Electronic Supplementary Material

Beta cell function and mass in individuals with and without remission of type 2 diabetes after Roux-en-Y Gastric Bypass

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ESM Methods

Participants

General exclusion criteria included pregnancy or the wish to become pregnant, breastfeeding, age <18 years, renal dysfunction (eGFR <40 ml/min/1.73m²), previous treatment with synthetic exendin-4 or dipeptidyl-peptidase IV inhibitors. To maximize contrast between the groups, people on monotherapy with metformin were excluded.

Combined arginine stimulation and oral glucose tolerance test

Participants underwent a combined arginine stimulation test (ARGT) and oral glucose tolerance test (OGTT) to determine beta cell function, performed in the morning after a 12 h fast. Depending on their daily glucose profiles, people in the non-remission group were asked to temporarily stop or reduce their glucose-lowering medication, including insulin, by up to 50% for 24 h before the tests to ensure glucose levels between 5 and 12 mmol/l at the start of the function tests (ESM Methods table 1). Insulin and other glucose-lowering medication were not used in the morning of the function tests. Five minutes before the function tests, blood was collected to determine baseline fasting glucose and C-peptide levels. Participants then received 5 g arginine hydrochloride intravenously and blood was collected at 2, 3, 4, 5 and 20 minutes after arginine administration to determine glucose and C-peptide levels. Subsequently, participants were asked to drink a maximum of 75 g of anhydrous glucose in 250 ml over a course of 5 minutes followed by blood collection at 0, 10, 20, 30, 45, 60, 90, 120 and 180 minutes after glucose administration to determine glucose and C-peptide levels. Not all participants could tolerate 75 g anhydrous glucose after RYGB, therefore participants drank the maximum tolerable amount of glucose until 75 g.

To determine the acute C-peptide response to arginine, the $AUC_{C\text{-}peptide}$ in the first five min of the ARGT was calculated and corrected for fasting glucose level, since the stimulated C-peptide response to arginine depends on glucose levels. For the OGTT, Area Under Curves (AUCs) for C-peptide ($AUC_{C\text{-}peptide}$), glucose ($AUC_{glucose}$) and the ratio between both ($AUC_{C\text{-}}$

$_{peptide}$/$AUC_{glucose}$) were calculated over the whole course of 180 min. The $AUC_{C\text{-}peptide}$, the $AUC_{C\text{-}peptide}$/$AUC_{glucose}$ and HOMA2-%β based on fasting glucose and C-peptide were used to assess beta cell function. In addition, the baseline fasting glucose and C-peptide levels were used to assess the HOMA indices for insulin sensitivity represented by HOMA2-%S.

PET/CT acquisition

Abdominal PET images (5 bed positions; 7 min/bed position) were obtained one hour post injection using the Siemens Biograph 40 mCT time-of-flight PET/CT scanner to quantify the total pancreatic tracer uptake as a measure for beta cell mass. Simultaneously, a low-dose CT scan without contrast agent was performed for anatomical identification of the pancreas and attenuation correction (transaxial matrix: 512×512 (0.98 mm voxel size), CT slide width: 3 mm). The PET images were reconstructed according to the following settings: three iterations, 21 subsets and post-reconstruction Gaussian filter of 3 mm in full width at half maximum. The PET image size was 200x200 (4.07 mm pixel size).

Quantitative image analysis

PET/CT images were analysed using PMOD version 4.4 software (PMOD Technologies, Zurich, Switzerland). First all scans were evaluated on possible artefacts by a nuclear medicine physician. To determine beta cell mass, anatomical volume of interests (VOIs) were manually drawn in the pancreas, kidneys and proximal duodenum. VOIs of the pancreas and proximal duodenum were drawn to include all uptake on PET images of the upper abdomen using the CT as a reference in case of misalignments. The VOI of the pancreas contained the entire volume of the pancreas. Since clearance of radiolabelled exendin via the kidneys results in high renal tracer uptake, VOIs of the kidneys were dilated by 8 mm to account for spillover activity. Subsequently, VOIs of the proximal duodenum and the dilated kidneys were subtracted from the VOI of the pancreas to correct for a possible overlap in anatomical location. In this way, overestimation of the pancreatic tracer uptake was prevented. The average uptake of radiolabelled exendin (kBq/ml) in the corrected pancreas was calculated.

To determine the total pancreatic uptake of radiolabelled exendin (kBq/MBq), representing beta cell mass, the average uptake of radiolabelled exendin (kBq/ml) was multiplied by the pancreatic volume (ml) and adjusted for injected dose corrected for decay (MBq).

Tables

ESM Table 1. Glucose-lowering medication use in individuals without diabetes remission after RYGB

Participant	Short-acting insulin	Long-acting insulin	Biguanides	SGLT-2 inhibitors	GLP-1R agonists	SU-derivates
1	✓	-	-	-	-	-
2	✓	✓	-	-	-	-
3	✓	✓	✓	-	-	✓
4	-	✓	✓	-	-	-
5	✓	✓	-	-	-	-
6	-	✓	-	-	-	✓
7	-	-	-	-	-	✓
8	-	✓	-	-	-	-
9	✓	-	-	-	-	-
	5/9	6/9	2/9	0/9	0/9	3/9

Abbreviations: SGLT-2, sodium-glucose co-transporter 2; GLP-1R, glucagon-like peptide 1 receptor; SU, sulfonylureas. The symbol "✓"depicts the use of a particular type of glucose-lowering medication. Data of individuals with remission of type 2 diabetes are not presented in this table since they did not use any glucose-lowering medication after RYGB.

ESM Table 2. Clinical baseline characteristics

Variable	Remission of T2D (n=8)	No remission of T2D (n=9)	p-value
Sex (female), n (%)	8 (100)	7 (78)	0.16
Age at inclusion (years)	50 ± 9	57 ± 7	0.12
Time between RYGB and inclusion (years)	2.2 ± 1.0	2.7 ± 0.9	0.27
Diabetes duration before RYGB (years)	8.1 ± 5.7	18.2 ± 8.2	<0.01
Insulin use before RYGB, n (%)	5 (63)	8 (89)	0.20
TBWL%	30.5 ± 6.5	30.3 ± 8.8	0.95
EWL%	83 ± 25	85 ± 30	0.91
Height (cm)	167 ± 7.5	173 ± 16	0.31
BMI (kg/m²)			
before RYGB	38 [37-47]	40 [37-46]	0.74
after RYGB	29 [24-32]**	27 [25-33]**	1.00
Weight (kg)			
before RYGB	103 [98-136]	119 [106-138]	0.42
after RYGB (kg)	80 ± 18**	85 ± 19***	0.54
Fasting glucose (mmol/l)			
before RYGB	9.4 ± 1.9	12.8 ± 4.8	0.10
after RYGB	5.8 ± 1.0**	9.5 ± 2.5	0.002
Fasting C-peptide (nmol/l)			
before RYGB	0.96 [0.68-1.3]	0.32 [0.25-0.73]	0.02
after RYGB	0.71 ± 0.18	0.36 ± 0.14	<0.001
HbA_{1c} (mmol/mol)			
before RYGB	59 ± 5.5	75 ± 15	0.01
after RYGB	39 ± 5.5***	63 ± 11	<0.0001
Creatinine	54 ± 10	54 ± 6	0.96
ALT	33 ± 12	46 ± 16	0.08
AST	29 ± 10	29 ± 11	1.00

Data are presented as mean ± SD, median [IQR], or n (%), as appropriate. Abbreviations: BMI, body mass index; TBWL%, total body weight loss; EWL%, excess weight loss; HbA_{1c}, haemoglobin A_{1c}; ALT, alanine amino transferase; AST, aspartate aminotransferase. The following symbols *, **, *** reflect $p<0.05$, $p<0.01$, <0.001, respectively, versus pre-surgery situation.

ESM Table 3. Changes in HOMA indices before and after RYGB within the participant groups

	Participant group	Before RYGB	After RYGB	P-value
HOMA2-%S	**Remission**	36 [27-47]	56 [51-59]	0.063
	Non-remission	103 [22-150]	94 [73-193]	0.94
HOMA2-%B	**Remission**	62 ± 12	103 ± 32	0.013
	Non-remission	25 ± 15	26 ± 9.9	0.66

Data are presented as mean ± SD and median [IQR], as appropriate. Abbreviations: HOMA2-%S, HOMA2 insulin sensitivity; HOMA2-%B, HOMA2 beta cell function. All HOMA2 parameters were calculated using the updated HOMA2 calculator.

ESM Table 4. The effect of diabetes duration on total pancreatic tracer uptake

Tests of Between-Subjects Effects

Dependent Variable: Total pancreatic tracer uptake

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.29[a]	2.00	2.14	0.90	0.43
Intercept	30.90	1.00	30.90	12.91	<0.001
Diabetes duration	0.90	1.00	0.90	0.37	0.55
Participant group (remission/no remission)	4.11	1.00	4.11	1.72	0.21
Error	28.73	12.00	2.39		
Total	249.54	15.00			
Corrected Total	33.02	14.00			

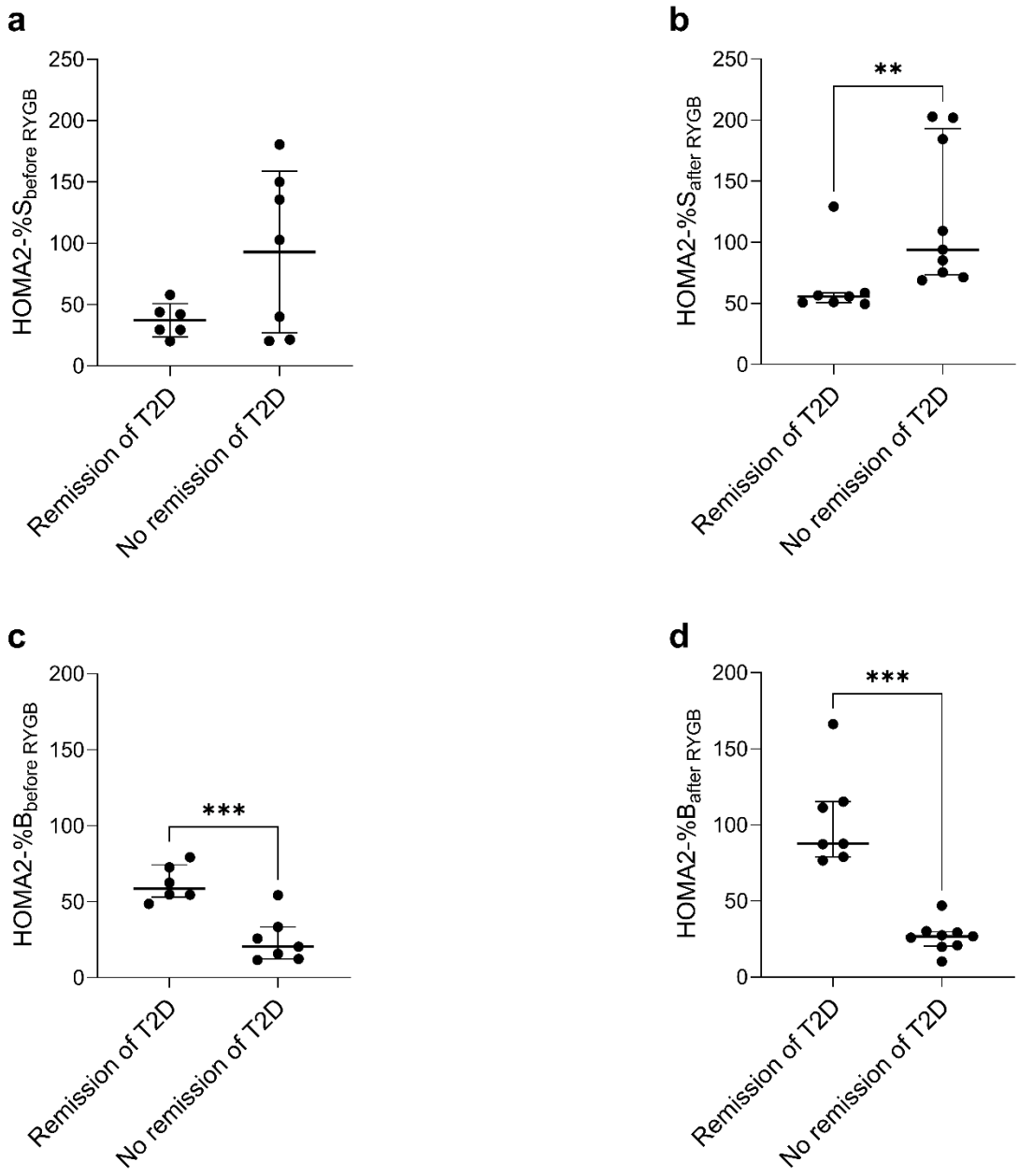
a. R Squared = 0.13 (Adjusted R Squared = -0.015)

ESM Table 5. Mean differences in beta cell mass between the two groups

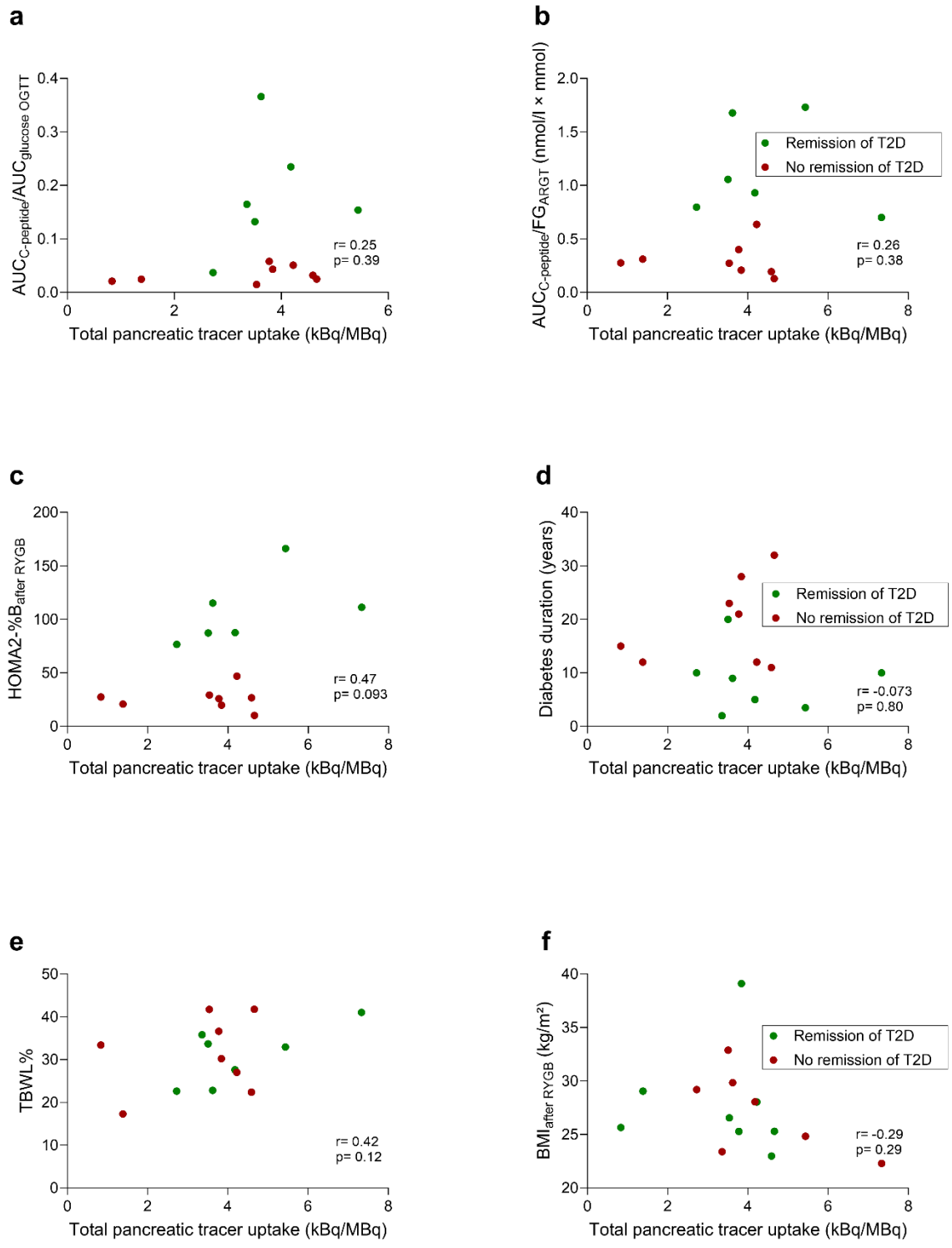
	Remission	Non-remission	p-value
Mean ± SD of total pancreatic tracer uptake (kBq/MBq)	4.3 ± 1.6	3.4 ± 1.4	0.25
95% CI of the mean (kBq/MBq)	[2.8 - 5.8]	[2.1 - 4.6]	-

Data are presented as mean ± SD. The difference between the means is 1.0 ± 0.8 kBq/MBq and the 95% CI on the difference in means extends from -0.8 to 2.7 kBq/MBq. Abbreviations: CI, confidence interval; SD, standard deviation.

Figures



ESM Fig. 1. HOMA2 indices before and after RYGB Insulin sensitivity, represented by HOMA2-%S, of participants with and without remission of type 2 diabetes (a) before and (b) after RYGB. Beta cell function, represented by HOMA2-%B, of participants with and without remission of type 2 diabetes (c) before and (d) after RYGB. Data presented as median and interquartile ranges. The following symbols *, **, *** reflect $p<0.05$, $p<0.01$, <0.001, respectively.



ESM Fig. 2. Correlations between total pancreatic uptake and (a) beta cell function derived from the OGTT; (b) beta cell function derived from the ARGT; (c) beta cell function represented by HOMA2-%B; (d) type 2 diabetes duration; (e) TBWL% and (f) BMI after RYGB. Green and red dots represent individuals with and without remission of type 2 diabetes after RYGB, respectively.