Combined GLP-1, Oxyntomodulin, and Peptide YY Improves Body Weight and Glycemia in Obesity and Prediabetes/Type 2 Diabetes: A Randomized, Single-Blinded, Placebo-Controlled Study

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OBJECTIVE

Roux-en-Y gastric bypass (RYGB) augments postprandial secretion of glucagon-like peptide 1 (GLP-1), oxyntomodulin (OXM), and peptide YY (PYY). Subcutaneous infusion of these hormones ("GOP"), mimicking postprandial levels, reduces energy intake. Our objective was to study the effects of GOP on glycemia and body weight when given for 4 weeks to patients with diabetes and obesity.

RESEARCH DESIGN AND METHODS

In this single-blinded mechanistic study, obese patients with prediabetes/diabetes were randomized to GOP (*n* = 15) or saline (*n* = 11) infusion for 4 weeks. We also studied 21 patients who had undergone RYGB and 22 patients who followed a very low-calorie diet (VLCD) as unblinded comparators. Outcomes measured were *1*) body weight, *2*) fructosamine levels, *3*) glucose and insulin during a mixed meal test (MMT), *4*) energy expenditure (EE), *5*) energy intake (EI), and *6*) mean glucose and measures of glucose variability during continuous glucose monitoring.

RESULTS

GOP infusion was well tolerated over the 4-week period. There was a greater weight loss (*P* = 0.025) with GOP (mean change −4.4 [95% CI −5.3, −3.5] kg) versus saline (−2.5 [−4.1, −0.9] kg). GOP led to a greater improvement (*P* = 0.0026) in fructosamine (−44.1 [−62.7, −25.5] μmol/L) versus saline (−11.7 [−18.9, −4.5] μmol/L). Despite a smaller weight loss compared with RYGB and VLCD, GOP led to superior glucose tolerance after a mixed-meal stimulus and reduced glycemic variability compared with RYGB and VLCD.

CONCLUSIONS

GOP infusion improves glycemia and reduces body weight. It achieves superior glucose tolerance and reduced glucose variability compared with RYGB and VLCD. GOP is a viable alternative for the treatment of diabetes with favorable effects on body weight.

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Bariatric surgery, in particular Roux-en-Y gastric bypass (RYGB), remains the most efficacious treatment for obesity and type 2 diabetes (T2DM). Glycemic improvement after RYGB is superior to intensive medical management and is sustained, with diabetes remission rates of 26–29% at 5 years (1). Mechanistic studies have shown an early improvement in hepatic insulin sensitivity and later in peripheral insulin sensitivity as well as β-cell function (2). Proposed mechanisms include early postsurgical calorie restriction; postprandial secretion of the satiety gut hormones glucagon-like peptide 1 (GLP-1), oxyntomodulin (OXM), and peptide YY (PYY), reduced secretion of the orexigenic hormone ghrelin, bypass of the proximal small bowel and reduced secretion of an "anti-incretin," changes in bile acid metabolism, changes in gut microbiota composition, reprogramming of intestinal glucose metabolism, and increase in energy expenditure, among others (3). However, the exact contributions from each mechanism are not clear.

GLP-1, OXM, and PYY are released from the L cells of the small intestine and colon, and their postprandial secretion is augmented several-fold after RYGB (4,5). GLP-1 is insulinotropic and reduces food intake. Its analogs are an established treatment for diabetes (6) as well as obesity (7). OXM is a dual agonist of the GLP-1 and glucagon receptors (8), which reduces food intake (9) and increases energy expenditure (10), leading to weight loss (11). PYY acts to reduce appetite and food intake postprandially (12,13). Combinations of GLP-1 and PYY (14,15), OXM and PYY (16), and GLP-1 and glucagon (17) have synergistic effects on food intake.

Replicating the postprandial gut hormone levels after RYGB is possible using a continuous subcutaneous infusion of combination of GLP-1, OXM, and PYY ("GOP"). This is safe, acceptable, and led to a reduction in total ad libitum energy intake compared with placebo over a 10.5-h infusion (5). A continuous subcutaneous infusion obviates the development of sharp peaks and leads to a gradual rise toward steady gut hormone plasma levels (5), which enhances tolerability. If needed to improve tolerability, the infusion can be stopped or the rate reduced, with hormone levels responding within 1 h because of their short half-lives. This is not possible with injections of analogs possessing extended pharmacokinetics. Lastly, continuous subcutaneous infusions are commonplace in diabetes treatment, demonstrating its practicality in daily life.

We therefore hypothesized that a GOP infusion, given for 4 weeks in "free living" conditions, would reduce body weight and improve glycemia compared with a placebo of 0.9% saline (hereafter referred to as Saline) infusion. To compare the metabolic effects of GOP with those of RYGB, we studied a group of patients who underwent surgery. To compare the effects of GOP with simple dietary restriction, we studied a group of patients who followed an 800 kcal/day very low-calorie diet (VLCD).

RESEARCH DESIGN AND METHODS

Study Design and Participants

This mechanistic study took place at the National Institute for Health Research (NIHR) Imperial Clinical Research Unit Facility at Hammersmith Hospital, London, from July 2016 to October 2018. It was a single-blinded randomized controlled study comparing two infusion groups (GOP or Saline) in patients with obesity and prediabetes or T2DM. Two further similar nonblinded groups of patients undergoing RYGB and patients following a VLCD diet were recruited. Potential volunteers for the GOP, Saline, and VLCD groups were recruited from clinics at the Imperial Weight Centre (IWC) or from newspaper advertising, whereas patients already listed for surgery at the IWC were recruited to form the RYGB group.

Inclusion and Exclusion Criteria

Key eligibility criteria were male or female participants aged between 18 and 70 years, meeting the U.K. National Health Service (NHS) criteria for bariatric surgery, and with a diagnosis of prediabetes (impaired fasting glucose, impaired glucose tolerance, or HbA_{1c} of 6.0–6.4% [42–47 mmol/mol]) or T2DM according to World Health Organization criteria. Patients who had diabetes had a stable HbA_{1c} of ≤9.0% (75 mmol/mol) either on diet or a single oral hypoglycemic agent. Key exclusion criteria were any comorbidities or medications that could compromise the validity and safety aspects of the study, a current history of smoking, pregnancy, and a history of eating disorders or restrained eating habits as assessed by SCOFF and the Dutch Eating Behavior Questionnaire (DEBQ) questionnaires (18–20).

Interventions

Infusion Groups (GOP or Saline)

GLP-1 (7-36) amide, PYY (3-36) amide, and OXM were manufactured to Good Manufacturing Practice standards (AmbioPharm). They were mixed under sterile conditions and freeze-dried in single vials. Visually identical vials containing freeze-dried 0.9% Saline were also manufactured. Vials were reconstituted with sterile water for injections and added to 0.9% Saline. Reconstituted peptides were verified to be stable for over 12 h at 37°C by high-performance liquid chromatography (Supplementary Fig. 1). A Cane Crono Infusion pump (Applied Medical Technology) and an infusion set (Medtronic) was used to deliver the subcutaneous infusion of GOP at a dose of 4/4/0.4 pmol/kg/min, respectively (5). Volunteers were allocated to receive the GOP or Saline infusion for 28 days by simple randomization performed by an independent investigator. Volunteers' usual treatment for diabetes was suspended for the duration of the study. Only volunteers remained blinded to the nature of the infusion from allocation until the end of the 28-day infusion period. They were instructed to run the infusion for 12 h/day, beginning 1 h before breakfast and disconnecting after their last meal of the day. On study days, the infusions were commenced at least 2 h before any study procedures. All volunteers also received dietetic advice on healthy eating and weight loss from a qualified dietitian.

RYGB Group

Volunteers undergoing RYGB attended the research unit for a baseline visit before surgery and were reviewed at 2, 4, and 12 weeks after surgery. RYGB surgery was performed laparoscopically according to standardized techniques by three designated surgeons at IWC.

VLCD Group

Volunteers attended the research unit for a baseline visit, before starting a complete meal replacement VLCD of 800 kcal/day for 4 weeks (Cambridge Weight Plan). They were reviewed by a dietitian prediet and then weekly until completion.

Method Details

Anthropometry and Body Composition

Body weight and body composition were measured using a Tanita T8148.

Mixed Meal Test

A standardized mixed meal test (MMT) (14 g protein, 12.9 g fat, 39.6 g carbohydrates, 330 kcal, 137.5 mL) (Ensure Compact, Abbott) was administered before and at the end of each intervention. Blood for glucose, insulin, and glucagon levels was sampled at $t = -30$, 0, 15, 30, 60, 120, 180, and 240 min from the time of meal ingestion via an indwelling cannula placed in the antecubital fossa. Estimates of hepatic insulin sensitivity (based on the interactive 24-variable homeostasis model assessment [iHOMA2%S]) were derived from fasting glucose and insulin levels (21).

Continuous Glucose Monitoring

Subjects were fitted with a blinded continuous glucose monitoring (CGM) device (Dexcom G4 Platinum) before their baseline visit and on week 3 of the intervention. Data were collected for 7 days under free living conditions and were analyzed using the easyGV calculator (http://www.phc.ox.ac.uk/research/technology-outputs/easygv) for measures of glycemic variability (GV).

Indirect Calorimetry and Three-Dimensional Accelerometry for Energy Expenditure

Indirect calorimetry using a ventilated hood system (GEM Nutrition) was used to measure resting energy expenditure (REE) and diet-induced thermogenesis (DIT) (5). After the individual rested for 30 min in a recliner chair, REE was measured in the fasted state for 15–20 min. The energy expenditure (EE) measurement was repeated at 30 min after the ingestion of the MMT to estimate DIT. Activity-related EE (AEE) was estimated using an Actiheart device (CamNTech).

Ad Libitum and 24-h Energy Intake Measurement

Ad libitum energy intake (EI) studies (5) were performed at baseline and at 4 weeks after the infusions but at 12 weeks after RYGB (because this group could not tolerate solid meals at 4 weeks and had resumed normal eating by 12 weeks). To estimate free living 24-h EI, volunteers were asked to fill in 72-h food diaries and mean intakes over 24 h calculated using Dietplan7 (Forestfield Software).

Blood Sampling and Assays

Blood for fructosamine was collected in clot activator tubes and analyzed using the colorimetric method (within-batch coefficient of variation [CV] $<1\%$, between-batch CV $<2.5\%$ at between 260 and 500 μmol/L; Sandwell and West Birmingham Hospitals NHS Trust). Blood for gut hormones was collected in lithium heparin tubes containing Aprotinin (Nordic Pharma) and the dipeptidyl peptidase 4 inhibitor Diprotin A (Enzo Life Sciences). Samples were placed on ice, centrifuged at 4°C within 10 min of collection, and stored at −80°C until analysis. Active GLP-1 levels were measured by the Milliplex magnetic bead-based multianalyte, metabolic panel, four-plex immunoassay (Millipore). Total PYY was measured by an in-house radioimmunoassay. OXM was measured using a specific and sensitive mass-spectrometry validated immunoassay (Holst Laboratory, University of Copenhagen). The intra- and interassay CV for active GLP-1, PYY, and OXM assays was $<10\%$, and the lowest limit of detection was 0.8 pmol/L for GLP-1, 8.7 pmol/L for PYY, and 5 pmol/L for OXM. Glucagon was measured using an ELISA (Mercodia AB), with a detection limit of 1.5 pmol/L. Blood for glucose was collected in fluoride oxalate tubes and for insulin and lipids in plain tubes. Glucose, insulin, and lipid levels were measured by NW London Pathology (CVs $<5\%$, $<10\%$, $<5\%$, respectively) (ARCHITECH, Abbott).

Outcomes and Statistical Analysis

The primary outcome of the study was the change in body weight at 4 weeks. Secondary outcomes were change in glycemia, as assessed by fructosamine measurement, change in ad libitum and 24-h total EI, fasting and postprandial glucose and insulin levels in response to a mixed meal test, GV as assessed by CGM; and energy expenditure. We calculated that 20 subjects per group would give an 85% power to detect a minimal clinically significant difference in weight loss of 2.5 kg between Saline and GOP at 4 weeks (SD 2.6 kg based on pilot data). With regards to fructosamine, a sample size of ~13 volunteers per group would provide 95% power (α 5%, SD 28 μmol/L, two-tailed t test) to detect a change of ~32 μmol/L, equivalent to a minimal clinically significant HbA_{1c} change of 0.5%.

Analysis was performed based on a per-protocol approach. The primary comparison between GOP and Saline and the secondary comparisons of GOP versus RYGB and GOP versus VLCD were made using an unpaired Student t test for the primary and secondary outcomes, and a P value of <0.05 was considered significant. Glycemic parameters, metabolic profiles in response to an MMT, energy balance parameters, and lipid parameters were assessed using the same methodology for the primary outcome analysis. To look for potential baseline effects for fructosamine, ANCOVA was used for the corresponding tests of GOP versus RYGB and GOP versus VLCD incorporating baseline fructosamine as a covariate.

Additional sensitivity tests investigated outliers highlighted during normality testing to estimate outlier influence. A sensitivity analysis showed that the conclusions were robust with and without imputation. Postprandial insulin and glucose levels from $t = 0$–240 min were analyzed using an area under the curve (AUC_{0-240}) derived using the trapezoidal method. Where values were missing at $t = 0$, the preceding $t = -30$ value was carried forward. Software packages used for analysis were R 3.5 (R Foundation) and GraphPad Prism 8.0.1 (GraphPad Software).

Study Approvals

The described study is part of a series of experimental medicine studies on the mechanisms of bariatric surgery (ClinicalTrials.gov NCT01945840). This was not a clinical trial of an investigational medicinal product, confirmed after a protocol review with the U.K. Medicines and Healthcare products Regulatory Agency. Ethical approval was obtained from the U.K. NHS Health Research Authority West London National Research Ethics Committee (reference 13/LO/1510). Substantial amendments to study methods were approved in July 2014 (to increase HbA_{1c} cutoff for inclusion), December 2014 (refinements to study visit procedures), June 2015 (increased upper limit for age to 70), October 2015 (added CGM to study procedures), December 2015 (added food preferences study, not reported here), May 2016 (removed upper limit for BMI for inclusion, previously 50 kg/m^2), and December 2017 (to include future arms to the study, not reported here). All participants provided written informed consent, and the study was conducted according to the principles of the Declaration of Helsinki.

RESULTS

Baseline Characteristics

The disposition of volunteers in the study is shown in Supplementary Fig. 2. Data

were available for 21 subjects who underwent RYGB, 22 who followed the VLCD, 15 who were infused GOP, and 11 who received Saline. Nine were excluded from the analysis set (six from GOP and three from Saline) due to technical issues with the infusion pump, inability to attend study visits, and unrelated conditions occurring during the study. Baseline characteristics are listed in Table 1. The primary comparison of GOP versus Saline found no significant differences in baseline characteristics. RYGB and VLCD volunteers were similar in weight to those in the GOP group but had lower baseline fructosamine levels. Most volunteers had diabetes (68–87%), and baseline treatments were for the most part evenly split between diet-only and metformin monotherapy.

Gut Hormone Levels During GOP Are Maintained at Postprandial Levels Observed 4 Weeks After RYGB

The fasting and postprandial levels of GLP-1, OXM, and PYY after an MMT are shown in Supplementary Fig. 3. Target levels of GLP-1, OXM, and PYY equivalent to postprandial peak levels after RYGB were achieved and maintained using a GOP infusion at doses of 4/4/0.4 pmol/kg/min, respectively.

GOP Leads to Significantly More Weight Loss Than Saline

GOP was significantly more effective than Saline in reducing weight, at 4.4 kg (percentage change from baseline 4.0%), vs. Saline at 2.5 kg (2.0%) (see Table 2). The reductions in weight were 10.3 kg (8.8%) for RYGB and 8.3 kg (7.6%) for VLCD. RYGB and VLCD subjects both lost significantly more weight than GOP subjects. Reductions in fat mass and muscle mass proportionate with body weight reductions were recorded with all interventions (Table 2).

Glycemia Improves After GOP, RYGB, and VLCD Interventions

Fructosamine was used as the biomarker for average glycemia because of its more rapid response to a glucose-lowering intervention compared with glycated hemoglobin. The mean absolute reduction in fructosamine after 4 weeks' GOP (Table 3) was 44.1 μmol/L compared with Saline at 11.7 μmol/L. The difference in treatment effect was 32.4 μmol/L (P = 0.0026, unpaired t test). A subsequent sensitivity analysis to explore any potential baseline effect on the results using ANCOVA indicated a similar statistically significant difference in treatment effect between GOP and Saline (P = 0.0001). As expected, RYGB led to a significant reduction in fructosamine (34.0 μmol/L), as did VLCD (28.5 μmol/L). There was no significant difference in treatment effects when comparing GOP and RYGB or GOP and VLCD.

Fasting glucose was reduced significantly both by GOP and Saline infusion, but the treatment effect with GOP was significantly larger than with Saline (Table 3). Fasting glucose fell also after RYGB and VLCD. GOP had a significantly better treatment effect on fasting glucose compared with RYGB. The treatment effects of GOP versus VLCD on fasting glucose were not significantly different (Table 3).

To assess the impact of the interventions on free living glycemia, we measured mean glucose and mean amplitude glucose change (MAG, which is an index of GV that measures summed absolute differences between sequential readings divided by the time between the first and last glucose measurement) from CGM records taken at baseline and during the 3rd week of each intervention (Supplementary Fig. 4). GOP significantly reduced mean glucose by 3.6 mmol/L, whereas the Saline group saw no significant change in mean glucose (Table 3). RYGB also reduced

Table 1—Baseline characteristics of the studied patients

	GOP (n = 15)	Saline (n = 11)	P value[a]	RYGB (n = 21)	P value[b]	VLCD (n = 22)	P value[c]
Age (years)	55.9 (8.5)	53.5 (8.5)	0.50	48.2 (13.2)	0.06	47.0 (10.2)	0.01
Sex, n			1.00		0.02		0.51
Female	6	5		17		12	
Male	9	6		4		10	
Weight (kg)	112.6 (26.7)	119.2 (25.1)	0.53	117.8 (25.3)	0.56	109.0 (20.5)	0.65
BMI (kg/m^2)	38.4 (6.9)	39.2 (5.4)	0.74	43.2 (6.2)	0.03	39.1 (4.3)	0.70
Diagnosis			0.61		1.00		0.26
T2DM, n (%)	13 (87)	8 (73)		18 (86)		15 (68)	
Prediabetic, n (%)[d]	2 (13)	3 (27)		3 (14)		7 (32)	
Treatment			1.00		0.31		0.51
Diet only, n (%)	8 (53)	6 (55)		7 (33)		9 (41)	
Metformin only, n (%)	7 (47)	5 (45)		13 (62)		13 (59)	
DPP-4 inhibitor only, n (%)	0	0		1 (5)		0 (0)	
Fructosamine (μmol/L)	304.6 (66.5)	278.1 (42.3)	0.26	250.3 (35.9)	0.003	252.4 (37.2)	0.004
Fasting glucose (mmol/L)	9.9 (4.7)	7.7 (1.5)	0.15	8.4 (2.0)	0.19	7.9 (2.7)	0.11
Fasting insulin (mIU/L)	16.3 (10.9)	14.4 (7.3)	0.63	19.7 (6.7)	0.25	18.0 (11.6)	0.66
iHOMA2%S	49.9 (21.3)	57.1 (21.7)	0.41	40.2 (17.0)	0.14	47.8 (18.3)	0.74
Total cholesterol (mmol/L)	4.8 (0.7)	5.1 (1.3)	0.49	4.3 (1.1)	0.09	5.0 (1.1)	0.65
LDL cholesterol (mmol/L)	2.9 (0.7)	3.1 (1.2)	0.63	2.5 (0.9)	0.14	3.1 (0.9)	0.48
HDL cholesterol (mmol/L)	1.1 (0.3)	1.3 (0.3)	0.12	1.1 (0.2)	0.56	1.0 (0.2)	0.21
Triglycerides (mmol/L)	1.7 (0.9)	1.5 (0.4)	0.44	1.5 (0.5)	0.50	2.1 (2.3)	0.57

Data are displayed as mean (SD), except where indicated. DPP-4, dipeptidyl peptidase 4; iHOMA2%S, interactive 24-variable homeostasis model assessment percentage insulin sensitivity. [a]GOP vs. Saline. [b]GOP vs. RYGB. [c]GOP vs. VLCD (unpaired two-tailed t test for continuous data or Fisher two-tailed exact probability test for categorical data). [d]Includes impaired glucose tolerance, impaired fasting glucose, or HbA_{1c} of 6.0–6.4% (42–48 mmol/mol).

Table 2—GOP leads to significantly more weight loss than Saline

	Primary comparison (GOP vs. Saline)		Secondary comparisons (GOP vs. RYGB, GOP vs. VLCD)	
	GOP	Saline	RYGB	VLCD
Weight (kg)				
Baseline	112.6	119.3	117.8	109.0
Week 4	108.2	116.8	107.6	100.8
ΔWeek 4	−4.4 (−5.4, −3.5)	−2.5 (−4.1, −0.9)	−10.3 (−11.8, −8.8)	−8.3 (−9.5, −7.1)
ΔWeek 4 vs. GOP		1.9 (0.3, 3.5)*	−5.9 (−7.5, −4.2)	−3.9 (−5.3, −2.4)
Weight change (%)				
ΔWeek 4	−4.0 (−4.8, −3.3)	−2.0 (−3.3, −0.8)	−8.8 (−10.0, −7.7)	−7.6 (−8.3, −6.8)
ΔWeek 4 vs. GOP		2.0 (0.7, 3.3)**	−4.8 (−6.2, −3.5)	−3.6 (−4.6, −2.5)
Fat mass (kg)				
Baseline	43.4	48.3	55.5	44.4
Week 4	40.9	47.0	49.4	39.3
ΔWeek 4	−2.4 (−3.2, −1.7)	−1.3 (−2.9, 0.3)	−6.1 (−7.1, −5.1)	−5.1 (−6.3, −3.9)
ΔWeek 4 vs. GOP		1.1 (−0.4, 2.7)	−3.6 (−4.9, −2.3)	−2.7 (−4.2, −1.1)
Muscle mass (kg)				
Baseline	63.3	67.3	59.3	60.9
Week 4	61.6	66.4	55.7	57.8
ΔWeek 4	−1.7 (−2.8, −0.7)	−0.9 (−1.7, −0.2)	−3.6 (−5.0, −2.3)	−3.0 (−3.8, −2.3)
ΔWeek 4 vs. GOP		0.8 (−0.5, 2.1)	−1.9 (−3.6, −0.3)	−1.3 (−2.5, −0.1)

Data are displayed as means (95% CI) for comparisons against baseline and for comparison of treatment effects between groups. ΔWeek 4, treatment effect between baseline and week 4; ΔWeek 4 vs. GOP, difference in treatment effect compared with the GOP arm. *$P < 0.05$; **$P < 0.01$ for primary comparison (unpaired t test) of treatment effect between the GOP and Saline arms.

mean glucose by 2.5 mmol/L. A comparison of GOP versus Saline showed that the treatment effect was significantly better with GOP. VLCD patients did not undergo CGM.

In terms of GV, there was a significant reduction in MAG with GOP whereas the Saline group saw no significant change in MAG (Table 3). The difference in treatment effect on MAG between GOP and Saline was significant. There was no significant reduction of MAG with RYGB. When GOP was compared with RYGB, the difference in treatment effects on this parameter was significant.

The Glucose and Insulin Dynamics During MMT Differ Between Interventions

Given that average glycemia was improved by both GOP and RYGB, but GV was improved by GOP but not RYGB, we analyzed glucose and insulin dynamics during an MMT before and after interventions. During the postintervention MMT in RYGB, there was a marked postprandial glucose peak at 30 min, reflecting rapid transit and absorption in the jejunum (Fig. 1*A*), accompanied by a large insulin peak at 60 min (Fig. 1*B*) leading to glucose disposal and a subsequent fall in glucose. In contrast, the glycemic response with GOP was nearly flat (Fig. 1*E*), accompanied by a relatively small insulinotropic response (Fig. 1*F*). VLCD led to an overall reduction of glucose levels by ~2 mmol/L compared with baseline (Fig. 1*C*), but there was no change in the insulin secretion profile (Fig. 1*D*).

The postprandial glucose excursion during the MMT, as measured by the glucose (Glu) $AUC_{0–240}$, was compared between baseline and after 4 weeks of each intervention. There was a profound reduction of Glu $AUC_{0–240}$ after GOP, whereas there was no significant change after Saline. GOP led to a reduction in the insulin (Ins) $AUC_{0–240}$, and again, there was no significant change in Ins $AUC_{0–240}$ after Saline (Supplementary Table 2). RYGB led to a significant overall reduction in Glu $AUC_{0–240}$, but the treatment effect was similar between GOP and RYGB. However, RYGB caused an increase in Ins $AUC_{0–240}$ in comparison with the fall with GOP (Supplementary Table 2). We found a significant reduction in Glu $AUC_{0–240}$ with VLCD, but the treatment effect was significantly better with GOP than VLCD. VLCD also led to a reduction in Ins $AUC_{0–240}$ (Supplementary Table 2).

We also examined the dynamics of glucagon secretion in response to the MMT in the GOP and RYGB groups. Fasting glucagon levels were not altered before and after interventions. GOP volunteers exhibited a postprandial elevation in glucagon secretion at baseline. When given GOP, this postprandial elevation was suppressed. In comparison, RYGB exhibited a peak in glucagon secretion both before and after the intervention (Fig. 1*I*).

GOP and RYGB Reduce EI and REE

Ad libitum EI was reduced significantly after 4 weeks of GOP by a mean of 292.7 kcal vs. Saline at 168.5 kcal (Supplementary Table 1), with no statistically significant difference in treatment effects between the GOP and Saline arms. The RYGB group was assessed for ad libitum EI at week 12: as expected, they ate less compared with baseline. Ad libitum EI was not assessed in the VLCD group because they were on a calorie restriction a priori. The 24-h EI estimated from food diaries are consistent with the above findings. GOP led to a significant reduction in 24-h EI, as did Saline, although the difference between GOP and Saline was not statistically significant. RYGB led to a numerically larger reduction in 24-h EI by 957.7 kcal/24 h, but this was not significantly larger than GOP. The VLCD group ate 822.7 kcal/24 h on average (i.e., they were compliant with the specified calorie restriction). REE was significantly reduced in the GOP group and in the RYGB group but not in any other treatment group (Supplementary Table 1). When corrected for body weight or fat-free mass, no significant treatment effects

Table 3—Glycemia improves after GOP, RYGB, and VLCD interventions

	Primary comparison (GOP vs. Saline)		Secondary comparisons (GOP vs. RYGB, GOP vs. VLCD)	
	GOP	Saline	RYGB	VLCD
Fructosamine (μmol/L)				
Baseline	304.6	278.1	250.3	252.4
Week 4	260.5	266.4	216.4	223.9
ΔWeek 4	−44.1 (−62.7, −25.5)	−11.7 (−18.9, −4.5)	−34.0 (−45.6, −22.4)	−28.5 (−40.4, −16.7)
ΔWeek 4 vs. GOP		32.4 (12.9, 51.9)**	10.2 (−9.8, 30.2)	15.6 (−6.6, 35.8)
Fructosamine (μmol/L)[a]				
ΔWeek 4	−44.1 (−57.2, −31.1)	−11.7 (−15.6, −7.9)	N/A	N/A
ΔWeek 4 vs. GOP		33.2 (19.0, 45.8)***	N/A	N/A
Fasting glucose (mmol/L)				
Baseline	9.9	7.7	8.3	7.9
Week 4	5.8	6.7	5.8	5.9
ΔWeek 4	−4.1 (−6.3, −1.9)	−1.0 (−1.7, −0.3)	−2.5 (−3.3, −1.7)	−2.0 (−3.0, −1.0)
ΔWeek 4 vs. GOP		3.1 (0.8, 5.3)*	1.6 (0.7, 3.9)	2.0 (−0.3, 4.5)
Fasting insulin (mIU/L)				
Baseline	16.3	14.4	19.7	18.0
Week 4	18.2	12.0	12.3	10.1
ΔWeek 4	1.9 (−0.8, 4.6)	−2.4 (−5.2, 0.4)	−7.4 (−10.4, −4.3)	−7.9 (−11.2, −4.6)
ΔWeek 4 vs. GOP		−4.8 (−8.0, −0.5)*	−9.2 (−13.4, −5.1)	−9.8 (−14.3, −5.3)
Mean glucose from CGM (mmol/L)				
Baseline	10.4	7.4	8.5	N/A
Week 4	6.9	71	6.0	N/A
ΔWeek 4	−3.6 (−5.2, −1.9)	−0.4 (−1.4, 0.7)	−2.5 (−3.6, −1.4)	N/A
ΔWeek 4 vs. GOP		3.2 (1.1, 5.3)**	1.1 (−0.9, 3.0)	N/A
MAG				
Baseline	2.3	1.7	1.7	N/A
Week 4	1.55	1.6	1.6	N/A
ΔWeek 4	−0.75 (−1.08, −0.41)	−0.1 (−0.5, 0.2)	−0.1 (−0.5, 0.3)	N/A
ΔWeek 4 vs. GOP		0.6 (0.2, 1.1)*	0.6 (0.1, 1.1)	N/A

Data are displayed as means (95% CI) for comparisons against baseline and for comparison of treatment effects between groups. N/A, not applicable; ΔWeek 4, treatment effect between baseline and week 4; ΔWeek 4 vs. GOP, difference in treatment effect compared with GOP arm. [a]Additional results based on sensitivity analysis (unpaired *t* test) of fitted values derived from ANCOVA model, adjusting for baseline fructosamine levels and any potential interaction between baseline and treatment effect. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ for primary comparison of treatment effect (unpaired *t* test) between GOP and Saline arms.

were observed in all groups. No significant treatment effects were observed for diet-induced thermogenesis, apart from a small reduction in Saline. AEE was significantly reduced after RYGB but not in the other groups.

Safety and Tolerability

Overall, GOP infusion was well tolerated with no significant change in nausea scores during the infusion. Some of the participants in the infusion groups experienced a temporary, mild erythema around the infusion sites resolving with improvements in set insertion technique. We did not observe hypoglycemic episodes in the GOP volunteers during the infusion, but four volunteers in the RYGB group had a documented glucose level of <4.0 mmol/L (Supplementary Table 3).

CONCLUSIONS

GOP infusion at home was feasible and well tolerated over a 4-week period. It led to a substantial mean weight loss of 4.4 kg. The GLP-1/glucagon dual agonist MEDI0382 (22) and the GLP-1 agonist semaglutide (23) led to mean weight losses of 3.8 kg and 1 kg, respectively, at approximately similar time points, although these studies were double-blinded with larger sample sizes. Our previous study showed a 32% reduction in ad libitum EI with an acute GOP infusion (5), and this study shows that the effect persists over 4 weeks with no tachyphylaxis. However, the treatment effects vis-à-vis EI in this study were not statistically different between the GOP and Saline groups, likely due to small sample sizes. The differential effects on body weight loss from GOP (4.0%) compared with RYGB (8.8%) and VLCD (7.6%) parallel the reductions in 24-h EI of 30.6%, 49.2%, and 53.6% in the respective groups. Our earlier study showed no change in REE when GOP was given acutely (5). There was a decrease in REE after GOP and RYGB, as in the Schmidt et al. (24) study, but this disappeared after adjustment for body weight or fat-free mass. A small reduction in AEE after RYGB was seen, which is likely because patients were still recovering from surgery. The effects of the interventions on body weight are therefore principally driven by the changes in EI.

GOP also led to improvements in fructosamine comparable with RYGB and VLCD, both of which can lead to diabetes remission (1,25). GOP achieved these improvements with a smaller weight loss compared with RYGB and VLCD. The average measures of glycemia mask a marked difference in glucose/insulin dynamics between interventions. GOP led to a significant reduction in GV compared with RYGB. During the MMT, after RYGB there was a rapid spike in glucose levels, followed by a peak in insulin secretion from the combination of hyperglycemia and insulinotropy from GLP-1 and possibly glucagon (26). In

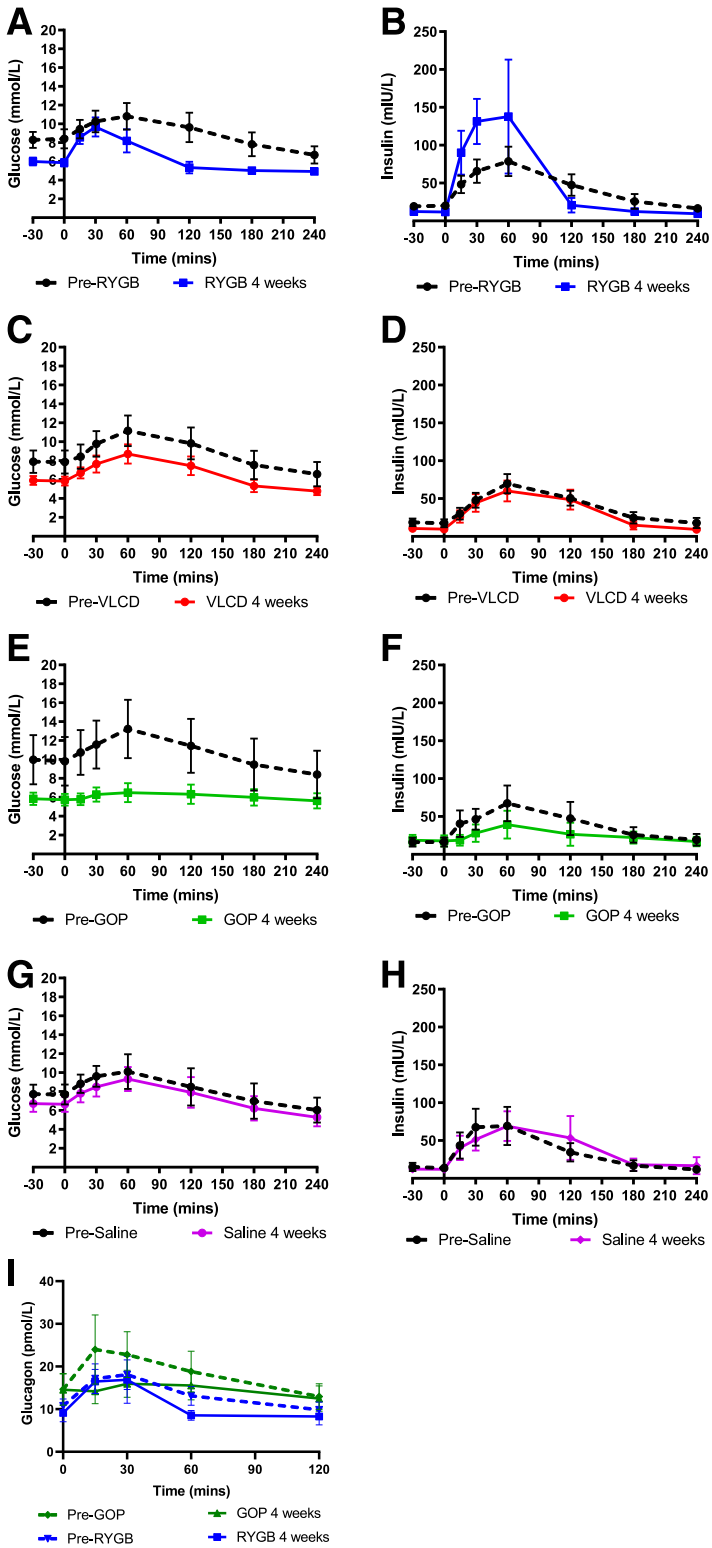

Figure 1—Dynamics of glucose (*A*, *C*, *E*, and *G*) and insulin (*B*, *D*, *F*, and *H*) during an MMT test differ between interventions. Pre- and post-RYGB intervention (*A* and *B*); pre- and post-VLCD intervention (*C* and *D*); pre- and post-GOP intervention (*E* and *F*); and pre- and post-Saline intervention (*G* and *H*). *I*: Glucagon responses to MMT pre- and post-GOP infusion compared with the RYGB group. Means and 95% CI plotted.

certain patients, this phenomenon may lead to an "overswing" hypoglycemia. Hypoglycemia after bariatric surgery is an increasingly recognized clinical problem, and this is linked to increased GV after RYGB (26). In contrast, GOP leads to nearly static glucose level and a minimal increase in insulin. This may be due to a delay in gastric emptying induced by GLP-1 and OXM (not directly tested here), although this phenomenon is usually subject to a rapid tachyphylaxis (27). That GOP could directly improve insulin sensitivity is also possible, although this was not directly tested in this study. Another explanation might be the flattened postprandial glucagon response with GOP (mediated by GLP-1 and OXM), thus leading to suppression of hepatic glucose output (28). With regards to VLCD, although there was some improvement in glucose tolerance, GOP was superior in this regard.

The limitations of our study are as follows. We infused only for 12 of 24 h. However, this pattern of elevation of gut hormones during the day is likely to be seen in RYGB patients, who tend to eat frequent small meals during the day, thus leading to consistent gut hormone elevations, and whose gut hormone levels fall back to baseline during the night. Although GOP replicates postsurgical postprandial gut hormone levels, it does not replicate anatomical changes. GOP does not replicate any changes in a notional "anti-incretin" from the bypassed gut (29) or any changes in ghrelin. The study is limited by a relatively small sample size and short duration. It was not powered to show differences in the secondary end points or in safety and tolerability characteristics. Lastly, the study was not designed to examine the contribution of the individual hormones to the overall effect.

We conclude that the postprandial elevations in GLP-1, OXM, and PYY after RYGB may be responsible for the glycemic improvements and some of the weight loss benefits from surgery. There may be other contributions from surgical anatomical changes that lead to larger weight loss. GOP achieves superior glucose tolerance to VLCD, reduces glucose variability, and lowers the risk of provoking hypoglycemia compared with RYGB. Together, this suggests that "triple agonism" with GLP-1, OXM, and PYY is a viable alternative to RYGB for the

treatment of diabetes, with favorable effects on body weight, in patients who may not be able to have bariatric surgery.

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