SUPPLEMENTARY MATERIAL

Time of Dosing

Time of administration was determined by a subject-specific linear regression model, which included (a) the fasting plasma glucose concentration, (b) an individual plasma glucose concentration declining slope after the postprandial peak, and (c) intercept plasma glucose concentration. Aiming at administering dasiglucagon 5-10 minutes before plasma glucose crossed below fasting levels, we calculated the predicted time of administration based on the following equation: $\text{Predicted time of administration} = (a - c) / b$.

Randomization and Blinding

Ten sets with three unique random integers in each were randomly generated, representing placebo, 80, and 200 µg dasiglucagon. Included participants were randomly assigned to one of ten sets using www.random.org by an unblinded staff member. Apart from assigning and dispensing the treatments, this staff member was not involved in the study. The randomization sequence is illustrated in Supplementary Fig. 2. Participants and investigators were blinded to the order of the treatment sequence and interventions during trial days. An unblinded clinical assistant carried out the dilution of the dasiglucagon under sterile conditions and immediately delivered the syringe containing either placebo or trial drug directly to the investigator on the trial day (approximately 30 minutes before administration). Syringes with placebo or trial drug were indistinguishable; all three syringes – placebo or treatment – had the same color, viscosity, and volume (0.4 mL). Vials containing dasiglucagon (1 mL, 4 mg/mL) or placebo (1 mL) in a liquid formulation were supplied by Zealand Pharma A/S in a carton packaged and labeled in the local language, indicating the content.

Analyses

Serum acetaminophen concentrations were measured by a photometric method (Atellica CH 930, Siemens Healthineers, Ballerup, Denmark). Plasma samples were extracted at a final concentration of 70% ethanol before glucagon, GIP, GLP-1, and pancreatic polypeptide measurements and 75% ethanol before GLP-2 measurements. Intact endogenous glucagon was assayed using a sandwich ELISA utilizing both N and C-terminal-specific antibodies (presented in Supplementary Table 3), demonstrated not to react with dasiglucagon. This assay from Mercodia (Uppsala Sweden) followed the so-called sequential protocol, meaning that its cross-reaction with intestinal proglucagon-derived peptides (glicentin, oxyntomodulin), the responses of which are dramatically elevated in these patients, is minimal. Total GIP concentration was measured with a radioimmunoassay using an antibody directed towards the C-terminal (code no. 80867), which reacts fully with intact GIP and N-terminally truncated forms. Total GLP-1 was measured using a radioimmunoassay (antibody code no 89390) specific for the C-terminal of the GLP-1 molecule and reacting equally with intact GLP-1 and the primary (N-terminally truncated) metabolite. Pancreatic polypeptide was measured using a mid-region-specific antibody (code no. HYB 347-07). Intact GLP-2 was measured using an antibody directed against the N-terminus of GLP-2. The limit of detection for all radioimmunoassays was below 1.0 pmol/L, and the intra-assay coefficient of variation is below 10%. Plasma norepinephrine and epinephrine were measured using an enzyme-linked immunosorbent assay (Biotech Equity, Karlstad, Sweden). Serum was analyzed for growth hormone on the IDS-iSYS Multi-Discipline Automated System® (cat. no. IS-3700; ImmunoDiagnosticSystems, Frankfurt am Main, Germany), which uses an automated immunoassay and chemiluminescence method, having a limit of detection of 0.050 ng/mL. Plasma cortisol was measured using competitive electrochemiluminescence immunoassay.

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table 1 − Baseline characteristics

	Median (IQR)
Characteristics	
Sex (male/female)	2/8
Age (years)	45.5 (38; 48)
Body weight (kg)	97.4 (72; 100)
BMI (kg/m^2)	34.6 (26; 36)
Fasting	
HbA_{1c} (%)	5.1 (4.9; 5.3)
HbA_{1c} (mmol/mol)	32 (30; 34)
HOMA2-IR	0.8 (0.4; 1.2)
Glucose (mmol/L)	5.0 (4.7; 5.4)
Insulin (pmol/L)	39.8 (29; 64)
C-peptide (pmol/L)	417 (338; 536)
CGM	
Number of days CGM worn	17.4 (16.1; 18.7)
Mean glucose (mmol/L)	6.1 5.9; 6.7)
Coefficient of Variance (CV%)	29.2 (25.1; 32.0)
Time spent in hypoglycemia (min/day)	
IG < 3.9 mmol/L	29.9 (20.0; 72.6)
IG < 3.0 mmol/L	3.4 (1.7; 10.6)
Hypoglycemic events (events/day) (≥ 15 min)	
IG < 3.9 mmol/L	0.9 (0.8; 1.9)
IG < 3.0 mmol/L	0.2 (0.2; 0.5)
Since operation	
Time (years)	9.3 (7; 11)
Weight loss (kg)	53.9 (45; 57)
BMI loss (units)	19.0 (16; 22)
EBMIL (%)	68.8 (62; 96)
TWL (%)	67.7 (47; 76)

Supplementary Table 1 − Baseline characteristics of the Roux-en-Y gastric bypass-operated participants. CGM, continuous glucose monitoring; IG, interstitial glucose concentration; EBMIL, excess BMI loss; TWL, total weight loss; HbA_{1c}, glycosylated hemoglobin; HOMA2-

IR, homeostasis model assessment II of insulin resistance. Data are presented as median with IQR in parenthesis except for gender distribution (male/female). EBMIL was determined as: [(preoperative BMI – BMI at screening) / (preoperative BMI – 25)] × 100.

Supplementary Table 2 – **Glucose and insulin measures from 240-minute liquid mixed meal tests**

	Placebo	80 µg dasiglucagon	200 µg dasiglucagon
Glucose			
Fasting (mmol/L)	5.0 ± 0.1	5.2 ± 0.2	5.2 ± 0.2
Peak (before administration, mmol/L)	13.2 ± 0.8	12.5 ± 0.8*	13.2 ± 0.7
Peak (after administration, mmol/L)	5.0 ± 0.1	6.5 ± 0.2**	7.1 ± 0.4***
Nadir (mmol/L)	3.0 ± 0.2	3.9 ± 0.3**	4.5 ± 0.3***
Time of nadir (min)	118 (96; 178)	185 (110; 235)	205 (155; 235)
Time in hyperglycemia (after administration PG > 7.8 mmol/L, min)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 6.3)
Time in hypoglycemia (PG < 3.9 mmol/L, min)	70.0 (38.8; 80.0)	0.0 (0.0; 58.8)*	0.0 (0.0; 11.3)**
Time in serious hypoglycemia (PG < 3.0 mmol/L, min)	5.0 (0.0; 26.3)	0.0 (0.0; 0.0)$^{P = 0.066}$	0.0 (0.0; 0.0)*
AUC (min × mmol/L)	1424 ± 73	1522 ± 90**	1622 ± 101**
bsAUC (min × mmol/L)	139 (55; 391)	164 (122; 483)	235 (160; 609)**
$AUC_{administration}$ (min × mmol/L)	633 ± 31	744± 32**	845 ± 49**
Insulin			
Fasting (pmol/L)	45 ± 6	45 ± 6	46 ± 6
Peak (pmol/L)	2712 ± 459	2663 ± 451	2704 ± 497
bsAUC (min × nmol/L)	112 (82; 156)	118 (71; 180)	126 (74; 169)
$AUC_{administration}$ (min × nmol/L)	18 (15; 36)	30 (18; 55)*	37 (21; 130)**
C-peptide			
Fasting (pmol/L)	458 ± 44	452 ± 41	463 ± 48

Peak (pmol/L)	5730 ± 542	5749 ± 552	5769 ± 593
bsAUC (min × nmol/L)	395 ± 42	438 ± 45	461 ± 49
$AUC_{administration}$ (min × nmol/L)	223 ± 24	268 ± 29*	291 ± 30**
ISR			
Fasting (pmol/kg/min)	7 ± 2	7 ± 2	7 ± 2
Peak (pmol/kg/min)	25 ± 1	25 ± 1	24 ± 1
bsAUC (min × pmol/L)	1040 ± 129	1549 ± 156***	1244 ± 147
$AUC_{administration}$ (min × pmol/L)	87 ± 9	164 ± 38	233 ± 34**
HOMA2-IR	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
QUICKI	0.37 ± 0.0	0.37 ± 0.0	0.36 ± 0.0
Matsuda index	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1

Supplementary Table 2 − Glucose and insulin levels during the 240-minute mixed meal tests with the administration of placebo, 80, and 200 µg dasiglucagon in 10 Roux-en-Y gastric bypass-operated individuals. bsAUC, baseline-subtracted area under the curve; $AUC_{administration}$, area under the curve calculated from the time of drug administration to 240 minutes after the mixed meal test; ISR, insulin secretion rate; HOMA2-IR, homeostatic model assessment II of insulin resistance; QUICKI, quantitative insulin sensitivity check index. Data are presented as mean ± standard error of the mean or, when skewed, median with IQR in parenthesis. Significant differences from placebo are presented as * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).

Supplementary Table 3 – Adverse events

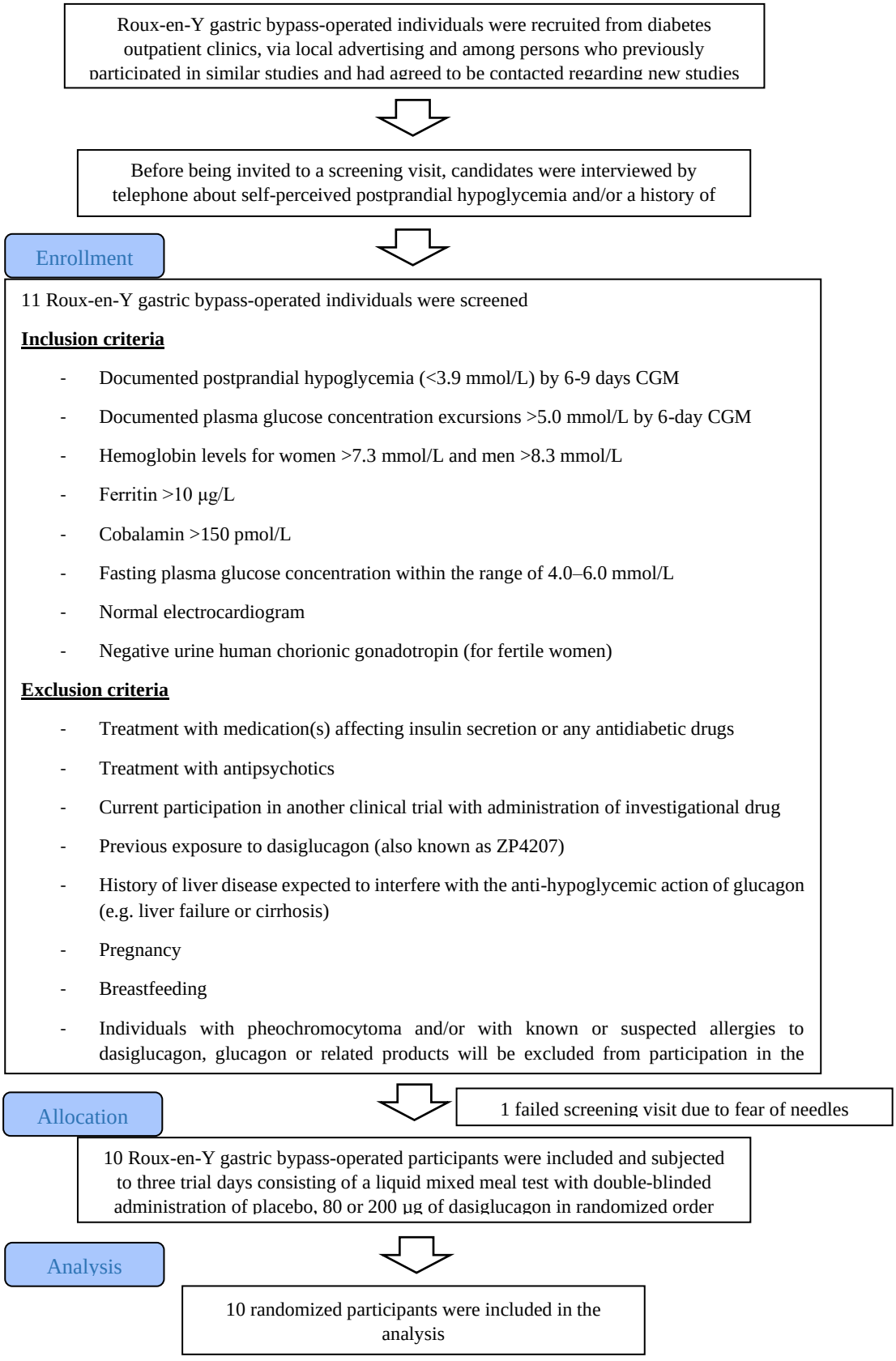
	Placebo	80 µg Dasiglucagon	200 µg Dasiglucagon	Total
No. of participants,	10	10	10	10
No. of participants (no. of events)	1 (1)	1 (1)	4 (8)	4 (10)
Events	1	1	8	10
Nausea	0 (0)	0 (0)	3 (3)	3 (3)
Vomiting	0 (0)	0 (0)	2 (2)	2 (2)
Headache	1 (1)	1 (1)	0 (0)	2 (2)
Dizziness	0 (0)	0 (0)	1 (1)	1 (1)
Sweatiness	0 (0)	0 (0)	1 (1)	1 (1)
Abdominal pain	0 (0)	0 (0)	1 (1)	1 (1)
SAEs (no. of events)	0 (0)	0 (0)	0 (0)	0 (0)
AEs leading to discontinuation of the study (no. of events)	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**

Supplementary Table 3 − Data are presented as the number of participants experiencing adverse events with the number of events in parenthesis. AE, adverse event; SAE, serious adverse event.

Supplementary Table 4 – **Energy content and macronutrient distribution of the liquid mixed meal content**

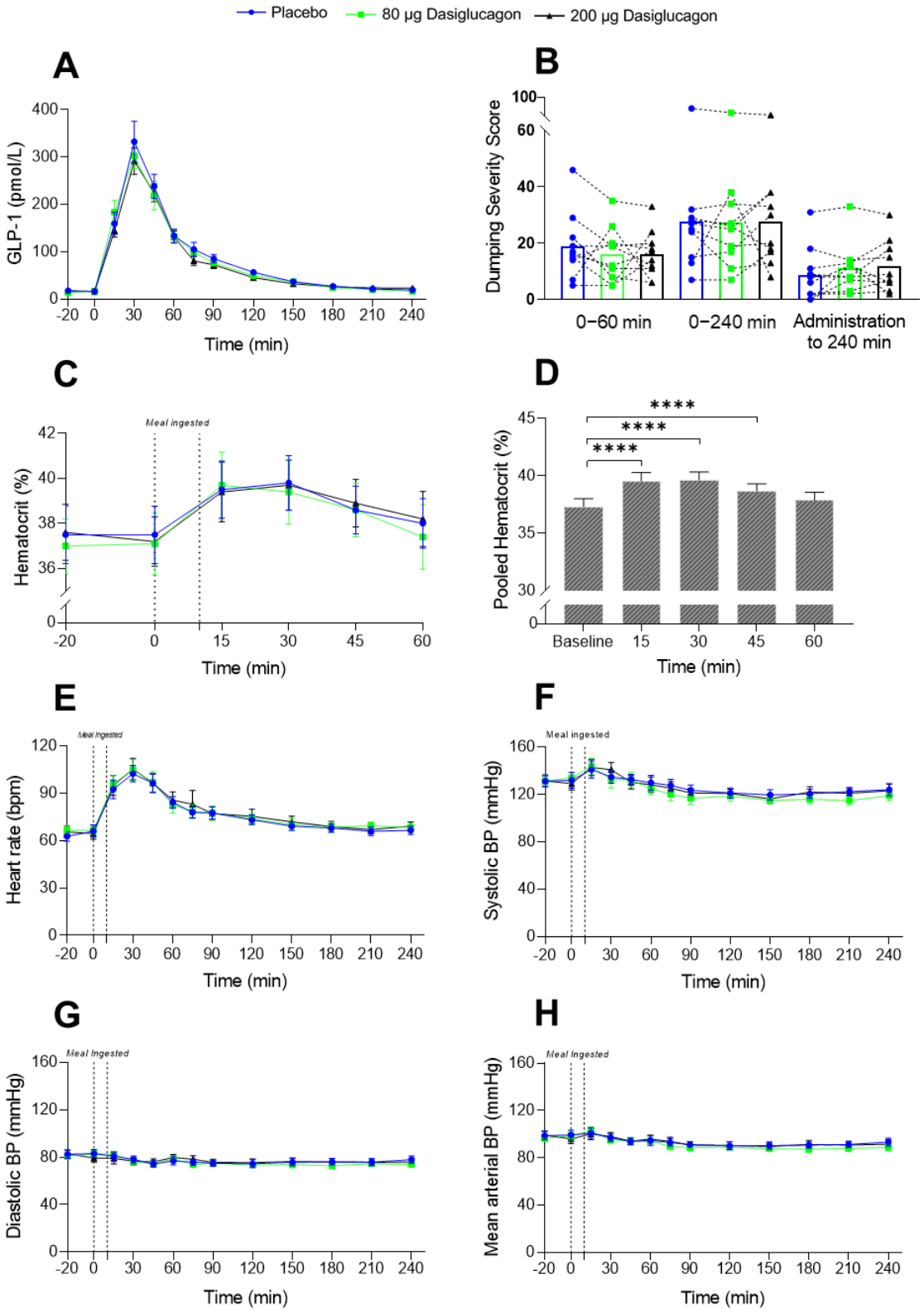
	Median (IQR)
Nutricia Compact (mL)	241 (177; 247)
Nutricia (kcal)	579 (426; 594)
Percentage of TDEE consumed (%)	28.6 (26; 29)
Macronutrient distribution	
Fat (g)	22 (16; 23)
Saturated (g)	1.7 (1.3; 1.8)
Monounsaturated (g)	11.0 (8.1; 11.3)
Polyunsaturated (g)	5.2 (3.8; 5.3)
Carbohydrate (g)	72 (53; 73)
Monosaccharides (g)	28.9 (21.3; 29.7)
Polysaccharides (g)	27.6 (20.3; 28.3)
Protein (g)	23 (17; 24)

Supplementary Table 4 − Energy intake per mixed meal test was based on 5.98 kcal/kg body weight per participant. Data are presented as median with IQR in parenthesis. TDEE, total daily energy expenditure calculated from the Mifflin-Jeor Formula (39).


Roux-en-Y gastric bypass-operated individuals were recruited from diabetes outpatient clinics, via local advertising and among persons who previously participated in similar studies and had agreed to be contacted regarding new studies
Before being invited to a screening visit, candidates were interviewed by telephone about self-perceived postprandial hypoglycemia and/or a history of
Enrollment
11 Roux-en-Y gastric bypass-operated individuals were screened
Inclusion criteria
- Documented postprandial hypoglycemia (<3.9 mmol/L) by 6-9 days CGM
- Documented plasma glucose concentration excursions >5.0 mmol/L by 6-day CGM
- Hemoglobin levels for women >7.3 mmol/L and men >8.3 mmol/L
- Ferritin >10 µg/L
- Cobalamin >150 pmol/L
- Fasting plasma glucose concentration within the range of 4.0–6.0 mmol/L
- Normal electrocardiogram
- Negative urine human chorionic gonadotropin (for fertile women)
Exclusion criteria
- Treatment with medication(s) affecting insulin secretion or any antidiabetic drugs
- Treatment with antipsychotics
- Current participation in another clinical trial with administration of investigational drug
- Previous exposure to dasiglucagon (also known as ZP4207)
- History of liver disease expected to interfere with the anti-hypoglycemic action of glucagon (e.g. liver failure or cirrhosis)
- Pregnancy
- Breastfeeding
- Individuals with pheochromocytoma and/or with known or suspected allergies to dasiglucagon, glucagon or related products will be excluded from participation in the
1 failed screening visit due to fear of needles
Allocation
10 Roux-en-Y gastric bypass-operated participants were included and subjected to three trial days consisting of a liquid mixed meal test with double-blinded administration of placebo, 80 or 200 µg of dasiglucagon in randomized order
Analysis
10 randomized participants were included in the analysis

Supplementary Fig. 1 –CONSORT flowchart including the recruitment process and inclusion and exclusion criteria used for enrollment in the study. CGM, continuous glucose monitoring.

Supplementary Fig. 2 – The six possible treatment sequences. Three-treatment, three-period crossover trial of dasiglucagon. 1:1:1:1:1:1 randomization.



Supplementary Fig. 3 – Total plasma glucagon-like peptide 1 (GLP-1) concentration (mean ± SEM) (A) and dumping symptoms (Dumping Severity Score) (B). The presence of dumping

syndrome was evaluated by hematocrit (C, D) and heart rate (E) during the first 60 minutes of the mixed meal test. Figure (D) represents grouped hematocrit pooled from all trial days before drug administration. No change in systolic (F), diastolic (G), and mean arterial (H) blood pressure (BP) was observed. Blue circles: placebo; green triangles: 80 µg of dasiglucagon; black squares: 200 µg of dasiglucagon. Data are presented as mean ± SEM. Significant differences from baseline are indicated by asterisks (****$P < 0.0001$).