	0.225
HOMA-IR	7.0 ± 6.9	3.6 ± 2.7	0.001	5.50 ± 2.8	2.8 ± 1.40	0.001	6.2 ± 2.8	3.9 ± 2.9	0.009	0.099
Matsuda index	3.8 ± 2.1	5.9 ± 2.9	0.008	3.7 ± 1.8	6.0 ± 2.8	0.001	2.9 ± 1.4	5.9 ± 5.2	0.035	0.151
Glucose iAUC (mg/dL*min)	129,607 ± 45861*	135,439 ± 25,456	0.5016	134,844 ± 39,102	107,845 ± 28,038	0.001	189,305 ± 83,535*	143,777 ± 47,599	0.110	0.011
Insulin iAUC (μIU/mL*min)	4370 ± 3200	3577 ± 3001	0.073	5395 ± 2895	3580 ± 1916	0.009	6766 ± 3278	5276 ± 4218	0.106	0.351
C-peptide iAUC (mmol/L*min)	1976 ± 1437	1671 ± 1783	0.091	1602 ± 1175	1780 ± 1346	0.774	3038 ± 1930	3617 ± 3121	0.989	0.373

Note: Data are presented as mean ±SD with the exception of sex (%). Baseline differences between groups in continuous variables were evaluated using ANOVA or Kruskal–Wallis tests (significant difference $p < 0.05$ between 2 groups denoted with *). Differences in sex distribution were assessed with the chi-square test. Differences between baseline and Week 11 values were evaluated by group using a paired Student's t-test or Wilcoxon rank sum test. Differences between groups at Week 11 were evaluated using ANCOVA (adjusting for the baseline value and age or baseline glucose iAUC as appropriate). Bonferroni's adjustment was used for post hoc pairwise comparisons. Abbreviations: FFM, fat free mass; FM, fat mass; HOMA-IR, homeostatic model assessment of insulin resistance; iAUC, incremental area under the curve; REE, resting energy expenditure; RQ, respiratory quotient; RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; VLED, very low-energy diet.

postprandial GIP concentrations were lowest in the RYGB group. We found an inverse correlation between the change in glucagon iAUC and the change in Matsuda index in the RYGB group only, suggesting that factors unique to the RYGB procedure could impact the relationship between postprandial glucagon and insulin sensitivity. Additionally, an increase in glucagon iAUC was associated with an increase in REE, independent of treatment type. Finally, an increase in fasting GIP concentrations was correlated with an increase in HOMA-IR, a marker for hepatic insulin resistance. These findings highlight the distinct hormonal responses following different obesity treatments and may inspire future research to investigate their implications for weight regain in this population.

Our first objective was to compare GIP and glucagon concentrations across groups at Week 11. Postprandial GIP was significantly lower after RYGB than after SG. To our knowledge, this is the first study to compare postprandial GIP concentrations across three obesity treatment groups with comparable weight loss. One previous study found lower GIP concentrations 2 years after RYGB compared to SG and intensive medical treatment; however, the medical treatment group lost significantly less weight compared to the surgical groups [11]. Another study also reported lower postprandial GIP after RYGB compared to SG and non-surgical controls, though this study was cross-sectional and included patients with at least a 12-month postoperative follow-up [22]. These findings suggest that the lower postprandial GIP observed after RYGB compared to SG is sustained over time, supporting the durability of our results. This sustained reduction in postprandial GIP may result from nutrients bypassing the duodenum after RYGB, where GIP-secreting cells are concentrated. Factors related to the RYGB procedure, such as biliopancreatic (BP) limb length, can further influence GIP secretion, leading to some heterogeneity in GIP concentrations post RYGB [23]. Whether this heterogeneity can explain different treatment responses to RYGB remains unclear, given we did not have individual BP limb measurements. Nevertheless, our findings support a distinct effect of RYGB on postprandial GIP concentrations compared to SG, which may contribute to postoperative outcomes.

After weight loss, fasting glucagon and glucagon tAUC were higher in the RYGB group compared to the SG group. This indicates that elevated glucagon concentrations in the RYGB group were mainly driven by fasting levels. Several studies have also reported higher fasting and/or postprandial glucagon following RYGB compared to SG [14, 24–26], although the reasons remain unclear. Fasting glucagon concentrations are influenced by weight loss, as well as by glucose and insulin concentrations. Adjusting for baseline glucose iAUC, which was higher in the RYGB group, did not alter the group effect on fasting glucagon. Further research is needed to determine whether elevated fasting glucagon concentrations after RYGB versus SG are clinically meaningful or contribute to long-term weight loss maintenance.

Our analysis found that an increase in glucagon iAUC was associated with a decrease in Matsuda index, observed only in the RYGB group. This inverse association between postprandial glucagon and insulin sensitivity may reflect differences in nutrient uptake and absorption after RYGB compared to SG or diet-induced weight loss. Glucagon is secreted in response to amino acids, and one study showed significantly faster amino acid absorption in RYGB patients compared to SG patients and nonoperated controls [22]. RYGB is also associated with accelerated gastric emptying and glucose absorption compared to SG and nonoperated controls, potentially affecting glucose and insulin concentrations [14, 22]. Beyond pancreatic secretion, the change in glucagon iAUC may reflect changes in intestinally derived glucagon and/or proglucagon peptide, as reported after RYGB in some studies [24, 27, 28]. This suggests that postprandial factors regulating glucagon secretion and suppression are altered following RYGB. In individuals where glucagon secretion exceeds suppression, this may be associated with reduced postprandial insulin sensitivity. Other studies have reported a similar inverse

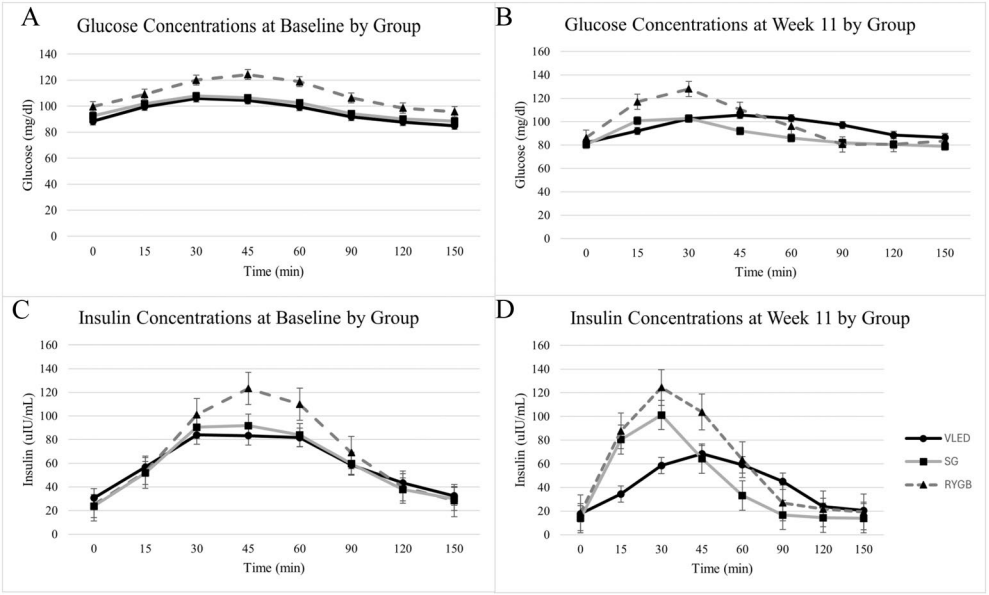

FIGURE 3 | Glucose and insulin concentrations during a liquid meal challenge at baseline and Week 11, by group. (A) Glucose concentrations at baseline by group. (B) Glucose concentrations at Week 11 by group. (C) Insulin concentrations at baseline by group. (D) Insulin concentrations at Week 11 by group. Data are presented as mean ± SEM. RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; VLED, very low-energy diet.

TABLE 2 | GIP and glucagon at baseline and Week 11, by group.

	VLED ($n=15$)			SG ($n=15$)			RYGB ($n=14$)			
Variables	**Baseline**	**Week 11**	**Baseline vs. Week 11 (p value)**	**Baseline**	**Week 11**	**Baseline vs. Week 11 (p value)**	**Baseline**	**Week 11**	**Baseline vs. Week 11 (p value)**	**Week 11 group (p value)**
Fasting GIP (pg/mL)	6.2 ± 2.5	4.5 ± 2.4	0.050	6.4 ± 5.5	5.4 ± 3.0	0.889	6.2 ± 4.6	5.6 ± 3.1	0.879	0.544
GIP iAUC (pg/mL*min)	5810 ± 2934	6270 ± 1027	0.123	5006 ± 2795	7272 ± 4445	0.067	5624 ± 2920	4610 ± 2515	0.178	0.032
GIP tAUC (pg/mL*min)	6181 ± 2973	6538 ± 1016	0.178	5389 ± 3028	7597 ± 4596	0.071	5993 ± 3115	4943 ± 2587	0.204	0.036
Fasting glucagon (pM)	18.8 ± 15.0	18.6 ± 11.6	0.695	18.1 ± 11.7	14.9 ± 8.3	0.463	20.2 ± 9.5	19.7 ± 7.9	0.818	0.031
Glucagon iAUC (pM*min)	118 ± 204	96 ± 170	0.033	251 ± 356	258 ± 275	0.904	332 ± 326	155 ± 382	0.056	0.071
Glucagon tAUC (pM*min)	1307 ± 875	1218 ± 704	0.581	1336 ± 910	1154 ± 684	0.610	1542 ± 698	1137 ± 436	0.061	0.013

Note: Data are presented as mean ±SD. Differences between groups at Week 11 were evaluated using ANCOVA (adjusted for baseline value and age or baseline glucose iAUC as appropriate). Bonferroni's adjustment was used for post hoc pairwise comparisons.

Abbreviations: GIP, glucose-dependent insulinotropic polypeptide; iAUC, incremental area under the curve; RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; tAUC, total area under the curve; VLED, very low-energy diet.

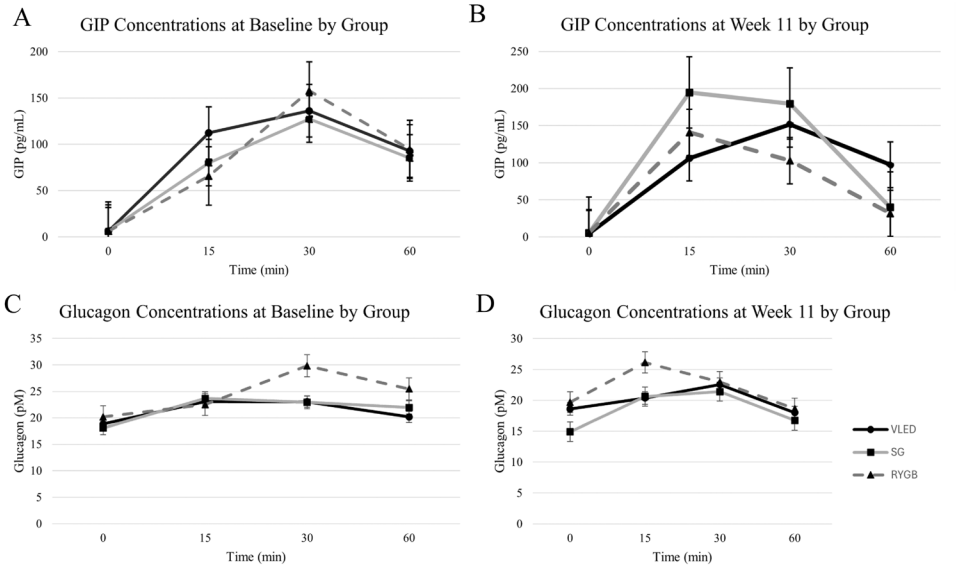

FIGURE 4 | GIP and glucagon concentrations during a liquid meal challenge at baseline and Week 11, by group. (A) GIP concentrations at baseline by group. (B) GIP concentrations at Week 11 by group. (C) Glucagon concentrations at baseline by group. (D) Glucagon concentrations at Week 11 by group. Data are presented as mean ± SEM. GIP, glucose-dependent insulinotropic polypeptide; RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; VLED, very low-energy diet.

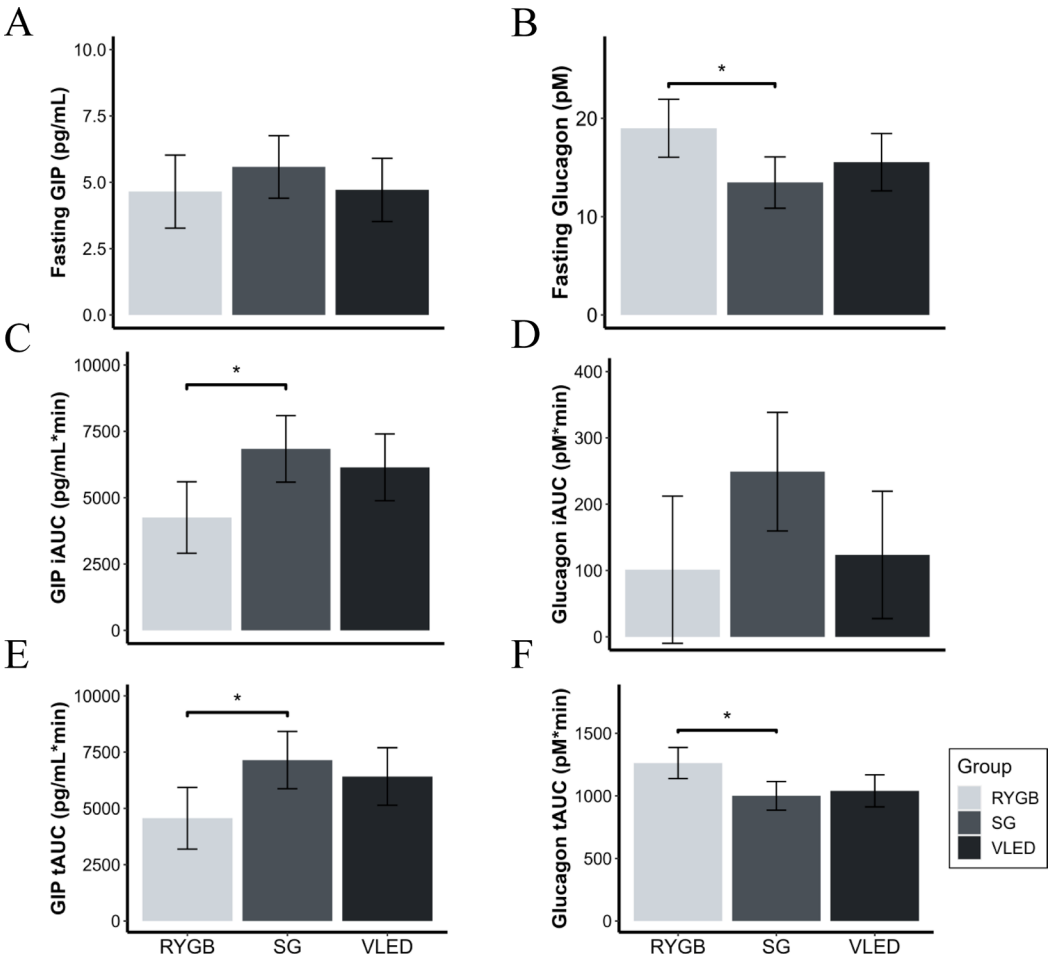

FIGURE 5 | GIP and glucagon comparisons between groups at Week 11. Bonferroni's adjustment was used for post hoc pairwise comparisons. Week 11 values are adjusted for baseline values and covariates (±SEM). *$p < 0.05$. (A) Fasting GIP after weight loss. (B) Fasting glucagon after weight loss. (C) GIP iAUC after weight loss. (D) Glucagon iAUC after weight loss. (E) GIP tAUC after weight loss. (F) Glucagon tAUC after weight loss. GIP, glucose-dependent insulinotropic polypeptide; iAUC, incremental area under the curve; RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; tAUC, total area under the curve; VLED, very low-energy diet.

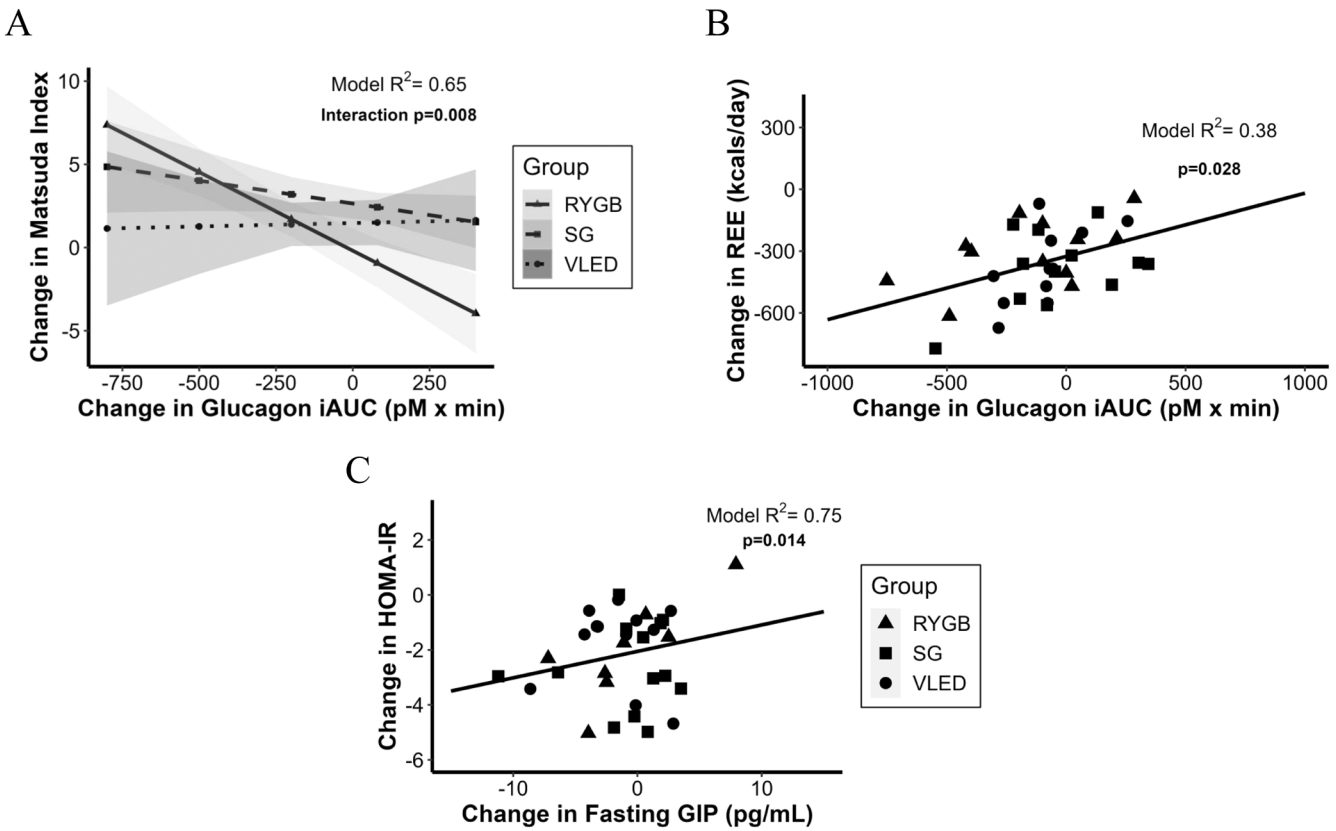

FIGURE 6 | Associations between changes in glucagon, GIP, insulin sensitivity indices, and resting energy expenditure. Each figure panel shows the results from a different regression model. R^2 and p values are shown for the interaction in panel A or the association of the hormone with the outcome in panels B and C. Covariates included age and baseline glucose iAUC (panel A), baseline fat free mass, age, and group (panel B), and age, baseline glucose iAUC, sex, and group (panel C). (A) Change in glucagon iAUC on the change in Matsuda index. (B) Change in glucagon iAUC on the change in resting energy expenditure. (C) Change in fasting GIP on the change in HOMA-IR. GIP, glucose-dependent insulinotropic polypeptide; HOMA-IR, homeostatic model assessment of insulin resistance; iAUC, incremental area under the curve; REE, resting energy expenditure; RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; VLED, very low-energy diet.

association, though they used nonphysiological methods or lacked surgical comparisons [29–31]. Further research is needed to understand the role of endogenous postprandial glucagon on insulin sensitivity after RYGB, ideally using nonoral insulin sensitivity indices to minimize confounding by gut-related processes.

An increase in glucagon iAUC was also associated with an increase in REE across all groups. Glucagon has been shown to elevate EE in humans independently of brown adipose tissue [32, 33], through the actions of the hepatic farnesoid X receptor and fibroblast growth factor 21 (Fgf21) [12, 34, 35]. In hepatocytes lacking glucagon receptors, both Fgf21 expression and circulating levels are reduced. Similarly, Fgf21 knock-out mice do not experience glucagon-receptor-mediated increases in EE. Our results are novel because prior research on glucagon and REE neither used physiological glucagon concentrations nor examined different weight loss contexts. This result may be applicable for RYGB mechanisms of action, given RYGB induces greater weight loss compared to SG under real-world conditions [36].

An increase in fasting GIP was associated with an increase in HOMA-IR. These results offer new insights into the role of GIP in the fasting state, as most incretin research focuses on the postprandial period when glucose concentrations are elevated. A Mendelian randomization analysis [37] showed that fasting GIP is positively associated with HOMA-IR, independently of BMI, GLP-1, and ghrelin. Furthermore, a Korean epidemiological study found that fasting GIP is associated with T2DM development [38]. Our results suggest that an increase in fasting GIP after weight loss, regardless of the treatment method, may be a marker of increased insulin resistance. However, more research is needed to understand if and how increases in fasting GIP are mechanistically linked to worsening insulin resistance.

This study has several strengths and limitations. Participants underwent similar body composition changes during the intervention, allowing us to compare the effects of different bariatric procedures versus diet alone on GIP and glucagon without confounding by factors related to differential weight loss. Participants in each group also consumed a similar diet composition during the study, which is important because fat intake can promote GIP gene expression and secretion [39]. The dietary control and similar weight loss across groups are remarkable for studies comparing hormones following surgical versus diet-induced weight loss.

Despite its strengths, this study has notable limitations. First, the small sample sizes and nonrandomized study design limit generalizability. This limitation is compounded by a post hoc power analysis, which suggests the study was underpowered to detect between-group differences in GIP and glucagon. Additionally, two patients with T2DM underwent RYGB, introducing imbalance in baseline comorbidities. Another limitation within the RYGB group is variability in BP limb length. BP lengths ranged from 60 to 100 cm, but individual measurements were unavailable. Since shorter BP limbs are associated with greater GIP responses [40], these findings may not generalize to patients with longer BP limbs. Finally, the results may not be applicable with longer follow-up. Additional limitations relate to the hormone measurements. Glucagon was measured using an assay with high cross-reactivity to glicentin, meaning it is uncertain that glucagon was solely responsible for the results. Additionally, GIP and glucagon concentrations were only measured during the first hour of the meal challenge due to budgetary constraints. This limits our interpretations of the hormones to the early postprandial period. We also did not measure gastric emptying rates, which are known to change after surgery and affect GI hormone secretion. Lastly, the liquid meal challenge was a low-glycemic drink which might limit the validity of estimating postprandial insulin sensitivity. The hormonal responses might have been different with other meals, as glucagon- and GIP-secreting cells are sensitive to protein and fat, respectively [41].

In conclusion, RYGB had lower GIP and greater glucagon concentrations compared to the other weight loss treatments. Increased fasting GIP after weight loss may be a marker for insulin resistance in people treated for severe obesity. Increased postprandial glucagon after weight loss was associated with higher REE overall and lower insulin sensitivity in the RYGB group. Larger studies should investigate whether GIP and glucagon influence weight loss maintenance for individuals with severe obesity.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Individual participant data that underlie the results reported in this article can be shared with other researchers after deidentification (text, tables, and figures) up to 5 years after publication. These researchers need to have a specific aim for the data that has been approved by an independent review committee identified for this purpose. The data can also be shared for the purpose of meta-analysis.

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