*Original Article*

# Reduced Incretin Effect in Type 2 Diabetes

## Cause or Consequence of the Diabetic State?

**Filip K. Knop,[1,2] Tina Vilsbøll,[1] Patricia V. Højberg,[1] Steen Larsen,[3] Sten Madsbad,[4] Aage Vølund,[5] Jens J. Holst,[2] and Thure Krarup[1]**

**We aimed to investigate whether the reduced incretin effect observed in patients with type 2 diabetes is a primary event in the pathogenesis of type 2 diabetes or a consequence of the diabetic state. Eight patients with chronic pancreatitis and secondary diabetes (A1C mean [range] of 6.9% [6.2–8.0]), eight patients with chronic pancreatitis and normal glucose tolerance (NGT; 5.3 [4.9–5.7]), eight patients with type 2 diabetes (6.9 [6.2–8.0]); and eight healthy subjects (5.5 [5.1–5.8]) were studied. Blood was sampled over 4 h on 2 separate days after a 50-g oral glucose load and an isoglycemic intravenous glucose infusion, respectively. The incretin effect (100% × [β-cell secretory response to oral glucose tolerance test − intravenous β-cell secretory response]/β-cell secretory response to oral glucose tolerance test) was significantly ($P < 0.05$) reduced (means ± SE) in patients with chronic pancreatitis and secondary diabetes (31 ± 4%) compared with patients with chronic pancreatitis and NGT (68 ± 3) and healthy subjects (60 ± 4), respectively. In the type 2 diabetes group, the incretin effect amounted to 36 ± 6%, significantly ($P < 0.05$) lower than in chronic pancreatitis patients with NGT and in healthy subjects, respectively. These results suggest that the reduced incretin effect is not a primary event in the development of type 2 diabetes, but rather a consequence of the diabetic state. *Diabetes* 56: 1951–1959, 2007**

The phenomenon that oral glucose elicits a higher insulin response than intravenous glucose at identical plasma glucose (PG) profiles (isoglycemia) is called the incretin effect. The incretin effect is conveyed by the two incretin hormones glucagon-like peptide (GLP-1) 1 and glucose-dependent insulinotropic polypeptide (GIP) (1). Both hormones are secreted from the small intestine in response to ingestion of nutrients (1,2). They are highly insulinotropic in a strictly glucose-dependent fashion (1). In type 2 diabetes, the incretin effect has been shown to be markedly reduced (3). This incretin defect is accompanied by a reduced GLP-1 response to a mixed meal (4,5), a decreased insulinotropic potency of GLP-1 (6), and an almost complete loss of late-phase insulin secretion in response to GIP (7). In addition, we have recently shown that the suppression of glucagon secretion is impaired during oral glucose tolerance tests (OGTTs) as opposed to isoglycemic intravenous glucose infusion in patients with type 2 diabetes (8). The pathophysiological background for the reduced incretin effect observed in patients with type 2 diabetes is unclear. In the current study we exploited the fact that patients with chronic pancreatitis eventually develop diabetes secondary to their inflammatory condition in the pancreas (9–12). If patients with chronic pancreatitis and secondary diabetes exhibit reduced incretin effect and patients with chronic pancreatitis and normal glucose tolerance (NGT) are normal in that regard, it is likely that the reduced incretin effect is a consequence of the diabetic state. On the other hand, if the incretin effect is preserved independently of the diabetic state of patients with chronic pancreatitis, the incretin defect in type 2 diabetes could be a primary pathogenetic defect. Therefore, in the current study, the classical isoglycemic technique was applied to gauge the incretin effect in patients with chronic pancreatitis and NGT and in patients with chronic pancreatitis and secondary diabetes. Healthy subjects and patients with type 2 diabetes were studied for comparison.

## RESEARCH DESIGN AND METHODS

Group 1 consisted of eight patients with chronic pancreatitis and secondary diabetes diagnosed according to criteria of the World Health Organization (13). Subject characteristics are shown in Table 1. In group 1 subjects, diabetes developed after the diagnosis of chronic pancreatitis had been established, and none of the patients had first-degree relatives with diabetes. Five were treated with diet alone, two with a sulfonylurea, and one with metformin and a sulfonylurea. Five patients in this group suffered from exocrine pancreatic insufficiency and were treated with pancreatic enzyme supplementation to alleviate steatorrhea. The etiology of chronic pancreatitis was judged to be alcoholism in seven patients and idiopathic in one patient.

Group 2 consisted of eight patients with chronic pancreatitis and NGT (PG concentration at 120 min after 75-g OGTT [$PG_{120\ min}$] of <7.8 mmol/l) without any family history of diabetes. Three patients in this group suffered from exocrine pancreatic insufficiency and were treated with pancreatic enzyme supplementation on a daily basis. The etiology of chronic pancreatitis was judged to be alcoholism in four patients and idiopathic in the rest.

All patients with chronic pancreatitis (groups 1 and 2) were without clinical and/or biochemical signs (amylase, C-reactive protein, and leukocyte

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

Address correspondence and reprint requests to Filip Krag Knop, MD, Department of Internal Medicine, Gentofte Hospital, University of Copenhagen, Niels Andersens Vej 65, DK-2900 Hellerup, Denmark. E-mail: filipknop@dadlnet.dk.

Received for publication 23 January 2007 and accepted in revised form 3 May 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 18 May 2007. DOI: 10.2337/db07-0100.

AUC, area under the curve; FPG, fasting plasma glucose; GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide; HOMA, homeostasis model assessment; HOMA-IR, HOMA for insulin resistance; ICA, islet cell autoantibody; ISR, insulin secretion rate; NGT, normal glucose tolerance; OGTT; oral glucose tolerance test; PG, plasma glucose.

TABLE 1
Anthropometric data for patients with chronic pancreatitis and secondary diabetes, patients with chronic pancreatitis and NGT, patients with type 2 diabetes, and healthy subjects

| | Patients with chronic pancreatitis and secondary diabetes | Patients with chronic pancreatitis and NGT | Patients with type 2 diabetes | Healthy subjects |
|---|---|---|---|---|
| *n* (F/M) | 2/6 | 2/6 | 2/6 | 2/6 |
| Age (years) | 56 (44–65) | 50 (45–59) | 62 (51–75) | 57 (44–69) |
| BMI (kg/m$^2$) | 22 (15–29) | 22 (18–29) | 24 (21–26) | 22 (20–26) |
| Waist-to-hip ratio | 1.0 (0.9–1.1) | 1.0 (0.9–1.0) | 1.0 (0.9–1.0) | 1.0 (0.9–1.0) |
| FPG (mmol/l) | 8.0 (4.8–13.5) | 5.2 (4.9–5.6) | 8.1 (6.2–10.4) | 5.2 (4.9–5.6) |
| A1C (%) | 6.9 (6.2–8.0) | 5.5 (5.1–5.6) | 6.8 (6.2–8.7) | 5.5 (5.1–5.8) |

Data are means (range).

counts within normal limits) of acute inflammatory activity in the pancreas. None of the patients drank alcohol on a daily basis, and there were no clinical or biochemical signs (albumin, aspartate aminotransferase/alanine aminotransferase, alkaline phosphatase, and international normalized ratio within normal limits × 1.5) of affected liver function. The diagnostic criteria of chronic pancreatitis were according to Layer et al. (12), and all chronic pancreatitis subjects had unequivocal morphological changes of the pancreas evident by ultrasonography, computed tomography scan, magnetic resonance cholangiopancreatography, or endoscopic retrograde cholangiopancreatography according to the Cambridge classification (14).

Group 3 consisted of eight patients with type 2 diabetes diagnosed according to the criteria of the World Health Organization (13). Four were treated with diet, three with a sulfonylurea, and one with a sulfonylurea in combination with metformin.

Group 4 consisted of eight healthy subjects without a family history of diabetes. All had NGT according to a 75-g OGTT performed immediately before inclusion in the study.

All participants were negative with regard to islet cell autoantibodies (ICAs) and GAD-65 autoantibodies, except for one subject in group 2 and one subject in group 4, both of whom had elevated GAD-65 autoantibodies (23.0 and 27.3 IU/l, respectively; normal: <9.5). Both had NGT ($PG_{120\ min}$ 6.4 and 4.3 mmol/l), normal fasting plasma glucose (FPG) (5.8 and 5.3 mmol/l), normal A1C (5.9 and 5.3%), and normal ICA. Otherwise, all subjects had normal clinical and biochemical parameters. None of the subjects took drugs likely to

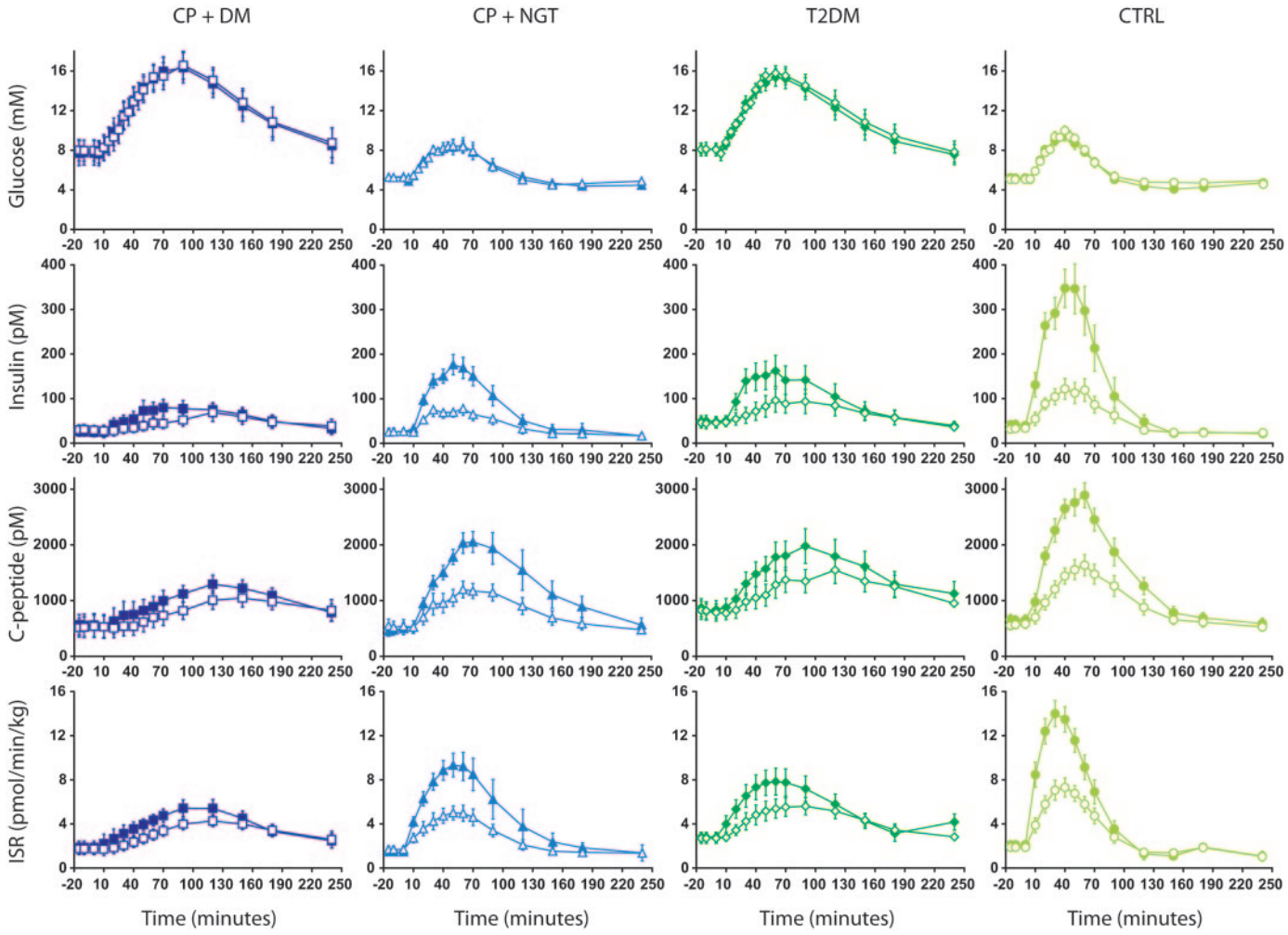


**FIG. 1. PG, plasma insulin, plasma C-peptide, and ISR in patients with chronic pancreatitis and NGT (triangles), patients with chronic pancreatitis and secondary diabetes (squares), healthy subjects (circles), and patients with type 2 diabetes (diamonds) after 50-g OGTT (filled symbols) and isoglycemic intravenous glucose infusion (open symbols), respectively. *Significant differences ($P < 0.05$) between the individual OGTT and isoglycemic intravenous glucose infusion curves (repeated-measures ANOVA). CP, chronic pancreatitis; CTRL, control; T2DM, type 2 diabetes.**

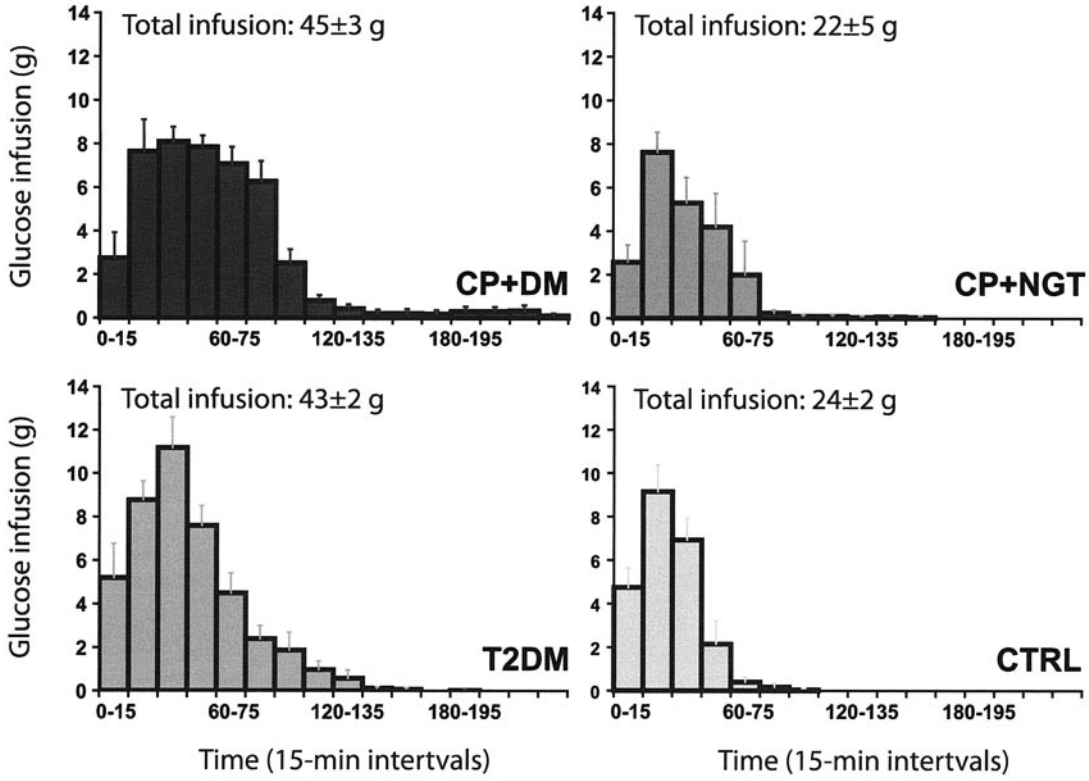


**FIG. 2. Mean glucose infusion rates per 15 min used during the isoglycemic intravenous glucose infusion in the four groups. Total amounts of glucose needed to copy the 50-g OGTT curves are indicated. CP, chronic pancreatitis; CTRL, control; T2DM, type 2 diabetes.**

differentially affect the responses of insulin, glucagon, or incretin hormones, respectively, to oral compared with intravenous glucose, and none had impaired renal function (they had normal plasma creatinine levels and no albuminuria). Seven of the healthy control subjects and the eight patients with type 2 diabetes had also participated in a recently published study and were reanalyzed for the current study.

All subjects agreed to participate after receiving oral and written information. The study design was approved by the scientific-ethical committee of the County of Copenhagen in March 2004 (registration no. KA 04034), and the study was conducted according to the principles of the Helsinki Declaration II.

**Methods.** All subjects were studied on two occasions separated by at least 24 h. Before each occasion the patients with diabetes had not taken their oral antidiabetic therapy, if any, for a period of no less than 1 week. Otherwise, the participants continued their normal lifestyle. On both occasions the subjects were studied in a recumbent position after an overnight (10-h) fast.

On day 1 a cannula was inserted in the retrograde direction in a dorsal hand vein. The cannulated hand was placed in a heating box (42°C) throughout the experiment for collection of arterialized blood samples. The subjects ingested 50 g of water-free glucose dissolved in 400 ml water over 5 min (0–5 min). Arterialized blood was drawn at time −15, −10, 0, 15, 30, 45, 60, 90, 120, 150, 180, and 240 min and distributed into chilled tubes containing EDTA plus aprotinin (Trasylol at 500 kIU/ml blood; Bayer, Leverkusen, Germany) and a specific dipeptidyl peptidase IV inhibitor (valine-pyrrolidide, final concentration of 0.01 mmol/l; a gift from Dr. R.B. Carr, Novo Nordisk, Bagsværd, Denmark) for analysis of glucagon, GLP-1, and GIP. For analysis of insulin and C-peptide, blood was distributed into chilled tubes containing heparin plus aprotinin (500 KIU/ml blood). All tubes were immediately cooled on ice and then centrifuged for 20 min at 4,000 rounds per min (rpm) and 4°C. Plasma for GLP-1 and GIP analysis was stored at −20°C, and plasma for insulin and C-peptide analysis 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.

On day 2 we inserted a retrograde cannula in a dorsal hand vein for blood sampling (42°C) and a cannula in the contralateral cubital vein for glucose infusion. An isoglycemic intravenous glucose infusion (20% wt/vol) was performed, aiming at a duplication of the PG profile determined on day 1. Blood was sampled as on day 1, except for more frequent PG sampling.

**Analyses.** PG concentrations were measured by the glucose oxidase method, using a glucose analyzer (2300 Stat Plus analyzer; YSI, Yellow Springs, OH). Plasma samples were assayed for total GLP-1 immunoreactivity, as previously described (15), using a radioimmunoassay (antiserum no. 89390) that is specific for the COOH terminus of the GLP-1 molecule and that reacts equally with intact GLP-1 and the primary ($NH_2$-terminally truncated) metabolite. Intact GLP-1 was measured using an enzyme-linked immunosorbent assay (16). The assay was a two-site sandwich assay using two monoclonal antibodies: GLP-1F5 as a catching antibody (COOH-terminally directed) and Mab26.1 as a detecting antibody ($NH_2$-terminally directed) (17).

Total GIP was measured using the COOH-terminally directed antiserum R65 (18,19), which reacts fully with intact GIP and the $NH_2$-terminally truncated metabolite. Intact, biologically active GIP was measured using antiserum no. 98171 (20).

The glucagon assay was directed against the COOH terminus of the glucagon molecule (antibody code no. 4305) and, therefore, measured glucagon of mainly pancreatic origin (21). Neither glicentin nor oxyntomodulin cross-react, but proglucagon 1-61, which is mainly formed in the pancreas, did react fully in this assay (22,23). Plasma insulin and C-peptide concentrations were measured using AutoDelfia time-resolved fluoroimmunoassay (Wallac Oy, Turku, Finland) (24).

**Calculations and statistical analyses.** All results are means ± SE. Area under the curve (AUC) values were calculated using the trapezoidal rule and are presented as the incremental values (baseline levels subtracted) if nothing else is stated. Incretin effect values were calculated by relating the difference in integrated β-cell secretory responses (insulin, C-peptide, and insulin secretion rate [ISR]) between stimulation with oral and isoglycemic intravenous glucose to the response after oral glucose, which was taken as 100% (3). The following formula was used: 100% × (integrated response to OGTT − intravenous integrated response)/integrated response to OGTT. ISR was calculated by deconvolution of measured C-peptide concentrations and application of population-based parameters for C-peptide kinetics as described previously (24–26). ISR is expressed as picomoles of insulin secreted per minute per kilogram body weight. The homeostatic model assessment (HOMA) was used to assess insulin resistance (HOMA-IR) (27). Comparisons of experiments in which the data were distributed normally were made with two-tailed Student's $t$ test (paired within groups, unpaired between groups). 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. Within-group comparisons of oral and intravenous profiles were made using a mixed-model ANOVA for repeated measures (SAS/STAT Proc Mixed, version 8.2; SAS Institute, Cary, NC). Differences between the groups, with

TABLE 2
Integrated β-cell secretory responses (insulin, C-peptide, and ISR) to oral glucose (50 g in 400 ml $H_2O$) and adjustable (isoglycemic) intravenous glucose infusion (20% wt/vol), and calculated incretin effects (percent of the β-cell secretory response after oral glucose) in patients with chronic pancreatitis and secondary diabetes, patients with chronic pancreatitis and NGT, patients with type 2 diabetes, and healthy subjects

| | Patients with chronic pancreatitis and secondary diabetes* | Patients with chronic pancreatitis and NGT† | Patients with type 2 diabetes‡ | Healthy subjects§ |
|---|---|---|---|---|
| *n* (F/M) | 2/6 | 2/6 | 2/6 | 2/6 |
| Integrated β-cell secretory responses | | | | |
| Insulin (nmol/l per 4 h) | | | | |
| Oral | 8.8 ± 1.7§ | 10.9 ± 1.8 | 12.1 ± 2.3 | 18.4 ± 3.1† |
| Intravenous | 5.5 ± 1.3 | 3.0 ± 0.7 | 6.8 ± 1.8 | 5.2 ± 1.7 |
| Oral-intravenous | 3.4 ± 1.0†§¶ | 7.8 ± 1.5*¶ | 5.3 ± 1.6§¶ | 13.1 ± 3.0*‡¶ |
| C-peptide (nmol/l per 4 h | | | | |
| Oral | 127 ± 20 | 186 ± 37 | 154 ± 28 | 186 ± 21 |
| Intravenous | 87 ± 13 | 62 ± 14 | 96 ± 14 | 88 ± 11 |
| Oral-intravenous | 40 ± 11*†§ | 124 ± 29*‡¶ | 58 ± 18†¶ | 98 ± 20*¶ |
| ISR (nmol/l per kg) | | | | |
| Oral | 687 ± 110 | 698 ± 169 | 783 ± 127 | 649 ± 61 |
| Intravenous | 455 ± 61† | 222 ± 53*‡ | 511 ± 114† | 305 ± 39 |
| Oral-intravenous | 166 ± 63*†§ | 476 ± 135*‡ | 272 ± 68¶ | 345 ± 55*¶ |
| Relative incretin effects (%) | | | | |
| Insulin | 36 ± 7† | 68 ± 7*‡ | 44 ± 9† | 73 ± 6*‡ |
| C-peptide | 26 ± 7† | 67 ± 6*‡ | 31 ± 9† | 52 ± 7*‡ |
| ISR | 31 ± 5† | 68 ± 6*‡ | 34 ± 7† | 53 ± 5*‡ |
| Average | 31 ± 4† | 68 ± 3*‡ | 36 ± 6† | 60 ± 4*‡ |

Data are means ± SE. Significant differences ($P < 0.05$) between responses to oral glucose and isoglycemic intravenous glucose infusion within each group (¶) and significant differences ($P < 0.05$) in integrated β-cell secretory responses and incretin effects, respectively, between the four groups are indicated. *Compared with patients with chronic pancreatitis and secondary diabetes. †Compared with patients with chronic pancreatitis and NGT. ‡Compared with patients with type 2 diabetes. §Compared with healthy subjects.

respect to incretin effects, were calculated using a one-way ANOVA with post hoc analysis. $P < 0.05$ was considered to be statistically significant.

## RESULTS

**Glucose.** No significant differences in FPG between days 1 and 2 were observed in any of the four groups. No significant differences between FPG in the two diabetic groups or between the two glucose-tolerant groups were evident, whereas mean FPG in both diabetic groups were higher ($P < 0.0001$) than the corresponding values in both glucose-tolerant groups. As illustrated in Fig. 1 the PG responses to oral glucose displayed the characteristic differences between normal glucose-tolerant subjects and diabetic subjects. The oral glucose curves were mimicked during the adjustable intravenous glucose infusions (with no significant differences between the two glucose curves in any of the groups) using glucose infusion rates as illustrated in Fig. 2.

**Insulin, C-peptide ISR, incretin effect, and HOMA.** There were no significant differences between fasting values on days 1 and 2 for plasma insulin or C-peptide in any of the four groups, and no differences between the groups were observed. Significant differences in the dynamic time courses for insulin, C-peptide, and ISR (Fig. 1), respectively, between day 1 and day 2 were observed in all groups ($P < 0.0014$). As shown in Table 2, integrated β-cell secretory responses (incremental AUC values for insulin, C-peptide, and ISR, respectively) were greater in all four groups during the OGTT compared with isoglycemic intravenous infusion ($P < 0.05$). Significant differences in integrated β-cell secretory responses (and in integrated responses to oral − integrated responses to intravenous glucose) between the groups are indicated in Table 2. Incretin effects calculated from the three integrated β-cell secretory responses are shown in Table 2. As indicated, no differences in incretin effects between the two groups of diabetic patients or between the two groups of glucose-tolerant subjects were observed, whereas each value for the individual diabetic group differed significantly from the corresponding value of each glucose-tolerant group ($P < 0.05$). No differences in HOMA-IR were observed between days 1 and 2 in any of the four groups.

**GLP-1.** Time courses for total and intact GLP-1 are shown in Fig. 3. No significant differences in basal values between days 1 and 2 were observed in any of the four groups. Basal levels in the chronic pancreatitis plus diabetes group and in the type 2 diabetes group were significantly higher compared with the control group. In all groups significant increases in both total and intact forms were observed after the OGTT. During the isoglycemic intravenous glucose infusion, no significant responses occurred in any of the groups. The AUC for total GLP-1 was significantly bigger during the OGTT compared with the isoglycemic intravenous infusion in all four groups, but with respect to the AUC for intact GLP-1, significant differences were only observed for the chronic pancreatitis plus diabetes group and the control group (Table 3). No differences in AUC between the four groups were observed.

**GIP.** Time courses for total and intact GIP are shown in Fig. 3. No significant differences in basal values between days 1 and 2 were observed in any of the four groups. In all groups significant increases in both total and intact forms were observed after the OGTT. During the isoglycemic intravenous glucose infusion, no significant responses occurred in any of the groups. The AUCs for total and intact GIP were significantly larger during the OGTT compared with isoglycemic intravenous infusion in all four groups (Table 3). Patients with chronic

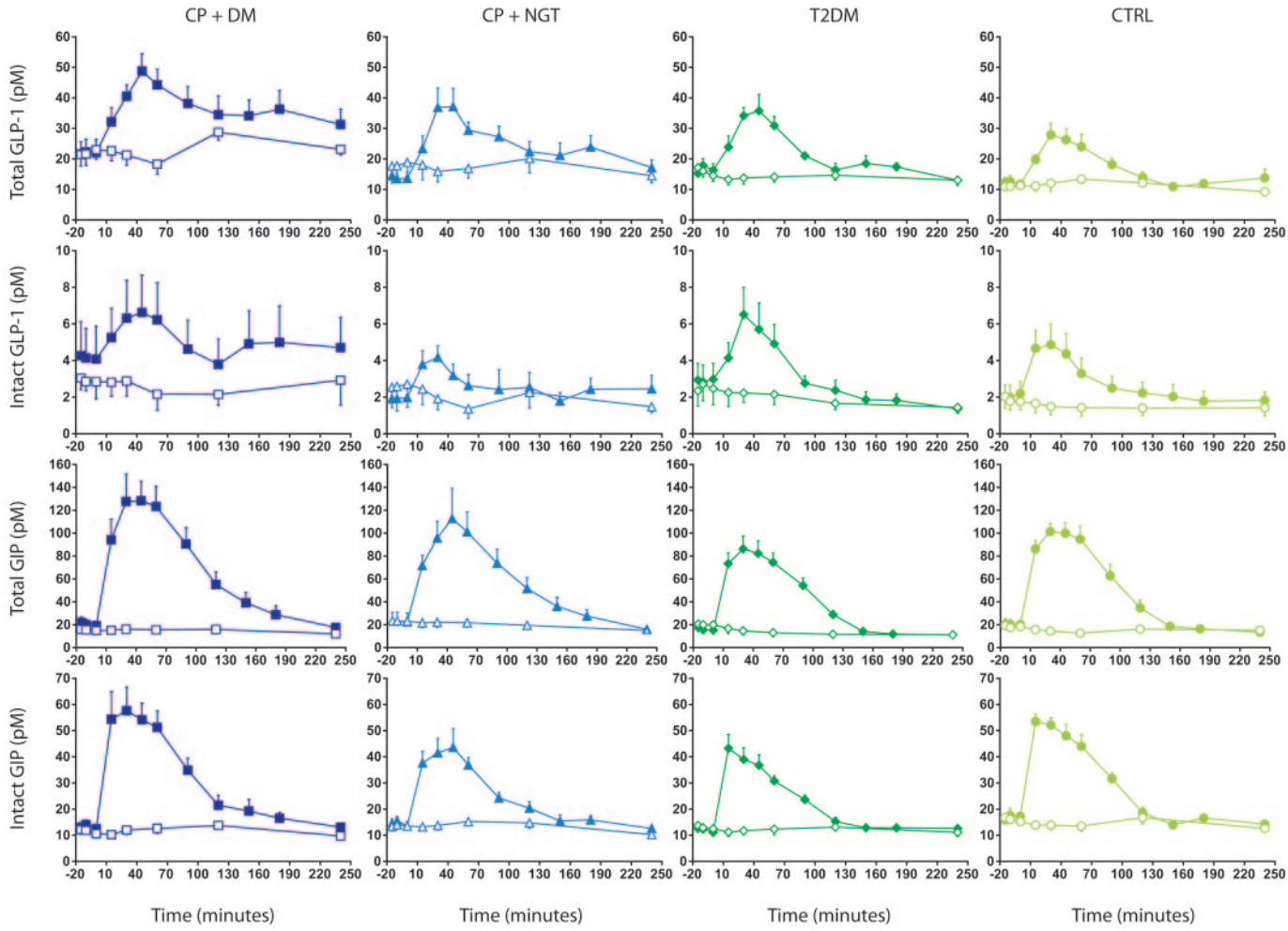


**FIG. 3. Total GLP-1, intact GLP-1, total GIP, and intact GIP in patients with chronic pancreatitis and NGT (triangles), patients with chronic pancreatitis and secondary diabetes (squares), healthy subjects (circles), and patients with type 2 diabetes (diamonds) after 50-g OGTT (filled symbols), and isoglycemic intravenous glucose infusion (open symbols), respectively. *Significant differences ($P < 0.05$) between the individual OGTT and isoglycemic intravenous glucose infusion curves (repeated-measures ANOVA). CP, chronic pancreatitis; CTRL, control; T2DM, type 2 diabetes.**

pancreatitis plus diabetes displayed significantly larger responses during OGTT compared with the other groups. Otherwise, no differences between the groups were observed.

**Glucagon.** Time courses for plasma glucagon are presented in Fig. 4. Similar basal values on the two experimental days were observed in all of the four groups ($P$ = NS). As expected, patients with type 2 diabetes exhibited significantly higher basal levels compared with the control group (9.5 ± 0.9 vs. 7.6 ± 0.6 pmol/l, $P = 0.01$). Otherwise no significant differences between the groups were observed. In the control group equal suppression of plasma glucagon concentrations was observed on both experimental days with similar nadirs of 5.4 ± 0.4 and 5.3 ± 0.5 pmol/l. As illustrated in Fig. 4, decremental AUC values during the 1st hour of the two experimental days in the control group amounted to −67 ± 19 and −92 ± 28 pmol/l per 1 h, respectively ($P$ = NS). In the remaining three groups (chronic pancreatitis plus diabetes, chronic pancreatitis plus NGT, and type 2 diabetes), complete lack of suppression of plasma glucagon was observed during the initial 60 min after the OGTT (23 ± 34, −13 ± 30, and −3 ± 18 pmol/l per 1 h; $P$ = NS), but normal suppression was observed during the isoglycemic intravenous glucose infusion (−43 ± 22, −88 ± 26, and −75 ± 22 pmol/l per 1 h; $P$ = NS) compared with the control group (Fig. 4).

## DISCUSSION

With the current study, we confirm the observation made by Nauck et al. (3) in 1986 that the incretin effect is reduced in patients with type 2 diabetes, and we conclude that this deficiency is most likely a consequence of the diabetic state and not a primary pathogenic trait leading to type 2 diabetes.

Until now, the incretin defect in patients with type 2 diabetes has been considered a possible candidate for a primary deficiency in type 2 diabetes. However, Nauck et al. (28) estimated the incretin effect in first-degree relatives of patients with type 2 diabetes and found it to be similar to that of matched healthy subjects, suggesting the deficiency to be a consequence of the diabetic state. In support of this, the incretin effect has been found to be reduced in individuals with type 1 diabetes (positive ICA) and normal fasting glucose levels (29). Recently, the incretin effect was shown to be affected in subjects who had impaired glucose tolerance and who were therefore at high risk for developing type 2 diabetes (30). This obser-

TABLE 3
Integrated incretin hormone responses (total and intact GLP-1 and total and intact GIP) to oral glucose (50 g in 400 ml $H_2O$) and adjustable (isoglycemic) intravenous glucose infusion (20% wt/vol) in patients with chronic pancreatitis and secondary diabetes, patients with chronic pancreatitis and NGT, patients with type 2 diabetes, and healthy subjects

| Integrated incretin hormone responses | Patients with chronic pancreatitis and diabetes | Patients with chronic pancreatitis and NGT | Patients with type 2 diabetes | Healthy subjects |
|---|---|---|---|---|
| *n* (F/M) | 8/6 | 8/6 | 8/6 | 8/6 |
| Total GLP-1 (nmol/l × 4 h) | | | | |
| Oral | 3.3 ± 0.4 | 2.6 ± 0.6 | 1.1 ± 0.5 | 1.0 ± 0.3 |
| Intravenous | 0.5 ± 0.4 | −0.1 ± 1.0 | −0.5 ± 0.1 | 0.1 ± 0.1 |
| Oral-intravenous | 3.0 ± 0.8* | 2.7 ± 1.2* | 1.6 ± 0.4* | 0.9 ± 0.3* |
| Intact GLP-1 (pmol/l × 4 h) | | | | |
| Oral | 211 ± 138 | 161 ± 60 | 11 ± 130 | 138 ± 39 |
| Intravenous | −103 ± 58 | −176 ± 213 | −167 ± 157 | −98 ± 28 |
| Oral-intravenous | 314 ± 144* | 336 ± 207 | 177 ± 129 | 235 ± 53* |
| Total GIP (nmol/l × 4 h) | | | | |
| Oral | 10.6 ± 2.0† | 7.7 ± 2.3 | 5.4 ± 0.5‡ | 6.3 ± 1.1‡ |
| Intravenous | −0.2 ± 0.3 | −1.0 ± 0.5 | −1.8 ± 0.7 | −0.8 ± 0.3 |
| Oral-intravenous | 10.8 ± 2.0* | 8.7 ± 2.1* | 7.2 ± 0.8* | 7.1 ± 1.0* |
| Intact GIP (pmol/l × 4 h) | | | | |
| Oral | 4.0 ± 0.7§ | 2.2 ± 0.5‡ | 2.2 ± 0.3‡ | 2.5 ± 0.5‡ |
| Intravenous | 0.1 ± 0.2 | 0.0 ± 0.3 | −0.2 ± 0.3 | −0.8 ± 0.5 |
| Oral-intravenous | 3.8 ± 0.7* | 2.2 ± 0.5* | 2.4 ± 0.3* | 3.2 ± 0.3* |

Data are means ± SE. Significant differences between incretin responses to oral glucose and isoglycemic intravenous glucose infusion within each group ($P < 0.05$) and significant differences in incretin responses between the four groups are indicated . *$P < 0.05$; †$P < 0.05$ compared with patients with type 2 diabetes and healthy subjects; ‡$P < 0.05$ compared with patients with chronic pancreatitis and diabetes; §$P < 0.05$ compared with patients with chronic pancreatitis and NGT, patients with type 2 diabetes, and healthy subjects.

vation could imply a primary role for the reduced incretin effect in type 2 diabetes, but on the other hand, the finding could also represent an early consequence of the chronic mild hyperglycemia of impaired glucose tolerance. Thus, no firm conclusion can be drawn from the existing literature.

Type 2 diabetes is characterized by a severely reduced insulinotropic effect of GIP, especially on the late-phase insulin response, compared with healthy control subjects. The effect of GIP has also been evaluated in first-degree relatives of patients with type 2 diabetes (31) and found to be reduced compared with healthy subjects, pointing toward an early, possibly genetic defect. On the other hand, we found a similar lack of GIP effect in patients with chronic pancreatitis and secondary diabetes (32), and Meier et al. (33) showed that the GIP effect was preserved in women who had a history of gestational diabetes and who were therefore at high risk of developing type 2 diabetes. It was therefore of interest to investigate whether patients with secondary diabetes exhibit a similar loss of incretin effect as patients with type 2 diabetes, which would indicate that the loss might be a secondary rather than a primary event in the pathogenesis of type 2 diabetes. Furthermore, we sought to investigate whether our recent finding that the regulation of α-cell secretion in patients with type 2 diabetes is different during an OGTT and isoglycemic intravenous glucose infusion (8) could be demonstrated in patients with chronic pancreatitis (with and without secondary diabetes).

Chronic pancreatitis is a chronic inflammatory condition in the pancreas that results in a progressive destruction of the pancreatic cells, with subsequent development of exocrine and endocrine pancreatic insufficiency (12). No good estimates for the time from diagnosis of chronic pancreatitis to the onset of secondary diabetes have been published, but in a large Danish cohort, one-third of the patients with chronic pancreatitis had NGT, one-third had impaired glucose tolerance or non–insulin-dependent diabetes, and one-third had insulin-dependent diabetes (11), in accordance with other published findings (34–36). These data suggest that the development of secondary glucose intolerance represents a continuum, worsening with the duration of chronic pancreatitis.

In the current study, the two groups of patients with chronic pancreatitis differed with respect to glucose tolerance, but they were very similar with regard to other chronic pancreatitis–related pathologies. All patients in the chronic pancreatitis plus diabetes group had relatively well-regulated glucose homeostasis on diet and/or oral antidiabetic drugs, suggesting the preservation of a substantial number of functional β-cells on which the incretin hormones could exert their actions. Therefore, a difference in incretin effect between the two groups of patients with chronic pancreatitis is most likely to be attributed to their different glycemic control. No matter how the incretin effect was calculated, a lower incretin effect was seen in patients with chronic pancreatitis plus diabetes (decreased to the level of patients with type 2 diabetes or even lower) compared with patients with chronic pancreatitis plus NGT, who exhibited an incretin effect similar to that of healthy subjects (Table 2). The abnormality is not likely to be explained by a decrease in β-cell mass. This notion is supported by the observation that the insulin and C-peptide responses to isoglycemic intravenous glucose challenges were similar in the four groups, indicating that all groups have a number of β-cells sufficient to respond equally to intravenous glucose. Furthermore, in a previous study, we found that insulin and C-peptide responses to a 15-mmol/l hyperglycemic clamp could be completely normalized in patients with type 2 diabetes (compared with matched healthy control subjects) after intravenous infusion of a supraphysiological amount of GLP-1 (7), and preliminary data from our group suggest that the same is true for patients with chronic pancreatitis and secondary diabetes compared with patients with chronic pancreatitis

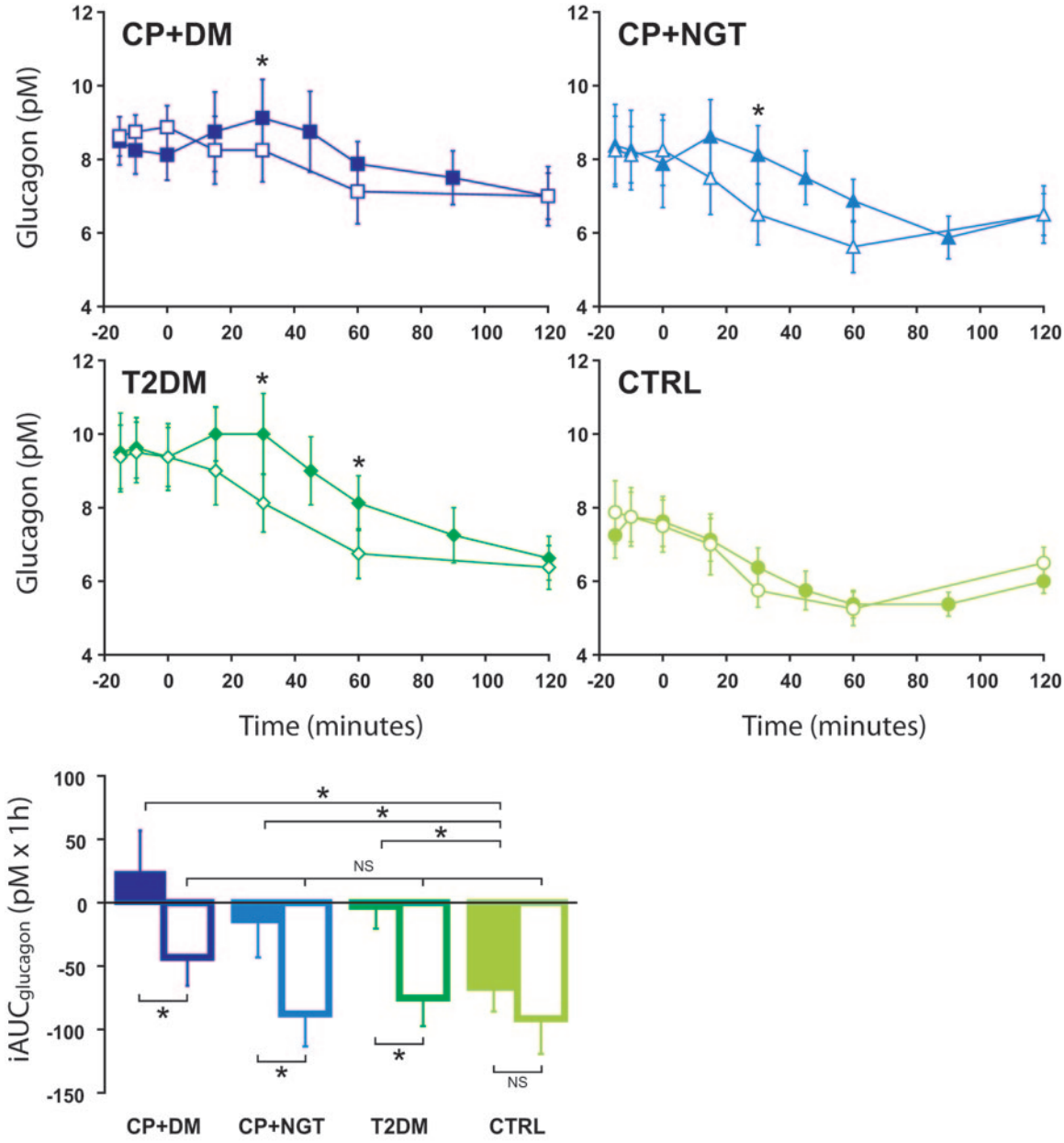


**FIG. 4. Plasma glucagon in patients with chronic pancreatitis and NGT (triangles), patients with chronic pancreatitis and secondary diabetes (squares), healthy subjects (circles), and patients with type 2 diabetes (diamonds) after a 50-g OGTT (filled symbols) and isoglycemic intravenous glucose infusion (open symbols), respectively. Lower panel: Incremental AUC values for plasma glucagon ($iAUC_{glucagon}$) during the first hour of 50-g OGTT (filled bars) and isoglycemic intravenous glucose infusion (open bars), respectively, in the four groups. *Significant differences ($P < 0.05$). CP, chronic pancreatitis; CTRL, control; T2DM, type 2 diabetes.**

and NGT (F.K.K., T.V., P.V.H., S.L., S.M., J.J.H., T.K., personal communication).

These observations suggest that patients with type 2 diabetes and patients with chronic pancreatitis and secondary diabetes do have a substantial residual β-cell secretory capacity that only needs the right stimulus to be recruited. Because the diabetes resulting from chronic pancreatitis is secondary, and therefore presumably nongenetic, we propose that the diabetic state results in a dysfunction of the β-cells, making them resistant to the insulinotropic effects of the incretin hormones. One might speculate whether it is possible to reestablish the incretin effect in patients with diabetes by near-normalization of PG for a longer period. That this notion might be feasible is supported by our recent finding that in patients with type 2 diabetes, 4 weeks of strict glycemic control during insulin treatment improved β-cell responsiveness to GLP-1 (37).

To further evaluate mechanisms underlying the reduced incretin effect, we measured intact (indicators of the endocrine impact on the β-cells) and total (indicators of the overall levels of secretion) plasma concentrations of GIP and GLP-1, respectively, during both experimental days. Baseline levels of GLP-1 were found to be increased in diabetic patients (chronic pancreatitis plus diabetes group and type 2 diabetes group) compared with healthy control subjects (Fig. 3). This supports the notion of a feed-forward cycle where high PG enhances GLP-1 secretion (4,38). In all groups the responses (AUC) of the total forms were significantly higher during oral glucose com-

pared with isoglycemic intravenous glucose infusion, as were the responses of intact GIP. Responses of intact GLP-1 were significantly greater during oral glucose compared with isoglycemic intravenous glucose only in the chronic pancreatitis plus diabetes group and in the control group. The corresponding differences in the remaining groups failed to reach statistical significance. The latter observation is probably attributable to the fact that because GLP-1 is subject to degradation by dipeptidyl peptidase IV almost immediately on its release (39), only 10–15% of intact GLP-1 actually reaches the systemic circulation (40), thereby reducing the possibility of detecting the response in the peripheral circulation. Interestingly, no differences in incretin hormone responses between the four groups could explain the different magnitude of the incretin effect in glucose-tolerant and -intolerant subjects, respectively. Therefore, studies investigating possible differences in the effects of the incretin hormones in patients with chronic pancreatitis plus NGT and chronic pancreatitis plus diabetes are warranted.

Finally, with regard to our recent results showing that the regulation of α-cell secretion in patients with type 2 diabetes is different during oral glucose and isoglycemic intravenous glucose infusion, we investigated whether this finding could be reproduced in patients with chronic pancreatitis and diabetes and therefore could be attributed to the diabetic state per se or whether it might be a primary event leading to type 2 diabetes. Interestingly, we observed that glucagon secretion was differentially regulated during oral glucose and isoglycemic intravenous glucose infusion, respectively, in patients with chronic pancreatitis and secondary diabetes and, to a lesser extent, in patients with chronic pancreatitis and NGT. This suggests that the inflammatory condition in the pancreas blunts the glucagon suppression during the OGTT independently of normal insulin secretion and normal glucose homeostasis. Thus, we suspect that (as indicated in studies of patients with acute pancreatitis) (41,42) the α-cells are very sensitive to inflammation (more sensitive than the β-cells) and that hyperglycemia worsens the ability of the α-cells to further suppress glucagon during the OGTT. However, the phenomenon that different glucagon responses are elicited by the OGTT and isoglycemic intravenous glucose infusion, respectively, in patients with type 2 diabetes and in those with chronic pancreatitis is currently unclear, and further studies are clearly needed to establish the underlying mechanisms.

In conclusion, the current study suggests that reduced incretin effect in type 2 diabetes is a consequence of the diabetic state rather than a primary event leading to type 2 diabetes.

## ACKNOWLEDGMENTS

This study was supported by grants from the Danish Diabetes Association, Novo Nordisk, the Danish Medical Research Council, the Else and Svend Madsen's Foundation, and the Chief Physician Johan Boserup and Lise Boserup's Foundation.

We are grateful to our volunteers, whose availability made this work possible, and to Birgitte Bischoff, Nina Kjeldsen, Jytte Purtoft, Charlotte Rasmussen, Susanne Reimer, and Lone B. Thielsen for technical assistance.

## REFERENCES

1. Holst JJ: On the physiology of GIP and GLP-1. *Horm Metab Res* 36:747–754, 2004
2. Dube PE, Brubaker PL: Nutrient, neural and endocrine control of glucagon-like peptide secretion. *Horm Metab Res* 36:755–760, 2004
3. Nauck M, Stockmann F, Ebert R, Creutzfeldt W: Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 29:46–52, 1986
4. Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, Holst JJ: Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab* 86:3717–3723, 2001
5. Vilsboll T, Krarup T, Deacon CF, Madsbad S, Holst JJ: Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 50:609–613, 2001
6. Kjems LL, Holst JJ, Volund A, Madsbad S: The influence of GLP-1 on glucose-stimulated insulin secretion: effects on beta-cell sensitivity in type 2 and nondiabetic subjects. *Diabetes* 52:380–386, 2003
7. Vilsboll T, Krarup T, Madsbad S, Holst JJ: Defective amplification of the late phase insulin response to glucose by GIP in obese type II diabetic patients. *Diabetologia* 45:1111–1119, 2002
8. Knop FK, Vilsboll T, Madsbad S, Krarup T, Holst JJ: Lack of suppression of glucagon during OGTT but not during isoglycemic IVGTT in patients with type 2 diabetes (Abstract). *Diabetes* 55 (Suppl. 1): 2006
9. Nyboe Andersen B, Krarup T, Thorsgaard Pedersen NT, Faber OK, Hagen C, Worning H: B cell function in patients with chronic pancreatitis and its relation to exocrine pancreatic function. *Diabetologia* 23:86–89, 1982
10. Domschke S, Stock KP, Pichl J, Schneider MU, Domschke W: Beta-cell reserve capacity in chronic pancreatitis. *Hepatogastroenterology* 32:27–30, 1985
11. Larsen S, Hilsted J, Tronier B, Worning H: Metabolic control and B cell function in patients with insulin-dependent diabetes mellitus secondary to chronic pancreatitis. *Metabolism* 36:964–967, 1987
12. Layer P, Yamamoto H, Kalthoff L, Clain JE, Bakken LJ, Dimagno EP: The different courses of early-onset and late-onset idiopathic and alcoholic chronic pancreatitis. *Gastroenterology* 107:1481–1487, 1994
13. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 25 (Suppl. 1):S5–S20, 2002
14. Axon AT, Classen M, Cotton PB, Cremer M, Freeny PC, Lees WR: Pancreatography in chronic pancreatitis: international definitions. *Gut* 25:1107–1112, 1984
15. Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ: Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. *Diabetes* 43:535–539, 1994
16. Vilsboll T, Krarup T, Sonne J, Madsbad S, Volund A, Juul AG, Holst JJ: Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. *J Clin Endocrinol Metab* 88:2706–2713, 2003
17. Wilken M, Larsen FS, Buckley D, Holst JJ: New highly specific immunoassays for glucagon-like peptide-1 (GLP-1) (Abstract). *Diabetologia* 42 (Suppl. 1):A196 1999
18. Krarup T, Holst JJ: The heterogeneity of gastric inhibitory polypeptide in porcine and human gastrointestinal mucosa evaluated with five different antisera. *Regul Pept* 9:35–46, 1984
19. Krarup T, Madsbad S, Moody AJ, Regeur L, Faber OK, Holst JJ, Sestoft L: Diminished immunoreactive gastric inhibitory polypeptide response to a meal in newly diagnosed type I (insulin-dependent) diabetics. *J Clin Endocrinol Metab* 56:1306–1312, 1983
20. Deacon CF, Nauck MA, Meier J, Hucking K, Holst JJ: Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. *J Clin Endocrinol Metab* 85:3575–3581, 2000
21. Orskov C, Jeppesen J, Madsbad S, Holst JJ: Proglucagon products in plasma of noninsulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. *J Clin Invest* 87:415–423, 1991
22. Baldissera FGA, Holst JJ: Glucagon-related peptides in the human gastrointestinal mucosa. *Diabetologia* 26:223–228, 1984
23. Holst JJ, Pedersen JH, Baldissera F, Stadil F: Circulating glucagon after total pancreatectomy in man. *Diabetologia* 25:396–399, 1983
24. Kjems LL, Christiansen E, Volund A, Bergman RN, Madsbad S: Validation of methods for measurement of insulin secretion in humans in vivo. *Diabetes* 49:580–588, 2000
25. Kjems LL, Volund A, Madsbad S: Quantification of beta-cell function during IVGTT in Type II and non-diabetic subjects: assessment of insulin secretion by mathematical methods. *Diabetologia* 44:1339–1348, 2001

26. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368–377, 1992
27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
28. Nauck MA, El Ouaghlidi A, Gabrys B, Hucking K, Holst JJ, Deacon CF, Gallwitz B, Schmidt WE, Meier JJ: Secretion of incretin hormones (GIP and GLP-1) and incretin effect after oral glucose in first-degree relatives of patients with type 2 diabetes. *Regul Pept* 122:209–217, 2004
29. Greenbaum CJ, Prigeon RL, D'Alessio DA: Impaired beta-cell function, incretin effect, and glucagon suppression in patients with type 1 diabetes who have normal fasting glucose. *Diabetes* 51:951–957, 2002
30. Muscelli E, Mari A, Natali A, Astiarraga BD, Camastra S, Frascerra S, Holst JJ, Ferrannini E: Impact of incretin hormones on beta-cell function in subjects with normal or impaired glucose tolerance. *Am J Physiol Endocrinol Metab* 291:E1144–E1150, 2006
31. Meier J, Hucking K, Holst J, Deacon C, Schmiegel W, Nauck M: Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. *Diabetes* 50:2497–2504, 2001
32. Vilsboll T, Knop FK, Krarup T, Johansen A, Madsbad S, Larsen S, Hansen T, Pedersen O, Holst JJ: The pathophysiology of diabetes involves a defective amplification of the late-phase insulin response to glucose by glucose-dependent insulinotropic polypeptide-regardless of etiology and phenotype. *J Clin Endocrinol Metab* 88:4897–4903, 2003
33. Meier JJ, Gallwitz B, Askenas M, Vollmer K, Deacon CF, Holst JJ, Schmidt WE, Nauck MA: Secretion of incretin hormones and the insulinotropic effect of gastric inhibitory polypeptide in women with a history of gestational diabetes. *Diabetologia* 48:1872–1881, 2005
34. James O, Agnew JE, Bouchier IA: Chronic pancreatitis in England: changing picture? *Br Med J* 2:34–38, 1974
35. Lankisch PG, Otto J, Erkelenz I, Lembcke B: Pancreatic calcifications: no indicator of severe exocrine pancreatic insufficiency. *Gastroenterology* 90:617–621, 1986
36. Linde J, Nilsson LH, Barany FR: Diabetes and hypoglycemia in chronic pancreatitis. *Scand J Gastroenterol* 12:369–373, 1977
37. Hojberg PV, Zander M, Vilsboll T, Knop FK, Krarup T, Holst JJ, Madsbad S: Effect of 4 weeks of near-normalization of blood glucose on beta-cell sensitivity to glucose and GLP-1 in type 2 diabetic patients. *Diabetes* 54 (Suppl. 1):A362, 2005
38. Hansen L, Hartmann B, Mineo H, Holst JJ: Glucagon-like peptide-1 secretion is influenced by perfusate glucose concentration and by a feedback mechanism involving somatostatin in isolated perfused porcine ileum. *Regul Pept* 118:11–18, 2004
39. Hansen L, Deacon CF, Orskov C, Holst JJ: Glucagon-like peptide-1-(7–36)amide is transformed to glucagon-like peptide-1-(9–36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. *Endocrinology* 140:5356–5363, 1999
40. Holst JJ, Deacon CF: Glucagon-like peptide-1 mediates the therapeutic actions of DPP-IV inhibitors. *Diabetologia* 48:612–615, 2005
41. Donowitz M, Hendler R, Spiro HM, Binder HJ, Felig P: Glucagon secretion in acute and chronic pancreatitis. *Ann Intern Med* 83:778–781, 1975
42. Solomon SS, Duckworth WC, Jallepalli P, Bobal MA, Iyer R: The glucose intolerance of acute pancreatitis: hormonal response to arginine. *Diabetes* 29:22–26, 1980