Increased postprandial responses of GLP-1 and GIP in patients with chronic pancreatitis and steatorrhea following pancreatic enzyme substitution

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1Department of Internal Medicine F, Gentofte Hospital, University of Copenhagen, Hellerup; 2Department of Internal Medicine M, Glostrup Hospital, University of Copenhagen, Glostrup; 3Department of Biostatistics, Novo Nordisk, Bagsvaerd; 4Department of Endocrinology, Hvidovre Hospital, University of Copenhagen, Hvidovre; and 5Department of Medical Physiology, The Panum Institute, University of Copenhagen, Copenhagen, Denmark

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Increased postprandial responses of GLP-1 and GIP in patients with chronic pancreatitis and steatorrhea following pancreatic enzyme substitution. Am J Physiol Endocrinol Metab 292: E324–E330, 2007. First published September 5, 2006; doi:10.1152/ajpendo.00059.2006.—We aimed to investigate how assimilation of nutrients affects the postprandial responses of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinoctropic polypeptide (GIP) and to evaluate the effect of pancreatic enzyme substitution (PES) on insulin secretion in patients with chronic pancreatitis (CP) and pancreatic exocrine insufficiency (PEI). Eight male patients with CP and PEI were studied. Blood was sampled frequently on two separate days after ingestion of a liquid meal with and without PES, respectively. Eight healthy male subjects served as a control group. β-Cell responsiveness was estimated as changes in insulin secretion rates in response to changes in postprandial plasma glucose (PG). There was no difference in the PG incremental area under curve (AUC) for patients with and without PES [406 ± 100 vs. 425 ± 80 mM·4 h (mean ± SE), P = 0.8]. The response of total GLP-1 was higher after PES (AUC: 7.8 ± 1.2 vs. 5.3 ± 0.6 nM·4 h, P = 0.01), as was the response of total GIP (AUC: 32.7 ± 7.5 vs. 21.1 ± 8.3 nM·4 h, P = 0.01). Concurrently, both plasma insulin, plasma C-peptide, and total insulin secretion increased after PES (AUC: 17.7 ± 4.2 vs. 13.6 ± 2.9 nM·4 h, P = 0.02; 237 ± 31.4 vs. 200 ± 27.4 nM·4 h, P = 0.005; and 595 ± 82 vs. 497 ± 80 pmol·kg−1·4 h, P = 0.01, respectively). β-Cell responsiveness to glucose was not significantly different on the two study days for patients with CP. These results suggest that the secretion of GLP-1 and GIP is under influence of the digestion and absorption of nutrients in the small intestine and that PES increases insulin secretion.

pancreatic exocrine insufficiency; glucagon-like peptide-1; glucose-dependent insulinoctropic polypeptide

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) and glucose-dependent insulinoctropic polypeptide (GIP) are insulinoctropic intestinal peptide hormones, the so-called “incretin hormones”. Both are secreted in response to oral ingestion of nutrients with lipids and simple carbohydrates being the most potent stimulators of secretion (11, 16). The incretin hormones are responsible for up to 70% of the insulin secretion during oral ingestion of glucose (30). GLP-1 is secreted from the endocrine mucosal L cells, found predominantly in the distal part of the small intestine, whereas GIP is released from the endocrine mucosal K cells, situated predominantly in the duodenum and proximal jejenum. The mechanisms underlying the release of the incretin hormones are poorly understood. Studies using α-glucosidase inhibitors or the gastrointestinal lipase inhibitor orlistat, which increase the transit of sucrose and fat, respectively, to the ileum suggest that the secretion of incretin hormones is related to the presence of nutrients in the lumen of the small intestine (6, 33). In 1980, Ebert and Creutzfeldt (12) showed that pancreatic enzyme substitution (PES) could improve the postprandial response of GIP in patients with chronic pancreatitis (CP) and pancreatic exocrine insufficiency (PEI), suggesting a relationship between digestion and absorption of nutrients and the secretion of GIP. To our knowledge, no data on postprandial responses of GLP-1 in patients with CP exist. Therefore, the present study aimed to address the following questions. 1) Is GLP-1 secretion related to the mere presence of nutrients in the lumen of the intestine, or is assimilation of nutrients also involved? 2) Is the postprandial response of GLP-1 normal in patients with CP? 3) Will PES increase the postprandial response of GLP-1 and GIP and thereby increase insulin secretion during a meal in patients with CP and PEI? Thus we hypothesized that an increased secretion of one or both of the two incretin hormones following a meal supplemented with PES would increase insulin secretion and thereby improve glucose tolerance. However, exact assessing of postprandial β-cell function is complicated, since the absolute insulin secretion differs in situations with different plasma glucose (PG) levels. Nevertheless, measurements of plasma C-peptide concentrations make it possible to reconstruct insulin secretion rates (ISR) during a meal. Therefore, in the present study, we used a C-peptide-based mathematical model to evaluate β-cell function independent of the PG level by describing changes in ISR relative to changes in PG concentrations.

MATERIALS AND METHODS

Patients. Eight male patients with CP and PEI and eight individually matched (sex, BMI, and age) healthy control subjects without family history of diabetes mellitus (DM) were studied (Table 1). All patients with CP were without signs of acute inflammatory activity in the pancreas. The etiology of CP was judged to be alcoholism in seven patients and idiopathic in one patient. None of the patients drank alcohol on a daily basis, and there were no clinical or biochemical signs of affected liver function. The diagnostic criteria of
CP were according to Layer et al. (28), and all CP subjects had reduced meal-stimulated duodenal concentrations of lipase and amylase and hyperexcreted fat in the stool (Table 1). Additionally, unequivocal morphological changes of the pancreas were evident by ultrasonography, CT scan, magnetic resonance cholangiopancreatography, or endoscopic retrograde cholangiopancreatography according to the Cambridge classification (4). All patients were being treated with PES on a daily basis to alleviate steatorrhea (Table 1).

The study was designed primarily to examine the postprandial incretin responses with and without PES, respectively, in patients with CP and PEI and secondarily to establish whether the postprandial incretin responses of patients with CP and PEI were normal compared with healthy control subjects. The patients with CP were studied on two separate occasions. On day 1, after an overnight (10-h) fast, the subjects were studied in a seated position with a cannula inserted in the retrograde direction in a dorsal hand vein for collection of arterialized blood samples. The cannulated hand was placed in a heating box (42°C) throughout the experiment. The patients ingested a liquid meal consisting of 100 g of NAN 1 [2,170 kJ: 9.5% protein (2.8% casein and 6.7% whey), 58% lactose, and 27.7% fat (11.3% saturated, 10% monounsaturated, and 4.7% polyunsaturated); Nestlé Nutrition, Nunspeet, The Netherlands] dissolved in 300 ml of H2O, over the first 15 min of the experiment, and consumed two capsules of PES (Creon, 25,000 U; Solvay Pharma, Herlev, Denmark) with the first sip of the meal. Each capsule contained 18 kIU pancreatic amylase, 25 kIU pancreatic lipase, and 1 kIU pancreatic protease in enterosoluble-coated granules. The capsules dissolve in the stomach, whereas the acid-protected granules dissolve when they reach the duodenum (8). At least 28 kIU lipase must be taken to abolish steatorrhea, giving rise to a concentration of ∼25 kIU lipase/liter in the duodenum (19, 27). In healthy subjects, ingestion of a standard meal results in secretion of 300 kIU pancreatic lipase to the duodenum, resulting in an intraduodenal concentration of 600 kIU/liter (20). PES administration to healthy subjects is therefore meaningless.

Arterialized blood was drawn 15, 10, and 0 min before and 15, 30, 45, 60, 75, 90, 120, 150, 180, and 240 min after ingestion of the liquid meal. Blood was distributed into chilled tubes containing heparin or EDTA plus aprotinin (500 kIU/ml blood TrasyloL; Bayer, Leverkusen, Germany) and a specific dipetidyl peptidase IV (DPP-IV) inhibitor (valine-tyrosolide, final concentration 0.01 mM; Novo Nordisk, Bagsvaerd, Denmark) for analyses of peptides and free fatty acids (FFA). These tubes were immediately cooled on ice. For serum triglyceride (TG) analysis, blood was distributed into empty tubes and kept at room temperature to coagulate. The tubes were centrifuged for 20 min at 3,000 rpm and 4°C. Plasma for GIP, GLP-1, glucagon, and FFA analyses and serum for TG analyses were stored at −20°C, and plasma for insulin and C-peptide analyses was stored at −80°C until analysis. For bedside measurement of PG, blood was distributed into fluoride tubes and centrifuged immediately for 2 min at 10,000 rpm at room temperature.

The patients abstained from taking their regular PES for a wash-out period of 3 days before the second experimental day. On that day (henceforth day 2), the patients were studied as described above but without ingestion of the two capsules of PES. The control subjects were studied once, following the same protocol as the patients on the second experimental day.

Analysis. PG concentrations were measured during the experiments by the glucose oxidase method, using a glucose analyzer (model YSI 2300 STAT plus analyzer; Yellow Springs Instrument, Yellow Springs, OH).

Plasma samples were assayed for total GLP-1 immunoreactivity, as previously described by Örskov et al. (32), using a radioimmunoassay (antiserum no. 89390), which is specific for the COOH terminus of the GLP-1 molecule and reacts equally with intact GLP-1 and the primary (NH2-terminally truncated) metabolite with a detection limit of 1.0 pM. Intact GLP-1 was measured using an enzyme-linked immunosorbent assay, as previously described (31, 38). The assay is a two-site sandwich assay using two monoclonal antibodies: GLP-1F5 as catching antibody (COOH-terminally directed) and MAb26.1 as detecting antibody (NH2-terminally directed). The detection limit for the assay is 1.0 pM.

All subjects agreed to participate after receiving oral and written information.

### Methods

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### Table 1. Subject characteristics and parameters of exocrine pancreatic function

<table>
<thead>
<tr>
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<th>Patients with Chronic Pancreatitis (8 males)</th>
<th>Healthy Control Subjects (8 males)</th>
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<tbody>
<tr>
<td><strong>Subject characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>57 (48–66)</td>
<td>58 (45–69)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21 (15–27)</td>
<td>23 (20–26)</td>
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<td>Waist-to-hip ratio</td>
<td>1.0 (0.90–1.04)</td>
<td>1.0 (0.97–1.02)</td>
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<tr>
<td>FPG, mM</td>
<td>6.2 (5.1–7.7)</td>
<td>5.4 (4.5–6.0)</td>
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<tr>
<td>Hb A₁c, %</td>
<td>6.0 (4.8–6.8)</td>
<td>5.3 (4.9–5.5)</td>
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†Meal-stimulated duodenal concentration of digestive enzymes (kU/l):
- Lipase; normal range: 264–1,330 kU/l
- Amylase; normal range: 47–269 kU/l
- Pancreatic amylase (mmol/24 h); normal range: <25
- Enzymatic treatment (kEP/c/day):
  - Amylase: 66±13 kU/l
  - Lipase: 88±19 kU/l
  - Protease: 4±1 kU/l

*Subject characteristics are reported as means with ranges in parentheses.
†Parameters of exocrine pancreatic function are reported as means ± SE. FPG, fasting plasma glucose; NA, not applicable.
Total GIP was measured, as described previously, using the COOH-terminally directed antiserum R65 (24, 25), which reacts fully with intact GIP, and the NH₂-terminally truncated metabolite. Intact, biologically active GIP was measured as previously described (7), using antiserum no. 98171. The detection limits of the assays are 2.0 pM.

Plasma insulin and C-peptide concentrations were measured using commercial AutoDELFIA Time-resolved fluoroimmunoassay (Wallac Oy, Turku, Finland), as previously described (38).

The glucagon assay is directed against the COOH terminus of the glucagon molecule (antibody code no. 4305) and therefore measures glucagon of mainly pancreatic origin (31).

Plasma FFA and serum TG were quantified using enzymatic colorimetric methods (Wako, Richmond, VA, and Roche Diagnostics, Basel, Switzerland, respectively).

Calculations and statistical analysis. All results are expressed as means ± SE. Area under the curve (AUC) values were calculated using the trapezoidal rule and are presented as the incremental values if nothing else is stated.

The parameter of β-cell responsiveness was derived from mathematical analyses of PG and C-peptide concentrations (5, 18, 21, 23). First, ISR was calculated by deconvolution of measured C-peptide concentrations as described previously (21, 23, 35). ISR is presented by cubic splines and expressed as picomoles per minute per kilogram of body weight. Changes in ISR in response to changes in PG during the meal express the efficacy by which changes in PG concentrations stimulate insulin secretion. This β-cell index (β-cell responsiveness to glucose) is the sum of the β-cell sensitivity to glucose per se and potentiating factors (nonglucose secretagogues, incretin hormones, neurotransmitters, and cephalic reflexes) that modulate the dose-response relationship between insulin secretion and PG. For each subject, β-cell responsiveness was evaluated by plotting ISR against PG at individual time points to establish the dose relationship on each experimental day. The slope of the individual line was used as an index of β-cell response to glucose (and potentiating factors). The steeper the line, the more sensitive the β-cell is to changes in PG concentration. A change in ISR induced by a change in PG of 1 mM is expressed as picomoles of insulin secreted per minute per kilogram of body weight.

The homeostatic model assessment (HOMA) was used to obtain a quantitative assessment of insulin resistance (HOMA-IR) (29).

According to the design of the study, the primary end points were to test effects of PES within the group of patients with CP and to compare the responses with those of healthy subjects. These comparisons were performed directly by standard statistical methods. Comparisons of experimental determinations in which the data were distributed normally were made with a two-tailed t-test (paired within the group of patients with CP, unpaired between patients with CP and healthy control subjects). For data that did not follow a normal distribution, the significance of differences between groups was tested using the Mann-Whitney U-test, and for within-subject comparisons the Wilcoxon test for paired differences was used. Time course measurements were compared using repeated-measures ANOVA. A P value of <0.05 was considered to be statistically significant.

The study design was approved by the Scientific-Ethical Committee of the County of Copenhagen, February 2004 (registration no. in the committee: KA 02144), and the study was conducted according to the principles of the Helsinki Declaration II.

RESULTS

Glucose. No differences in FPG between patients on day 1 and 2 or between control subjects and patients (on day 1 or day 2) were observed. PG concentrations increased during the first 60–75 min, with peak values of 10.3 ± 0.3 mM, 9.6 ± 0.5 mM and 7.1 ± 0.2 mM for day 1, day 2, and control subjects, respectively (Fig. 1). AUC values did not differ significantly between day 1 and day 2 (406 ± 100 vs. 425 ± 80 mM·h, P = 0.8) for the patients but were greater than in healthy control subjects (84 ± 29 mM·h) on both days (P = 0.001 and 0.008, respectively).

GLP-1. Time courses for both total and intact GLP-1 are shown in Fig. 2. No significant differences in basal values were observed. Total GLP-1 concentrations increased following the meal to reach maximum values after 30, 45, and 45 min in the three data sets (day 1, day 2, and controls), respectively. The corresponding time points for the intact form of GLP-1 were
30, 60, and 45 min, respectively. At the end of the experiments, total and intact concentrations were returning toward basal levels in all three sets of data. AUC for total GLP-1 was significantly greater on day 1 compared with day 2 (7.8 ± 1.2 vs. 5.3 ± 0.6 nM·h, P = 0.01). The difference in AUC for intact GLP-1 (2.9 ± 1.1 vs. 2.5 ± 1.1 nM·h, P = 0.3) was not statistically significant. No significant AUC differences were observed between the patients (on day 1 or 2) and the healthy control subjects (total GLP-1 AUC: 5.1 ± 0.7; intact GLP-1 AUC: 1.6 ± 0.5 nM·h), indicating a preserved post-prandial response of GLP-1 in patients with CP.

**GIP.** No significant differences in basal values were observed. Time courses for both total and intact GIP are shown in Fig. 2. Both intact and total GIP concentrations increased following the meal to reach maximum values after 60 (day 1) and 45 min (day 2), respectively [control group: 30 (intact) and 60 min (total)]. Maximum value of total GIP was significantly higher during day 1 compared with day 2 (375 ± 77 vs. 270 ± 84 pM, P = 0.04). Total and intact concentrations had returned to their basal values by 4 h after meal ingestion in all three sets of data (Fig. 2). AUC for total and intact GIP on day 1 were higher compared with day 2 (32.7 ± 7.5 vs. 21.1 ± 8.3 nM·h, P = 0.01; and 12.3 ± 2.9 vs. 7.5 ± 2.3 nM·h, P = 0.04, respectively). No significant AUC differences were observed between the patients (on day 1 or 2) and the healthy control subjects (total GIP AUC: 33.4 ± 5.6 nM·h; intact GIP AUC: 11.3 ± 2.2 nM·h), indicating a preserved secretion of GIP among the patients.

**Insulin and C-peptide.** There were no differences between fasting values on day 1 and day 2 for plasma insulin or C-peptide, but the fasting C-peptide value for healthy control subjects was higher than the fasting value for day 2 (603 ± 117 vs. 292 ± 58 pM, P = 0.03) and tended to be higher than the fasting value for day 1 (603 ± 117 vs. 344 ± 60 pM, P = 0.07). Maximum concentrations of both peptides were attained earlier in the control subjects than in the patients (Fig. 1). After the meal, total AUC differed significantly between day 1 and day 2 for both insulin (17.7 ± 4.2 vs. 13.6 ± 2.9 nM·h, P = 0.02) and C-peptide (237 ± 31.4 vs. 200 ± 27.4 nM·h, P = 0.005). No significant AUC differences were observed between the patients (day 1 or 2) and the healthy control subjects (insulin AUC: 12.2 ± 3.3 nM·h; C-peptide AUC: 131 ± 17.1 nM·h).

**Insulin secretion and β-cell responsiveness.** The dynamic profiles of the average ISR differed among patients and healthy control subjects (Fig. 3). Nevertheless, the total and incremental amounts of insulin secreted during the meal were not different between the two groups. In the patients with CP, the total (860 ± 109 vs. 726 ± 94 pmol·kg<sup>−1</sup>·h<sup>−1</sup>; P = 0.003) and incremental (595 ± 82 vs. 497 ± 80 pmol·kg<sup>−1</sup>·h<sup>−1</sup>; P = 0.01) amounts of insulin secreted were highest on the day with PES (day 1).

β-Cell responsiveness to changes in PG concentrations during the meal in patients with CP, expressed as the slope of the linear relationship between the calculated ISR and the concomitant PG concentration, was not statistically significantly dif-
differences were observed between the experiments in all three sets of data. No significant AUC FFAs concentrations were approaching basal levels at the end of day 1 or day 2 (0.94 ± 0.19 vs. 0.78 ± 0.12 pmol·min⁻¹·kg⁻¹·mM⁻¹, *P = 0.3*). β-Cell responsiveness was significantly higher in the healthy control subjects compared with patients with CP (both on day 1 and day 2) (3.0 ± 0.4 pmol·min⁻¹·kg⁻¹·mM⁻¹, *P = 0.0003 and 0.0001*, respectively).

According to HOMA-IR, no difference in insulin sensitivity was observed either between day 1 and day 2 among the patients with CP or between the group of healthy control subjects and patients with CP on day 1 and day 2, respectively.

Glucagon. Glucagon concentrations on day 1 (with PES) increased to maximum values (10.4 ± 1.4 pM) 45 min after ingestion of the meal and returned to basal values (8.4 ± 1.2 pM) after 120 min. This pattern was very similar to the one observed among the healthy control subjects. No change (flat line) from basal values (7.8 ± 0.3 pM) was observed in the patients on day 2 (without PES). There was a significant increase in total AUC<sub>0-90 min</sub> for plasma glucagon on day 1 compared with day 2 (881 ± 105 vs. 748 ± 73 pM·h; *P = 0.03*), but no significant AUC differences were seen between the patients (day 1 or 2) and the healthy control subjects (total AUC<sub>0-90 min</sub>: 834 ± 111 pM·h; *P = 0.93 and 0.29*, respectively).

FFA. A physiological insulin-induced inhibition of lipolysis was observed as a decrease in FFA following ingestion of the liquid meal in all three sets of data. The basal concentrations in the group of patients with CP (0.59 ± 0.11 and 0.52 ± 0.07 mM for day 1 and day 2, respectively) decreased to nadirs of 0.17 ± 0.06 and 0.16 ± 0.07 mM, respectively, 120 min after ingestion of the two meals. The corresponding values for the healthy control group were 0.37 ± 0.08 mM (basal) and 0.13 ± 0.02 mM (nadir; 75 min after ingestion), respectively. FFA concentrations were approaching basal levels at the end of the experiments in all three sets of data. No significant AUC differences were observed between day 1 and day 2 (−74.3 ± 21.2 vs. −60.8 ± 10.9 mM·h, *P = 0.6*) or between the control subjects (−31.3 ± 17.0 mM·h) and day 1 (*P = 0.14*) and day 2 (*P = 0.17*), respectively.

TG. A tendency toward increased AUC for TG on day 1 compared with day 2 (43.0 ± 20.4 vs. 2.5 ± 17.9 mM·h·P = 0.2) was observed as a sign of increased assimilation of fat after ingestion of the meal with PES. A significant AUC difference was observed between day 2 (without PES) and the control group (2.5 ± 17.9 vs. 79.7 ± 19.6 mM·h·P = 0.02) but not between day 1 and the control group.

**DISCUSSION**

The present study reveals a preserved postprandial GLP-1 response in patients with CP and PEI. By supplementing the meal with PES, we were able to show a relationship between assimilation of nutrients and the level of postprandial GLP-1 secretion. A similar relationship has previously been described for GIP (12). In the present study, patients with CP and established PEI participated. By supplementing the day 1 meal with PES, the digestive capacity was increased compared with day 2 supported by increased concentrations of TG and glucagon. The rapid GLP-1 response to a meal (13) has led some authors to suspect that secretion of GLP-1 is mediated not only through direct luminal contact of nutrients with lower gut mucosal endocrine L cells but also through indirect neuroendocrine mechanisms (11). With or without the involvement of these mechanisms, our study supports the notion that GLP-1 secretion is stimulated by the presence of nutrients in the gut in combination with the level of assimilation of such nutrients.

In this study, we measured both the intact and the total (intact + cleaved) forms of GIP and GLP-1. The total concentrations are indicators of the overall levels of secretion, whereas the intact levels are indicators of the exact concentrations of active hormones in the peripheral circulation and, therefore, the impact on the β-cells. Plasma levels of both intact and total GIP, as well as total GLP-1, increased significantly after PES, but there was only a trend toward increased levels of intact GLP-1. The latter observation is probably due to the fact that, because GLP-1 is subject to degradation by DPP-IV almost immediately upon its release (15), only 10–15% of intact GLP-1 actually reaches the systemic circulation (17), thereby reducing the possibility of detecting the response in the peripheral circulation. In the present study, 25% of the measurements for intact GLP-1 fell below the 1 pM detection limit. These undetectable GLP-1 concentrations occurred mainly during the basal state and during the last hour of each experiment. In contrast, the total GLP-1 response will reflect the actual rate of secretion of the L cells and the subsequent activation of sensory afferent nerves that, by reflex, may stimulate the pancreatic β-cells. In the present study, the postprandial response of total GLP-1 in patients with CP following PES exceeded the postprandial response in the healthy control subjects, whereas the response for total GIP following PES was the same in the two groups. This discrepancy is best explained by the different anatomic localization of K and L cells, suggesting that the liquid meal stimulates the L cells of the distal part of the small intestine to a greater extent in patients with CP and PEI than in healthy subjects. The reason for this could be attributed to the fact that the assimilation of nutrients in patients with CP and PEI is greatly reduced, giving rise to a larger amount of digested (with PES) or undigested (without PES) nutrients in the distal part of the small intestine.

We hypothesized that an increased response of one or both of the two incretin hormones on the day with PES would
enhance β-cell responsiveness, but surprisingly only a minor and nonsignificant improvement in β-cell responsiveness was observed. To obtain a well-defined estimate of the effect of glucose and nonglucose stimulation on insulin secretion, we applied a two-step approach. First, we estimated ISR by deconvolution (21, 23, 35) to quantitate prehepatic insulin secretion. Thereafter, to estimate the changes in ISR in response to changes in PG concentrations during the meal, we cross-correlated the ISR with the concomitant PG concentration. The slope of the line was used as an index of β-cell responsiveness (5, 18, 21, 23). β-Cell responsiveness is a composite index, and its strength is that it provides an overall measure of postprandial β-cell responsiveness. Its weakness is that it does not allow glucose effect per se and nonglucose potentiation of postprandial insulin secretion, i.e., by incretin hormones, to be individually evaluated.

We found an increased insulin secretion during the meal on day 1 (with PES) compared with day 2 (relative increase: 20%), but without a concomitant improvement in glucose tolerance. Nevertheless, a trend of increased β-cell responsiveness was observed following PES (relative increase: 21%, \( P = 0.3 \)). When we scrutinized our data, it was revealed that this trend was carried out exclusively by the single patient with CP and normal glucose tolerance (1.5 vs. 0.8 pmol·min\(^{-1}\)·kg\(^{-1}\)·mM\(^{-1}\)). No difference in β-cell responsiveness was observed in patients with reduced glucose tolerance (0.85 vs 0.78, pmol·min\(^{-1}\)·kg\(^{-1}\)·mM\(^{-1}\), \( P = 0.6 \)). This is somewhat a contrast to the observation made by Ebert and Creutzfeldt (12) showing a robust and significant increase in the postprandial insulin-to-glucose ratio in patients with CP and PEI following PES. This could be attributed to the fact that none of the patients with CP in the study by Ebert and Creutzfeldt had “overt” diabetes even though all the patients had pathological oral glucose tolerance (the 60- + 120-min sum of blood glucose levels after ingestion of 75-g glucose being above 18 mM). Interestingly, Fölsch et al. (14) studied rats with exocrine pancreatic atrophy and found that the ability of the incretin hormones to elicit insulin secretion more or less depends on a normal glucose tolerance and not the exocrine status of the pancreas. The current data cannot elucidate in detail the mechanisms underlying the greater insulin response following administration of PES, but it is likely explained by the increase in digestive capacity and the enhanced absorption of nutrients. The increased insulin secretion might also explain why the glucose tolerance was similar on day 1 and day 2 even though assimilation was enhanced after PES.

Our results indicate that the significantly increased incretin hormone responses following PES administration had no significant effect on β-cell responsiveness to changes in PG concentrations. How is that explained when both total and intact GIP and total GLP-1 responses were increased? In a previous study (36), we demonstrated a severely impaired GIP action on insulin secretion in patients with CP and secondary DM. Therefore, the modulating effect of GIP on β-cell responsiveness in these particular patients might be minor or absent, since all patients except one displayed abnormal glucose tolerances. Furthermore, a decreased potency of GLP-1, as observed in patients with T2DM (22), might have contributed.

Even though the study design is straightforward, the weaknesses of our study need to be discussed. The progressive nature of CP results in a substantial number of patients with PEI also having secondary glucose intolerance (3, 9, 26). It has been shown that patients with T2DM have reduced postprandial responses of GLP-1 compared with healthy control subjects (34, 37). From this point of view, one can argue that our results would be more conclusive if the patients with CP and PEI had displayed normal glucose tolerance, but since very few patients with CP and PEI maintain a normal glucose tolerance (3, 9, 26) this study was set up to examine the relation between PEI and postprandial GLP-1 responses regardless of endocrine status. Our control group was deliberately chosen to consist of glucose-tolerant subjects, making it possible to evaluate whether the incretin responses of patients with CP and PEI were different from those of healthy subjects. The control group was examined for only 1 day (without PES) because of the unlikelihood that adding enzymes to subjects with normal endogenous enzyme secretion would affect these much higher enzyme concentrations and, therefore, any motor or secretory function in the gastrointestinal tract. Another limitation of our study is the lack of data on gastric emptying rates. One can speculate that the observed postprandial rise in incretin levels can be ascribed to mechanisms of PES inflicting changes in gastric emptying rates. Against this is the observation made by Ebert and Creutzfeldt (12) that PES does not change the GIP response to oral glucose in patients with CP and PEI. Last, we wanted to study the patients with CP as close as possible to their habitual gastrointestinal condition (10). Therefore, the patients were examined with PES during the first experimental day (day 1). The wash-out period of 3 days was chosen to leave time enough for steatorrhea to develop before day 2. However, it cannot be excluded, even though it is unlikely, that this design had an influence on the results.

With the present study, we show that the postprandial response of GLP-1 is preserved in patients with CP and PEI compared with matched healthy control subjects. We confirm the observations made by Ebert and Creutzfeldt in 1980, suggesting a relationship between assimilation of nutrients and the postprandial response of GIP (14) and show that the same mechanisms seem to be in play with regard to GLP-1. Thus the secretion of incretin hormones seems to be related not only to the mere presence of nutrients in the small intestine but also to the assimilation of such nutrients.

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