The GLP-1 receptor agonist lixisenatide reduces postprandial glucose in patients with diabetes secondary to total pancreatectomy

a randomised, placebo-controlled, double-blinded crossover trial

Juel, Caroline T.B.; Lund, Asger; Andersen, Maria M.; Hansen, Carsten P.; Storkholm, Jan H.; Rehfeld, Jens F.; van Hall, Gerrit; Hartmann, Bolette; Wewer Albrechtsen, Nicolai J.; Holst, Jens J.; Vilsbøll, Tina; Knop, Filip K.

Published in:
Diabetologia

DOI:
10.1007/s00125-020-05158-9

Publication date:
2020

Document version
Publisher's PDF, also known as Version of record

Diabetologia (2020) 63:1285–1298
https://doi.org/10.1007/s00125-020-05158-9

ARTICLE

The GLP-1 receptor agonist lixisenatide reduces postprandial glucose in patients with diabetes secondary to total pancreatectomy: a randomised, placebo-controlled, double-blinded crossover trial

Caroline T. B. Juel[1] · Asger Lund[1] · Maria M. Andersen[1] · Carsten P. Hansen[2] · Jan H. Storkholm[2] · Jens F. Rehfeld[3] · Gerrit van Hall[3,4] · Bolette Hartmann[5] · Nicolai J. Wewer Albrechtsen[3,5] · Jens J. Holst[5,6] · Tina Vilsbøll[1,7] · Filip K. Knop[1,6,7,8]

Received: 25 November 2019 / Accepted: 11 March 2020 / Published online: 11 May 2020

Abstract

Aims/hypothesis Treatment of diabetes secondary to total pancreatectomy remains a challenge and insulin constitutes the only glucose-lowering treatment for these patients. We hypothesised that the glucagon-like peptide 1 (GLP-1) receptor agonist lixisenatide would improve postprandial glucose tolerance in totally pancreatectomised patients.

Methods In a double-blinded, randomised, crossover study, 12 totally pancreatectomised individuals (age: 65.0 ± 9.5 mean±SD years; BMI: 22.9 ± 3.9 kg/m^2) and 12 healthy control individuals (age 66.1 ± 7.6 years; BMI: 24.0 ± 2.9 kg/m^2) underwent two 3 h liquid mixed-meal tests (with paracetamol for assessment of gastric emptying) after single-dose injection of 20 μg of lixisenatide or placebo. Basal insulin was given the night before each experimental day; no insulin was given during study days.

Results Compared with placebo, lixisenatide reduced postprandial plasma glucose excursions in the pancreatectomy group (baseline-subtracted AUC [bsAUC] [mean±SEM]: 548 ± 125 vs 1447 ± 95 mmol/l × min, $p < 0.001$) and in the control group (−126 ± 12 vs 222 ± 51 mmol/l × min, $p < 0.001$). In the pancreatectomy group a mean peak glucose concentration of 23.3 ± 1.0 mmol/l was reached at time point 134 ± 11 min with placebo, compared with a mean peak glucose concentration of 18 ± 1.4 mmol/l ($p = 0.008$) at time point 148 ± 13 min ($p = 0.375$) with lixisenatide. In the control group a mean peak concentration of 8.2 ± 0.4 mmol/l was reached at time point 70 ± 13 min with placebo, compared with a mean peak concentration of 5.5 ± 0.1 mmol/l ($p < 0.001$) at time point 8 ± 25 min ($p = 0.054$) with lixisenatide. Lixisenatide also reduced gastric emptying and postprandial glucagon responses in the pancreatectomy group (66 ± 84 vs 1190 ± 311 pmol/l × min, $p = 0.008$) and in the control group (141 ± 100 vs 190 ± 100 pmol/l × min, $p = 0.034$). In the pancreatectomy group, C-peptide was undetectable in plasma. In the control group, postprandial plasma C-peptide responses were reduced with lixisenatide (18 ± 17 vs 189 ± 31 nmol/l × min, $p < 0.001$).

Conclusions/interpretation The GLP-1 receptor agonist lixisenatide reduces postprandial plasma glucose excursions in totally pancreatectomised patients. The mode of action seems to involve deceleration of gastric emptying and reduced postprandial responses of gut-derived glucagon.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00125-020-05158-9) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

✉ Filip K. Knop
filip.krag.knop.01@regionh.dk

[1] Center for Clinical Metabolic Research, Gentofte Hospital, University of Copenhagen, Gentofte Hospitalsvej 7, 3rd floor, DK-2900 Hellerup, Denmark

[2] Department of Surgery and Transplantation, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

[3] Department of Clinical Biochemistry, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

[4] Clinical Metabolomics Core Facility, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

[5] Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

[6] Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

[7] Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

[8] Steno Diabetes Center Copenhagen, Gentofte, Denmark

Research in context

What is already known about this subject?

- Owing to a lack of pancreatic insulin and glucagon secretion, treatment of diabetes secondary to total pancreatectomy remains a challenge and exogenous insulin constitutes the only recommended glucose-lowering treatment for these patients
- In addition to its insulinotropic and glucagonostatic effects in the pancreas, the gut-derived incretin hormone glucagon-like peptide 1 (GLP-1) is known to have pleiotropic extrapancreatic effects; however, these have been difficult to disentangle from the potent effects of GLP-1 on the endocrine pancreas
- We have recently shown that totally pancreatectomised individuals are characterised by secretion of gut-derived glucagon following OGTT, but the effect of GLP-1 on gut-derived glucagon secretion is unknown

What is the key question?

- How does the GLP-1 receptor agonist lixisenatide affect postprandial glucose excursions in individuals with diabetes secondary to total pancreatectomy?

What are the new findings?

- A single dose of the GLP-1 receptor agonist lixisenatide significantly reduces postprandial plasma glucose excursions in totally pancreatectomised individuals
- Plasma glucagon levels, assessed by an MS-validated monoclonal antibody-based glucagon ELISA, increased significantly after meal ingestion in totally pancreatectomised individuals; single-dose lixisenatide abolished the postprandial glucagon response in totally pancreatectomised patients
- Lixisenatide also reduced gastric emptying in totally pancreatectomised individuals, which, together with its suppressive effect on postprandial glucagon responses, may contribute to the postprandial glucose-lowering effect of lixisenatide in these patients

How might this impact on clinical practice in the foreseeable future?

- Our results illustrate that GLP-1 receptor activation can lower postprandial plasma glucose excursions independently of its potent effects on the endocrine pancreas (probably via deceleration of gastric emptying and perhaps via suppression of gut-derived glucagon secretion), and, thus, offers the possibility of utilising GLP-1 receptor activation in the management of postprandial hyperglycaemia in totally pancreatectomised individuals

Trial registration ClinicalTrials.gov NCT02640118.
Funding This study was funded by an unrestricted investigator-initiated study grant from Sanofi. Support was also received from from the Novo Nordisk Foundation Center for Basic Metabolic Research, the A.P. Møller Foundation for the Advancement of Medical Science and the Faculty of Health and Medical Sciences, University of Copenhagen.

Keywords Extrapancreatic glucagon · Gastric emptying · GLP-1 · GLP-1 receptor agonist · Glucagon · Postprandial glucose metabolism · Secondary diabetes · Total pancreatectomy

Abbreviations

bsAUC	Baseline-subtracted AUC
CCK	Cholecystokinin
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide 1
R_a	Rate of appearance
R_d	Rate of disappearance
REE	Resting energy expenditure
VAS	Visual analogue scales

Introduction

The gut hormone glucagon-like peptide 1 (GLP-1) plays an essential role in regulation of glucose homeostasis. In the pancreas, GLP-1 potentiates glucose-induced insulin secretion [1] and suppresses glucagon secretion [2], promoting glucose disposal and inhibiting hepatic glucose production [3].

Extrapancreatic effects of GLP-1 include decreased gastrointestinal motility [4], lowering of postprandial plasma triacylglycerol concentrations [5], promotion of satiety [6, 7, 8] and weight loss [8], lowering of systolic blood pressure and cardiovascular and kidney protective effects during chronic administration in patients with type 2 diabetes [9, 10]. Also, an association between high basal concentrations of GLP-1 and increased resting energy expenditure (REE) has been reported [11]. The complex and pleiotropic effects of GLP-1 combined with its potent effects on the pancreas make it difficult to disentangle pancreatic and extrapancreatic effects. For example, regarding gastric emptying, appetite, food intake and energy expenditure, it is difficult to discern whether the mechanisms involve pancreatic effects (on insulin and glucagon secretion) or exclusively rely on direct extrapancreatic effects.

The present randomised, placebo-controlled, double-blinded, crossover investigation of postprandial effects of the short-acting GLP-1 receptor agonist lixisenatide in totally pancreatectomised individuals and matched healthy control participants was undertaken with two overall objectives: (1) To disentangle extrapancreatic effects from pancreatic effects of exogenous short-acting GLP-1 receptor agonist administration during a meal test by comparing the two groups, and (2) to evaluate whether lixisenatide reduces postprandial glucose excursions in patients with diabetes secondary to total pancreatectomy [12]; their diabetes is often referred to as erratic, with frequent episodes of hypoglycaemia and hyperglycaemia, making it difficult to control, especially postprandially. To meet these objectives, we subjected totally pancreatectomised individuals and matched healthy control participants to standardised liquid mixed-meal tests following randomised, double-blinded, single-dose injections of lixisenatide or placebo.

Methods

Research design and methods The primary endpoint was the effect of the short-acting GLP-1 receptor agonist lixisenatide on postprandial plasma glucose excursions (as assessed by baseline-subtracted AUC [bsAUC] from time −30 to 180 min) during a standardised liquid mixed-meal test) compared with placebo in totally pancreatectomised patients. Pre-specified secondary endpoints were C-peptide, glucose and glycerol kinetics, gastric emptying assessed by paracetamol absorption, glucagon, cholecystokinin (CCK), gastrin, glucose-dependent insulinotropic polypeptide (GIP), GLP-1, blood pressure, appetite, food intake, diuresis and REE. The study was conducted at Gentofte Hospital, University of Copenhagen, Hellerup, Denmark, in accordance with the Helsinki Declaration (seventh revision, 2013), registered at ClinicalTrials.gov (identifier NCT02640118) and approved by the Ethics Committee of the Capital Region of Denmark (registration no. H-15004078).

Study participants Twelve totally pancreatectomised individuals (three women; age: [mean±SD] 65.0 ± 9.5 years; BMI: 22.9 ± 3.9 kg/m^2) and 12 matched healthy control participants (four women; age 66.1 ± 7.6 years; BMI: 24.0 ± 2.9 kg/m^2) with no family history of diabetes were included in this study between August 2015 and July 2016 (Tables 1 and 2). Matching criteria were age ± 20% and BMI ± 20%. All participants gave informed consent to participate. A study flow chart is presented in Electronic supplementary material (ESM) Fig. 1.

Experimental procedures After a screening visit, participants were examined on two study days within 2 months, separated by at least 72 h. The participants were instructed to refrain from strenuous physical exercise and any intake of alcohol for 2 days prior to the screening visit and experimental days. The pancreatectomised participants were instructed to take their normal daily insulin doses the day before but not to take any insulin in the morning of the experimental day. On both experimental days, the participants met in our clinical research facility after an overnight (10 h) fast and were placed in a recumbent position in a hospital bed. Cannulas were inserted in cubital veins, one for infusion of stable isotopes and one in a contralateral vein for collection of arterialised blood (hand and forearm wrapped in a heating pad [~50°C] throughout the experimental day). The two experimental days were performed in randomised order planned according to the website www.random.org. The experiments were double-blinded. An employee at the research unit, not involved in the project, was responsible for handing out the correct experimental medicine (lixisenatide pen or similar-looking placebo pen) to the physician in charge of the experimental days. After collection of basal blood samples, an infusion of stable isotope-labelled glucose ([6,6-$^2H_2$]glucose; priming dose of 17.6 μmol kg^{-1} × ϒ/5, where ϒ stands for fasting plasma glucose in mmol/l, and continuous infusion of 0.6 μmol kg^{-1} min^{-1}) and glycerol ([1,1,2,3,3-$^2H_5$]glycerol; priming dose of 2.0 μmol/kg and continuous infusion of 0.1 μmol kg^{-1} min^{-1}) (Cambridge Isotope Laboratories, Tewksbury, MA, USA) was initiated at time −120 min. Isotope enrichment at different time points was calculated by subtracting basal enrichment. The tracer/tracee ratio for glucose is depicted in ESM Fig. 2. At time −30 min, either 20 μg of lixisenatide or placebo was injected into the abdominal subcutis. The presence of lixisenatide in plasma was verified using an in-house RIA (data not shown). After 2 h of tracer infusions (to obtain tracer steady state), at time 0 min, the participants ingested a 200 ml liquid meal (containing 1650 kJ [394 kcal]; 50% from carbohydrate, 15% from protein and 35% from fat) consisting of glucose (47.2 g + 2.8 g [U-$^{13}C_6$]glucose), rapeseed

Table 1 Clinical characteristics of the totally pancreatectomised individuals

Patient no.	Sex (M/F)	Time since operation (years)	BMI (kg/m^2)	Insulin treatment (U)	Reason for surgery	Other treatment
1	M	6.0	29.4	Insulin detemir 24 + 14 Insulin aspart 7 + 7 + 7	Neuroendocrine tumour	Creon 25,000 × 5; plus Creon 10,000 to snacks between meals Paracetamol 665 mg × 6 Quinine 100 mg × 2 Lanzoprazole 30 mg × 1 Vitamin D 25 μg × 1 Glucosamine 400 mg × 3 Oxycodone hydrochloride 5 mg × 2
2	M	6.8	25.3	Insulin degludec 0 + 12 Insulin aspart 12 + 10 + 18	Adenocarcinoma	Creon 25,000 × 2; Creon 35,000 × 1; plus Creon 10,000 to snacks between meals Pantoprazole 40 mg × 1
3	F	6.4	21.3	Insulin detemir 12 + 28 Insulin aspart 10 + 10 + 10	Adenocarcinoma	Creon 25,000 × 3; plus Creon 10,000 to snacks between meals Pantoprazole 40 mg × 1 Amlodipine 10 mg × 2 Bendroflumethiazide (2.5 mg) + Potassium chloride (573 mg) × 2
4	M	6.3	25.7	Insulin glargine 8 + 12 Insulin aspart 10 + 0 + 10	Adenocarcinoma	Creon 40,000 × 3; plus Creon 10,000 to snacks between meals Pantoprazole 40 mg × 2
5	F	2.3	17.1	Insulin detemir 9 + 6 Insulin aspart 4 + 2 + 4	IPMN	Creon 25,000 × 3
6	M	2.3	20.9	Insulin detemir 12 + 0 Insulin aspart 5 + 5 + 5	Adenocarcinoma	Creon 80,000 × 3; plus Creon 25,000 × 2 to snacks between meals Pantoprazole 40 mg × 2 Mirtazapine 15 mg × 1 Citalopram 40 mg × 1 Magnesium 360 mg × 1 Loperamide 2 mg p.n.
7	M	0.3	22.0	Insulin detemir 14 + 6 Insulin aspart 2 + 2 + 2	Pancreatitis	Creon 75,000 × 3 Tramadol 50 mg × 2 Pantoprazole 40 mg × 2 Melatonin 3 mg × 1
8	M	1.2	24.0	Insulin detemir 20 + 9 Insulin aspart 6 + 6 + 6	Adenocarcinoma	Creon 50,000 × 3; plus Creon 50,000 to snacks between meals Promethazine 25 mg × 1 Pantoprazole 40 mg × 2 Losartan potassium 100 mg × 1 Bendroflumethiazide 2.5 mg × 2 Amlodipine 5 mg × 1
9	M	4.0	19.5	Insulin detemir 17 + 7 Insulin aspart 7 + 4 + 4	Pancreatitis	Creon 25,000 × 3 Pantoprazole 40 mg × 2 Buprenorphine 0.2 mg × 3
10	F	1.3	17.8	Insulin detemir 8 + 5 Insulin aspart 4 + 6 + 8	Adenocarcinoma	Creon 25,000 × 4; plus Creon 25,000 to snacks between meals Pantoprazole 20 mg × 1 Loperamide 4 mg × 1
11	M	1.0	23.2	Insulin detemir 16 + 12 Insulin aspart 8 + 8 + 8	Adenocarcinoma	Creon 25,000 × 3; plus Creon 25,000 to snacks between meals Centyl 2.5 mg × 1 Pantoprazole 40 mg × 2
12	M	0.4	28.5	Insulin detemir 15 + 15 Insulin aspart 6 + 6 + 6	IPMN	Creon 25,000 × 3; plus Creon 25,000 to snacks between meals Bendroflumethiazide 2.5 mg × 1 Amlodipine 10 mg × 1 Ramipril 10 mg × 1 Spironolactone 25 mg × 1

Creon 10,000: 8000 amylase units, 10,000 lipase units and 600 protease units; Creon 25,000: 18,000 amylase units, 25,000 lipase units and 1000 protease units; Creon 40,000: 25,000 amylase units, 40,000 lipase units and 1600 protease units

IPMN, intraductal papillary mucinous neoplasm

Table 2 Clinical characteristics of the healthy control participants

Control participant no.	Sex (M/F)	BMI (kg/m^2)	Treatment
1	M	29.4	Fluticasone 50 μg + salmeterol 500 μg × 1 Tiotropium bromide 18 mg × 1
2	M	18.3	–
3	F	20.7	Budesonide 160 μg + formoterol fumarate dihydrate 4.5 μg × 1 Losartan potassium 50 mg × 1
4	F	22.5	Zoledronate inj. 5 mg × 1 year
5	M	24.5	–
6	M	22.9	–
7	F	23.0	–
8	M	24.5	–
9	M	26.6	–
10	M	24.8	–
11	M	24.6	Simvastatin 40 mg × 1 Enalapril 20 mg × 1
12	F	26.8	–

F, female; M, male

oil [14.1 g], whey protein [15.2 g] and 1.5 g paracetamol) over 10 min. Pancreatectomised patients were given a standard pancreatic enzyme supplement (Creon 25,000 lipase units, Abbott, Orifarm, Odense, Denmark) with the meal. Pulse rate and blood pressure were measured at time −120 min and every 30 min thereafter. Indirect calorimetry was performed over 15 min at time −90, 30 and 150 min for the measurement of REE. At time 0, 30, 60, 90, 120, 150 and 180 min, hunger, satiety, fullness and prospective food consumption were assessed by visual analogue scales (VAS). At time 180 min, the participants were offered a standardised ad libitum meal consisting of minced meat, pasta, corn, carrots, peppers, cream and salt and pepper (50 energy [E]% carbohydrate, 37 E% fat, 13 E% protein). Participants were instructed to eat until they felt comfortably satisfied. The meal was consumed within a maximum of 30 min. Pancreatectomised patients were instructed to take their regular pancreatic enzyme supplementation, half their normal basal insulin dose and prandial insulin (according to plasma glucose levels) together with the ad libitum meal. At the end of the ad libitum meal, time spent eating, weight and total amount of energy consumed were noted. The participants also evaluated the taste, smell, visual appeal, aftertaste and overall palatability of the meal using standardised VAS. Blood samples were drawn 120, 45, 30 and 15 min before and 10, 20, 30, 50, 70, 90, 120, 150, 180 min after ingestion of the liquid meal. The cannula was flushed with 1 ml of saline (54 mmol/l NaCl) after every sample was drawn. For bedside measurement of plasma glucose, blood was collected in sodium fluoride tubes and centrifuged immediately at 7400 *g* for 2 min at room temperature. For the plasma analysis of tracers, lixisenatide, GIP, GLP-1, glucagon, gastrin and CCK, blood was collected in chilled tubes (on ice) containing EDTA and a specific dipeptidyl peptidase 4 inhibitor (valine pyrrolidide, 0.01 mmol/l final concentration; a gift from Novo Nordisk, Måløv, Denmark). For analysis of insulin and C-peptide in serum, blood was sampled in plain tubes for coagulation (20 min at room temperature). For plasma analysis of paracetamol, blood was collected in chilled tubes (on ice) containing lithium–heparin. All tubes were centrifuged for 20 min at 1200 *g* and 4°C. Plasma samples for GIP, GLP-1, glucagon, CCK, gastrin and tracer analyses were stored at −20°C. Serum samples for insulin and C-peptide analyses, plasma samples for paracetamol and buffy coat from the first three EDTA tubes were stored at −80°C until analysis.

Analyses Plasma glucose concentrations were measured at the bedside using the glucose oxidase method (Yellow Springs Instrument Model 2300 STAT Plus Analyzer, Yellow Springs, OH, USA). Plasma glucagon concentrations were assayed with a sandwich ELISA using N- and C-terminal wrapping monoclonal antibodies (intra-assay CV 5.0%) (Mercodia, Uppsala, Sweden) as previously described [13, 14]. Serum C-peptide concentrations were measured with a two-site sandwich immunoassay using direct chemiluminescent technology (intra-assay CV 19%) (Siemens Healthcare, Ballerup, Denmark) for the ADVIA Centaur XP. Plasma enrichment of [6,6-$^2H_2$]glucose (intra-assay CV 3.2%), [U-$^{13}C_6$]glucose (intra-assay CV 3.5%) and [1,1,2,3,3-$^2H_5$]glycerol (intra-assay CV 3.1%) was determined using LC-MS/MS as previously described [15]. Amidated gastrin (intra-assay CV 5.0–11.4%), CCK, total

GIP, total GLP-1 and lixisenatide were measured with RIAs (intra-assay CV 5.0%) as previously described [16–18]. REE was measured by indirect calorimetry using a tight facemask connected to the calorimeter, which measures the gas exchange breath by breath via an O_2 alkali cell and an infrared CO_2 sensor (CCM Express, Medical Graphics Corporation, St Paul, MN, USA). The calorimeter was calibrated immediately before every measurement session. Metabolic rates are presented as averages of measures carried out every 10th second within a 12 min period. Gastric emptying was measured according to the paracetamol absorption test [19, 20], appetite, hunger and satiety was evaluated using VAS [21], and food intake was measured during the ad libitum meal test as previously described [22].

Calculations and statistical analysis Our sample size calculations were based on a previous study looking at differences in peak postprandial glucose values in patients with type 1 diabetes and no residual beta cell function and infusions of either saline or GLP-1 [18]. According to our calculations, eight participants were needed to detect a difference in postprandial glucose values of at least 6.0 mmol/l with a two-sided 5% significance level and a power of 80%. As our experimental protocol differs from the above, to avoid type 2 errors and to ensure a power of >80%, we enrolled 12 pancreatectomised

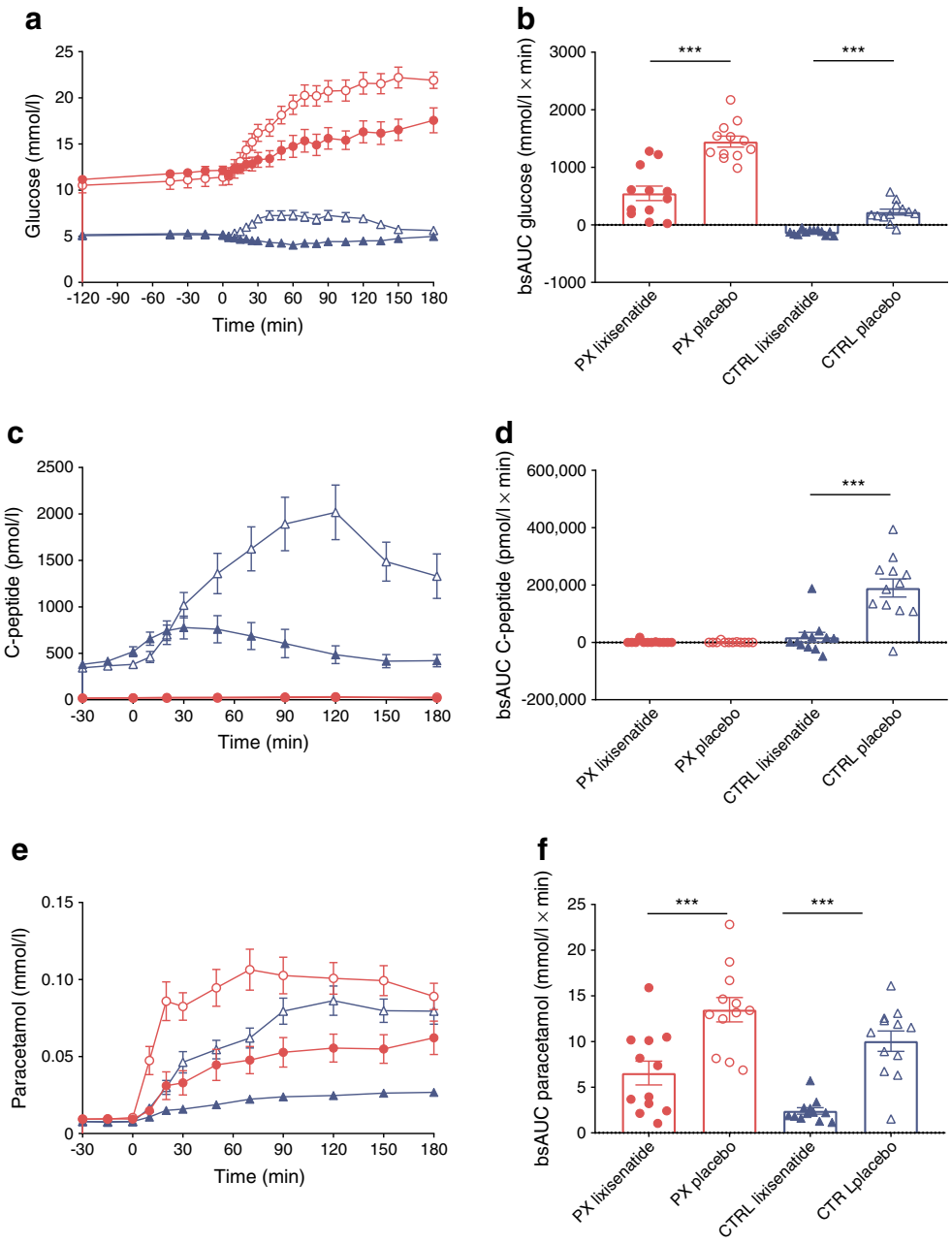


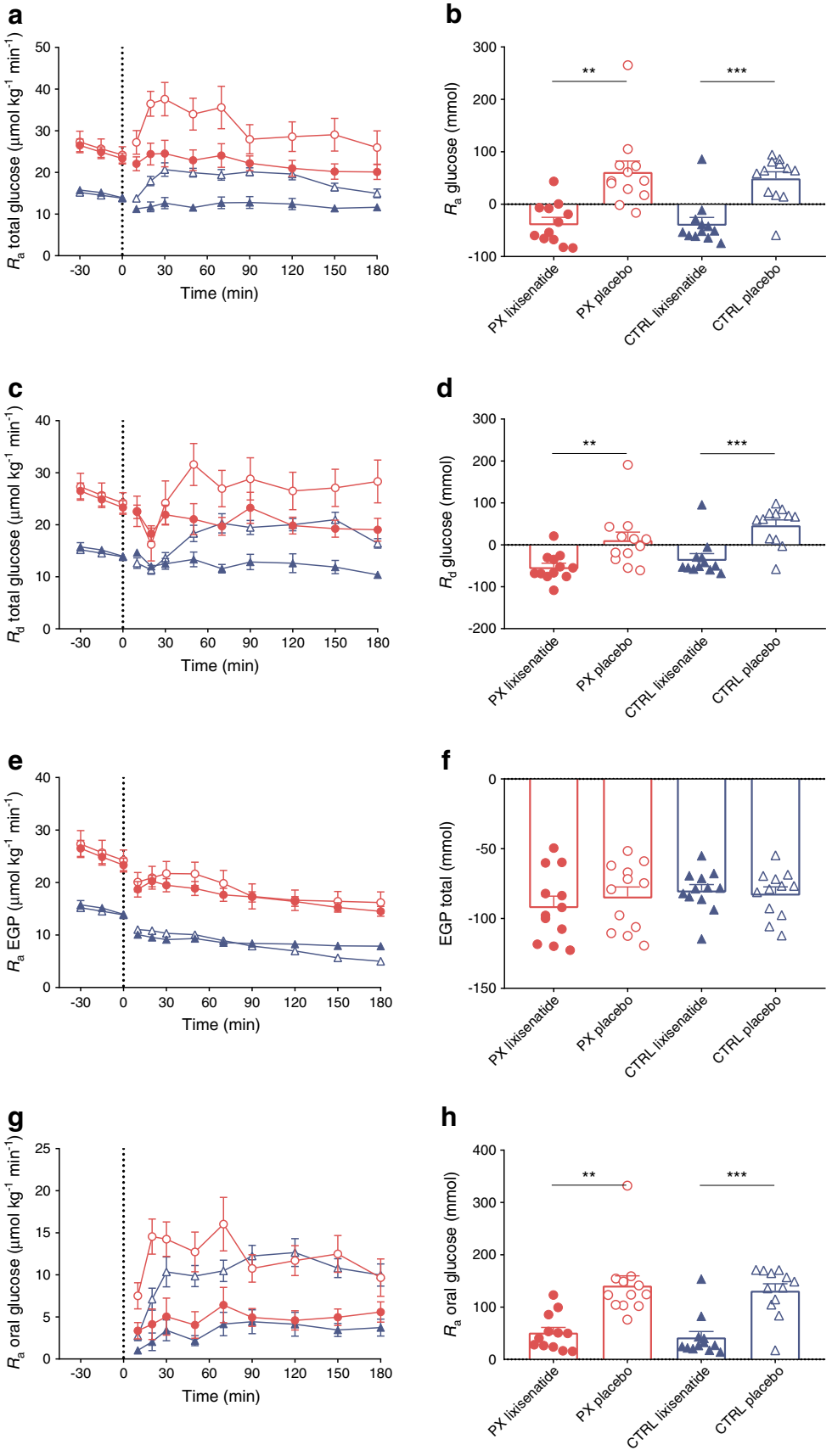
Fig. 1 Plasma glucose (**a**), C-peptide (**c**), and paracetamol (**e**) concentrations and corresponding bsAUC results (**b**, **d**, **f**, respectively) during a liquid mixed-meal test (initiated at time 0 min) in 12 totally pancreatectomised individuals (PX) (red curves/circles/bar outlines) and 12 healthy control participants (CTRL) (blue curves/triangles/bar outlines) with preceding (−30 min) single-dose administration of 20 μg lixisenatide (filled symbols) or placebo (open symbols). ***$p < 0.001$

Table 3 Glucose, hormones and paracetamol

Variable	Totally pancreatectomised patients			Healthy control participants		
	Lixisenatide	Placebo	*p* value	Lixisenatide	Placebo	*p* value
Glucose						
Mean baseline (mmol/l)	12.0 ± 0.5	11.3 ± 0.9	0.372	5.2 ± 0.1	5.1 ± 0.1	0.321
C_{max} (mmol/l)	18 ± 1.4	23.3 ± 1	0.008	5.5 ± 0.1	8.2 ± 0.4	<0.001
T_{max} (min)	148 ± 13	134 ± 11.1	0.375	8 ± 5.2	70 ± 13	0.054
AUC (mmol/l × min)	2715 ± 179	3475 ± 177	0.006	813 ± 13	1153 ± 57	<0.001
bsAUC (mmol/l × min)	547 ± 125	1446 ± 95	<0.001	−125 ± 12	222 ± 51	<0.001
C-peptide						
Mean baseline (nmol/l)	20.3 ± 3.9	17.8 ± 2.9	0.527	438 ± 38	401 ± 27.2	0.039
C_{max} (nmol/l)	32.8 ± 15.2	29.5 ± 10.2	0.581	873 ± 134	2240 ± 282	<0.001
T_{max} (min)	−7.5 ± 15.3	0 ± 20.6	0.191	30 ± 8	99.2 ± 12.7	<0.001
AUC (nmol/l × min)	5.3 ± 2.2	4.2 ± 0.9	0.42	105 ± 19	261 ± 34	<0.001
bsAUC (nmol/l × min)	1.6 ± 1.4	0.9 ± 0.8	0.34	17.8 ± 16.9	189 ± 31	<0.001
Paracetamol						
C_{max} (mmol/l)	0.1 ± 0.01	0.1 ± 0.01	<0.001	0.0 ± 0	0.1 ± 0	0.230
T_{max} (min)	124 ± 14.6	95.8 ± 15.4	0.126	148 ± 14.6	135 ± 10.1	<0.001
AUC (mmol/l × min)	8.4 ± 1.3	16.5 ± 1.6	<0.001	3.9 ± 0.3	11.7 ± 1.2	<0.001
bsAUC (mmol/l × min)	6.5 ± 1.3	13.5 ± 1.3	<0.001	2.4 ± 0.4	10 ± 1.1	<0.001
Glucagon						
Mean baseline (pmol/l)	3.3 ± 0.8	2.8 ± 0.7	0.133	7.6 ± 1.2	6.5 ± 1.1	0.064
C_{max} (pmol/l)	6.3 ± 1.4	14.1 ± 2.7	0.011	14.5 ± 3.34	10.7 ± 1.7	0.755
T_{max} (min)	51.3 ± 21.9	73.3 ± 15.2	0.497	53.3 ± 21.2	25 ± 7.4	0.116
AUC (pmol/l × min)	620 ± 161	1479 ± 270	0.003	1283 ± 159	1253 ± 225	0.815
bsAUC (pmol/l × min)	66.3 ± 84.2	1190 ± 311	0.008	141 ± 100	190 ± 99.5	0.034
CCK						
Mean baseline (pmol/l)	1.0 ± 0.2	0.8 ± 0.1	0.194	0.9 ± 0.1	1.0 ± 0.1	0.718
C_{max} (pmol/l)	3.9 ± 1.1	3.5 ± 0.7	0.599	2.7 ± 0.5	3.9 ± 0.6	0.071
T_{max} (min)	45 ± 11.1	35.8 ± 6.1	0.366	75.8 ± 22.7	68 ± 16.2	0.776
AUC (pmol/l × min)	297 ± 83.4	297 ± 44.9	0.993	267 ± 53.5	362 ± 43.9	0.094
bsAUC (pmol/l × min)	123 ± 75.8	150 ± 35.5	0.698	105 ± 46.7	140 ± 31.8	0.127
Gastrin						
Mean baseline (pmol/l)	18.6 ± 5.6	19.2 ± 5.9	0.199	7.5 ± 0.4	78 ± 0.5	0.210
C_{max} (pmol/l)	20.8 ± 6.3	25 ± 7.6	0.028	10 ± 0.6	12 ± 0.9	0.046
T_{max} (min)	−12.9 ± 5.6	44.6 ± 20.5	0.024	57.9 ± 18.9	77.1 ± 21.8	0.497
AUC (pmol/l × min)	2939 ± 882	3487 ± 1059	0.032	1522 ± 82	1788 ± 116	0.055
bsAUC (pmol/l × min)	−401 ± 162	26.5 ± 129	0.027	176 ± 73.3	393 ± 102	0.089
GLP-1						
Mean baseline (pmol/l)	17.9 ± 2.0	18.1 ± 1.7	0.914	11.6 ± 1.2	11.2 ± 1.3	0.821
C_{max} (pmol/l)	50.4 ± 10.2	143.4 ± 41.2	0.038	18.8 ± 3.1	27.8 ± 3.9	0.046
T_{max} (min)	66.7 ± 21.4	42.5 ± 12.3	0.343	15.8 ± 8.9	53.3 ± 13.8	0.045
AUC (pmol/l × min)	4240 ± 582	8865 ± 1599	0.011	1609 ± 168	3020 ± 283	<0.001
bsAUC (pmol/l × min)	1025 ± 592	5615 ± 1412	0.016	−481 ± 186	1005 ± 297	0.006
GIP						
Mean baseline (pmol/l)	15.9 ± 3.1	16.6 ± 2.8	0.553	10.2 ± 1.0	9.0 ± 1.8	0.609
C_{max} (pmol/l)	67.9 ± 13.7	112.2 ± 19.9	0.430	18.9 ± 2.5	77.1 ± 9.4	<0.001
T_{max} (min)	105 ± 17.6	71.7 ± 15	0.248	13.3 ± 13.9	93.3 ± 16.4	0.008
AUC (pmol/l × min)	6143 ± 1319	12,714 ± 2243	0.003	1508 ± 410	9735 ± 1392	<0.001
bsAUC (pmol/l × min)	3278 ± 941	9729 ± 1828	0.003	−322 ± 445	8115 ± 1357	<0.001

Plasma/serum baseline concentration, maximal concentration (C_{max}) and time to peak concentration (T_{max}) of glucose, C-peptide, glucagon, GLP-1, GIP, CCK, gastrin and paracetamol during a liquid mixed-meal test with 1.5 g paracetamol after single-dose injection of 20 μg of lixisenatide or placebo in totally pancreatectomised individuals ($n = 12$) and healthy control participants ($n = 12$). Data are mean ± SEM. Statistical analysis was performed by paired two-sample Student's *t* test (two-tailed) within groups

individuals and 12 matched healthy individuals. Data are presented as mean±SEM unless otherwise stated. AUC values were calculated using the trapezoid rule and are presented as bsAUC unless otherwise stated. Glucose rate of appearance (R_a) and glucose rate of disappearance (R_d) were calculated from changes in glucose enrichment using the one-compartment, fixed-volume, non-steady-state model of Steele [23] and modified for use with stable isotopes and a pool fraction of 70 ml/kg. Group differences in baseline characteristics and AUCs were evaluated using two-sample Student's *t* test, paired tests within groups and unpaired tests between groups. A *p* value of ≤0.05 was accepted as


a
R_a total glucose (μmol kg^{-1} min^{-1})
Time (min)
b
R_a glucose (mmol)
**
***
PX lixisenatide
PX placebo
CTRL lixisenatide
CTRL placebo
c
R_d total glucose (μmol kg^{-1} min^{-1})
Time (min)
d
R_d glucose (mmol)
**
***
PX lixisenatide
PX placebo
CTRL lixisenatide
CTRL placebo
e
R_a EGP (μmol kg^{-1} min^{-1})
Time (min)
f
EGP total (mmol)
PX lixisenatide
PX placebo
CTRL lixisenatide
CTRL placebo
g
R_a oral glucose (μmol kg^{-1} min^{-1})
Time (min)
h
R_a oral glucose (mmol)
**
***
PX lixisenatide
PX placebo
CTRL lixisenatide
CTRL placebo

◀ **Fig. 2** Glucose kinetics. Total glucose rate of appearance (*Ra*) (**a**), total glucose rate of disappearance (*Rd*) (**c**), endogenous glucose production (EGP) *Ra* (**e**) and oral glucose *Ra* (**g**) during the liquid mixed-meal test (initiated at time 0 min) in 12 totally pancreatectomised individuals (PX) (red curves/circles/bar outlines) and 12 healthy control participants (CTRL) (blue curves/triangles/bar outlines) with preceding (−30 min) single-dose administration of 20 μg lixisenatide (closed symbols) or placebo (open symbols). The corresponding bsAUC results (**b**, **d**, **f**, **h**) are also shown. $^{**}p < 0.01$, $^{***}p < 0.001$

statistically significant. GraphPad Prism, version 7 (La Jolla, CA, USA) was used for statistical evaluation and generating graphs of results.

Results

Plasma glucose Basal concentrations of glucose were higher in the pancreatectomy group compared with the control group (Fig. 1a). During the OGTT, a peak mean concentration of 23.3 ± 1.0 mmol/l was reached at time point 134 ± 11 min in the pancreatectomy group with placebo compared with a peak mean glucose concentration of 18 ± 1.4 mmol/l ($p = 0.008$) at time point 148 ± 13 min ($p = 0.375$) with lixisenatide. In the control group, a peak mean concentration of 8.2 ± 0.4 mmol/l was reached at time point 70 ± 13 min on the placebo day compared with a peak mean concentration of 5.5 ± 0.1 mmol/l ($p < 0.001$) at time point 8 ± 25 min ($p = 0.054$) with lixisenatide. Postprandial glucose excursions (bsAUCs) were reduced with lixisenatide compared with placebo in both the totally pancreatectomised participants (548 ± 125 vs 1447 ± 95 mmol/l × min, $p < 0.001$) and in the healthy control participants (−126 ± 12 vs 222 ± 51 mmol/l × min, $p < 0.001$) (Fig. 1b and Table 3).

C-peptide Serum C-peptide concentrations in the totally pancreatectomised participants were below the detection limit of the assay (<16 pmol/l) on both experimental days at all time points. However, two patients (one who was operated because of a neuroendocrine tumour [patient 1] and another because of an adenocarcinoma [patient 8]) showed very low levels of C-peptide (lower than what is needed to define C-peptide negativity [24]) with peak values of 35 pmol/l at time 90 min (patient 1) and 199 pmol/l at time 120 min (patient 8) after lixisenatide administration. Other endpoints, including glucagon, were not different in these patients compared with the mean group values, and thus data from these patients were kept in the dataset for the following analyses. In the healthy control participants, postprandial C-peptide responses were reduced with lixisenatide compared with placebo (17.8 ± 16.9 vs 189 ± 31 nmol/l × min, $p < 0.001$) (Fig. 1c,d and Table 3).

Glucose and glycerol kinetics Baseline levels of total glucose R_a and total glucose R_d were higher in the pancreatectomy group compared with the control group ($p < 0.001$ for both measures) (Fig. 2a,c). In both groups, total glucose R_a was

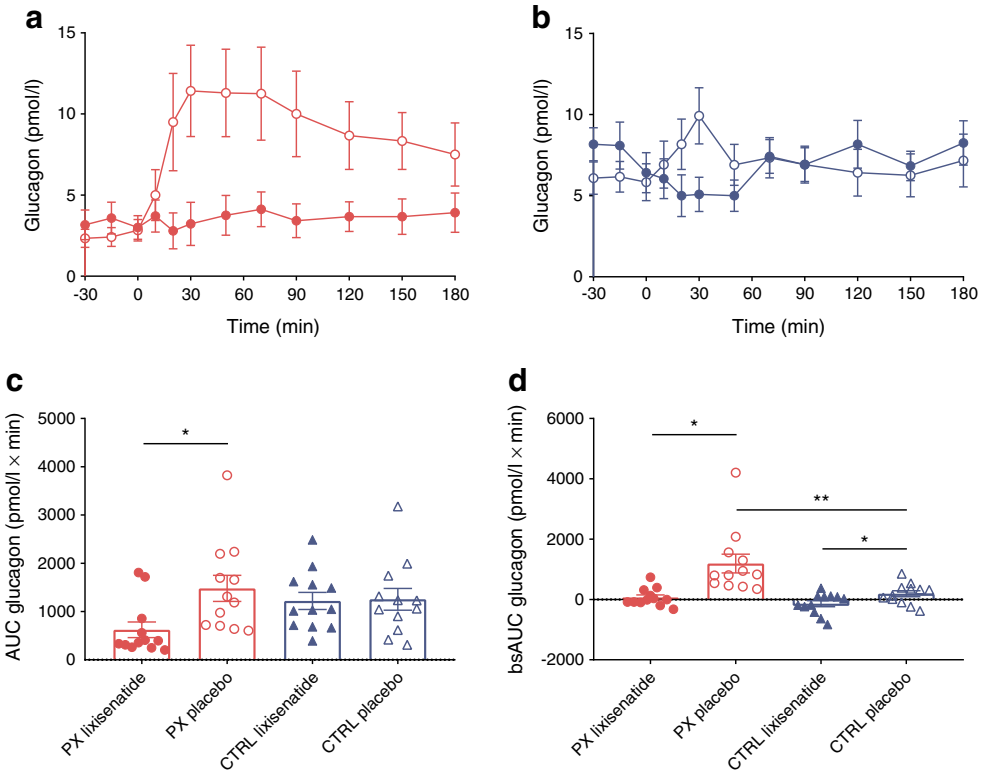


Fig. 3 Plasma glucagon concentrations (**a**, **b**) and plasma glucagon responses (**c**, **d**) during liquid mixed−meal test (initiated at time 0 min) in 12 totally pancreatectomised participants (PX) (red curves/circles/bar outlines) and 12 healthy control participants (CTRL) (blue curves/triangles/bar outlines) with preceding (−30 min) single-dose administration of 20 μg lixisenatide (filled symbols) or placebo (open symbols). AUCs (**c**) and baseline-subtracted AUCs (bsAUC) (**d**) are also shown. $^{*}p < 0.05$, $^{**}p < 0.01$

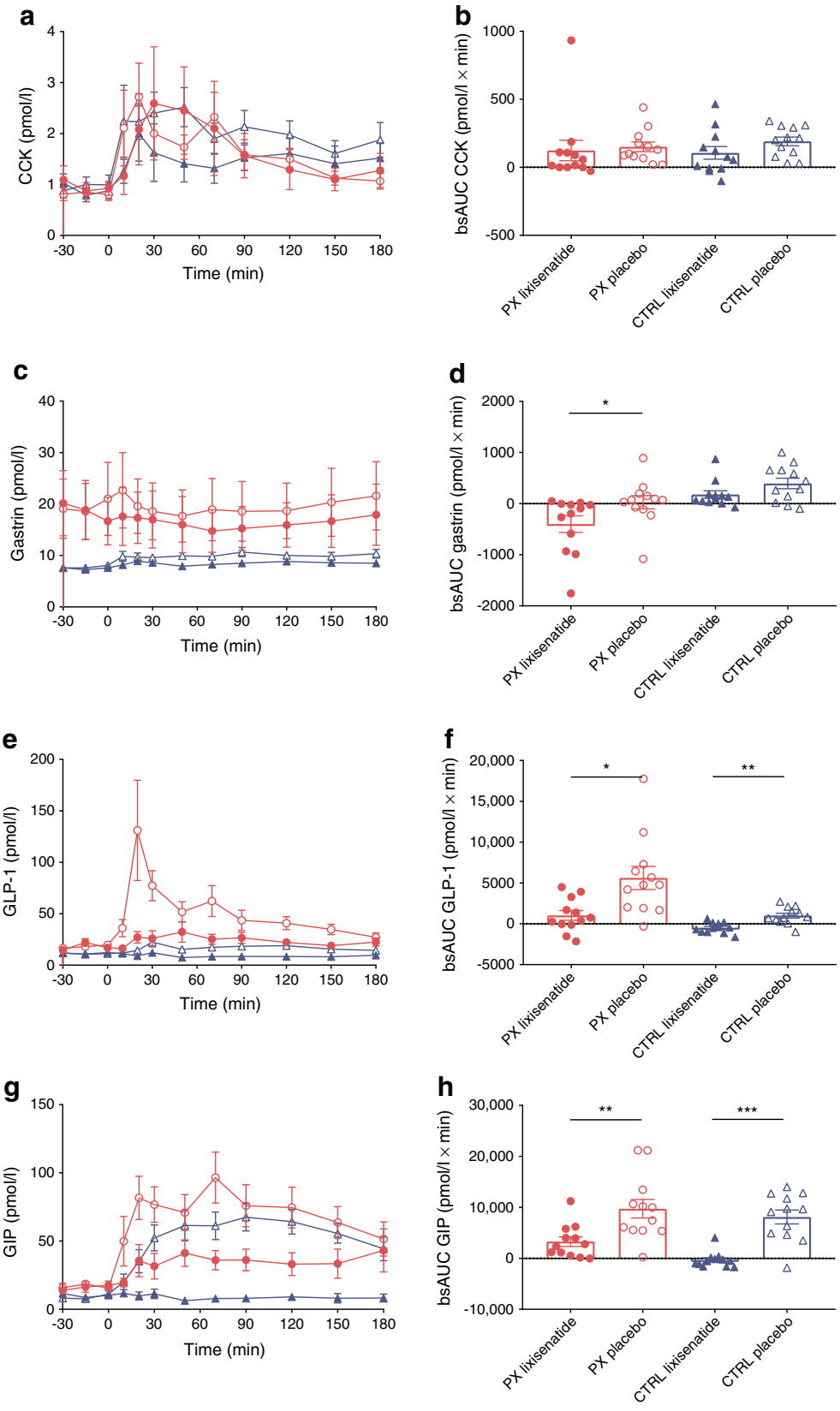


Fig. 4 Plasma CCK (**a**), gastrin (**c**), GLP-1 (**e**) and GIP (**g**) concentrations and corresponding baseline-subtracted AUC (bsAUC) results (**b**, **d**, **f**, **h**, respectively) during a liquid mixed-meal test (initiated at time 0 min) in 12 totally pancreatectomised individuals (PX) (red curves/circles/bar outlines) and 12 healthy control participants (CTRL) (blue curves/triangles/bar outlines) with preceding (−30 min) single-dose administration of 20 μg lixisenatide (filled symbols) or placebo (open symbols). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

higher with placebo compared with lixisenatide (pancreatectomy group, bsAUC 61.3 ± 20 vs −36.5 ± 11 mmol, $p = 0.003$; control group, bsAUC 49.2 ± 12 vs −37.6 ± 12 mmol, $p < 0.001$) (Fig. 2b). Total glucose R_d was also higher with

placebo in both groups (pancreatectomy group, bsAUC 11.1 ± 19 vs −53.4 ± 9.4 mmol, $p = 0.008$; control group, bsAUC 46.5 ± 13 vs −33.8 ± 12 mmol, $p < 0.001$) (Fig. 2d). There was no significant difference in postprandial endogenous glucose production between lixisenatide and placebo (pancreatectomy group, bsAUC −91.1 ± 7.2 vs −84.3 ± 6.8 mmol, $p = 0.371$; healthy control group, bsAUC −79.9 ± 4.3 vs −82.2 ± 4.8 mmol, $p = 0.659$) (Fig. 2f). R_a of oral glucose was lower with lixisenatide in both groups (pancreatectomy group, bsAUC 51.0 ± 10 vs 141 ± 18 mmol, $p = 0.003$; healthy control group, bsAUC 42.0 ± 11 vs 131 ± 13 mmol, $p < 0.001$) (Fig. 2h).

Baseline concentrations of plasma glycerol were higher in the totally pancreatectomised participants than in the healthy control participants ($p < 0.001$). In the pancreatectomy group there was no significant difference in plasma glycerol levels between the two study days (bsAUC, $p = 0.284$). In the control group, plasma concentrations of glycerol were lower with placebo compared with lixisenatide (bsAUC −7284 ± 943 vs −3858 ± 1746 μmol/l × min, $p = 0.05$). There was no significant difference in levels of glycerol R_a (bsAUC, $p = 0.677$) or R_d (bsAUC, $p = 0.652$) between the two study days in the pancreatectomy group. Both glycerol R_a (bsAUC −21.7 ± 2.3 vs −5.4 ± 3.1 mmol, $p < 0.001$) and R_d (bsAUC −21.5 ± 2.3 vs −5.3 ± 2.9 mmol, $p < 0.001$) were lower with placebo compared with lixisenatide in the healthy control group (ESM Fig. 3).

Paracetamol Postprandial concentrations of serum paracetamol were reduced with lixisenatide compared with placebo in both the totally pancreatectomised participants (6.5 ± 1.3 vs 13.5 ± 1.3 mmol/l × min, $p < 0.001$) and the control group (2.4 ± 0.4 vs 10 ± 1.1 mmol/l × min, $p < 0.001$), illustrating a reduced rate of gastric emptying (nutrient entry into the small intestine) in both groups on the day with lixisenatide administration (Fig. 1e,f and Table 3).

Glucagon Basal plasma concentrations of glucagon in the pancreatectomy group were above the detection limit (1 pmol/l) of the applied ELISA but were lower than those in the control group (2.9 ± 0.5 vs 6.79 ± 0.8 pmol/l, $p = 0.005$) (Fig. 3). With placebo, the totally pancreatectomised patients exhibited larger glucagon responses (1190 ± 311 vs 190 ± 99.5 pmol/l × min, $p = 0.006$) and numerically higher peak concentrations (14.1 ± 2.7 vs 10.7 ± 1.7 pmol/l, $p = 0.298$) compared with the healthy control participants. Lixisenatide reduced postprandial glucagon responses (bsAUC) compared with placebo in the pancreatectomy group (66.3 ± 84.2 vs 1190 ± 311 pmol/l × min, $p = 0.008$) and the control group (141 ± 100 vs 190 ± 99.5 pmol/l × min, $p = 0.034$) (Fig. 3 and Table 3).

CCK and gastrin Baseline concentrations of CCK were similar between the pancreatectomy group and the control group (0.89 ± 0.1 vs 0.93 ± 0.1 pmol/l, $p = 0.84$) (Fig. 4a,b and Table 3). In both groups, a rise in postprandial CCK concentrations was observed, but there was no significant difference between study days in either of the two groups. Baseline concentrations of gastrin were higher in the pancreatectomy group (18.9 ± 3.9 vs 7.6 ± 0.3 pmol/l × min, $p = 0.063$) (Fig. 4c,d and Table 3). Lixisenatide lowered postprandial gastrin concentrations compared with placebo in both the pancreatectomy group (−401 ± 162 vs 26.5 ± 129 pmol/l × min, $p = 0.027$) and the control group (176 ± 75.3 vs 393 ± 102 pmol/l × min, $p = 0.089$), yet only significantly in the pancreatectomy group.

Lixisenatide, GLP-1 and GIP Injection of 20 μg of lixisenatide resulted in a plasma concentration of approximately 70 pmol/l measured 2 h after injection. Baseline concentrations of GLP-1 were higher in the pancreatectomy group compared with the control group (17.9 ± 1.3 vs 11.4 ± 0.8 pmol/l, $p = 0.002$). With placebo, the pancreatectomy group showed an eightfold higher peak value in GLP-1 concentration at the 20 min time point compared with the control group (122 ± 45 vs 14 ± 2.2 pmol/l, $p = 0.028$). In both the pancreatectomy group (1025 ± 592 vs 5615 ± 1412 pmol/l × min, $p = 0.016$) and the control group (−481 ± 186 vs 1005 ± 297 pmol/l × min, $p = 0.006$), postprandial GLP-1 responses (bsAUC) were reduced by lixisenatide compared with placebo (Fig. 4e,f and Table 3). Baseline concentrations of GIP were higher in the pancreatectomy group (16.3 ± 2.0 vs 9.6 ± 1.0 pmol/l, $p = 0.038$). Lixisenatide reduced postprandial GIP responses (bsAUC) compared with placebo in both the pancreatectomy group (3278 ± 941 vs 9729 ± 1828 pmol/l × min, $p = 0.003$) and the control group (−322 ± 445 vs 8115 ± 1357 pmol/l × min, $p < 0.001$) (Fig. 4g,h and Table 3).

Data on appetite scores, food intake (ESM Table 1, ESM Fig. 4), blood pressure, pulse (ESM Fig. 5) and REE are presented in the ESM Results.

Discussion

We assessed postprandial effects of single-dose treatment with the GLP-1 receptor agonist lixisenatide (vs placebo) in totally pancreatectomised patients and show (1) a clear reduction in postprandial plasma glucose excursions, (2) attenuation of robust postprandial plasma glucagon responses and (3) a deceleration of gastric emptying.

Total pancreatectomy entails dramatic anatomical changes, including resection of the distal 2–3 cm of the antrum and removal of the pyloric sphincter and the duodenum [12]. Using a mass spectrometry-validated [14] and highly specific glucagon sandwich ELISA targeting the N- and C-terminal

regions of glucagon simultaneously, we observed significantly lower basal plasma concentrations of glucagon in totally pancreatectomised participants, but—as we have previously described with an OGTT [25]—we saw robust postprandial plasma glucagon responses in these individuals. Interestingly, the rise in postprandial plasma glucagon concentrations in the totally pancreatectomised patients was eliminated by administration of a single dose of the GLP-1 receptor agonist lixisenatide. What is the explanation for postprandial glucagon secretion in totally pancreatectomised patients and how does lixisenatide eliminate this increase? Our previous findings showing grossly elevated glucagon responses to oral glucose and suppression of plasma glucagon concentrations after intravenous glucose in totally pancreatectomised patients support a gut-derived origin of glucagon in these individuals [25]. We believe that proglucagon-producing enteroendocrine cells in the gut (so-called L cells) may constitute a potential origin of glucagon secretion. L cells are found throughout the intestinal tract and are highly abundant in the jejunum [26]. Because of the altered gastrointestinal anatomy following Whipple's procedure with total pancreatectomy (not pylorus-preserving), nutrients are delivered directly from the stomach to the jejunum after ingestion. This unretarded and direct delivery of nutrients to L cell-rich segments of the small intestine causes massive stimulation of L cells, reflected by the eightfold greater postprandial peak plasma GLP-1 concentrations in our totally pancreatectomised patients. Also, other proglucagon-derived L cell products, such as oxyntomodulin, have been shown to circulate at much higher concentrations in these individuals [25]. It is generally believed that prohormone convertase 1/3 processes proglucagon to GLP-1, glucagon-like peptide 2 and glicentin, which is further processed to oxyntomodulin, in enteroendocrine L cells, and that prohormone convertase 2, processing proglucagon to glucagon, is restricted to the pancreatic alpha cells [27]. Currently, the mechanisms underlying gut-derived glucagon secretion remain obscure, but they may involve the presence of prohormone convertase 2 in proglucagon-producing enteroendocrine cells [25, 27] and/or unspecific processing by prohormone convertase 1/3. In totally pancreatectomised individuals, the latter may feasibly result in detectable concentrations of prohormone convertase 1/3 products owing to the massive postprandial stimulation of L cell-rich areas of the small intestines. The elimination of the postprandial glucagon response in our totally pancreatectomised participants by administration of lixisenatide may be driven by the deceleration of gastric emptying, reducing the flow of nutrients to the intestinal L cells. On that note, the lixisenatide-induced deceleration of gastric emptying (as previously observed in individuals with intact pancreas) in our totally pancreatectomised participants clearly must be categorised as an extrapancreatic effect of lixisenatide. Nevertheless, we cannot exclude a direct suppressive effect of lixisenatide on extrapancreatic glucagon secretion in our totally pancreatectomised volunteers.

When investigating glucose kinetics, we found that R_a of oral glucose and total glucose R_a and R_d were higher with placebo in both study groups. These findings underline the slowing effect lixisenatide has on gastric emptying and nutrient delivery to the gut. The higher baseline R_a and R_d of glycerol observed in the totally pancreatectomised participants indicate a higher rate of lipolysis in this group. These glycerol findings are in line with those of Lund et al [25] and are most likely explained by the insulin-deficient state of the pancreatectomy group since insulin inhibits lipolysis.

We show significantly higher baseline concentrations of gastrin in the totally pancreatectomised group compared with the control group, mainly driven by higher concentrations in two of the totally pancreatectomised patients. Lund et al found lower concentrations of gastrin in totally pancreatectomised individuals compared with healthy control individuals [25] and, similarly, Rehfeld et al found gastrin concentrations to be low or below the detection limit in patients who had undergone Whipple's surgery [28]. Notably, however, pancreatectomy using Whipple's operation at the time of the experiments by Rehfeld et al. would have involved total or almost total antrectomy [28]. Moreover, totally pancreatectomised patients today are usually treated with proton pump inhibitors, which stimulates antral G cell secretion of gastrin.

Diabetes secondary to total pancreatectomy is classified as a disease of the exocrine pancreas by the American Diabetes Association [29] and World Health Organization [30] (previously it has been classified as type 3c diabetes). This form of diabetes is often characterised by erratic fluctuations in plasma glucose concentrations and frequent episodes of hypoglycaemia and hyperglycaemia, with the latter constituting a profound problem postprandially for these patients. No specific treatment guidelines exist, but the primary target is to maintain $HbA_{1c} < 53$ mmol/l (<7%) to minimise the risk of microvascular complications [31, 32]. Currently, there are no randomised controlled trial data available to help develop guidelines on glucose-lowering treatments specific to this type of diabetes, and the standard insulin therapy used in these patients is based on empirical knowledge. Insulin replacement therapy in totally pancreatectomised individuals is a necessity, but it is associated with hypoglycaemia, limiting compliance and treatment success. In recent years, GLP-1 receptor agonists have been implemented in the treatment of patients with type 2 diabetes, but a demarcation of the pancreatic and extrapancreatic effects of these agents has been difficult to establish. The present study is, to our knowledge, the first to provide controlled evidence of an incretin-based—in fact, any—glucose-lowering drug for the treatment of hyperglycaemia in diabetes secondary to total pancreatectomy. Lixisenatide is a short-acting GLP-1 receptor agonist and therefore less prone to tachyphylaxis when it comes to gastric emptying [33], thus providing sustained reductions of postprandial glucose concentrations during long-term use [34, 35].

In the present study, single-dose lixisenatide significantly reduced postprandial plasma glucose excursions in individuals with diabetes secondary to total pancreatectomy. The mechanisms behind this observation may involve lixisenatide-induced deceleration of gastric emptying and/or attenuation of postprandial glucagon responses. The present results are unlikely to have an immediate impact on clinical practice; larger and longer-term clinical trials are warranted.

An important strength of this study is the double-blinded, placebo-controlled, crossover design, which reduces the influence of inter-individual confounding factors. However, some limitations need to be mentioned. First, we did not achieve complete steady state according to our tracer/tracee ratio at baseline (ESM Fig. 2), which may contribute to the decrease in the R_a of glucose in the fasting state observed in both the pancreatectomy group and the control group. However, this was evident on both experimental days and did not differ between study days in either of the groups and is therefore unlikely to explain the differences in glucose kinetics between the interventions. Second, totally pancreatectomised individuals are deficient in pancreatic enzymes and depend on supplements of amylase, protease and lipase in order to absorb nutrients sufficiently [36]. Therefore, in our study the pancreatectomised individuals ingested a single dose of Creon (enzyme supplement) just before the liquid meal on both study days. Nevertheless, we expect absorption to be different in the two study groups, which should be taken into account when comparing the two groups. Furthermore, we did not collect urine during the experiments. We expect that more glucose was excreted through urine during the meal test with placebo (highest plasma glucose concentrations) than with lixisenatide treatment in the totally pancreatectomised participants.

In conclusion, the short-acting GLP-1 receptor agonist lixisenatide reduced postprandial plasma glucose excursions in a cohort of totally pancreatectomised individuals. The mode of action may involve lixisenatide-induced deceleration of gastric emptying and/or elimination of postprandial responses of gut-derived glucagon in these patients.

Acknowledgements The authors would like to thank all study participants and acknowledge the laboratory assistance from S. M. Schmidt and I. al Nachar from Steno Diabetes Center Copenhagen, Gentofte Hospital, and A. von der Lieth from the Department of Clinical Biochemistry, Rigshospitalet, Copenhagen.

Some of the data reported here were presented as an abstract at the ADA's 77th Scientific Sessions and the 53rd EASD Annual Meeting in 2017.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding This study was supported by an unrestricted investigator-initiated study grant from Sanofi and grants from the A. P. Møller Foundation for the Advancement of Medical Science, the Novo Nordisk Foundation, and the Faculty of Health and Medical Sciences, University of Copenhagen. The lixisenatide and placebo pens used in this study were produced and sponsored by Sanofi. Sanofi was not involved in the design of the study; the collection, analysis, and interpretation of data; writing the report or the decision to submit the report for publication.

Duality of interest FKK has received lecture fees from, participated in advisory boards of, consulted for and/or received research grants from Sanofi (the manufacturer of lixisenatide) and Zealand Pharma (the inventor of lixisenatide). The remaining authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

Contribution statement CTBJ was involved in planning of the study, conducted the clinical experiments, was responsible for the statistical analyses and drafting the manuscript. AL was involved in the planning of the study, conception of the statistical analyses and was involved in drafting of the manuscript. MMA conducted clinical experiments. CPH and JHS performed total pancreatectomies and recruited patients. MMA, CPH and JHS furthermore took part in interpretation of data and revising the article critically for important intellectual content. JFR processed and analysed gastrin and CCK data. GvH processed and analysed glucose and glycerol tracer data. NJWA processed and analysed glucagon data. JJH and BH provided ELISA analysis of glucagon and RIA analyses of lixisenatide, GLP-1 and GIP. TV was involved in planning the study. FKK conceptualised the study and was involved in planning the study, interpretation of data and writing the manuscript. All authors contributed to interpretation of the data, critically reviewed and edited the manuscript and approved the version to be published. CTBJ and FKK are the guarantors of this work and, as such, had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Holst JJ (2004) On the physiology of GIP and GLP-1. Horm Metab Res 36(11–12):747–754. https://doi.org/10.1055/s-2004-826158
2. Hare KJ, Knop FK, Asmar M et al (2009) Preserved inhibitory potency of GLP-1 on glucagon secretion in type 2 diabetes mellitus. J Clin Endocrinol Metab 94(12):4679–4687. https://doi.org/10.1210/jc.2009-0921
3. Larsson H, Holst JJ, Ahrén B (1997) Glucagon-like peptide-1 reduces hepatic glucose production indirectly through insulin and glucagon in humans. Acta Physiol Scand 160(4):413–422. https://doi.org/10.1046/j.1365-201X.1997.00161.x
4. Meier JJ, Gallwitz B, Salmen S et al (2003) Normalization of glucose concentrations and deceleration of gastric emptying after

solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. J Clin Endocrinol Metab 88(6):2719–2725. https://doi.org/10.1210/jc.2003-030049
5. Whyte MB, Shojaee-Moradie F, Sharaf SE et al (2019) Lixisenatide reduces chylomicron triacylglycerol by increased clearance. J Clin Endocrinol Metab 104(2):359–368. https://doi.org/10.1210/jc.2018-01176
6. Little TJ, Pilichiewicz AN, Russo A et al (2006) Effects of intravenous glucagon-like peptide-1 on gastric emptying and intragastric distribution in healthy subjects: relationships with postprandial glycemic and insulinemic responses. J Clin Endocrinol Metab 91(5):1916–1923. https://doi.org/10.1210/jc.2005-2220
7. Flint A, Raben A, Astrup A, Holst JJ (1998) Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J Clin Invest 101(3):515–520. https://doi.org/10.1172/JCI990
8. Vilsbøll T, Christensen MJA et al (2012) Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials. BMJ 344:d7771. https://doi.org/10.1136/bmj.d7771
9. Wang B, Zhong J, Lin H et al (2013) Blood pressure-lowering effects of GLP-1 receptor agonists exenatide and liraglutide: a meta-analysis of clinical trials. Diabetes Obes Metab 15(8):737–749. https://doi.org/10.1111/dom.12085
10. Skov J (2014) Effects of GLP-1 in the kidney. Rev Endocr Metab Disord 15(3):197–207. https://doi.org/10.1007/s11154-014-9287-7
11. Pannacciulli N, Bunt JC, Koska J, Bogardus C, Krakoff J (2006) Higher fasting plasma concentrations of glucagon-like peptide 1 are associated with higher resting energy expenditure and fat oxidation rates in humans. Am J Clin Nutr 84(3):556–560. https://doi.org/10.1093/ajcn/84.3.556
12. Kulu Y, Schmied BM, Werner J, Museli P, Büchler MW, Schmidt J (2009) Total pancreatectomy for pancreatic cancer: indications and operative technique. Int Hepato-Pancreato-Biliary Assoc 11(6):469–475. https://doi.org/10.1111/j.1477-2574.2009.00085.x
13. Holst JJ, Wewer Albrechtsen NJ (2019) Methods and guidelines for measurement of glucagon in plasma. Int J Mol Sci 20(21):E5416
14. Wewer Albrechtsen NJ, Hartmann B, Veedfald S et al (2014) Hyperglucagonaemia analysed by glucagon sandwich ELISA: nonspecific interference or truly elevated levels? Diabetologia 57(9):1919–1926. https://doi.org/10.1007/s00125-014-3283-z
15. Bornø A, Foged L, Van Hall G (2014) Glucose and glycerol concentrations and their tracer enrichment measurements using liquid chromatography tandem mass spectrometry. J Mass Spectrom 49(10):980–988. https://doi.org/10.1002/jms.3407
16. Stadil F, Rehfeld JF (1973) Determination of gastrin in serum. An evaluation of the reliability of a radioimmunoassay. Scand J Gastroenterol 8(2):101–112
17. Rehfeld JF (1998) Accurate measurement of cholecystokinin in plasma. Clin Chem 44(5):991–1001. https://doi.org/10.1136/jcp.s1-8.1.26
18. Kielgast U, Holst JJ, Madsbad S (2011) Antidiabetic actions of endogenous and exogenous GLP-1 in type 1 diabetic patients with and without residual β-cell function. Diabetes 60(5):1599–1607. https://doi.org/10.2337/db10-1790
19. Medhus AW, Lofthus CM, Bredesen J, Husebye E (2001) Gastric emptying: the validity of the paracetamol absorption test adjusted for individual pharmacokinetics. Neurogastroenterol Motil 13(3):179–185. https://doi.org/10.1046/j.1365-2982.2001.00249.x
20. Willems M, Quartero AO, Numans ME (2001) How useful is paracetamol absorption as a marker of gastric emptying? A systematic literature study. Dig Dis Sci 46(10):2256–2262. https://doi.org/10.1023/A:1011935603893
21. Flint A, Raben A, Ersbøll AK, Holst JJ, Astrup A (2001) The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity. Int J Obes Relat Metab Disord 25(6):781–792. https://doi.org/10.1038/sj.ijo.0801627
22. Bergmann NC, Lund A, Gasbjerg LS et al (2019) Effects of combined GIP and GLP-1 infusion on energy intake, appetite and energy expenditure in overweight/obese individuals: a randomised, crossover study. Diabetologia 62(4):655–675. https://doi.org/10.1007/s00125-018-4810-0
23. Vella A, Rizza RA (2009) Application of isotopic techniques using constant specific activity or enrichment to the study of carbohydrate metabolism. Diabetes 58(10):2168–2174. https://doi.org/10.2337/db09-0318
24. Jones AG, Hattersley AT (2013) The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med 30(7):803–817. https://doi.org/10.1111/dme.12159
25. Lund A, Bagger JI, Albrechtsen NJW et al (2016) Evidence of extrapancreatic glucagon secretion in man. Diabetes 65(3):585–597. https://doi.org/10.2337/db15-1541
26. Jorsal T, Rhee NA, Pedersen J et al (2018) Enteroendocrine K and L cells in healthy and type 2 diabetic individuals. Diabetologia 61(2):284–294. https://doi.org/10.1007/s00125-017-4450-9
27. Baggio LL, Drucker DJ (2007) Biology of Incretins: GLP-1 and GIP. Gastroenterology 132(6):2131–2157. https://doi.org/10.1053/j.gastro.2007.03.054
28. Rehfeld JF, Stadil F, Baden H, Fischerman K (1975) The enteral insulin-stimulation after Whipple's operation. Diabetologia 11(3):207–210. https://doi.org/10.1007/BF00422323
29. ADA (2019) 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2019. Diabetes Care 42(Suppl 1):S13–S28. https://doi.org/10.2337/dc19-S002
30. World Health Organization (2019) Classification of diabetes mellitus. WHO, Geneva
31. Ewald N, Kaufmann C, Raspe A, Kloer HU, Bretzel RG, Hardt PD (2012) Prevalence of diabetes mellitus secondary to pancreatic diseases (type 3c). Diabetes Metab Res Rev 28(4):338–342. https://doi.org/10.1002/dmrr.2260
32. Makuc J (2016) Management of pancreatogenic diabetes: challenges and solutions. Diabetes, Metab Syndr Obes Targets Ther 9:311–315. https://doi.org/10.2147/DMSO.S99701
33. Nauck MA, Kemmeries G, Holst JJ, Meier JJ (2011) Rapid tachyphylaxis of the glucagon-like peptide 1-induced deceleration of gastric emptying in humans. Diabetes 60(5):1561–1565. https://doi.org/10.2337/db10-0474
34. Kapitza C, Forst T, Coester H-V, Poitiers F, Ruus P, Hincelin-Méry A (2013) Pharmacodynamic characteristics of lixisenatide once daily versus liraglutide once daily in patients with type 2 diabetes insufficiently controlled on metformin. Diabetes Obes Metab 15(7):642–649. https://doi.org/10.1111/dom.12076
35. Fonseca VA, Alvarado-Ruiz R, Raccah D, Boka G, Miossec P, Gerich JE, EFC6018 GetGoal-Mono Study Investigators (2012) Efficacy and safety of the once-daily GLP-1 receptor agonist lixisenatide in monotherapy. A randomized, double-blind, placebo-controlled trial in patients with type 2 diabetes (GetGoal-Mono). Diabetes Care 35(6):1225–1231. https://doi.org/10.2337/dc11-1935
36. Whitcomb DC, Lehman GA, Vasileva G et al (2010) Pancrelipase delayed-release capsules (CREON) for exocrine pancreatic insufficiency due to chronic pancreatitis or pancreatic surgery: a double-blind randomized trial. Am J Gastroenterol 105(10):2276–2286. https://doi.org/10.1038/ajg.2010.201