Supplementary Material for 'Roux-en-Y gastric bypass increases glycaemic variability and time in hypoglycaemia in patients with obesity and pre-diabetes/type 2 diabetes mellitus: a prospective cohort study'

Ibiyemi Ilesanmi, George Tharakan, Kleopatra Alexiadou, Preeshila Behary, Haya Alessimii, Candace Bovill-Taylor, Julia Kenkre, Sirazum Choudhury, Chedie Doyle, Sanjay Purkayastha, Alex Miras, Christos Tsironis, Harvinder Chahal, Stephen R. Bloom, Nick S. Oliver, Ahmed R. Ahmed, Bernard Khoo, Tricia M-M. Tan

Power Calculation and Statistical Analysis

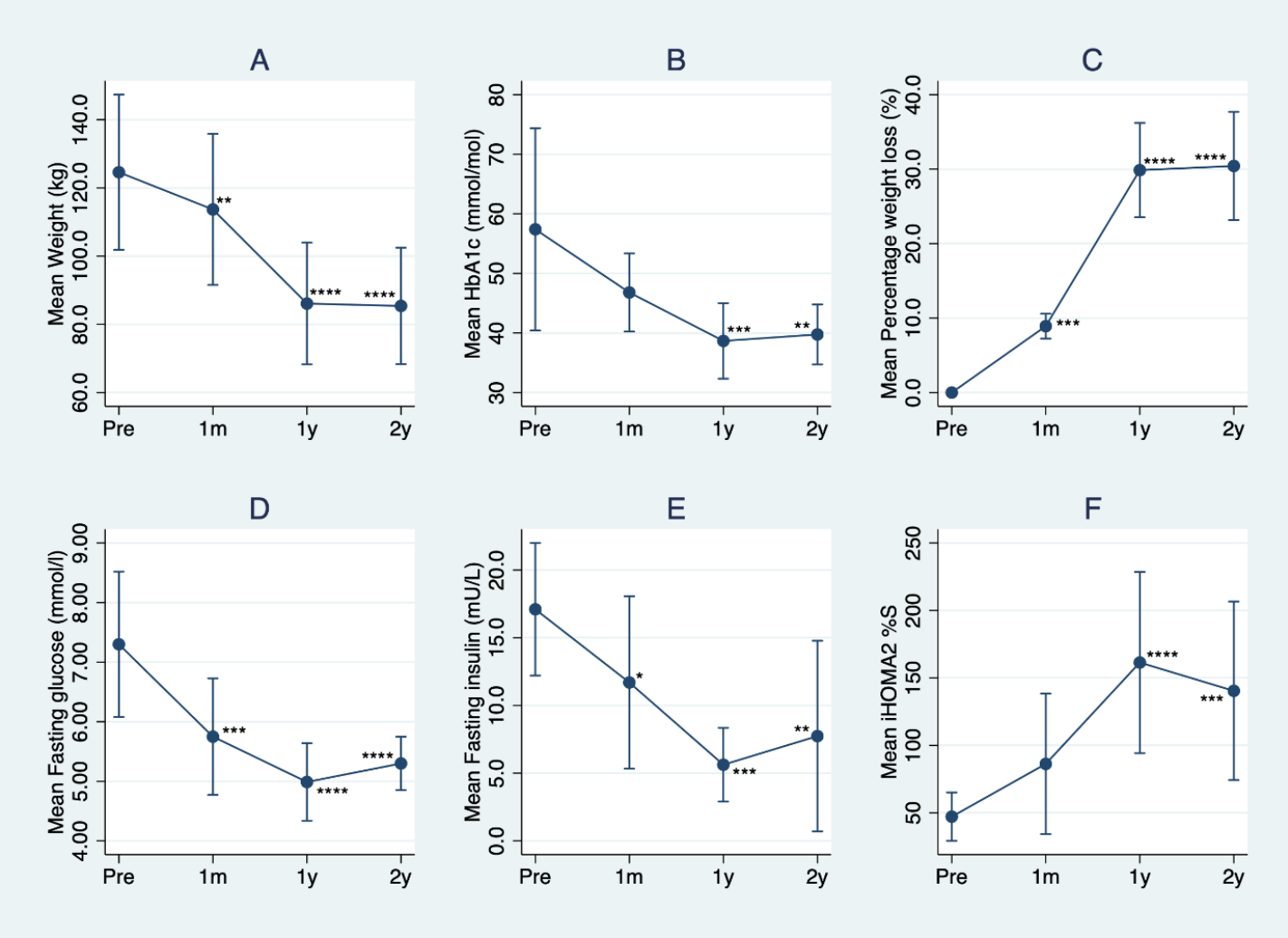
Data and statistical analysis used Prism 8.2.1 (GraphPad Software) and STATA 15.1 (STATACorp LLC). A power calculation for a repeated measures one-way ANOVA suggested that a sample size of 8 participants had 90% power at an alpha of 0.05 to detect clinically significant changes in mean CGM of 2.5 mmol/L [45 mg/dl], equivalent to a change in HbA1c of 17 mmol/mol, and percent coefficient of variation (%CV) of 10%, a difference which has been linked to significantly increased risks of hypoglycaemia during treatment of patients with type 2 diabetes (1). The trapezoid method was used to calculate area under curve over the 180 minute MMT study ($AUC_{0\text{-}180}$). A one-way repeated measures ANOVA with Bonferroni correction (reported as adjusted p-values) was used to compare GV metrics. For analysis of the MMT data, a linear mixed model repeated measures analysis was used, with Bonferroni correction. To examine the relationship of %TIR<3.0 mmol/L [54 mg/dl] and %TIR<3.9 mmol/L [70 mg/dl] with CGM and MMT parameters, the univariable association of these parameters was initially tested using Spearman correlation. A multivariable linear mixed model,

incorporating those parameters found to have statistically significant associations, was then used to examine the relationship of significantly correlated parameters with %TIR. For comparison of the %TIR values through time, a non-parametric Friedman test was used.

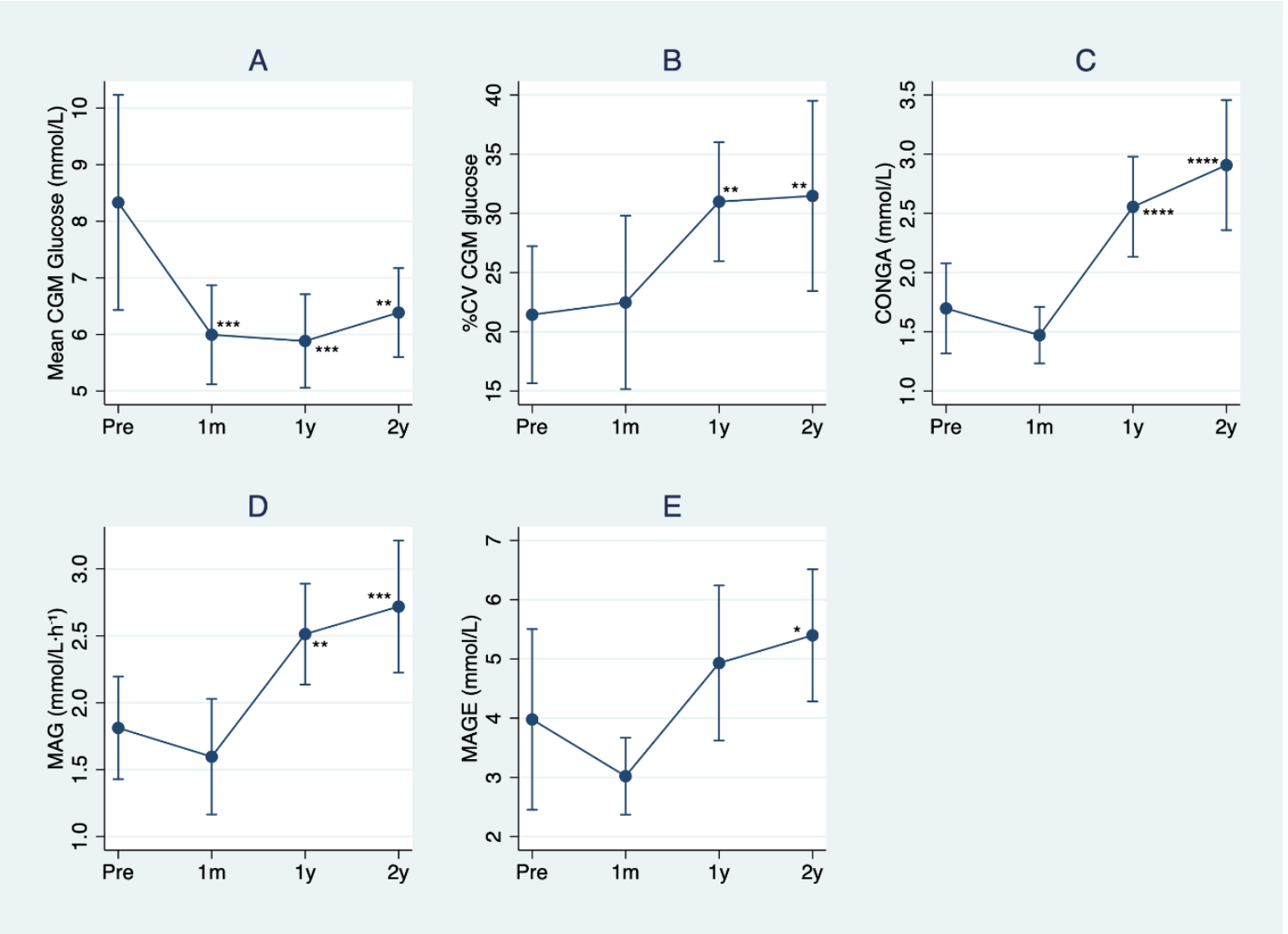
Assay Techniques

Blood for glucose was collected in fluoride oxalate tubes, for insulin in plain (serum) tubes, for gut hormones in Lithium heparin tubes containing Aprotinin (Nordic Pharma) and the DPP-IV inhibitor Diprotin A (Enzo Life Sciences). Glucose and insulin levels were measured by NW London Pathology (Abbott Architect; CVs <5%, <10% respectively). Active glucagon-like peptide-1 (GLP-1) levels were measured by a customised Milliplex magnetic bead-based multi-analyte, metabolic panel immunoassay (Millipore). The intra-assay and inter-assay coefficient of variation for active GLP-1 was <10%. The lowest limit of detection was 0.8 pmol/L. Glucagon was measured using an ELISA (Mercodia AB) with the high-stringency 'Alternative' protocol to eliminate cross-reaction with other proglucagon-derived peptides (2): there was a detection limit of 1.5 pmol/L and intra-assay and inter-assay coefficient of variation of <10%.

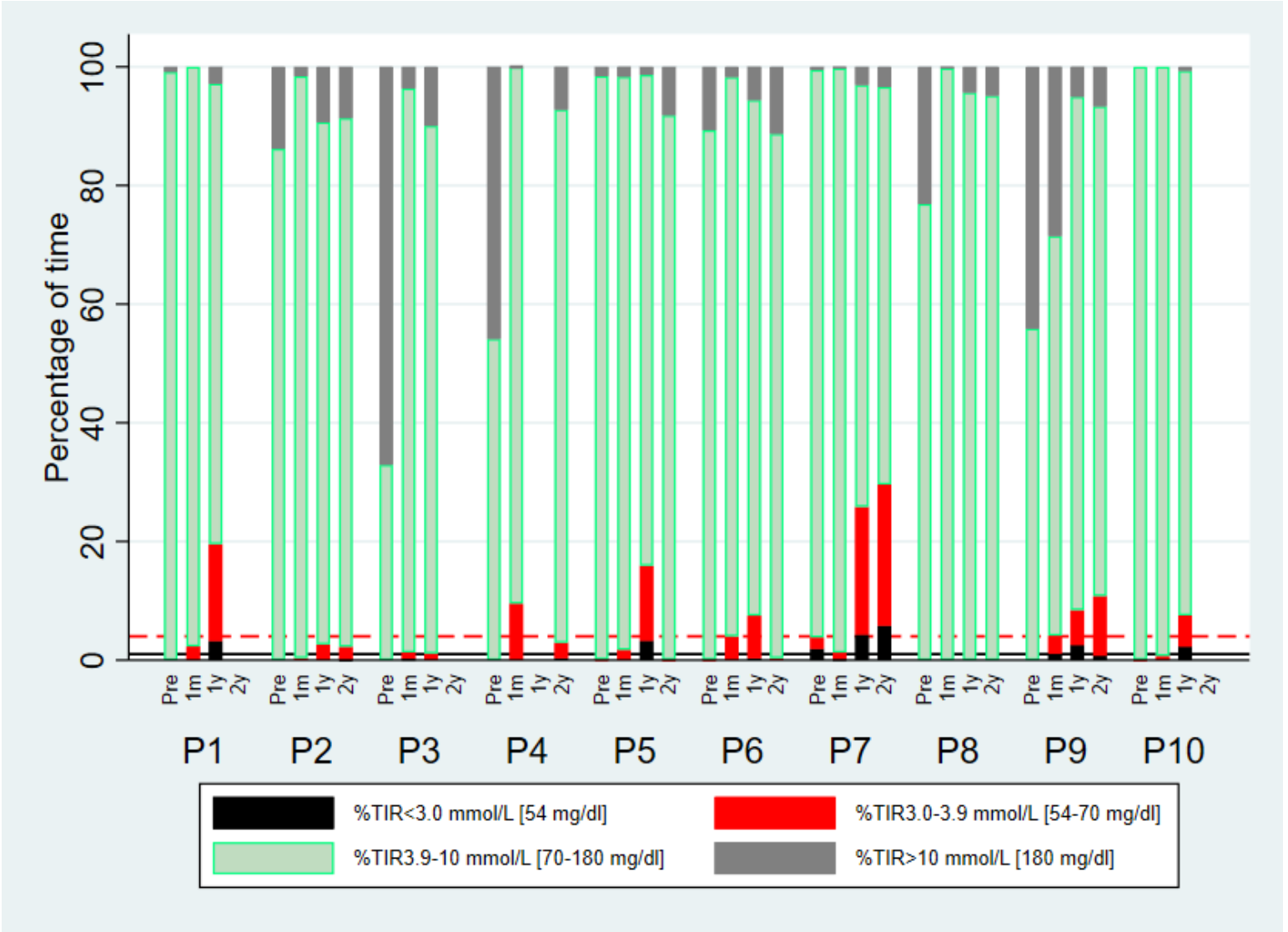
Supplementary Figures



Supplementary Figure 1: Plots of weight (A), HbA1c (B), percentage weight loss (C), fasting glucose (D), fasting insulin (E), 24-variable interactive homeostatic model assessment with default settings (iHOMA2 – (3) percentage insulin sensitivity (F) in prospective RYGB cohort over time (Pre: pre-surgery, 1m: 1 month post-surgery, 1y: 1 year post-surgery, 2y: 2 years post-surgery). Mean and SD plotted. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ for comparison with Pre (one-way repeated measures ANOVA with Bonferroni correction).



Supplementary Figure 2: Measures of mean glycaemia and glycaemic variability (4) in the RYGB cohort. Mean CGM glucose (A), percentage coefficient of variation (%CV – B), Continuous Overall Net Glycaemic Action (CONGA – C), Mean Absolute Glucose (MAG – D), Mean Amplitude of Glycaemic Excursions (MAGE – E) plotted as mean and SD over time (Pre: pre-surgery, 1m: 1 month post-surgery, 1y: 1 year post-surgery, 2y: 2 years post-surgery). One-way repeated measures ANOVA with Bonferroni correction for A-E. Adjusted p-value symbols: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ for comparison with Pre.



Supplementary Figure 3: Post-surgical evolution of percentage time in range (%TIR) (Y-axis) in the RYGB cohort <3.0 mmol/L [54 mg/dl] (black), 3.0-3.9 mmol/L [54-70 mg/dl] (red), 3.9-10.0 mmol/L [70-180 mg/dl] (light green outline), >10.0 mmol/L [180 mg/dl] (grey). These data are plotted as stacked bar graphs, grouped per participant (indicated by P1, P2 etc.) and the time-point for each bar graph is labelled next to the X-axis. Horizontal dashed red line indicates International Consensus for %TIR<3.9 target at <4% and horizontal black line indicates International Consensus for %TIR<3.0 target cut-off in patients with T2DM at <1%.

Supplementary Tables

Supplementary Table 1: Clinical and metabolic characteristics of the prospective Roux-en-Y Gastric Bypass (RYGB) cohort at the study time points. All participants were diagnosed with diabetes or pre-diabetes, and had RYGB surgery performed at the IWC between 2016 and 2018. All participants were followed up pre-surgery (Pre) and at 1m. Nine of the cohort were assessed at 1y. The remaining patient did not attend this assessment but returned for assessment at the 2y mark. A total of 9 participants were assessed at the 2y timepoint. One participant's data at the 2y timepoint was not analysed as she was pregnant at the time. Another participant's CGM data at 2y was not available due to failure of the CGM to collect enough data for adequate analysis, but the clinical and metabolic data have been included in the analysis. Data displayed as mean ± SD or median (range) for Percentage Time in Range. N/A, not applicable. iHOMA2, 24-variable interactive homeostatic model assessment using default settings, %B indicates estimated beta-cell function and %S indicates estimated insulin sensitivity (3). %TIR, Percentage Time in Range to 3 significant figures. One-way repeated measures ANOVA with Bonferroni correction used for parametric measures. Adjusted p-value symbols: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ for comparison with Pre. Friedman test used for comparison of %TIR values from Pre to 1y and 2y timepoints. † $p<0.05$, †† $p<0.01$

	Pre	**1m**	**1y**	**2y**
Number analysed	10	10	9	8
Treatment (Diet/Metformin)	9/1			
Age at surgery (yr)	50.2 ± 13.2	N/A	N/A	N/A
Gender (M/F)	2/8	2/8	2/7	2/6
Weight (kg)	124.6 ± 22.7	113.7 ± 22.2**	86.1 ± 17.8****	85.4 ± 17.1****
Weight loss (%)	0	8.9 ± 1.7***	29.9 ± 6.3****	30.4 ± 7.3****
HbA1c (IFCC mmol/mol)	57.4 ± 17.0	46.8 ± 6.6	38.7 ± 6.3***	39.8 ± 5.0**
HbA1c (converted to NGSP %)	7.4 ± 1.56	6.4 ± 0.60	5.7 ± 0.58***	5.8 ± 0.46**
Fasting glucose (mmol/L)	7.3 ± 1.2	5.8 ± 1.0***	5.0 ± 0.7****	5.3 ± 0.5****
Fasting insulin (mU/L)	17.1 ± 4.9	11.7 ± 6.4*	5.6 ± 2.7***	7.7 ± 7.0**
iHOMA2 %B	82.7 ± 17.9	96.8 ± 32.0	82.3 ± 44.2	83.3 ± 51.8
iHOMA2 %S	47.1 ± 17.9	86.3 ± 52.1	161.4 ± 67.2****	140 ± 66.2***
%TIR<3.0 mmol/L [54 mg/dl]	0 (0-1.91)	0 (0-1.09)	2.27 (0-4.30)†	0.07 (0-5.81)†
%TIR<3.9 mmol/L [70 mg/dl]	0 (0-3.83)	1.51 (0-9.52)	7.53 (0-25.8)†	2.24 (0-29.6)†
%TIR3.9-10.0 mmol/L [70-180 mg/dl]	87.7 (32.9-99.9)	97.2 (67.4-99.8)	86.9 (71.1-95.7)	89.1 (67.0-95.2)
%TIR>10.0 mmol/L [180 mg/dl]	12.3 (0-67.1)	0.92 (0-28.5)	4.33 (0.67-9.95)	6.97 (3.40-11.3)

Supplementary Table 2: Summary of fasting, maximal concentration (Cmax), time of maximal concentration (Tmax), area under curve over 180 min ($AUC_{0\text{-}180}$), incremental area under curve over 180 min (Inc $AUC_{0\text{-}180}$) in glucose, insulin, GLP-1, glucagon during MMT before surgery (Pre), 1 month (1m) and 1 year (1y) after surgery. Nadir glucose is defined as lowest glucose after the peak. Mean ± SD are shown except for Tmax which are noted as median (IQR). Linear mixed model (repeated measures) with Bonferroni correction. * indicates adjusted p-values for contrast between Pre and indicated timepoint after surgery where * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

		Pre	**1m**	**1y**
Glucose	Fasting (mmol/L)	7.1±1.1	5.8±1.0***	5.0±0.8****
	Cmax (mmol/L)	9.3±2.0	9.7±2.2	9.3±1.9
	Nadir glucose (mmol/L)	6.7±2.2	5.0±1.0*	4.1±0.6***
	Tmax (min)	30 (15-30)	30 (15-30)	30 (15-30)
	$AUC_{0\text{-}180}$ (mmol·min/L)	1437±324	1245±306*	1008±153****
	Inc $AUC_{0\text{-}180}$ (mmol·min/L)	231±107	300±134	247±89
Insulin	Fasting (mU/L)	16.1±3.9	10.2±5.0****	6.1±3.2****
	Cmax (mU/L)	71.8±30.8	122.0±62.8***	115.1±46.4**
	Tmax (min)	60 (30-60)	30 (30-60)	30 (15-30)
	$AUC_{0\text{-}180}$ (mU·min/L)	7757±2876	8822±5341	6440±3006
	Inc $AUC_{0\text{-}180}$ (mmol·min/L)	4877±2537	7143±4953	5417±2600
GLP-1	Fasting (pmol/L)	2.7±3.3	2.5±2.9	6.8±7.5*
	Cmax (pmol/L)	8.3±4.3	43.7±19.3****	61.6±19.8****
	Tmax (min)	30 (15-60)	30 (15-30)	15 (15-30)
	$AUC_{0\text{-}180}$ (pmol·min/L)	904±539	3532±1223****	4282±1396****
	Inc $AUC_{0\text{-}180}$ (mmol·min/L)	430±231	3150±1230****	3400±1409****
Glucagon	Fasting (pmol/L)	9.9±2.0	9.1±4.8	5.0±2.7**
	Cmax (pmol/L)	15.8±2.8	21.1±17.0	12.8±3.4
	Tmax (min)	30 (15-30)	30 (15-30)	30 (15-30)
	$AUC_{0\text{-}180}$ (pmol·min/L)	1941±241	1932±855	1288±383*
	Inc $AUC_{0\text{-}180}$ (mmol·min/L)	416±166	709±472	522±313

Supplementary References

1. Monnier L, Colette C, Wojtusciszyn A, Dejager S, Renard E, Molinari N, Owens DR: Toward Defining the Threshold Between Low and High Glucose Variability in Diabetes. Diabetes Care 2017;40:832-838
2. Alexiadou K, Cuenco J, Howard J, Wewer Albrechtsen NJ, Ilesanmi I, Kamocka A, Tharakan G, Behary P, Bech PR, Ahmed AR, Purkayastha S, Wheller R, Fleuret M, Holst JJ, Bloom SR, Khoo B, Tan TM: Proglucagon peptide secretion profiles in type 2 diabetes before and after bariatric surgery: 1-year prospective study. BMJ Open Diabetes Res Care 2020;8
3. Hill NR, Levy JC, Matthews DR: Expansion of the homeostasis model assessment of beta-cell function and insulin resistance to enable clinical trial outcome modeling through the interactive adjustment of physiology and treatment effects: iHOMA2. Diabetes Care 2013;36:2324-2330
4. Hill NR, Oliver NS, Choudhary P, Levy JC, Hindmarsh P, Matthews DR: Normal reference range for mean tissue glucose and glycemic variability derived from continuous glucose monitoring for subjects without diabetes in different ethnic groups. Diabetes Technol Ther 2011;13:921-928