

Supplementary Appendix

Manuscript: *Dasiglucagon Treatment of Postprandial Hypoglycemia After Gastric Bypass: a Randomized, Double-blind, Placebo-controlled Trial*

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Trial Oversight

The study was performed per principles of the Helsinki Declaration (Seventh revision, 2013) and in accordance with ICH-GCP monitored by an independent GCP-unit at Copenhagen University Hospital. The study was approved by the Danish Medicine Agency (reg. no. 2021020234 and 2021020809) and by the Scientific-Ethical Committee of the Capital Region of Denmark (reg. no. H-20078733) and registered at the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT no 2020-005241-16) and at ClinicalTrials.gov (identifier: NCT04836273). The study design is illustrated in Supplementary Figure S1. Before the outpatient treatment period, each participant was subjected to two mixed meal tests with either dasiglucagon administration or placebo (in a random and double-blind fashion). Thus, the participants were equally randomized into two 2-period, 2-treatment crossover sequences, hence in totally four separate randomization sequences (DP & PD for the mixed meal tests and DP & PD for the outpatient treatment; where ‘D’=dasiglucagon and ‘P’=placebo)

Trial Procedures

As illustrated in Figure S2, the participants were instructed to self-administer dasiglucagon/placebo at the very early onset of hypoglycemia (<3.9 mmol/L) as recorded by the continuous glucose monitor and (optimally) subsequently verified by finger prick measurement. The dosing instructions were as follows: After a postprandial glucose peak, the continuous glucose monitor would advise the participant of a sensor-detected level of interstitial glucose concentration levels below $4.2\text{--}3.9$ mmol/L. Instantly after being alerted by the continuous glucose monitor, the participants were instructed to confirm hypoglycemia by a finger prick measurement. If the finger prick measurement confirmed hypoglycemia (<3.9 mmol/L), participants were instructed to self-administer placebo/dasiglucagon and conduct an

ensuing post-dosing finger prick measurement 15 minutes later. If hypoglycemia was not confirmed by finger prick measurement, the participants were to await further continuous glucose monitor readings for the next 2 hours; for any continuous glucose monitor reading below 3.5 mmol/L, the participants were instructed to self-administer placebo/dasiglucagon regardless of the finger prick reading. Hence, when in doubt, the participants were instructed to rely on the continuous glucose monitor over the finger prick readings since the continuous glucose monitor captured time in hypoglycemia was the primary outcome of this study. Therefore self-administrations in some instances occurred above 3.9 mmol/L. Furthermore, during the mixed meal tests, the glucose levels, as assessed by finger prick measurements, were repeatedly higher compared with venous-derived plasma glucose concentration and sensor-detected interstitial glucose concentration, as assessed by the continuous glucose monitor. The investigators routinely contacted the participants to ensure the correct timing and safe self-administration of the investigational product. Generally, the dosing instructions were hard to memorize, and, therefore, the instructions were repeatedly rehearsed before, during, and after the mixed meal tests. Due to conflicting finger prick readings and the continuous glucose monitor leading to confusion as to when to self-administer, the participants needed sparring with the investigators several times a day at the beginning of the first treatment period. Putatively caused by our interest in the self-administrating placebo/dasiglucagon at the immediate onset of hypoglycemia (interstitial glucose concentration <3.9 mmol/L) – but by pursuing this very early onset of hypoglycemia, continuous glucose monitor notifications led to the cessation of daily activities to conduct finger prick measurements elevating the participation burden of the study. Thus, it was not uncommon for the continuous glucose monitor to notify hypoglycemia while the finger prick reading was well above 3.9 mmol/L, leading to omitting drug self-administration. Subsequently, the finger prick measurements

before and/or after dosing was not always obtainable. To combat the risk of skin contamination with an exogenous glucose-containing substance of the punctured finger, all participants were instructed to clean the skin of the puncture site (tip of the finger) before performing a finger prick measurement. Skin-containing reminiscence of glucose-dense substances has been demonstrated to cause a 3-fold increase in blood glucose concentration compared with a clean finger. Notably, per multiple participants' reports, by swinging the arm with the punctured fingertip and thus centrifugally forcing arterial blood into the dermal capillary, finger prick measurement could vary by 0.3–0.8 mmol/L. We speculate this phenomenon can be attributed to cold fingertips causing vasoconstriction and thus decreasing vascular perfusion at the dermal capillary plexus. All these factors caused varying finger prick measurements at the time of dosing. However, even though some finger prick measurement was well above 3.9 mmol, the mean finger prick measurement before all self-administrations in both arms was 3.7 mmol/L placebo: 95% CI (3.6 to 3.7); dasiglucagon: 95% CI (3.6 to 3.7). After self-administration, if self-monitoring blood glucose or interstitial glucose fell below 2.3 mmol/L or onset of marked neuroglycopenic symptoms (severe dizziness, speaking disabilities, blurred or double vision, or confusion), participants were instructed to ingest at least three dextrose tablets to increase blood glucose.

Although the Dexcom G6 device is calibration-free, to maximize continuous glucose monitoring accuracy, we encouraged the participants to calibrate in the morning before having breakfast (i.e., in the fasted state). Before analysis of continuous glucose monitor recordings, data were reviewed for pressure-induced sensor attenuations, which were determined by visual inspection of all data points and subsequently interpolated by the last observation carried forward method. Pressure-induced sensor attenuations were confirmed across all participants at

a median of 0.3% (interquartile range (IQR), 0.2; 0.4) of the total continuous glucose monitoring readings.

Experimental Days

The participants fasted overnight for at least 10 hours and were instructed to abstain from moderate-to-strenuous physical activities and consumption of alcohol 72 hours before each of the experimental days. Moreover, the participants were instructed to duplicate meal content and volume the day before the experimental days. The liquid mixed meal (5.98 kcal/kg body weight), consisting of 50 E% carbohydrates, 35 E% fat, and 15 E% protein (Nutricia Compact, 300 kcal (Nutricia A/S, Allerød, Denmark)), was divided evenly into three portions and consumed within ten minutes. The duration of the mixed meal test was 240 minutes, and the participants were instructed to self-administer dasiglucagon/placebo at the onset of hypoglycemia (<3.9 mmol/L). Blood, for plasma glucose measurement, was collected every 5 minutes and analyzed using a bedside glucose oxidase method (Yellow Springs Instrument [YSI] Model 2300 STAT plus analyzer, Yellow Springs, OH, USA). Blood samples for hormone analysis (insulin, C-peptide, glucose-dependent insulintropic polypeptide and glucagon-like peptide 1, pancreatic polypeptide, glucagon, dasiglucagon, growth hormone, epinephrine, norepinephrine, and cortisol) were collected at time=-20, 0, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 minutes. Additionally, blood pressure and heart rate were measured every 15 minutes throughout the mixed meal test, and the participants were asked to score their hypoglycemic symptoms according to the Edinburgh Hypoglycemia Symptom Scale. Area under the curve (AUC) was calculated using the trapezoidal model, and counter-regulatory hormone responses (glucagon, norepinephrine, epinephrine, pancreatic polypeptide, growth hormone, and cortisol) were evaluated as the integrated hormone response after drug

self-administration (i.e., $AUC_{administration}$). Blood for analysis of plasma glucose-dependent insulintropic polypeptide, glucagon-like peptide 1, pancreatic polypeptide, and glucose-dependent insulintropic polypeptide, glucagon, and dasiglucagon was collected in chilled EDTA tubes (on ice) and centrifuged immediately. Blood for serum analysis of growth hormone was collected in tubes containing clot activator and analyzed immediately. Blood for analysis of serum epinephrine and norepinephrine was collected in chilled EGTA tubes (on ice) and centrifuged 10 minutes after collection. Blood for analysis of plasma cortisol levels was collected in lithium-heparin tubes and centrifuged immediately. All tubes were centrifuged for 15–20 minutes at 2,000 g at 4°C before plasma/serum was transferred to storage tubes on ice and subsequently stored at either –20°C or –80°C.

Analyses

Self-monitoring blood glucose and continuous glucose monitoring measurement of glucose level were analyzed by the glucose oxidase method forming hydrogen peroxide. A mediator on the glucose strip or membrane replaces oxygen and transfers the electron from hydrogen peroxide to an electrode that measures the electrical current. Hence, the current is a surrogate marker for glucose concentration. Plasma samples were extracted at a final concentration of 70% ethanol before glucose-dependent insulintropic polypeptide, glucagon-like peptide 1, pancreatic polypeptide, glucagon, and dasiglucagon measurements: Total glucose-dependent insulintropic polypeptide concentration was measured with a radioimmunoassay using an antibody directed towards the C-terminal (code no. 80867), which reacts fully with intact glucose-dependent insulintropic polypeptide and N-terminally truncated forms. Total glucagon-like peptide 1 was measured using a radioimmunoassay (antibody code no 89390) specific for the C-terminal of the glucagon-like peptide 1 molecule and reacting equally with

intact glucagon-like peptide 1 and the primary (N-terminally truncated) metabolite. Pancreatic polypeptide was measured using a mid-region specific antibody (code no. HYB 347-07). Intact endogenous glucagon was assayed using a sandwich ELISA kit (cat no 10-1271-01, Mercodia, Uppsala, Sweden) utilizing both N and C-terminal-specific antibodies and demonstrated not to react with dasiglucagon. Dasiglucagon was measured using a radioimmunoassay (antibody code no 4830); First total glucagon (dasiglucagon and endogenous glucagon) was measured using the 4830-assay, which is directed toward the N-terminus of glucagon and cross-reacts with oxyntomodulin and approximately 20% with peptides extended from the N-terminus of glucagon, including glicentin. The assay has a detection limit of 2 pmol/L and an intra-assay coefficient of variation below 10%. Hereafter, total glucagon measured by the 4830-assay was subtracted with the endogenous glucagon (measured using the sandwich ELISA), resulting in a dasiglucagon concentration. The limit of detection for all radioimmunoassays is below 1.0 pmol/L, and the intra-assay coefficient of variation is below 10%. 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 norepinephrine and epinephrine were measured using an enzyme-linked immunosorbent assay (Biotech Equity, Karlstad, Sweden). Plasma cortisol was measured using a competitive electrochemiluminescence immunoassay using a Cobas8000 e801 (F. Hoffmann–La Roche, Ltd, Basel, Switzerland).

Supplementary Figures and Tables

Supplementary Figure S1

Legend text for Figure S1 – Study design. Symptoms of hypoglycemia, quality of life, and fear of hypoglycemia were scored by the end of each treatment period.

Supplementary Figure S2

Legend text for Figure S2 – Flowchart illustrating the algorithm used for self-administration of dasiglucagon and placebo, respectively, during the two four-week treatment periods.

Supplementary Figure S3

Legend text for Figure S3 – Quality of life and fear of hypoglycemia as assessed by the WHOQOL-BREF and the HFS-II. All questionnaires are presented as mean with 95% confidence interval (CI) and individual data points. WHOQOL-BREF is reported in four separate domains: physical, psychological, social, and environment domains (**A–D**), whereas HFS-II is presented in three separate domains (total score, HFS-W, and HFS-B) (**E–G**).

Table S1. Continuous Glucose Monitor Metrics Based on Total Continuous Glucose Monitor-Recording and Glycemic Intervals During Nighttime and Daytime During Four Weeks of Treatment With Dasiglucagon Compared With Four Weeks of Placebo.

	Placebo (n=24)	Dasiglucagon (n=24)	Estimated treatment difference	P value
Continuous glucose monitor metrics				
Mean glucose (mmol/L)	6.1 (6.0 to 6.3)	6.2 (6.0 to 6.3)	0.0 (−0.1 to 0.2)	<i>P</i> =0.49/0.65
Standard deviation (SD)	1.9 (1.7 to 2.0)	1.9 (1.7 to 2.0)	0.0 (−0.1 to 0.1)	<i>P</i> =0.63/0.78
Coefficient of variance (CV%)	30.1 (27.6 to 33.0)	30.1 (27.7 to 32.4)	−0.1 (−1.1 to 1.0)	<i>P</i> =0.92/0.97
Low blood glucose index (LBGI)*	2.1 [1.7; 3.0]	1.9 [1.6; 2.7]	−0.1 [−0.4; 0.0]	<i>P</i> =0.08/0.23
High blood glucose index (HBGI)	3.9 (3.2 to 4.6)	3.8 (3.2 to 4.4)	−0.1 (−0.4 to 0.1)	<i>P</i> =0.30/0.44
Continuous glucose monitor recording (days)	28.1 (27.7 to 28.6)	28.3 (28.0 to 28.6)	0.2 (−0.1 to 0.5)	<i>P</i> =0.23/0.42
Sensor reading (%)	92.4 (91.2 to 93.6)	92.4 (91.2 to 93.6)	0.0 (−0.2 to −0.3)	<i>P</i> =0.75/0.88
Daily calibrations	0.90 (0.9 to 0.9)	0.9 (0.9 to 0.9)	0.0 (0.0 to 0.0)	<i>P</i> =0.26/0.44
Hypoglycemic events (events/day) (≥ 15 minutes)				
Interstitial glucose <3.9 mmol/L	1.4 (1.0 to 1.7)	1.2 (0.9 to 1.5)	−0.2 (−0.4 to 0.1)	<i>P</i> =0.16/0.35
Interstitial glucose <3.0 mmol/L†	0.3 [0.2; 0.5]	0.2 [0.1; 0.4]	−0.1 [−0.2; −0.0]	<i>P</i> =0.002/ 0.01
24-hour (glycemic percentage of time (%))				
<3.0 mmol/L*	0.7 (0.5 to 1.0)	0.3 (0.2 to 0.5)	−0.4 (−0.6 to −0.2)	<i>P</i> <0.001/ 0.006

<3.9 mmol/L	3.7 (2.6 to 4.8)	2.4 (1.8 to 3.1)	-1.2 (-2.0 to -0.5)	<i>P</i> =0.002/ 0.01
3.0–3.9 mmol/L	2.9 (2.0 to 3.8)	2.1 (1.6 to 2.6)	-0.8 (-1.5 to -0.2)	<i>P</i> =0.01/ 0.049
Daytime (glycemic percentage of time (%))				
<3.0 mmol/L*	0.6 [0.3; 1.1]	0.3 [0.1; 0.7]	-0.3 [-0.6; -0.2]	<i>P</i> =0.0003/ 0.003
<3.9 mmol/L	4.1 (2.8 to 5.4)	2.8 (2.0 to 3.5)	-1.4 (-2.2 to -0.5)	<i>P</i> =0.004/ 0.02
3.9–10.0 mmol/L	89.6 (87.6 to 91.6)	90.8 (89.0 to 92.6)	1.2 (0.3 to 2.0)	<i>P</i> =0.01/ 0.046
>7.8 mmol/L	17.1 (14.9 to 19.3)	17.9 (15.3 to 20.)	0.8 (-0.7 to 2.2)	<i>P</i> =0.30/0.45
>10.0 mmol/L	6.3 (4.9 to 7.7)	6.5 (5.0 to 8.0)	0.2 (-0.5 to 0.9)	<i>P</i> =0.53/0.68
Nighttime (glycemic percentage of time (%))				
<3.0 mmol/L†	0.3 [0.0; 0.6]	0.1 [0.0; 0.7]	-0.1 [-1.8; 0.0]	<i>P</i> =0.01/0.05
<3.9 mmol/L*	1.7 [0.7; 3.0]	1.1 [0.3; 2.1]	-0.5 [-1.5; 0.0]	<i>P</i> =0.02/0.08
3.9–10.0 mmol/L	95.7 (93.9 to 97.6)	96.6 (95.1 to 98.0)	0.8 (-0.5 to 2.1)	<i>P</i> =0.20/40
>7.8 mmol/L	5.2 (2.8 to 7.7)	6.4 (3.8 to 9.1)	1.2 (-0.4 to 2.8)	<i>P</i> =0.14/32
>10.0 mmol/L	1.9 (0.7 to 3.0)	1.8 (0.8 to 2.9)	0.0 (-0.8 to 0.8)	<i>P</i> =0.99/1.0

Data are presented as mean with 95% CI, mean difference as estimated treatment difference (ETD) with 95% CI, and with corresponding *P* values shown as non-adjusted/adjusted. Multiplicity adjusted for false discovery rate by the Benjamini-Hochberg procedures and adjusted *P* values <0.05 are indicated with bold font. The low blood glucose index (LBGI) and the high blood glucose index (HBGI) were calculated using the EasyGV workbook. Daytime is 6:00_{A.M.}–12:00_{A.M.}, and nighttime is 12:00_{A.M.}–06:00_{A.M.}. 1 mmol/L=18.018 mg/dL.

*Owing to substantial skewed data distribution, these results are presented as median with IQR.

†These data were analyzed using a Wilcoxon Signed rank test owing to substantial skewed distribution and presented as median with IQR.

Table S2. Edinburgh hypoglycemia symptom scale, dietary intake, and self-administration frequency during treatment periods.

	Placebo (n=24)	Dasiglucagon (n=24)	<i>P</i> value
Edinburgh Hypoglycemia Symptom Scale			
Full score	37 [29; 51]	31 [27; 48]	<i>P</i> =0.006/ 0.03
Autonomic	13 [10; 16]	10 [7; 15]	<i>P</i> =0.02/0.06
Neuroglucopenic	20 [15; 28]	17 [14; 28]	<i>P</i> =0.02/0.07
Malaise	4 [3; 6]	4 [3; 6]	<i>P</i> =0.08/0.23
Dietary intake			
Energy (MJ/day)	9 (8 to 10)	8 (7 to 10)	<i>P</i> =0.23/0.44
Energy (kJ/kg body weight/day)	102 (90 to 114)	95 (84 to 106)	<i>P</i> =0.16/0.35
Protein (g/day)	80 (68 to 91)	79 (67 to 91)	<i>P</i> =0.96/0.99
Carbohydrate (g/day)	237 (205 to 269)	225 (191 to 260)	<i>P</i> =0.40/0.57
Fat (g/day)	87 (73 to 100)	78 (63 to 92)	<i>P</i> =0.09/0.25
Saturated (g/day)	31 (24 to 37)	28 (20 to 35)	<i>P</i> =0.30/0.47
Monounsaturated (g/day)	22 (17 to 26)	16 (13 to 19)	<i>P</i> =0.02/0.07
Polyunsaturated (g/day)	9 (6 to 11)	7 (5 to 9)	<i>P</i> =0.23/0.43

Dietary fibers (g/day)	21 (18 to 24)	21 (18 to 24)	<i>P</i> =0.85/0.95
Self-administration frequency			
Sum of total self-administrations	480	541	-
Sum of complete self-monitoring blood glucose measurements (i.e., before and after administration)	357	412	-
Self-administration frequency (pr/day)	0.7 [0.07; 0.97]	0.7 [0.07; 1.22]	<i>P</i> =0.24/0.46

The Edinburgh Hypoglycemia Symptom Scale is presented as median with IQR in brackets and dietary intake as mean with 95% CI. *P* values are shown as non-adjusted/adjusted. Multiplicity adjusted for false discovery rate by the Benjamini-Hochberg procedures and adjusted *P* values <0.05 are indicated with bold font. MJ; megajoule. 1 MJ=239.01 kcal.

Table S3. Overview of Adverse Events.

	During liquid mixed meal tests (0-240 minutes)		During outpatient treatment periods (2 × 4 weeks)		From signed consent (0 to 16 weeks)
	Placebo (n=19)	Dasiglucagon (n=19)	Placebo (n=24)	Dasiglucagon (n=24)	Total (n=24)
No. of participants (no. of events)					
All adverse events	3 (5)	2 (8)	6 (9)	11 (40)	17 (70)
Mild	3 (5)	2 (4)	5 (5)	7 (14)	12 (31)
Moderate	0	2 (4)	2 (3)	6 (24)	9 (34)
Severe	0	0	1 (1)	2 (2)	5 (5)
Serious adverse events	0	0	0	1 (1)*	1 (1)
Deaths	0	0	0	0	0
Adverse event leading to premature discontinuation of the trial product	0	0	0	0	0
No. of participants (no. of events)					
Nausea	2 (2)	2 (3)	2 (2)	7 (25)	9 (33)
Vomiting					
Headache	2 (2)		2 (2)	2 (2)	6 (6)
Dizziness		1 (1)		1 (4)	2 (5)
Perspiration		1 (1)			1 (1)
Abdominal pain				1 (1)	2 (2)
Skin irritation under continuous glucose monitor				1 (2)	2 (5)

Continuous glucose monitor		1 (1)		1 (1)
application site hematoma				
Fatigue		1 (1)	1 (1)	2 (2)
Feeling sick	1 (1)			1 (1)
Tachycardia	1 (1)		1 (1)	2 (2)
Diarrhea			1 (1)	1 (1)
Pneumonia			1 (1)	1 (1)
Severe hypoglycemia				2 (2)†
Chest and back pain			1 (1)	1 (1)
Covid-19 positive		1 (1)		2 (2)
Injection site hematoma		1 (1)		1 (1)
Reduced concentration ability		1 (1)		1 (1)
Tunnel vision	1 (1)			1 (1)
Spontaneous miscarriage*			1 (1)	1 (1)
Antecubital hematoma and pain in forearm	1 (1)			1 (1)

Adverse events are tabulated as the number of participants (number of events) and summarised during the liquid mixed meal tests, the treatment period, and from signed consent to end of trial (0–16 weeks).

*The trial participant was excluded immediately after a positive human chorionic gonadotropin test indicating pregnancy. Two weeks after the exclusion, the participant had a spontaneous miscarriage that required admission to the hospital. The incidence was deemed unrelated to trial participation by the principal investigator.

†The severe hypoglycemic events occurred in the washout period and during the follow-up period, where the continuous glucose monitor was discontinued, and thus, during cessation of continuous glucose monitor-guided self-administered placebo/dasiglucagon.

Table S4. Changes in Glucose, Pancreatic-, and Hypoglycemic Counterregulatory Hormones Responses During a 240-minute Liquid Mixed Meal Test of which Placebo or Dasiglucagon was Self-administered at the Onset of Hypoglycemia.

	Placebo (n=19*)	Dasiglucagon (n=19*)	Estimated treatment difference (ETD)	P value
Glucose				
Fasting (mmol/L)	4.8 (4.7 to 5.0)	5.0 (4.8 to 5.2)	−0.2 (0.1 to 0.3)	P=0.002/ 0.01
Nadir (mmol/L)	3.2 (2.9 to 3.5)	3.5 (3.4 to 3.7)	0.4 (0.2 to 0.5)	P=0.0007/ 0.002
Mean nadir (mmol/L)	3.2 (2.9 to 3.5)	3.5 (3.4 to 3.5)	0.4 (0.2 to 0.5)	P=0.0002/ 0.002
Peak (after self-administration, mmol/L)	4.8 (4.6 to 4.9)	6.5 (6.0 to 7.0)	1.8 (1.3 to 2.3)	P<0.0001/ 0.0005
Time in hyperglycemia (minutes after self-administration >7.8 mmol/L)	0 (0 to 0)	2 (−1 to 5)	2 (−1 to 5)	P=0.20/0.38
Insulin				
Fasting (pmol/L)	41 (32 to 51)	45 (34 to 57)	4 (−2 to 10)	P=0.19/0.38
Peak (pmol/L)	2364 (1630 to 3098)	2509 (1712 to 3306)	145 (−130 to 420)	P=0.28/0.44
AUC _{administration} (min × nmol/L)	6 (5 to 8)	12 (9 to 14)	5 (4 to 7)	P=0.0002/ 0.002
C-peptide				
Fasting (pmol/L)	430 (365 to 496)	460 (369 to 552)	30 (−5 to 65)	P=0.089/0.26
Peak (pmol/L)	4766 (3812 to 5720)	4894 (3921 to 5867)	128 (−225 to 482)	P=0.46/0.74
AUC _{administration} (min × nmol/L)	82 (66 to 97)	120 (100 to 140)	38 (26 to 50)	P<0.0001/ 0.0009

Glucagon-like peptide 1

Fasting (pmol/L) †	9 [6; 14]	8 [3; 14]	0 [-2; 0]	$P=0.16/0.35$
Peak (pmol/L)	140 (117 to 163)	136 (105 to 167)	-4 (-24 to 15)	$P=0.64/0.77$
AUC _{administration} (min × nmol/L)	1871 (1428 to 2314)	1852 (1414 to 2291)	-19 (-316 to 278)	$P=0.89/0.92$

Glucose-dependent insulinotropic polypeptide

Fasting (pmol/L)	13 (11 to 15)	12 (10 to 14)	-1 (-4 to 2)	$P=0.46/0.62$
Peak (pmol/L)	107 (91 to 123)	104 (88 to 119)	-3 (-12 to 6)	$P=0.46/0.61$
AUC _{administration} (min × nmol/L)	3 (2 to 3)	3 (2 to 3)	0 (0 to 0)	$P=0.78/0.85$

Norepinephrine

Fasting (ng/ml) †	0.2 [0.2; 0.2]	0.2 [0.1; 0.3]	-0.0 [-0.1; 0.0]	$P=0.22/0.43$
Peak (ng/ml)	0.4 (0.3 to 0.4)	0.4 (0.3 to 0.4)	-0.0 (-0.1 to 0.0)	$P=0.15/0.34$
AUC _{administration} (min × ng/ml)	7.2 (5.3 to 9.0)	7.2 (5.2 to 9.2)	0.0 (-1.1 to 1.1)	$P=0.98/0.99$

Epinephrine

Fasting (pg/ml)	20 (10 to 40)	40 (30 to 40)	0 (-10 to 10)	$P=0.69/0.84$
Peak (pg/ml)	30 (30 to 40)	40 (30 to 50)	0 (-10 to 13)	$P=0.68/0.84$
AUC _{administration} (min × pg/ml)	79 (47 to 110)	99 (63 to 134)	20 (-5 to 50)	$P=0.11/0.27$

Pancreatic polypeptide

Fasting (pmol/L) †	17 [11; 26]	12 [9; 21]	-5 [-11; 6]	$P=0.51/0.063$
Peak (pmol/L) †	87 [54; 123]	75 [57; 168]	-3 [-24; 24]	$P=0.58/0.71$
AUC _{administration} (min × nmol/L) †	4.5 [3.2; 7.6]	2.9 [1.8; 5.0]	-1.6 [-2.5; -0.3]	$P=0.0001/0.001$

Growth hormone

Fasting (ng/ml) †	1.3 [0.3; 2.3]	0.7 [0.3; 4.7]	−0.2 [−0.8; 0.4]	<i>P</i> =0.86/0.92
Peak (ng/ml)	3.7 (2.2 to 5.1)	4.6 (3.5 to 5.7)	1.0 (−0.2 to 2.1)	<i>P</i> =0.10/0.20
AUC _{administration} (min × ng/ml) †	45.1 [29.9; 135.4]	87.6 [52.9; 185.8]	53.3 [10.3; 150.4]	<i>P</i> =0.01/ 0.04

Cortisol

Fasting (nmol/L)	296 (247 to 346)	279 (245 to 312)	−18 (−62 to 27)	<i>P</i> =0.42/0.58
Peak (nmol/L)	432 (368 to 496)	475 (427 to 523)	43 (−8 to 95)	<i>P</i> =0.09/0.23
AUC _{administration} (min × mmol/L)	25 (20 to 30)	30 (25 to 35)	5 (1 to 10)	<i>P</i> =0.02/0.07

Systolic blood pressure (mmHg)

AUC _{administration} (min × mmHg/1000)	14 (12 to 16)	14 (12 to 16)	0 (−1 to 0)	<i>P</i> =0.74/0.86
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Diastolic blood pressure (mmHg)

AUC _{administration} (min × mmHg/1000)	9 (8 to 10)	9 (8 to 10)	0 (0 to 0)	<i>P</i> =0.89/0.93
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Heart rate (beats per minute (BPM))

AUC _{administration} (min × BPM/1000)	8 (7 to 10)	8 (7 to 9)	0 (0 to 1)	<i>P</i> =0.26/0.43
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Edinburgh Hypoglycemia Symptom Scale

Full score (0 to 240 min)	302 [268; 336]	308 [273; 333]	6 [−11; 24]	<i>P</i> =0.40/0.62
During hypoglycemia (administration to 240 min)	132 [97; 252]	125 [100; 145]	0 [−7; 5]	<i>P</i> =0.85/0.91

Data are presented as mean with 95% CI, mean difference as estimated treatment difference (ETD) with 95% CI, and with corresponding *P* values shown as non-adjusted/adjusted. *P* values are shown as non-adjusted/adjusted. Multiplicity adjusted for false discovery rate by the Benjamini-Hochberg procedures and adjusted *P* values <0.05 are indicated with bold font. AUC_{administration}, the area under the curve calculated from the time of placebo/dasiglucagon self-administration. The

mean nadir glucose denotes a mean of three consecutive plasma glucose measurements collected 5 minutes apart during the mixed meal test. 1 mmol/L = 18.018 mg/dL.

*Five participants did not complete the experimental days due to unsuccessful provocation of postprandial hypoglycemia.

†Owing to substantial skewed data distribution, these results are presented as median with IQR.