Asger Lund,[1,2] Jonatan I. Bagger,[1,2] Nicolai J. Wewer Albrechtsen,[2,3] Mikkel Christensen,[1,2] Magnus Grøndahl,[1] Bolette Hartmann,[2] Elisabeth R. Mathiesen,[4] Carsten P. Hansen,[5] Jan H. Storkholm,[5] Gerrit van Hall,[6] Jens F. Rehfeld,[7] Daniel Hornburg,[3] Felix Meissner,[3] Matthias Mann,[3,8] Steen Larsen,[1] Jens J. Holst,[2] Tina Vilsbøll,[1] and Filip K. Knop[1,2]

Evidence of Extrapancreatic Glucagon Secretion in Man

Diabetes 2016;65:585–597 | DOI: 10.2337/db15-1541

Glucagon is believed to be a pancreas-specific hormone, and hyperglucagonemia has been shown to contribute significantly to the hyperglycemic state of patients with diabetes. This hyperglucagonemia has been thought to arise from α-cell insensitivity to suppressive effects of glucose and insulin combined with reduced insulin secretion. We hypothesized that postabsorptive hyperglucagonemia represents a gut-dependent phenomenon and subjected 10 totally pancreatectomized patients and 10 healthy control subjects to a 75-g oral glucose tolerance test and a corresponding isoglycemic intravenous glucose infusion. We applied novel analytical methods of plasma glucagon (sandwich ELISA and mass spectrometry–based proteomics) and show that 29–amino acid glucagon circulates in patients without a pancreas and that glucose stimulation of the gastrointestinal tract elicits significant hyperglucagonemia in these patients. These findings emphasize the existence of extrapancreatic glucagon (perhaps originating from the gut) in man and suggest that it may play a role in diabetes secondary to total pancreatectomy.

Patients with diabetes are characterized not only by compromised insulin secretion and action but also by elevated plasma concentrations of the 29–amino acid peptide hormone glucagon, which hitherto has been considered a pancreas-derived hormone in humans (produced in and secreted from α-cells in the islet of Langerhans) (1). In patients with diabetes, plasma concentrations of glucagon are elevated in the fasting state and fail to decrease appropriately or even increase in response to an oral glucose tolerance test (OGTT) and show exaggerated increases in response to ingestion of a mixed meal (1,2). The elevated glucagon concentrations increase the hepatic glucose production and thereby contribute significantly to the fasting and postprandial hyperglycemia characterizing patients with diabetes. The etiology behind diabetic hyperglucagonemia is still controversial. Whereas oral intake of glucose elicits a hyperglucagonemic response, intravenous glucose administration causes suppression of plasma glucagon levels (3,4). A "lighter version" of this phenomenon has also been observed in healthy individuals after ingestion of larger oral glucose loads (4). Together, these findings led us to speculate that postprandial hyperglucagonemia could be gut derived and independent of the endocrine pancreas.

The notion of extrapancreatic glucagon secretion in man has been debated for years, and several studies looking at glucagon responses after total pancreatectomy in animals (5–10) and man (11–24) have been published. Overall, these investigations have reported very conflicting

[1]Center for Diabetes Research, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark
[2]The Novo Nordisk Foundation Center for Basic Metabolic Research and Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[3]Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, Martinsried, Germany
[4]Center for Pregnant Women with Diabetes, Department of Endocrinology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
[5]Department of Gastrointestinal Surgery, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
[6]Clinical Metabolomics Core Facility, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
[7]Department of Clinical Biochemistry, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
[8]The Novo Nordisk Foundation Center for Protein Research, Proteomics Program, University of Copenhagen, Copenhagen, Denmark
Corresponding author: Filip K. Knop, filipknop@dadlnet.dk.

Received 6 November 2015 and accepted 6 December 2015.
Clinical trial reg. no. NCT02006459, clinicaltrials.gov.
This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-1541/-/DC1.

results. One of the main challenges is that many of the glucagon assays suffer from shortcomings, and until recently, analytical methods have not been sufficiently sensitive or specific to justify decisive statements about the absence or presence of extrapancreatic fully processed 29–amino acid glucagon (24). Recently, however, sandwich ELISAs using a combination of C- and N-terminal antiglucagon antibodies, and thus in theory eliminating cross-reactivity with elongated or truncated forms of glucagon, have become available (25). Furthermore, technical advances have made it possible to detect low-abundant peptides, such as glucagon, in human plasma by mass spectrometry–based proteomics (26,27). Applying these novel techniques, we aimed to investigate whether postabsorptive hyperglucagonemia occurs independently of functioning pancreatic islets and plasma glucose and insulin concentrations by measuring plasma concentrations of 29–amino acid glucagon in totally pancreatectomized patients and healthy control subjects after 75-g OGTT and isoglycemic intravenous glucose infusion (IIGI).

RESEARCH DESIGN AND METHODS

Study Design

This study examined glucagon responses in totally pancreatectomized patients and healthy control subjects during OGTT and IIGI. The study was conducted at the Center for Diabetes Research (Gentofte Hospital), approved by the Ethics Committee of the Capital Region of Denmark (reg. no. H-1-2012-123), and conducted in accordance with the principles of the Declaration of Helsinki (Seventh Revision, 2013).

Study Participants

Ten totally pancreatectomized patients (age [mean ± SD]: 59.8 ± 9.9 years; BMI: 21.5 ± 4.3 kg/m^2; HbA_{1c}: 67.3 ± 11.0 mmol/mol; time since operation: 4.6 ± 4.5 years) and 10 age-, sex-, and BMI-matched healthy control subjects (age: 58.4 ± 5.0 years; BMI: 22.9 ± 2.4 kg/m^2; HbA_{1c}: 34.6 ± 6.2 mmol/mol) without any family history of diabetes were studied. Clinical characteristics of the totally pancreatectomized subjects are displayed in Table 1, and a schematic illustration of the anatomy before and after surgery (28) is depicted in Fig. 1.

Experimental Procedures

After an initial screening visit, participants were examined on two study days separated by at least 72 h. Subjects were studied after an overnight (10 h) fast, and patients were instructed to take their normal daily insulin doses the day before but not to take any insulin on the morning of the experimental day. On the 1st day, a 75-g OGTT was performed. Participants were placed in a recumbent position and cannulas were inserted in cubital veins, one for infusion of stable isotopes and one in the contralateral vein for collection of arterialized blood with the hand wrapped in a heating pad (~50°C). At time −120 min, an infusion of stable isotopes (Cambridge Isotope Laboratories, Tewksbury, MA) with [6,6-D_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 [1,1,2,3,3-D_5]glycerol (priming dose of 2.0 μmol × kg^{-1} and continuous infusion of 0.1 μmol × kg^{-1} × min^{-1}) was initiated. Over the first 10 min (time 0–10 min), the participants ingested 75 g glucose (71.5 g + 3.5 g [U-$^{13}C_6$] glucose) dissolved in 250 mL water. Blood samples were drawn 120, 45, 30, 15, and 0 min before and 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 120, 150, and 180 min after initiation of glucose ingestion. At the very end of the experiment, patients took half their normal basal insulin dose and prandial insulin according to blood glucose levels. At the 2nd experimental day, an IIGI (20% weight for volume) was performed, aiming at duplicating the plasma glucose profile attained in the same individual during the OGTT. Other procedures were as on the first study day, except for more frequent plasma glucose sampling (every 5 min) in order to adjust the glucose infusion rate to obtain isoglycemia. Plasma glucose concentrations were measured bedside using the glucose oxidase method (YSI 2300 STAT PLUS analyzer; YSI, Yellow Springs, OH). Plasma glucagon concentrations were assayed with a sandwich ELISA using N- and C-terminal–specific glucagon antibodies (Mercodia AB, Uppsala, Sweden) (25). Gel chromatography of plasma was performed as previously described (29). High-performance liquid chromatography (HPLC) and mass spectrometry–based proteomics were performed for the analysis of glucagon. C-peptide concentrations were measured using a two-sided electrochemiluminescence immunoassay (Siemens Healthcare, Ballerup, Denmark). Plasma enrichment of [6,6-D_2]glucose, [U-$^{13}C_6$]glucose, and [1,1,2,3,3-D_5] glycerol was determined using liquid chromatography–tandem mass spectrometry (LC-MS/MS) as previously described (30). Amidated gastrin and cholecystokinin (CCK), total glucose-dependent insulinotropic polypeptide (GIP), total glucagon-like peptide 1 (GLP-1), and GIP(1-30) were measured with radioimmunoassays (RIAs). Oxyntomodulin was measured using a sandwich ELISA. Further details about analytical procedures are available in the Supplementary Data.

Calculations and Statistical Analysis

Area under the curves (AUCs) were calculated using the trapezoidal rule. Gastrointestinal-mediated glucose disposal (GIGD) was calculated from the amount of glucose ingested during the OGTT related to the amount administered during the IIGI using the following formula: GIGD (%) = 100% × ($glucose_{OGTT}$ − $glucose_{IIGI}$)/$glucose_{OGTT}$ (31). Glucose rate of appearance (R_a) and rate of disappearance (R_d) were calculated from changes in glucose enrichment using the one-compartment, fixed-volume, non–steady-state model of Steele and modified for use with stable isotopes and a varying pool fraction ranging from 70 to 200 mL × kg^{-1}, taking into account the high blood glucose levels of totally pancreatectomized patients (32). Results are reported as

Table 1—Clinical characteristics of the totally pancreatectomized patients

Patient	Sex (M/F)	Years since operation	BMI (kg/m^2)	Insulin treatment (IU)	Reason for surgery	Other treatment
1	M	1.4	20.3	Insulin detemir 16 + 5 Insulin aspart 7 + 4 + 4	Pancreatitis	Creon* 65,000 ×3; plus 25,000 to in-between meal–snacks Buprenorphine 0.2 mg ×2 Cholecalciferol 20 μg ×1
2	M	4	29.3	Insulin detemir 26 + 14 Insulin aspart 8 + 6 + 8	Neuroendocrine tumor	Creon 45,000 + 50,000 + 50,000; plus 35,000 to in-between meal–snacks Lansoprazole 30 mg ×1 Cholecalciferol 20 μg ×1 Acetaminophen 500 mg ×6 Glucosamine 400 mg ×1
3	F	4.5	18.0	Insulin glargine 13 + 0 Insulin aspart 3 + 3 + 5	Adenocarcinoma	Creon 35,000 ×3; plus 35,000 to in-between meal–snacks Levothyroxine 100 mg ×2 Lansoprazole 30 mg ×1
4	F	1	17.5	Insulin detemir 9 + 6 Insulin aspart 4 + 2 + 3	Adenocarcinoma	Creon 25,000 ×3; plus 10,000 to in-between meal–snacks Pantoprazole 40 mg ×2 Metoprolol 50 mg ×1 Calcium 500 mg ×1 Cholecalciferol 10 μg ×1
5	M	4.3	25.0	Insulin degludec 0 + 10 Insulin aspart 10 + 10 + 5	Adenocarcinoma	Creon 25,000 ×3; plus 10,000 to in-between meal–snacks
6	F	4.3	23.6	Insulin detemir 12 + 24 Insulin aspart 7 + 7 + 7	Adenocarcinoma	Creon 35,000 ×3; plus 10,000 to in-between meal–snacks Pantoprazole 40 mg ×1 Bendroflumethiazide (1.25 mg) + kaliumchlorid (573 mg)
7	M	4	25.4	Insulin detemir 8 + 10 Insulin aspart 12 + 6 + 10	Adenocarcinoma	Creon 50,000 ×3; plus 35,000 to in-between meal–snacks Lansoprazole 30 mg ×2
8	F	3.7	17.7	Insulin detemir 20 + 6 Insulin aspart 3 + 2 + 2	Mucinous cystadenoma	Creon 40,000 ×3; plus 20,000 to in-between meal–snacks Hydroxocobalamin 1 mg ×1 every 2 months
9	M	17.0	16.4	Insulin glargine 0 + 15 Insulin aspart 6 + 8 + 6	Adenocarcinoma	Creon 75,000 ×3; plus 50,000–75,000 to in-between meal–snacks Acetaminophen 500 mg ×6 Morphine 30 mg ×2 Isosorbidmononitrate 60 mg ×1
10	F	2.0	21.9	Insulin detemir 12 + 6 Insulin aspart 4 + 4 + 4	Adenocarcinoma	Creon 80,000 ×3; plus 35,000 to in-between meal–snacks Pantoprazole 40 mg ×2 Loperamide 2 mg ×2

IU, international unit. *Creon (pancreas enzyme replacement therapy): 40,000: 25,000 EP-e amylase, 40,000 EP-e lipase, and 1,600 EP-e protease; Creon 25,000: 18,000 EP-e amylase, 25,000 EP-e lipase, and 1,000 EP-e protease; Creon 10,000: 8,000 EP-e amylase, 10,000 EP-e lipase, and 600 EP-e protease.

mean ± SEM unless otherwise stated. Group differences in baseline characteristics and AUCs were evaluated using two-sample Student *t* test (two-tailed). Linear mixed effect modeling was applied for analysis of repeated measures with data transformed according to distribution pattern. A homogeneous or heterogeneous autoregressive residual covariance structure for the repeated measurements was chosen according to the Akaike information criteria. Statistics were performed in GraphPad Prism version 6.0 (GraphPad Software, La Jolla, CA) and the statistical software package SAS version 9.3 (SAS Institute, Cary, NC). A two-sided

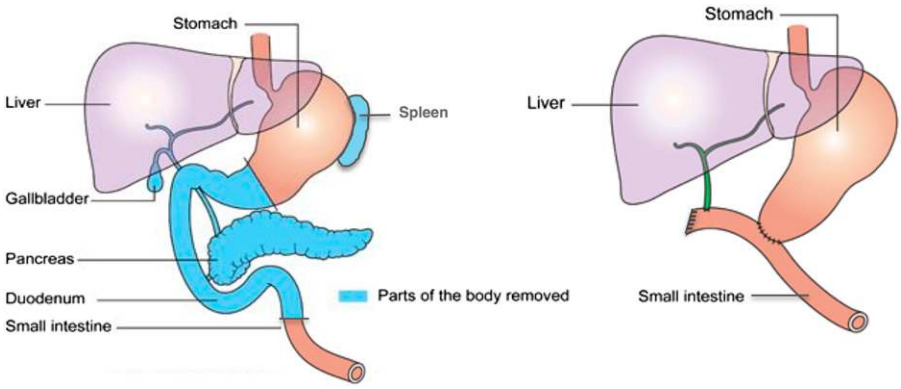

Figure 1—Illustration of the changed anatomy after total pancreatectomy operation. Left: The anatomy before operation, with the light blue–colored organs indicating the organs being removed during surgery. Right: The anatomy after surgery.

P value <0.05 was used to indicate statistically significant differences. Further details about calculations and statistical analysis are available in the Supplementary Data.

RESULTS

Plasma Glucose

After the 75-g OGTT, a mean peak concentration of 27.9 ± 1.4 mmol/L was reached at time point 150 min in the totally pancreatectomized patients compared with a mean peak concentration of 9.1 ± 0.5 mmol/L at time point 60 min in the healthy control subjects (Fig. 2*A*). The individually adjustable intravenous glucose infusions resulted in glucose excursions over time similar to those during the corresponding OGTT (isoglycemia) in both the pancreatectomy group and the control group (Fig. 2*A* and Table 2). The total amount of glucose infused during the IIGI, copying the plasma glucose excursions from the 75-g OGTT, amounted to 28.5 ± 1.8 g in the control group and 83.3 ± 3.1 g in the pancreatectomy group (142 ± 9 and 416 ± 19 mL of 20% weight for volume glucose solution, respectively; $P < 0.001$), corresponding to a GIGD of 62.0 ± 2.4% in the control group and −11.0 ± 5.0% in the pancreatectomy group ($P < 0.001$), both different from 0% ($P < 0.001$ and $P = 0.05$, respectively).

C-Peptide and Pancreatic Polypeptide

C-peptide and pancreatic polypeptide (PP) concentrations in the totally pancreatectomized patients were below detection limit of the assays (<16 and <0.5 pmol/L, respectively) at all time points on both days (Fig. 2*C* and *D* and Table 2), except for one patient (operated because of a neuroendocrine tumor in the pancreas) showing very low levels of C-peptide (lower than what is needed to define C-peptide negativity) in the last hour of both study days (41 pmol/L at time point 180 min during the OGTT and 37 pmol/L at time point 150 min during the IIGI). Other end points, including glucagon, were not different in this patient compared with the mean group values, and thus this patient was kept in for the following analyses. As expected, the control group responded with significantly larger C-peptide and PP responses during the OGTT compared with the IIGI.

Glucagon

In the totally pancreatectomized patients, basal concentrations of glucagon were above the detection limit (1 pmol/L) of the applied sandwich ELISA ($P < 0.001$), with levels tending to be lower compared with the healthy control subjects although without reaching statistical significance (Fig. 3*A* and Table 2). After OGTT, the totally pancreatectomized patients responded with significant secretion of glucagon, resulting in mean peak levels of 18.8 ± 3.0 pmol/L (at time point 30 min), whereas immediate suppression of glucagon concentrations was seen after initiation of the intravenous glucose infusion (Fig. 3*A* and *B*). The healthy control subjects responded with significant suppression of glucagon concentrations on both the OGTT day and the IIGI day, but glucagon suppression after oral glucose was delayed compared with during intravenous glucose infusion, in line with previous observations (4,33). To verify that the observed glucagon responses in the pancreatectomy group were in fact due to the presence of 29–amino acid glucagon, we performed affinity chromatography (Fig. 3*C*) and HPLC fractionation of pooled plasma samples from the OGTT day, from baseline, and from time point 30 min where glucagon concentrations peaked (Fig. 3*D*), followed by LC-MS/MS analysis (Fig. 3*E*). Peptide intensities for the glucagon sequence increased significantly ($P < 0.001$) from baseline to time point 30 min during the OGTT. LC-MS/MS analysis therefore confirmed that fully processed 29–amino acid glucagon was present at baseline and that concentrations increased considerably during OGTT in the totally pancreatectomized patients.

Glucose and Glycerol Kinetics

Baseline levels of glucose R_a and R_d were higher in the totally pancreatectomized patients compared with the

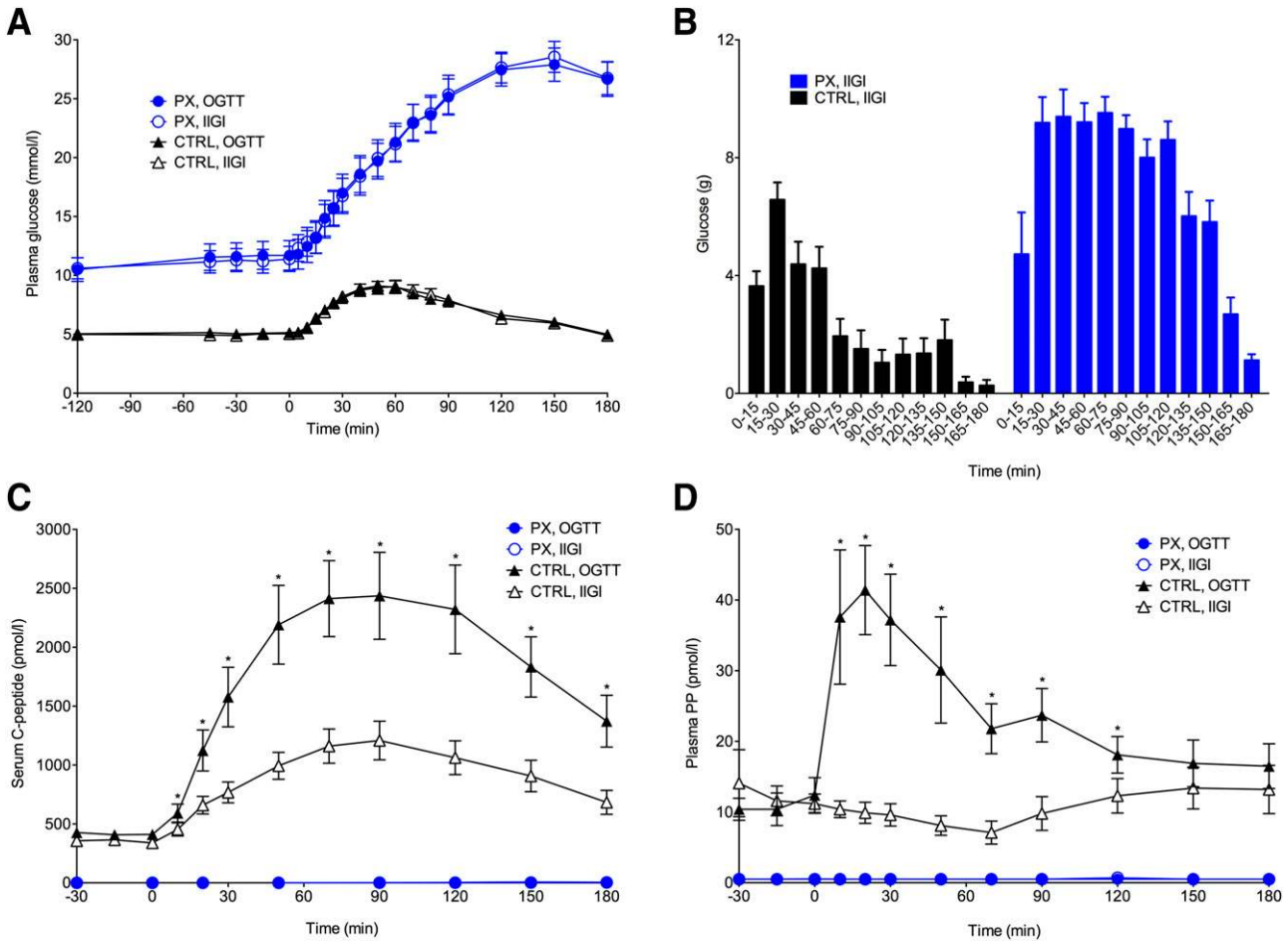

Figure 2—Plasma glucose concentrations (*A*), glucose infusions during IIGI depicted as bar graphs (*B*), and serum/plasma responses of C-peptide (*C*) and PP (*D*) during 75-g OGTT and IIGI, respectively, in totally pancreatectomized patients (PX) (n = 10) and healthy control subjects (CTRL) (n = 10). Data are mean ± SEM. Statistical analysis was performed by linear mixed effect modeling for analysis of repeated measures between days within groups. Asterisks (*) indicate significant ($P < 0.05$) differences.

healthy control subjects ($P < 0.001$, for both measures) (Fig. 4*A* and *B*). In the pancreatectomy group, R_d of glucose was higher during IIGI compared with OGTT (P = 0.005), driven partly by a higher glucose excretion in the urine during the IIGI (15.6 ± 2.9 vs. 12.3 ± 2.3 g; P = 0.01). R_a of glucose over time was also higher during IIGI compared with OGTT in the pancreatectomy group (P = 0.02). Baseline levels of endogenous R_a of glucose were higher in the totally pancreatectomized patients compared with the healthy control subjects ($P < 0.001$) and not different between study days in each group (Fig. 4*C*). Suppression of endogenous R_a was enhanced during IIGI compared with OGTT in both groups, reflecting a mean difference between the 2 days in endogenous glucose production (EGP) over 3 h of 16.5 ± 2.3 g in the pancreatectomy group and 6.0 g ± 1.0 g in the control group (P = 0.001). Basal concentrations of plasma glycerol were higher in the totally pancreatectomized patients compared with the healthy control subjects (146 ± 22 vs. 80 ± 13 μmol/L; P = 0.022), but within each group, no difference in responses over time between study days was observed (Fig. 4*D*). Plasma glycerol concentrations in the control group were significantly reduced (to ~50% of baseline levels) after both OGTT and IIGI, whereas no significant changes over time were seen in the pancreatectomy group. Mean levels of R_a and R_d, respectively, for glycerol (Fig. 4*E* and *F*) were higher in the pancreatectomy group compared with the control group, likely explained by the insulin-deficient state of the patients.

Gastrin and CCK

The control group responded with significant secretion of gastrin after OGTT, whereas no change from baseline was observed during IIGI (Fig. 5*A* and Table 2). In the pancreatectomy group, no differences in gastrin concentrations were observed over time, during either OGTT or IIGI (P = NS). However, total AUC (but not baseline-subtracted AUC) was greater during OGTT compared with IIGI. Gastrin responses over time during OGTT were greater in the control group compared with the pancreatectomy group (P = 0.01). Significantly higher responses of CCK were observed in

Table 2—Glucose and hormonal responses

	Healthy control subjects	Pancreatectomized patients	*P*
Glucose			
Mean baseline (mmol/L)	5.1 ± 0.1	11.5 ± 1.1	<0.001
AUC (mmol/L × 3 h)			
75-g OGTT	1,276 ± 39	4,122 ± 254	<0.001
IIGI	1,273 ± 36	4,154 ± 248	<0.001
P	0.79	0.10	
bsAUC (mmol/L × 3 h)			
75-g OGTT	358 ± 43	2,022 ± 111	<0.001
IIGI	373 ± 42	2,120 ± 133	<0.001
P	0.46	0.33	
C-peptide			
Mean baseline (pmol/L)	384.9 ± 38.2	≤16.0 ± 0.0	<0.001
AUC (nmol/L × 3 h)			
75-g OGTT	341.2 ± 44.8	3.1 ± 0.1	<0.001
IIGI	167.1 ± 19.3	2.9 ± 0.1	<0.001
P	<0.001	0.17	
bsAUC (nmol/L × 3 h)			
75-g OGTT	266.4 ± 38.8	0.2 ± 0.1	<0.001
IIGI	103.3 ± 14.1	0.01 ± 0.01	<0.001
P	<0.001	0.17	
PP			
Mean baseline (pmol/L)	16.6 ± 2.6	≤0.5 ± 0.0	<0.001
AUC (pmol/L × 3 h)			
75-g OGTT	4,331 ± 672	90.1 ± 0.1	<0.001
IIGI	1,927 ± 332	96.0 ± 4.6	<0.001
P	<0.001	0.22	
bsAUC (pmol/L × 3 h)			
75-g OGTT	350 ± 99	−0.9 ± 0.8	0.006
IIGI	−54 ± 95	3.2 ± 2.5	0.56
P	0.019	0.18	
Glucagon			
Mean baseline (pmol/L)	8.3 ± 1.5	5.4 ± 0.2	0.087
AUC (pmol/L × 3 h)			
75-g OGTT	1,019 ± 228	2,418 ± 231	<0.001
IIGI	867 ± 196	465 ± 56	0.075
P	0.23	<0.001	
bsAUC (pmol/L × 3 h)			
75-g OGTT	−367 ± 86	1,383 ± 170	<0.001
IIGI	−784 ± 139	−449 ± 72	<0.001
P	0.022	<0.001	
Gastrin			
Mean baseline (pmol/L)	6.8 ± 0.6	5.9 ± 1.5	0.58
AUC (pmol/L × 3 h)			
75-g OGTT	1,674 ± 138	1,088 ± 251	0.059
IIGI	1,107 ± 114	1,011 ± 253	0.74
P	<0.001	0.015	
bsAUC (pmol/L × 3 h)			
75-g OGTT	417 ± 114	40 ± 30	0.009
IIGI	−91 ± 56	−62 ± 53	0.71
P	0.011	0.15	
CCK			
Mean baseline (pmol/L)	0.7 ± 0.1	1.1 ± 0.2	0.095
AUC (pmol/L × 3 h)			
75-g OGTT	317 ± 30	363 ± 73	0.58
IIGI	169 ± 18	172 ± 27	0.95
P	<0.001	<0.001	
bsAUC (pmol/L × 3 h)			
75-g OGTT	189 ± 41	156 ± 62	0.67
IIGI	64 ± 17	−1.2 ± 29	0.073
P	0.08	0.03	

Continued on p. 591

Table 2—Continued

	Healthy control subjects	Pancreatectomized patients	*P*
GIP			
Mean baseline (pmol/L)	8.8 ± 1.0	11.8 ± 1.1	0.053
AUC (pmol/L × 3 h)			
75-g OGTT	7,432 ± 1,108	8,598 ± 1,374	0.52
IIGI	1,439 ± 164	2,086 ± 246	0.044
P	<0.001	<0.001	
bsAUC (pmol/L × 3 h)			
75-g OGTT	5,848 ± 1,006	6,366 ± 1,331	0.76
IIGI	−157 ± 117	58 ± 105	0.19
P	<0.001	0.001	
GLP-1			
Mean baseline (pmol/L)	14.2 ± 0.6	25.7 ± 2.8	0.0025
AUC (pmol/L × 3 h)			
75-g OGTT	4,454 ± 226	9,557 ± 1,170	0.0017
IIGI	2,500 ± 113	4,278 ± 424	0.0022
P	<0.001	<0.001	
bsAUC (pmol/L × 3 h)			
75-g OGTT	1,712 ± 305	4,799 ± 898	0.0076
IIGI	142 ± 135	−209 ± 218	0.19
P	<0.001	<0.001	
GIP(1-30)			
Mean baseline (pmol/L)	3.1 ± 0.4	2.4 ± 0.3	0.19
AUC (pmol/L × 3 h)			
75-g OGTT	650 ± 84	416 ± 50	0.061
IIGI	639 ± 62	414 ± 66	0.023
P	0.84	0.24	
bsAUC (pmol/L × 3 h)			
75-g OGTT	32 ± 12	−11 ± 16	0.06
IIGI	133 ± 84	−9 ± 10	0.13
P	0.25	0.98	
Oxyntomodulin			
Mean baseline (pmol/L)	8.7 ± 1.1	21.6 ± 1.8	<0.001
AUC (pmol/L × 3 h)			
75-g OGTT	4,590 ± 300	6,442 ± 505	0.007
IIGI	1,154 ± 61	3,816 ± 391	<0.001
P	<0.001	0.002	
bsAUC (pmol/L × 3 h)			
75-g OGTT	2,907 ± 328	2,590 ± 469	0.59
IIGI	−277 ± 152	−117 ± 274	0.62
P	<0.001	<0.001	

Responses of glucose, C-peptide, PP, glucagon, gastrin, CCK, GIP, GLP-1, GIP(1-30), and oxyntomodulin during a 75-g OGTT and IIGI in totally pancreatectomized patients (n = 10) and healthy control subjects (n = 10). Data are mean ± SEM. Statistical analysis was performed by two-sample Student t test (two-tailed), paired or unpaired. bsAUC, baseline-subtracted AUC.

both groups during OGTT compared with IIGI (Fig. 5*B* and Table 2). However, no significant differences in CCK responses over time were observed between the pancreatectomy group and the control group during OGTT (P = 0.13).

Incretin Hormones

Basal concentrations of GIP were similar on the two study days in each group but tended to be higher in the totally pancreatectomized patients compared with the healthy control subjects (Fig. 5*C* and Table 2). During OGTT, significant GIP responses were observed in both groups, with no significant differences between the two groups. Basal concentrations of GLP-1 (from enteroendocrine L cells) were significantly higher in the pancreatectomy group compared with the control group (Fig. 5*D* and Table 2). GLP-1 responses during OGTT were larger in the pancreatectomy group compared with the control group ($P < 0.001$), with a threefold higher peak value at time point 30 min (92 ± 18 vs. 30 ± 5 pmol/L; P = 0.008).

GIP(1-30)

Basal concentrations of GIP(1-30), argued to be a product of prohormone convertase 2 (PC2) processing of pro-GIP in pancreatic α-cells and therefore not secreted from the enteroendocrine K cells from where the major isoform GIP(1-42) is secreted (34), were similar between study days in each group and not different between groups (Table 2). Responses over time were similar

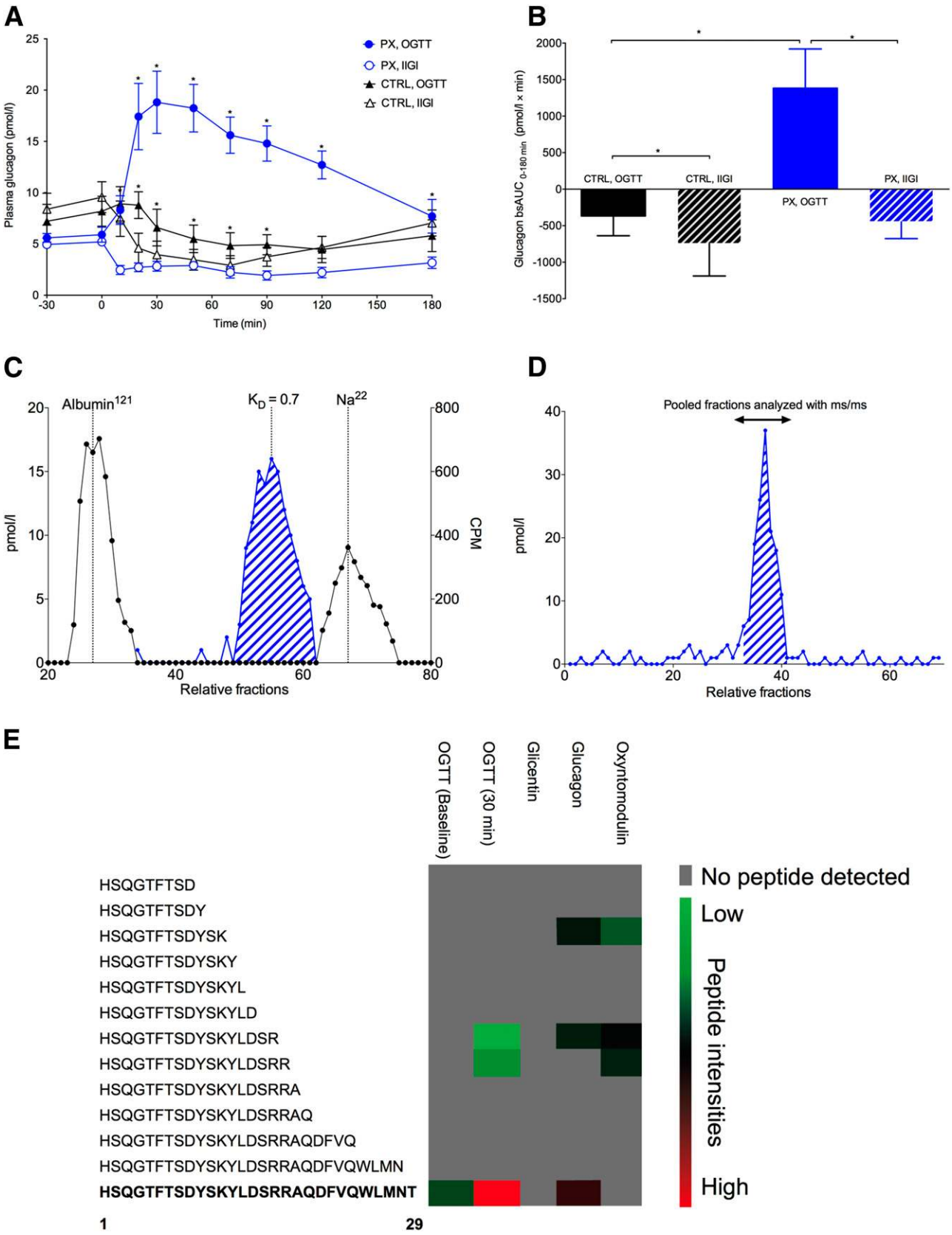

Figure 3—Plasma responses of glucagon (*A*) and glucagon expressed as baseline-subtracted AUC (bsAUC) (*B*) during 75-g OGTT and IIGI in totally pancreatectomized patients (PX) (n = 10) and healthy control subjects (CTRL) (n = 10). Gel chromatography of pooled plasma (*C*) from time point 30 min during the 75-g OGTT in the pancreatectomy group (n = 10) analyzed using a C-terminal–specific glucagon assay (blue) and quality calibrators (black). A single peak (blue hatched area) with similar coefficient of distribution (K_D) to that of the glucagon calibrator control was identified. HPLC profile of pooled plasma (*D*) from fasting and from time point 30 min during the 75-g OGTT from the

between days in both the pancreatectomy group and the control group (P = 0.59 and P = 0.49, respectively), and likewise no differences in responses over time were observed between groups during OGTT (P = 0.09) (Fig. 5*E*).

Oxyntomodulin

Basal concentrations of the enteroendocrine L-cell product oxyntomodulin were significantly higher in the pancreatectomy group compared with the control group (Fig. 5*F* and Table 2). AUC during OGTT was significantly higher in the pancreatectomy group compared with the control group; this was driven by the higher basal concentrations, as baseline-subtracted AUCs were not different (Table 2). No significant changes from baseline occurred during IIGI in either group.

DISCUSSION

This study demonstrates that 29–amino acid glucagon is circulating in man after total pancreatectomy and that glucagon secretion in these patients is stimulated by orally administered glucose and suppressed when glucose is administered intravenously. Furthermore, our data suggest that the hyperglucagonemic response to OGTT in totally pancreatectomized patients is involved in the pathogenesis of postabsorptive hyperglycemia, as EGP was markedly higher during OGTT compared with during intravenously administered glucose where glucagon values were suppressed.

Extrapancreatic glucagon secretion was proposed as early as 1948 by Sutherland and De Duve (5), who showed that in rabbits and dogs a "glycogenolytic substance" could be extracted not only from the pancreas but also from the upper two-thirds of the gastric mucosa. After the introduction of the first glucagon RIA in 1961, reports of measurable "immunoreactive glucagon" in insulin-deprived totally pancreatectomized animals and humans appeared (35). Since then, several investigations have been conducted in totally pancreatectomized animals and humans (8,12,14,15,17–20,22–24), reporting considerable species variation and providing conflicting conclusions regarding the presence or absence of extrapancreatic glucagon. One obvious reason for these conflicting results is that circulating glucagon concentrations are relatively low (picomolar range), demanding a high degree of assay sensitivity not met by the early assays and remaining a drawback for many glucagon assays (36). Furthermore, unclear specificity of the glucagon immunoassays relying on C-terminal or side-viewing antibodies toward the various circulating glucagon-like peptides remains a challenge for many of the currently applied glucagon assays (37).

In the current study, we used a recently developed sandwich ELISA targeting the N- and C-terminal regions of glucagon simultaneously whereby these specificity problems should be eliminated (25). In order to verify the accuracy of this assay, we analyzed plasma from totally pancreatectomized patients for glucagon by using a mass spectrometry–based platform. These results confirm that the glucagon responses observed are indeed genuine 29–amino acid glucagon. The absence of PP and C-peptide responses in the totally pancreatectomized patients confirms the completeness of pancreatectomy and thus eliminates the possibility that the observed glucagon responses might originate from remnant pancreatic tissue. Although the exact origin cannot be determined in this study, the fact that oral glucose stimulation resulted in significant hyperglucagonemic responses, whereas intravenous glucose infusion suppressed circulating glucagon levels, directs the attention to the gastrointestinal tract. Glucagon is a product of the preproglucagon gene (*GCG*), which is expressed by both pancreatic α-cells and specific enteroendocrine cells (L cells) of the intestinal mucosa and neurons within the nucleus of the solitary tract (38,39). *GCG* encodes the precursor hormone proglucagon, which after posttranslational processing yields different and multiple biologically active proglucagon-derived peptides depending on the presence and relative activity of the processing enzymes PC1/3 and PC2 (40). These processing enzymes have generally been considered to be tissue specific, with PC2 being expressed in the pancreatic α-cells, where it processes proglucagon into glucagon, glicentin-related PP, and a major proglucagon fragment, and PC1/3 being expressed in the enteroendocrine L cells, where it processes proglucagon into glicentin, oxyntomodulin, and the two glucagon-like peptides, GLP-1 and GLP-2. Interestingly, recent studies have revealed that activity of PC1/3 may also be present in pancreatic α-cells under certain circumstances (41,42) and that PC2 may also be present in enteroendocrine cells in the human gastrointestinal tract (43,44), including cells costaining for PC2 and the proglucagon product GLP-1 (F.K. Knop et al., unpublished data). It is therefore possible that the proglucagon-containing enteroendocrine L cells may be the origin of the extrapancreatic glucagon secretion observed here. Enteroendocrine L cells are found dispersed in the epithelium of the small and large

pancreatectomy group (n = 10), analyzed using a side-viewing glucagon assay (blue). The fractions containing glucagon-like peptides (blue hatched area) were subsequently pooled and subjected to tandem mass spectrometry–based proteomics (*E*). Peptide intensities ($\log_2$ transformed) are color coded from low (green) to high (red) concentration with a false discovery rate of <1%. The 29–amino acid sequence corresponding to that of glucagon is depicted in bold font. Data are mean ± SEM. Statistical analysis was performed by linear mixed effect modeling for analysis of repeated measures between days within groups, and two-sample Student t test (two-tailed), paired or unpaired, was used for analysis of bsAUC. Asterisks (*) indicate significant (P < 0.05) differences. CPM, counts per minute.

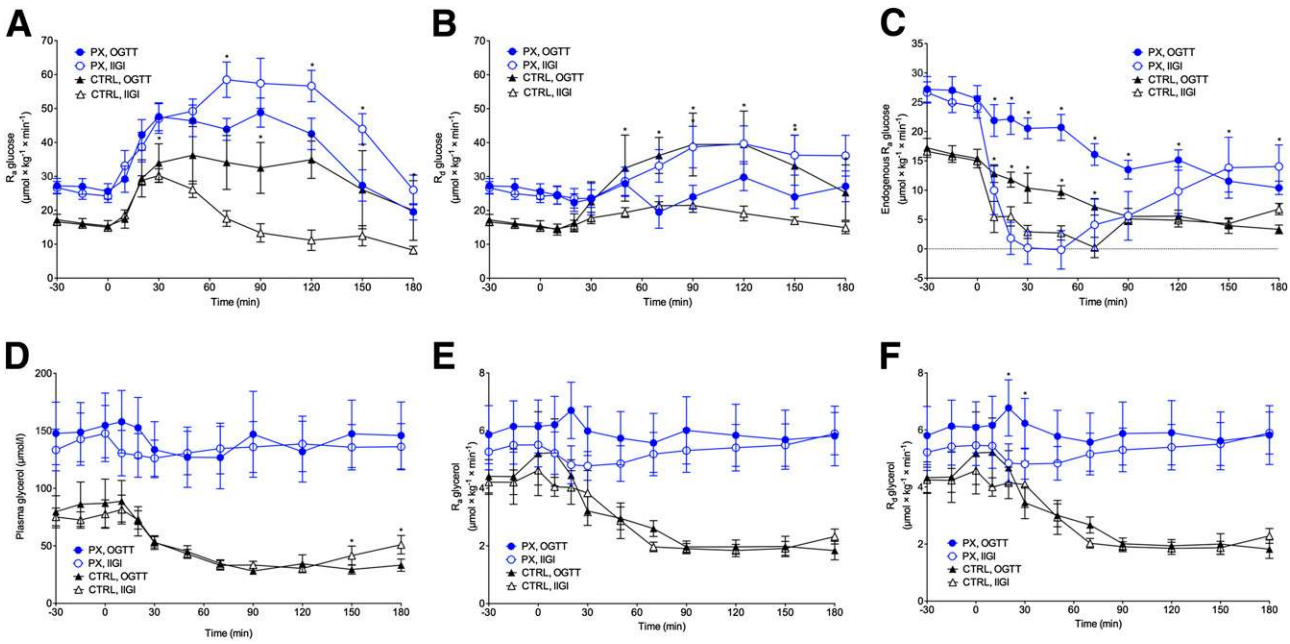

Figure 4—Glucose R_a (*A*), glucose R_d (*B*), endogenous R_a of glucose (*C*), concentrations of plasma glycerol (*D*), glycerol R_a (*E*), and glycerol R_d (*F*) during a 75-g OGTT and IIGI in totally pancreatectomized patients (PX) (n = 10) and healthy control subjects (CTRL) (n = 10). Data are mean ± SEM. Statistical analysis was performed by linear mixed effect modeling for analysis of repeated measures between days within groups. Asterisks (*) indicate significant ($P < 0.05$) differences.

intestines, with densities increasing from the duodenum to the large intestine (43). After total pancreatectomy, the gastrointestinal anatomy is dramatically changed, including the removal of the pyloric sphincter and the duodenum. Thus, after ingestion of a meal, nutrients are rerouted and delivered directly from the stomach to the jejunum, where L cells are more abundant, possibly explaining the significantly increased GLP-1 and oxyntomodulin concentrations observed in the totally pancreatectomized patients after OGTT. These responses are reminiscent of the levels seen in subjects after Roux-en-Y gastric bypass surgery (43,45) (a bariatric surgical procedure in which the small intestine is rearranged so that the nutrients are rerouted from a small gastric pouch to the more distal jejunum) (45). Interestingly, we found that fasting levels of GLP-1 and oxyntomodulin were significantly higher in the totally pancreatectomized patients compared with the healthy control subjects, a difference not typically observed after Roux-en-Y gastric bypass (45). This finding raises the interesting possibility of an increased recruitment of *GCG*-expressing cells in the pancreatectomized patients as a response to the removal of the pancreatic α-cells. In line with this, pancreatic α-cell hyperplasia is observed in total disruption of glucagon receptor signaling in mice (46,47) and during partial disruption of glucagon receptor signaling in humans (48). Furthermore, studies in mice with complete disruption of glucagon receptor signaling specifically in the liver have pointed to the existence of a circulating factor, which stimulates α-cell proliferation when glucagon signaling is abolished (49,50). Perhaps this factor is also formed in response to the surgical removal of the pancreas, and thus in the absence of pancreatic α-cells, the enteroendocrine L cells, expressing the *GCG* gene, might be upregulated instead. Accordingly, we hypothesize that when the L cells of the totally pancreatectomized patients are exposed to a large and rapid stimulation, as by the ingested glucose in this experiment, they respond not only with increased secretion of PC1/3 cleavage products (i.e., GLP-1 and oxyntomodulin) but also with secretion of PC2 cleavage products, resulting in significant glucagon responses. The fact that we did not observe increased GIP(1-30) levels in the pancreatectomized group suggests that a general upregulation of PC2 is not present [as an upregulation of PC2 in the enteroendocrine K cells should yield increased levels of GIP(1-30)], but this does not rule out the possibility of a selective upregulation and/or activity of PC2 in the enteroendocrine L cells.

We found an increased EGP during OGTT compared with during IIGI in the totally pancreatectomized patients, which might be a consequence of the postprandial hyperglucagonemia. The pancreatectomized patients had a higher R_d of glucose during the IIGI day compared with the OGTT day despite isoglycemic conditions and no difference in insulin treatment on the two experimental days. But this difference in R_d was driven primarily by a difference in glucosuria between the 2 days, most likely explained by the larger volume of liquid infused during IIGI compared with the volume ingested during OGTT. Interestingly, a higher rate of EGP was also observed in

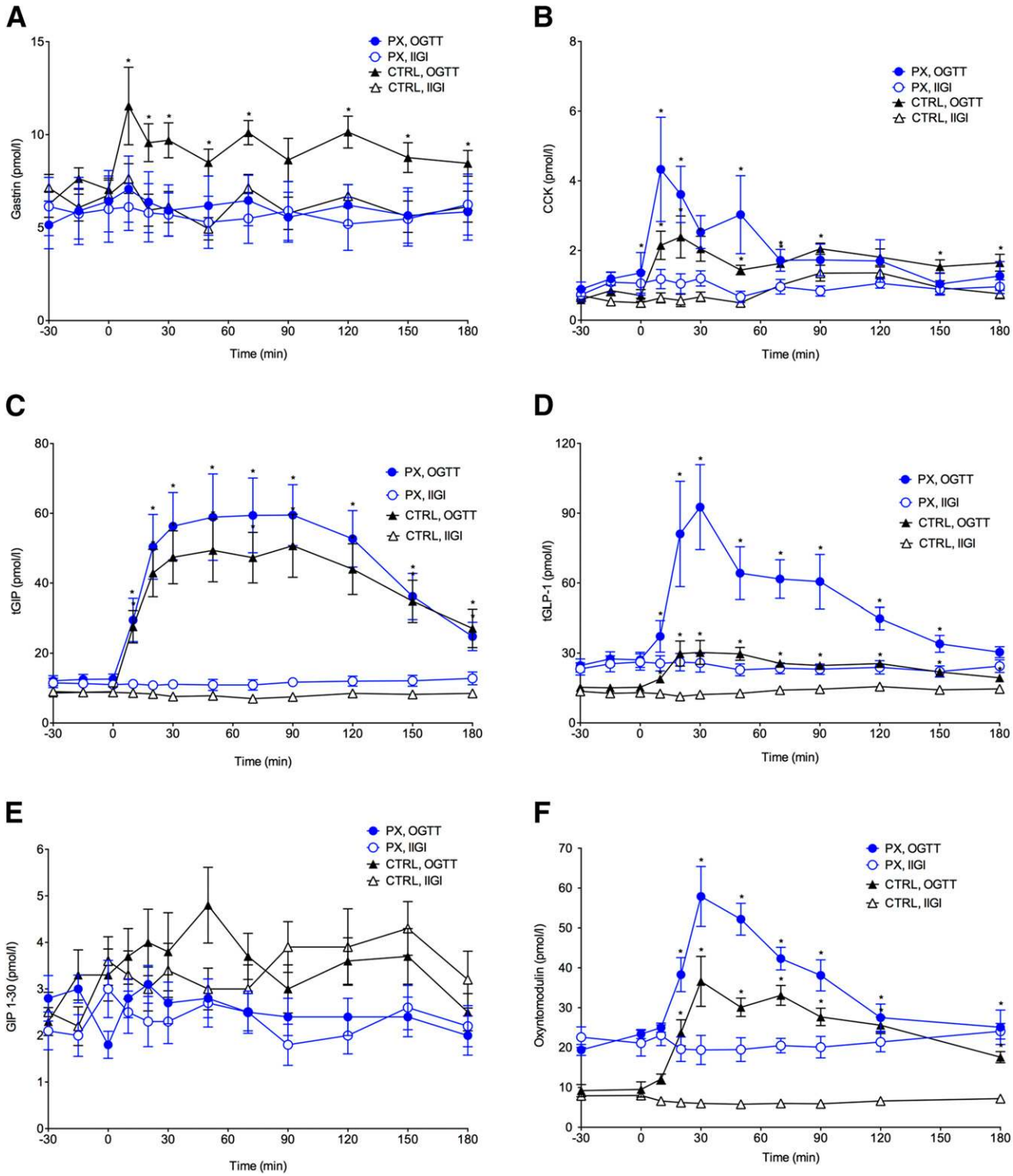

Figure 5—Plasma concentrations of gastrin (*A*), CCK (*B*), total (t) GIP (*C*), total GLP-1 (*D*), GIP(1-30) (*E*), and oxyntomodulin (*F*) during a 75-g OGTT and IIGI in totally pancreatectomized patients (PX) (n = 10) and healthy control subjects (CTRL) (n = 10). Data are mean ± SEM. Statistical analysis was performed by linear mixed effect modeling for analysis of repeated measures between days within groups. Asterisks (*) indicate significant ($P < 0.05$) differences.

the healthy control subjects during OGTT compared with IIGI (albeit the difference was considerably smaller than in the pancreatectomy group). This finding might seem counterintuitive, as significantly more insulin was secreted during the OGTT compared with the IIGI. The explanation could be that maximum insulin-mediated suppression of EGP in the healthy individuals was reached already with the insulin levels obtained during IIGI,

combined with stimulatory effects from the delayed suppression of glucagon observed during OGTT in these subjects. The higher R_a and R_d of glycerol, and, thus, the higher lipolysis rate in the pancreatectomy group compared with the control group, are likely explained by the insulin-deficient state of the patients.

In conclusion, the ascertainment of extrapancreatic glucagon and the postprandial hyperglucagonemia observed in totally pancreatectomized patients might have clinical and scientific implications. First, the present physicochemical proof that 29–amino acid glucagon is secreted from extrapancreatic tissue in humans changes the concept of glucagon being a pancreas-specific hormone and thus opens the way for a new explanation of postprandial hyperglucagonemia, as this might be a gut-dependent phenomenon. Second, the need for a larger amount of glucose during IIGI compared with the amount ingested during OGTT and the larger EGP during OGTT compared with IIGI in the pancreatectomy group reveals, in a setting of isoglycemia and without insulin in the equation, that the hyperglucagonemic response contributes to the postprandial hyperglycemia of the patients. And last, our findings suggest that gut-derived glucagon may play a hitherto unrecognized role in diabetes secondary to pancreatectomy, and possibly, although speculative, also in the pathophysiology of other conditions with paradoxical postprandial hyperglucagonemia, including type 2 diabetes.

Acknowledgments. The authors thank the participants for spending time on this project, and the authors are grateful for laboratory assistance from Sisse M. Schmidt, Sofie P. Olesen, Lene Albæk, and Alice Lieth of the University of Copenhagen. The authors also thank Julie L. Forman of the University of Copenhagen for providing statistical support and expertise.

Funding. This study was supported by the European Foundation for the Study of Diabetes–Merck Sharpe & Dohme (EFSD-MSD); The Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen; the European Molecular Biology Organization (EMBO); the European Foundation for the Study of Diabetes; the A.P. Møller Foundation for the Advancement of Medical Science; the Aase and Ejnar Danielsens Foundation; the Johan Boserup and Lise Boserup's Foundation; and the Faculty of Health and Medical Sciences, University of Copenhagen.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. A.L. conceptualized and planned the study as well as wrote applications for funding, conducted the clinical experiments, researched data, performed statistical analyses, and wrote the manuscript. J.I.B., M.C., and M.G. planned the study and helped with experiments. N.J.W.A. conducted ELISA and gel chromatography and performed mass spectrometry–based proteomic analysis of glucagon. B.H. conducted RIA analyses of GIP(1-30) and PP. E.R.M. recruited patients. C.P.H. and J.H.S. performed total pancreatectomies and recruited patients. G.v.H. processed and analyzed glucose and glycerol tracer data. J.F.R. processed and analyzed CCK and gastrin data. D.H., F.M., and M.M. performed mass spectrometry–based proteomic analysis of glucagon. S.L. and T.V. planned the study. J.J.H. provided ELISA analysis of glucagon and RIA analyses of glucagon, GLP-1, GIP, oxyntomodulin, GIP(1-30), and PP. F.K.K. conceptualized the study, wrote applications for funding, planned the study, and wrote the manuscript. All authors contributed to discussion and critically reviewed the manuscript. F.K.K. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. The main findings of this study were presented at the 75th Scientific Sessions of the American Diabetes Association, Boston, MA, 5–9 June 2015, and at the 51st Annual Meeting of the European Association for the Study of Diabetes, Stockholm, Sweden, 14–18 September 2015.

References

1. Unger RH, Orci L. Glucagon and the A cell: physiology and pathophysiology (first two parts). N Engl J Med 1981;304:1518–1524
2. Gromada J, Franklin I, Wollheim CB. Alpha-cells of the endocrine pancreas: 35 years of research but the enigma remains. Endocr Rev 2007; 28:84–116
3. Knop FK, Vilsbøll T, Madsbad S, Holst JJ, Krarup T. Inappropriate suppression of glucagon during OGTT but not during isoglycaemic i.v. glucose infusion contributes to the reduced incretin effect in type 2 diabetes mellitus. Diabetologia 2007;50:797–805
4. Meier JJ, Deacon CF, Schmidt WE, Holst JJ, Nauck MA. Suppression of glucagon secretion is lower after oral glucose administration than during intravenous glucose administration in human subjects. Diabetologia 2007;50: 806–813
5. Sutherland EW, De Duve C. Origin and distribution of the hyperglycemic-glycogenolytic factor of the pancreas. J Biol Chem 1948;175:663–674
6. Vranic M, Pek S, Kawamori R. Increased "glucagon immunoreactivity" in plasma of totally depancreatized dogs. Diabetes 1974;23:905–912
7. Muller WA, Girardier L, Seydoux J, Berger M, Renold AE, Vranic M. Extrapancreatic glucagon and glucagonlike immunoreactivity in depancreatized dogs. A quantitative assessment of secretion rates and anatomical delineation of sources. J Clin Invest 1978;62:124–132
8. Gotoh M, Monden M, Okamura J, Mori T, Shima K. Insulin and glucagon secretion after pancreatectomies. Correlation of secretion and hormonal contents of remaining pancreas. Diabetes 1989;38:861–867
9. Matsuyama T, Foà PP. Plasma glucose, insulin, pancreatic, and enteroglucagon levels in normal and depancreatized dogs. Proc Soc Exp Biol Med 1974;147:97–102
10. Doi K, Prentki M, Yip C, Muller WA, Jeanrenaud B, Vranic M. Identical biological effects of pancreatic glucagon and a purified moiety of canine gastric immunoreactive glucagon. J Clin Invest 1979;63:525–531
11. Unger RH, Ketterer H, Eisentraut AM. Distribution of immunoassayable glucagon in gastrointestinal tissues. Metabolism 1966;15:865–867
12. Boden G, Master RW, Rezvani I, Palmer JP, Lobe TE, Owen OE. Glucagon deficiency and hyperaminoacidemia after total pancreatectomy. J Clin Invest 1980;65:706–716
13. Barnes AJ, Bloom SR. Pancreatectomised man: a model for diabetes without glucagon. Lancet 1976;1:219–221
14. Dammann HG, Besterman HS, Bloom SR, Schreiber HW. Gut-hormone profile in totally pancreatectomised patients. Gut 1981;22:103–107
15. Holst JJ, Pedersen JH, Baldissera F, Stadil F. Circulating glucagon after total pancreatectomy in man. Diabetologia 1983;25:396–399
16. Villanueva ML, Hedo JA, Marco J. Plasma glucagon immunoreactivity in a totally pancreatectomized patient. Diabetologia 1976;12:613–616
17. Kåresen R, Tronier B, Aune S. Immunoreactive glucagon and insulin C-peptide in man after resection of the pancreas and total pancreatectomy. Am J Surg 1980;140:272–276
18. Sudo T, Suzuki T, Tobe T. Changes in plasma glucagon after total pancreatectomy in man. Gastroenterol Jpn 1980;15:464–468
19. Yasui K. Effects of total pancreatectomy on the secretion of gut glucagon in humans. Jpn J Surg 1983;13:122–129
20. Polonsky KS, Herold KC, Gilden JL, et al. Glucose counterregulation in patients after pancreatectomy. Comparison with other clinical forms of diabetes. Diabetes 1984;33:1112–1119

21. Bajorunas DR, Fortner JG, Jaspan J, Sherwin RS. Total pancreatectomy increases the metabolic response to glucagon in humans. J Clin Endocrinol Metab 1986;63:439–446
22. Bajorunas DR, Fortner JG, Jaspan JB. Glucagon immunoreactivity and chromatographic profiles in pancreatectomized humans. Paradoxical response to oral glucose. Diabetes 1986;35:886–893
23. Ohtsuka K, Nimura Y, Yasui K. Paradoxical elevations of plasma glucagon levels in patients after pancreatectomy or gastrectomy. Jpn J Surg 1986;16:1–7
24. Tanjoh K, Tomita R, Fukuzawa M, Hayashi N. Peculiar glucagon processing in the intestine is the genesis of the paradoxical rise of serum pancreatic glucagon in patients after total pancreatectomy. Hepatogastroenterology 2003;50:535–540
25. Albrechtsen NJW, Hartmann B, Veedfald S, et al. Hyperglucagonaemia analysed by glucagon sandwich ELISA: nonspecific interference or truly elevated levels? Diabetologia 2014;57:1919–1926
26. Meissner F, Scheltema RA, Mollenkopf H-J, Mann M. Direct proteomic quantification of the secretome of activated immune cells. Science 2013;340:475–478
27. Scheltema RA, Hauschild J-P, Lange O, et al. The Q Exactive HF, a Benchtop mass spectrometer with a pre-filter, high-performance quadrupole and an ultra-high-field Orbitrap analyzer. Mol Cell Proteomics 2014;13:3698–3708
28. Storkholm JH, Hansen CP. Mesenterico-portal vein resection in patients with pancreatico-duodenal cancer is safe and may increase survival. Dan Med J 2014;61:A4757
29. Holst JJ. Molecular heterogeneity of glucagon in normal subjects and in patients with glucagon-producing tumours. Diabetologia 1983;24:359–365
30. Bornø A, Foged L, van Hall G. Glucose and glycerol concentrations and their tracer enrichment measurements using liquid chromatography tandem mass spectrometry. J Mass Spectrom 2014;49:980–988
31. Hare KJ, Vilsbøll T, Holst JJ, Knop FK. Inappropriate glucagon response after oral compared with isoglycemic intravenous glucose administration in patients with type 1 diabetes. Am J Physiol Endocrinol Metab 2010;298:E832–E837
32. Radziuk J, Norwich KH, Vranic M. Experimental validation of measurements of glucose turnover in nonsteady state. Am J Physiol 1978;234:E84–E93
33. Bagger JI, Knop FK, Lund A, Holst JJ, Vilsbøll T. Glucagon responses to increasing oral loads of glucose and corresponding isoglycaemic intravenous glucose infusions in patients with type 2 diabetes and healthy individuals. Diabetologia 2014;57:1720–1725
34. Fujita Y, Wideman RD, Asadi A, et al. Glucose-dependent insulinotropic polypeptide is expressed in pancreatic islet alpha-cells and promotes insulin secretion. Gastroenterology 2010;138:1966–1975
35. Holst JJ. Extrapancreatic glucagons. Digestion 1978;17:168–190
36. Dunning BE, Gerich JE. The role of alpha-cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications. Endocr Rev 2007;28:253–283
37. Bak MJ, Albrechtsen NW, Pedersen J, et al. Specificity and sensitivity of commercially available assays for glucagon and oxyntomodulin measurement in humans. Eur J Endocrinol 2014;170:529–538
38. Sandoval DA, D'Alessio DA. Physiology of proglucagon peptides: role of glucagon and GLP-1 in health and disease. Physiol Rev 2015;95:513–548
39. Vrang N, Grove K. The brainstem preproglucagon system in a non-human primate (*Macaca mulatta*). Brain Res 2011;1397:28–37
40. Campbell JE, Drucker DJ. Islet α cells and glucagon–critical regulators of energy homeostasis. Nat Rev Endocrinol 2015;11:329–338
41. Kilimnik G, Kim A, Steiner DF, Friedman TC, Hara M. Intraislet production of GLP-1 by activation of prohormone convertase 1/3 in pancreatic α-cells in mouse models of ß-cell regeneration. Islets 2010;2:149–155
42. Marchetti P, Lupi R, Bugliani M, et al. A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets. Diabetologia 2012;55:3262–3272
43. Claridge LE, Valiyapurayil NV, Nguyen NQ, Rayner CK, Wu T, Burgess JE, et al. Regional variation in expression of enzymes that synthesize incretin hormones and glucagon in lean and morbidly obese humans. Diabetes 2015;64 (Suppl. 1):A528–529
44. Rhee NA, Wahlgren CD, Pedersen J, et al. Effect of Roux-en-Y gastric bypass on the distribution and hormone expression of small-intestinal enteroendocrine cells in obese patients with type 2 diabetes. Diabetologia 2015;58:2254–2258
45. Jørgensen NB, Dirksen C, Bojsen-Møller KN, et al. Exaggerated glucagon-like peptide 1 response is important for improved β-cell function and glucose tolerance after Roux-en-Y gastric bypass in patients with type 2 diabetes. Diabetes 2013;62:3044–3052
46. Gelling RW, Du XQ, Dichmann DS, et al. Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice. Proc Natl Acad Sci USA 2003;100:1438–1443
47. Vuguin PM, Kedees MH, Cui L, et al. Ablation of the glucagon receptor gene increases fetal lethality and produces alterations in islet development and maturation. Endocrinology 2006;147:3995–4006
48. Zhou C, Dhall D, Nissen NN, Chen C-R, Yu R. Homozygous P86S mutation of the human glucagon receptor is associated with hyperglucagonemia, alpha cell hyperplasia, and islet cell tumor. Pancreas 2009;38:941–946
49. Solloway MJ, Madjidi A, Gu C, et al. Glucagon couples hepatic amino acid catabolism to mTOR-dependent regulation of α-cell mass. Cell Reports 2015;12:495–510
50. Longuet C, Robledo AM, Dean ED, et al. Liver-specific disruption of the murine glucagon receptor produces α-cell hyperplasia: evidence for a circulating α-cell growth factor. Diabetes 2013;62:1196–1205