

SUPPLEMENTARY DATA

Combined GLP-1, oxyntomodulin and peptide YY improves body weight and glycaemia in obesity and prediabetes/type 2 diabetes: a randomized single-blinded placebo controlled study

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Supplemental Results

GOP infusion is well tolerated over 4 weeks

Rested blood pressure and pulse were recorded in the fasted state, in all volunteers during the study visits. Nausea was subjectively assessed using a Visual Scale Analogue scale, both in the fasted (t=0) and fed state (t=30 and 240 minutes from the ingestion of Ensure) at the baseline visit and after 4 weeks of each intervention (23). Treatment-emergent adverse effects were recorded in the case record files for each volunteer. Significant improvements from baseline in fasting blood pressure were noted within the VLCD (systolic and diastolic) and GOP (systolic) groups at the end of 4 weeks. No significant improvements in fasting BP were observed in the RYGB and Saline groups (Table S3). Comparing the two primary analysis groups indicated no significant mean [95% CI] difference in effect between the two groups for both systolic (4.8 [-1.3, 10.9]) mmHg and diastolic (-1.2 [-6.9, 4.5]) mmHg fasting BP. A significantly reduced pulse rate was observed in the VLCD group but no other groups (Table S3). Nausea, as assessed by visual analogue scores in the fasted state and after feeding were unchanged following the GOP infusion. In contrast, after surgery, the volunteers experienced a significant increase in post-prandial nausea (Table S3). Some of the participants in the infusion groups experienced a temporary, mild erythema around the infusion sites; however, these resolved with additional training in infusion set insertion technique. We did not observe any hypoglycemic episodes in any of the GOP volunteers during the infusion on any study days. Four volunteers in the RYGB group had a documented glucose level of <4.0 mmol/L.

All interventions reduce fasting total cholesterol

Fasting lipid profiles were measured for each intervention group before and after the interventions. As noted in Table S4, all interventions led to a statistically significant reduction in total cholesterol from baseline. This was mainly driven by significant reductions in LDL cholesterol (seen with the GOP, Saline, VLCD interventions, but not with the RYGB intervention). The Saline and RYGB intervention groups also had statistically significant reductions in HDL cholesterol. The GOP intervention led to a statistically significant reduction in triglyceride levels which was not seen in other interventions. No statistically significant differences in treatment effects between GOP and the other interventions were observed.

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Table S1. GOP reduces Energy Intake and Resting Energy Expenditure. Data displayed as Means [95% CI] for comparisons against baseline and for comparison of treatment effects between groups. BL, baseline; W4, week 4; ΔW4, treatment effect between baseline and week 4; ΔW4 vs GOP, difference in treatment effect compared to GOP arm. N/A not applicable. ^aAssessed at week 12 for the RYGB group. REE, resting energy expenditure. DIT, diet-induced thermogenesis. AEE, activity-related energy expenditure.

		Primary Comparison (GOP vs Saline)		Secondary Comparisons (GOP vs RYGB, GOP vs VLCD)	
		GOP	SALINE	RYGB	VLCD
Ad Libitum Energy Intake (kcal)	BL	785.1	1002.6	655.1	N/A
	W4	492.4	834.1	378.3 ^a	N/A
	ΔW4	-292.7 [-389.5, -196.0]	-168.5 [-300.5, -36.5]	-276.8 [-398.4, -155.3] ^a	N/A
	ΔW4 vs GOP		124.2 [-26.6, 275.0]	15.9 [-140.0, 171.8] ^a	N/A
24-hour EI (kcal)	BL	2073.2	2133.9	1882.6	2192.1
	W4	1269.5	1696.6	924.9 [#]	822.7
	ΔW4	-803.7 [-1239.6, -367.9]	-437.3 [-823.4, -51.2]	-957.7 [-1239.5, -675.9] ^a	-1369.4 [-2059.3, -679.5]
	ΔW4 vs GOP		366.4 [-212.3, 945.2]	-154.0 [-643.6, 335.6] ^a	-565.7 [-1374.3, 243.0]
REE (kcal/24 hours)	BL	1984.3	1936.2	1994.7	1829.7
	W4	1780.2	1891.4	1806.0	1705.6
	ΔW4	-204.1 [-356.2, -52.0]	-44.8 [-229.4, 139.9]	-188.8 [-363.8, -13.7]	-124.2 [-325.8, 77.5]
	ΔW4 vs GOP		159.4 [-65.0, 383.7]	15.4 [-221.4, 252.1]	80.0 [-184.1, 344.1]
DIT (kcal/24 hours)	BL	120.6	229.5	123.8	239.4
	W4	118.6	63.7	220.9	192.3
	ΔW4	-2.0 [-330.7, 326.7]	-165.8 [-307.4, -24.3]	97.1 [-86.2, 280.4]	-47.1 [-187.9, 93.8]
	ΔW4 vs GOP		-163.8 [-510.2, 182.5]	99.1 [-252.7, 450.8]	-45.1 [-354.5, 264.3]
AEE (kcal/24 hours)	BL	626.7	653.8	735.5	711.2
	W4	688.2	530.7	520.1	728.5
	ΔW4	61.5 [-98.5, 221.5]	-123.1 [-278.6, 32.4]	-215.4 [-426.9, -3.9]	17.3 [-155.6, 190.2]
	ΔW4 vs GOP		-184.6 [-414.9, 61.5]	-276.9 [-532.8, 21.0]	-44.2 [-279.1, 190.7]

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Table S2. GOP improves glucose tolerance and reduces the insulin excursion during the MMT. Data displayed as Means [95% CI] for comparisons against baseline and for comparison of treatment effects between groups. BL, baseline; W4, week 4; Δ W4, treatment effect between baseline and week 4; Δ W4 vs GOP, difference in treatment effect compared to GOP arm. N/A not applicable. ** p<0.01 for primary comparison (unpaired t-test) of treatment effect between GOP and Saline arms.

		Primary Comparison (GOP vs Saline)		Secondary Comparisons (GOP vs RYGB, GOP vs VLCD)	
		GOP	SALINE	RYGB	VLCD
Glucose AUC₀₋₂₄₀ (mmol/L·min)	BL	2584.1	1970.5	2168.3	2145.9
	W4	1471.6	1799.9	1528.2	1614.9
	Δ W4	-1112.6 [-1624.8, -600.3]	-170.5 [-362.1, 21.0]	-640.1 [-898.7, -381.5]	-531.0 [-818.4, -243.6]
	Δ W4 vs GOP		942.5 [407.6, 1476.6] **	472.5 [-37.6, 982.6]	582.0 [57.5, 1105.6]
Insulin AUC₀₋₂₄₀ (mU/L·min)	BL	9669.2	8811.1	10766.1	9831.5
	W4	6254.5	9724.8	12849.9	8261.0
	Δ W4	-3414.8 [-5983.7, -845.8]	913.6 [-463.7, 2291.0]	2083.8 [-933.1, 5100.7]	-1570.5 [-2906.6, -234.3]
	Δ W4 vs GOP		4402.6 [1520.5, 7136.2] **	5498.6 [1503.3, 9493.8]	-1844.3 [-704.3, 4392.9]

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Table S3. Safety Data. Means [95% CI] displayed. * p <0.05, ** p<0.01 for comparison between pre and following 4 weeks of each intervention, Student Paired t-test.

	GOP (n=15)		SALINE (n=11)		RYGB (n=21)		VLCD (n=22)	
	Pre	4 weeks	Pre	4 weeks	Pre	4 weeks	Pre	4 weeks
Fasting Systolic BP (mmHg)	132.0 [126.2, 137.8]	123.6 [117.8,129.3]**	128.1 [118.8, 137.5]	124.5 [113.7,135.2]	128.6 [122.0,135.2]	122.9 [116.4, 129.4]	132.3 [126.1, 138.6]	124.7 [120.3, 129.2]*
Fasting Diastolic BP (mmHg)	73.6 [68.5, 78.8]	71.5 [67.3, 75.7]	76.3 [70.0, 82.5]	72.9 [65.9, 80.0]	73.0 [68.8, 77.1]	72.2 [68.0, 76.4]	78.5 [74.2, 82.9]	72.5 [69.0, 76.0]**
Fasting Pulse rate (min⁻¹)	70.6 [65.2, 76.1]	69.5 [64.5, 74.6]	68.5 [60.9, 76.1]	69.1 [61.1, 77.1]	78.3 [72.7, 83.9]	74.0 [69.2, 78.7]	74.8 [71.0, 78.5]	70.2 [66.2, 74.2]**
Fasted Nausea VAS (mm)	3.1 [-1.6, 7.9]	9.5 [-2.05, 21.0]	2.9 [-1.6, 7.4]	2.0 [-2.0, 6.0]*	3.3 [0.3, 6.3]	4.2 [0.5, 7.9]	4.3 [1.2, 7.4]	4.0 [-0.7, 8.6]
Nausea AUC₀₋₂₄₀ (mm·min)	1604 [159, 3049]	2193 [-61, 4447]	758 [-305, 1821]	310.9 [-304, 926]	990 [61.5, 1919]	5222 [2475, 7970]**	1276 [450.2, 2101]	842.7 [397, 1288]
Treatment-emergent adverse events, n (%)								
Mild transient erythema	8 (53%)		3 (27%)		N/A		N/A	
Hypoglycemia (< 4.0 mmol/L)	0 (0%)		0 (0%)		4 (19%)		0 (0%)	

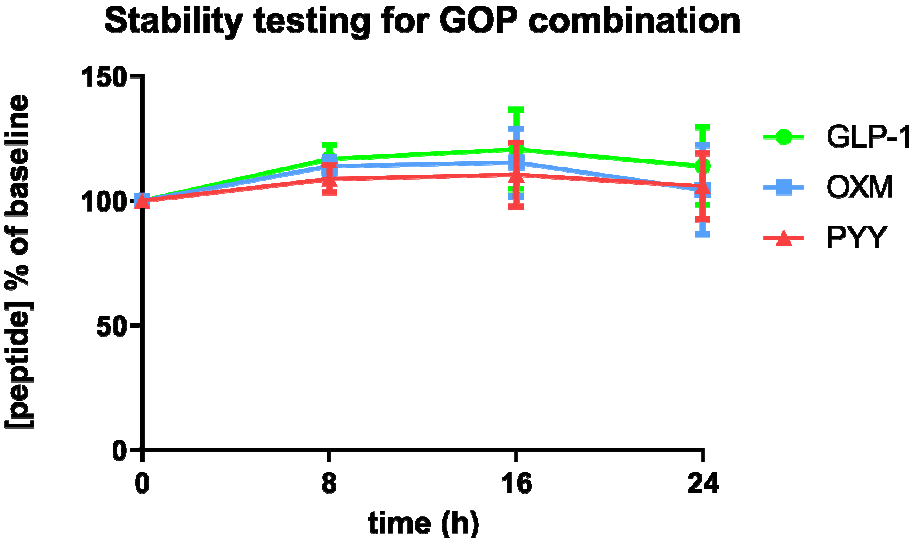
SUPPLEMENTARY DATA

Table S4. All interventions reduce fasting total cholesterol. Data displayed as Means [95% CI] for comparisons against baseline and for comparison of treatment effects between groups. BL, baseline; W4, week 4; Δ W4, treatment effect between baseline and week 4; Δ W4 vs GOP, difference in treatment effect compared to GOP arm.

		Primary Comparison (GOP vs Saline)		Secondary Comparisons (GOP vs RYGB, GOP vs VLCD)	
		GOP	SALINE	RYGB	VLCD
Total Cholesterol (mmol/L)	BL	4.8	5.1	4.3	5.0
	W4	4.2	4.5	3.7	3.8
	Δ W4	-0.7 [-1.0, -0.3]	-0.6 [-1.0, -0.3]	-0.6 [-1.1, -0.2]	-1.2 [-1.6, -0.8]
	Δ W4 vs GOP		0.0 [-0.4, 0.5]	0.1 [-0.5, 0.6]	-0.5 [-1.1, 0.0]
HDL cholesterol (mmol/L)	BL	1.1	1.3	1.1	1.0
	W4	1.0	1.2	0.9	0.9
	Δ W4	-0.1 [-0.2, 0.0]	-0.2 [-0.2, -0.1]	-0.2 [-0.3, -0.1]	-0.1 [-0.2, 0.0]
	Δ W4 vs GOP		-0.1 [-0.2, 0.1]	-0.1 [-0.3, 0.4]	0.0 [-0.1, 0.1]
LDL cholesterol (mmol/L)	BL	2.9	3.1	2.5	3.1
	W4	2.6	2.7	2.2	2.4
	Δ W4	-0.4 [-0.6, -0.1]	-0.4 [-0.7, -0.1]	-0.3 [-0.7, 0.1]	-0.7 [-1.0, -0.5]
	Δ W4 vs GOP		-0.0 [-0.4, 0.3]	0.0 [-0.3, 0.4]	-0.4 [-0.7, 0.0]
Triglycerides (mmol/L)	BL	1.7	1.5	1.5	2.1
	W4	1.2	1.3	1.3	1.1
	Δ W4	-0.5 [-0.8, -0.1]	-0.2 [-0.5, 0.2]	-0.2 [-0.6, 0.1]	-1.0 [-2.0, 0.1]
	Δ W4 vs GOP		0.3 [-0.2, 0.8]	0.2 [-0.2, 0.7]	-0.5 [-1.6, 0.5]

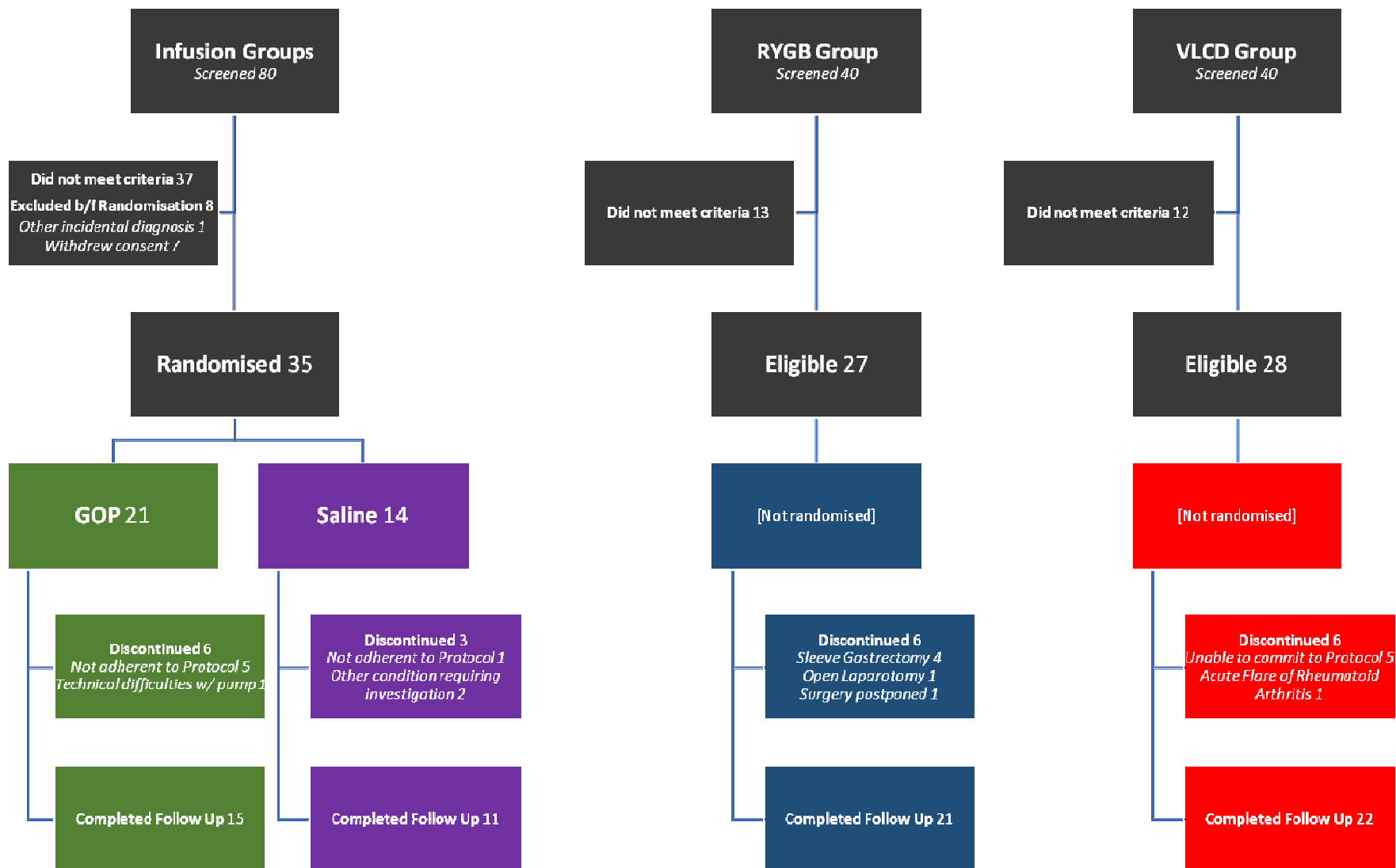
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Figure S1. Peptide stability in the GOP mixture when loaded into infusion syringes at room temperature as measured by HPLC. Means plotted with 95% CI.



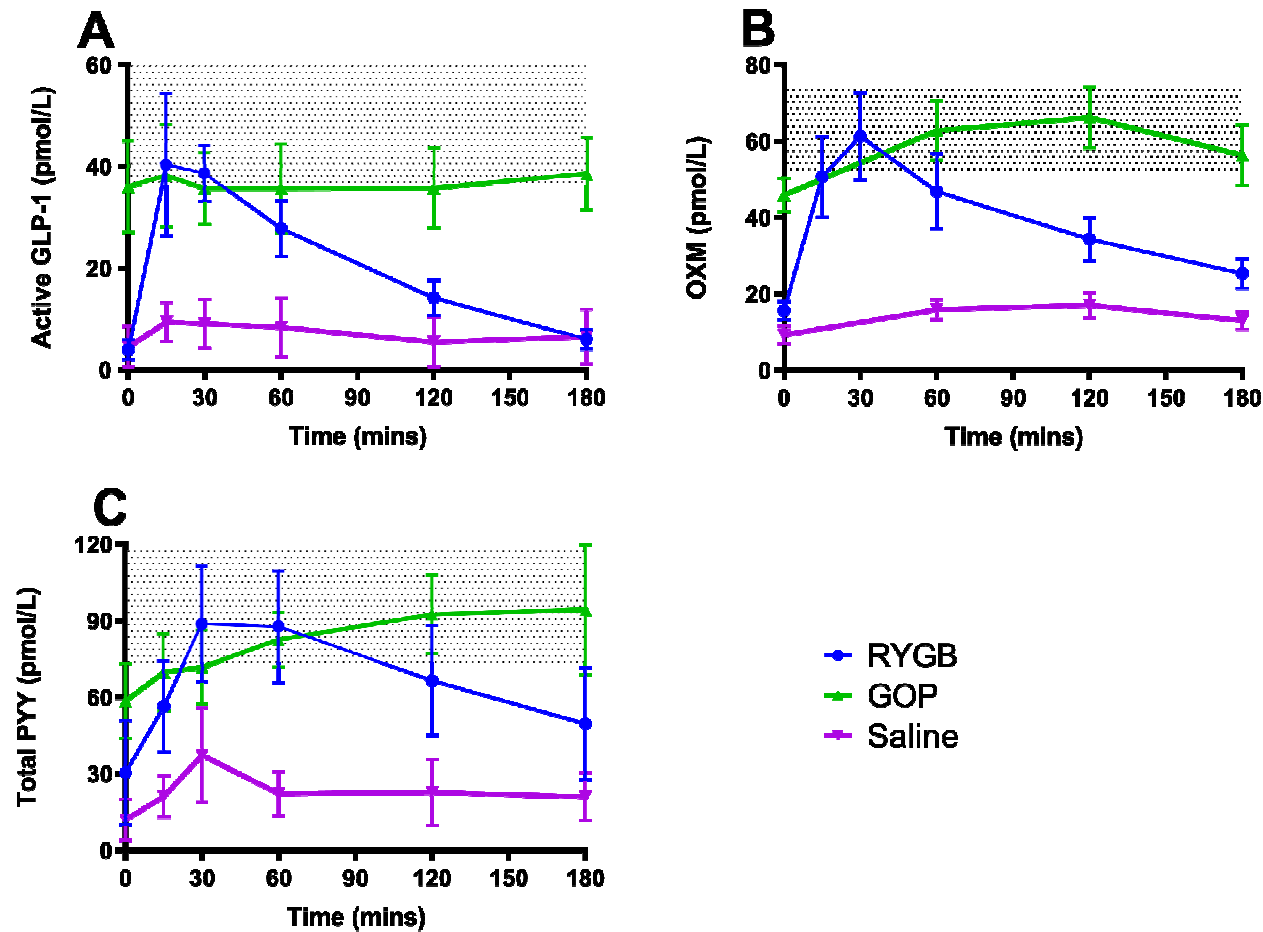
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Figure S2. CONSORT Diagram showing patient flow in the study



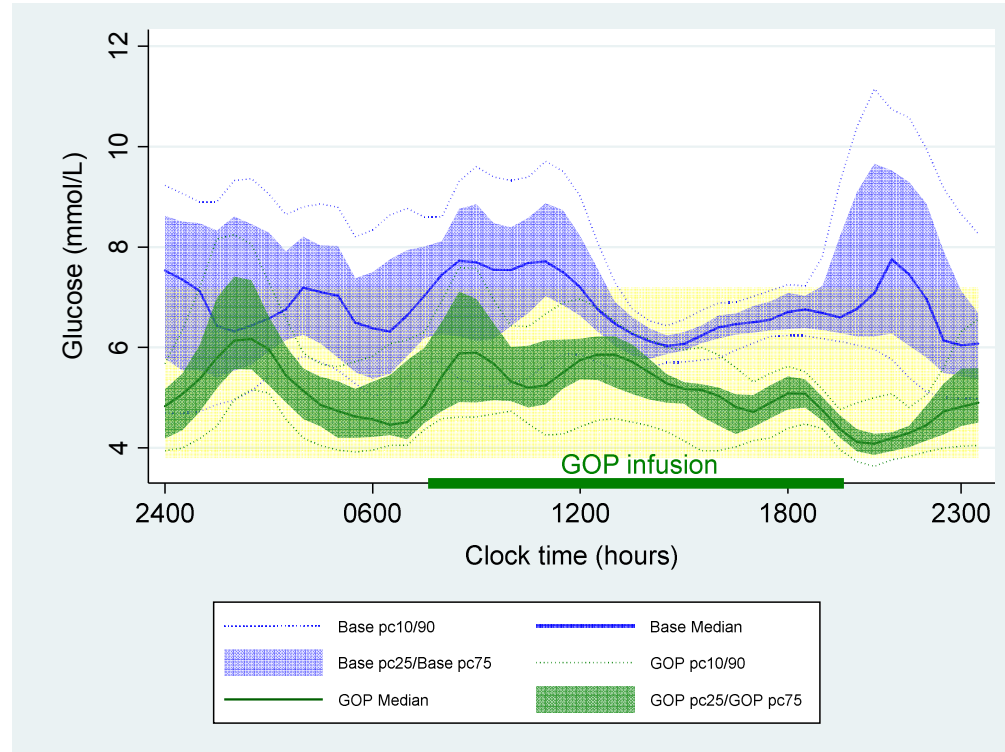
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Figure S3. Gut hormone levels after GOP infusion are maintained at post-prandial levels observed 4 weeks post RYGB (a) Active GLP-1; (b) OXM; (c) total PYY in response to an MMT test at week 4 of intervention. For the GOP group, the patients have been receiving the infusion for 120 minutes prior to the MMT test start time. Means plotted with 95% CI. Shaded areas denote the target zone for each hormone (95%CI for the peak values for each hormone).



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Figure S4. Representative examples of ambulatory glucose profiles from CGM for two volunteers at baseline and during GOP infusion. Y-axis represents the interstitial glucose values (mmol/L), X-axis represents clock time for each day. Blue lines/shading represents baseline recording (before infusion). Green lines/shading represents GOP infusion recording with thick green line on X-axis indicating time on infusion. Solid lines represent median glucose levels. Shading represents interquartile range of glucose readings. Dotted lines represent 10th and 90th percentiles of glucose readings. Yellow shading represents desired range of glucose between 3.8 to 7.2 mmol/L.



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