Defects in \( \alpha \)-Cell Function in Patients With Diabetes Due to Chronic Pancreatitis Compared With Patients With Type 2 Diabetes and Healthy Individuals

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**OBJECTIVE**

Diabetes frequently develops in patients with chronic pancreatitis. We examined the alterations in the glucagon response to hypoglycemia and to oral glucose administration in patients with diabetes due to chronic pancreatitis.

**RESEARCH DESIGN AND METHODS**

Ten patients with diabetes secondary to chronic pancreatitis were compared with 13 patients with type 2 diabetes and 10 healthy control subjects. A stepwise hypoglycemic clamp and an oral glucose tolerance test (OGTT) were performed.

**RESULTS**

Glucose levels during the OGTT were higher in patients with diabetes and chronic pancreatitis and lower in control subjects (\( P < 0.0001 \)). Insulin and C-peptide levels were reduced, and the glucose-induced suppression of glucagon was impaired in both groups with diabetes (all \( P < 0.0001 \) vs. control subjects). During hypoglycemia, glucagon concentrations were reduced in patients with chronic pancreatitis and with type 2 diabetes (\( P < 0.05 \)). The increase in glucagon during the clamp was inversely related to the glucose-induced glucagon suppression and positively related to \( \beta \)-cell function. Growth hormone responses to hypoglycemia were lower in patients with type 2 diabetes (\( P = 0.0002 \)) but not in patients with chronic pancreatitis.

**CONCLUSIONS**

\( \alpha \)-Cell responses to oral glucose ingestion and to hypoglycemia are disturbed in patients with diabetes and chronic pancreatitis and in patients with type 2 diabetes. The similarities between these defects suggest a common etiology.

Chronic pancreatitis is frequently complicated by the development of diabetes (1,2). This typically occurs at the advanced stages of the disease, alongside with pancreatic atrophy and fibrosis (2–4). Phenotypically, diabetes in patients with chronic pancreatitis is often characterized by insulin deficiency, while insulin resistance is held to be less prevalent (5). Furthermore, obesity is far less common in patients with diabetes secondary to chronic pancreatitis than in patients with type 2 diabetes, owing to the malnutrition resulting from the loss of exocrine tissue (1,6). The etiology of diabetes in patients with chronic pancreatitis mainly involves a loss of endocrine \( \beta \)-cells, which likely results from inflammatory destruction (4,7). We have previously demonstrated...
that pancreatic β-cell mass was reduced by ~45% in a group of patients with chronic pancreatitis, whereas the reduction in α-cell mass was less pronounced in this group (~35%) (4). Interestingly, the loss of β-cells seems to occur relatively late during the course of the disease. Thus, it appears that the endocrine islets exhibit greater resistance to the self-destruction of the pancreas than acinar or ductal cells. This observation is further supported by the fact that the endocrine islets are more resistant against the enzymatic digestion of the pancreas during the process of islet isolation for transplantation (8).

Although the impairment of insulin secretion in patients with diabetes due to chronic pancreatitis is relatively well established (7,9), much less is known about potential alterations in α-cell function. A recent study suggested higher rises of glucagon after a mixed-meal challenge in patients with chronic pancreatitis, thought to be a consequence of the loss of insulin-mediated inhibition of glucagon secretion (10). In contrast, glucagon secretion after arginine stimulation was found to be reduced in patients without diabetes with chronic pancreatitis (11). However, very little information is yet available about other physiological functions of glucagon, such as the counterregulatory response to hypoglycemia (12,13). Furthermore, it is not known to what extent the abnormalities in glucagon secretion in patients with diabetes secondary to chronic pancreatitis resemble those typically found in patients with type 2 diabetes.

In clinical practice, glucose-lowering treatment of patients with diabetes secondary to chronic pancreatitis can be challenging (2). This is partly due to the fact that oral glucose-lowering agents that act to improve insulin sensitivity (e.g., metformin) may not address the key pathophysiological problems, and other drugs (DPP-4 inhibitors and GLP-1 receptor agonists) are even contraindicated because of a potentially increased risk of pancreatitis (14). On the other hand, the use of insulinotropic agents, such as the sulphonylureas, or exogenous insulin replacement therapy is often affected with the induction of hypoglycemia. Although epidemiological evidence regarding the prevalence of hypoglycemia in patients with diabetes secondary to chronic pancreatitis is sparse, the prevalence of hypoglycemia in such patients seems to be relatively high (15). Because glucagon is the major safeguard against the development of hypoglycemia (16), an impairment in hypoglycemia-induced glucagon secretion might be found in such patients.

Therefore, in the current study, the hormonal counterregulatory response to hypoglycemia was examined in patients with diabetes secondary to chronic pancreatitis, patients with type 2 diabetes, and control subjects without diabetes. In addition, the glucagon response to oral glucose ingestion was examined and related to glucagon secretion during hypoglycemia.

RESEARCH DESIGN AND METHODS

Study Protocol, Ethics Committee Approval

The study protocol was approved by the ethics committee of the Medical Faculty, Ruhr-University Bochum (registration number 4134–11). Written informed consent was obtained from all participants.

 Patients With Diabetes Due to Chronic Pancreatitis, Patients With Type 2 Diabetes, and Healthy Control Subjects

Three groups of subjects were recruited into the current study: 1) patients with diabetes due to chronic pancreatitis, 2) patients with type 2 diabetes and no chronic pancreatitis, and 3) normal glucose-tolerant subjects without chronic pancreatitis. Chronic pancreatitis was diagnosed after clinical workup based on the presence of pancreatic calcifications, dilated pancreatic ducts, septation/lobulation due to fibrotic changes, exocrine pancreatic atrophy (all demonstrated by appropriate imaging procedures) (for details, see Supplementary Table 1), and pancreatic exocrine insufficiency requiring pancreas enzyme replacement therapy. The absence of chronic pancreatitis in the control groups (normal glucose-tolerant and type 2 diabetes) was verified by the absence of clinical signs and symptoms of chronic pancreatitis.

For all three groups, the following inclusion criteria were used: both sexes, age 18–75 years (inclusive), and BMI 17–45 kg/m² (inclusive). Exclusion criteria were as follows: pregnancy or breastfeeding, type 1 diabetes, renal functional impairment with a serum creatinine >2.0 mg/dL (177 μmol/L), severe liver disease, congestive heart failure New York Heart Association class III–IV, angina pectoris with recurrent ischemia, a history of acute myocardial infarction, percutaneous coronary angioplasty or coronary artery bypass graft, hemoglobinopathies or anemia (hemoglobin <9 g/dL), systemic treatment with glucocorticoids, C-reactive protein >100 mg/dL (normal value <5 mg/dL), previous pancreatic resective surgery, current infections (fever), a history of seizures, or unwillingness or lack of competence to comply with the requirements of the study protocol.

Study Design

In all participating subjects, a screening examination including the determination of a standard laboratory profile (hemoglobin, leukocytes, transaminases [ALT/AST], γ-glutamyl transpeptidase, creatinine, sodium, potassium, and C-reactive protein) was performed. If subjects met the inclusion criteria, they were invited to participate in two experiments: 1) a hyperinsulinemic, sequentially euglycemic and hypoglycemic clamp experiment and 2) an oral glucose tolerance test (OGTT) over 240 min. β-Blockers and oral glucose-lowering agents were discontinued for at least 3 days before the date of the clamp experiment. Long-acting insulin preparations were last allowed in the morning of the previous day, and short-acting insulin preparations were last allowed for dinner of the day before the experimental days.

Experimental Procedures

OGTT

After an overnight fast of at least 10 h, a large forearm vein was cannulated, and the cannula was kept patent by a slow drip of 0.9% saline. The body position was semirecumbent, with the body ~30° upright. After drawing basal blood samples at −5 and 0 min, an oral glucose drink (75 g glucose in 300 mL) was administered, and venous blood samples were collected over 240 min.

Hyperinsulinemic, Stepwise Hypoglycemic Clamp

The hyperinsulinemic, stepwise hypoglycemic clamp was commenced in the morning after an overnight (>10 h) fast. One large vein on each forearm was cannulated, and the cannulas were kept patent by a slow drip of 0.9% saline. Participants were constantly monitored for blood pressure, pulse, electrocardiogram, and pulse oxymetry (capillary oxygen saturation) using GE Healthcare Dash 3000 monitors. The body position was semirecumbent, with the body ~30° upright. After drawing
basal blood samples at −5 and 0 min, an infusion of insulin lispro (Eli Lilly and Company, Homburg, Germany) was started at a variable rate between 1 and 3 mU·kg⁻¹·min⁻¹. This insulin titration was allowed because of the wide spectrum of fasting plasma glucose values (Fig. 1) and the expected wide range of individual insulin sensitivities between lean patients with diabetes due to chronic pancreatitis and variable degrees of maldigestion on the one hand, and obese patients with type 2 diabetes and insulin resistance on the other hand. The choice of initial insulin infusion rate and any decision to change the insulin infusion rate was at the discretion of the investigator. Insulin infusion rates for the three groups studied are shown in Fig. 1E. Accurate achievement of glycemic targets was achieved by determining capillary plasma glucose at 5-min intervals from an earlobe made hyperemic using Finalgon, and by adjusting a variable intravenous administration of sterile 20% (weight for volume) glucose in water (Fig. 1D).

Three glucose concentrations were pre-specified as glycemic targets for the clamp experiment. First, euglycemia (85 mg/dL) was targeted and maintained for 30 min. Note that patients with diabetes (chronic pancreatitis and type 2 diabetes) had fasting hyperglycemia of varying degree, whereas normal glucose-tolerant subjects started at euglycemia (Fig. 1A). Next, a glycemic plateau of 65 mg/dL was targeted (it took variable time periods to reach this glucose concentration) and again maintained for 30 min. Last, a glycemic plateau of 45 mg/dL was targeted and maintained for 30 min. When this last hypoglycemic plateau had been maintained

**Figure 1**—Capillary plasma concentrations of glucose (A) and venous concentrations of insulin (C) and C-peptide (E) as well as exogenous glucose (B) and insulin infusion rates (D) during a hyperinsulinemic, sequentially euglycemic and hypoglycemic clamp experiment in patients with diabetes due to chronic pancreatitis (filled triangles), subjects with type 2 diabetes (filled circles), and healthy control subjects (open circles). Statistical analysis: repeated-measures ANCOVA reporting *P* values for significant differences between the three groups of patients (A), over time (B) and any significant interactions (AB). Baseline concentrations/values with placebo were imputed as a covariate. *P* < 0.05, significant difference at particular time points vs. subjects with diabetes due to chronic pancreatitis; †*P* < 0.05, significant difference at particular time points vs. healthy control subjects.
for 30 min, insulin infusions were stopped, a glucose bolus was injected to elevate glucose concentrations into at least the euglycemic range, glucose infusions were continued for at least 30 min, and a meal was served. Participating subjects were supervised until stable glucose concentrations were reached.

**Blood Specimens**

Blood was drawn from indwelling teflon cannulas inserted into forearm veins at the time points shown in Figs. 2 and 3 and processed as previously described (17). EDTA plasma (with the addition of aprotinin) was separated by centrifugation and stored deep-frozen at −80°C in portions of 0.5–1.0 mL.

**Laboratory Determinations**

Glucose was measured (glucose oxidase method) with a Super GL compact glucose analyzer (Hitado). Insulin and C-peptide were measured with an ECLIA (electrochemiluminescence) assay (System Elecsys 2010, Cobas E Immunoassay, Modular Analytics E170; Roche). Intra-assay coefficient of variation was —2%. Of note, the insulin immunoassay did not detect insulin lispro, and thus only measured endogenously produced insulin under the circumstances of this clamp experiment. Glucagon was measured by a radioimmunoassay. The assay uses a polyclonal antiserum (code number 4305) (18,19) that was raised in rabbits against natural porcine glucagon-linked NH2 terminally to albumin. The assay has a detection limit of 1 pmol/L and an intra-assay coefficient of variation of

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**Figure 2**—Venous plasma concentrations during a hyperinsulinemic, sequentially euglycemic and hypoglycemic clamp experiment of glucagon (A), human growth hormone (B), cortisol (C), adrenaline (D), and noradrenaline (E) in patients with diabetes due to chronic pancreatitis (filled triangles), subjects with type 2 diabetes (filled circles), and healthy control subjects (open circles). Statistical analysis: repeated-measures ANCOVA reporting P values for significant differences between the three groups of patients (A), over time (B) and any significant interactions (AB). Baseline concentrations/values with placebo were imputed as a covariate. *P < 0.05, significant difference at particular time points vs. subjects with diabetes due to chronic pancreatitis; †P < 0.05, significant difference at particular time points vs. healthy control subjects.
<6% and has been validated versus sandwich ELISA and by mass spectrometry (20). The determination of human growth hormone, cortisol, adrenaline, and noradrenaline was performed as previously described (21).

Calculations and Statistical Analysis

The primary end point of the study was the maximum glucagon concentration at the lowest glucose plateau (45 mg/dL) during the clamp. Insulin secretion was judged from a C-peptide/glucose ratio calculated 20 min after oral glucose ingestion, as previously described (22).

Sample size calculation was performed using ClinCalc (clincalc.com). In a previous study (23), glucagon increased in response to hypoglycemia by 20 ± 7 pmol/L (±SD). With an α error of 0.05 and a β error of 0.15, nine subjects per group would be necessary to detect a difference of 50% with 85% power.

The suppression of glucagon in response to oral glucose ingestion was judged from the glucagon levels after oral glucose administration subtracted by the basal glucagon concentrations.

Subject characteristics are reported as mean ± SD and results as mean ± SEM. Statistical calculations were performed as repeated-measures ANCOVA using Statistica version 5.0 (Statsoft Europe, Hamburg, Germany). Experimental conditions (the three groups of subjects examined) were used as independent fixed variables, and the respective baseline values of the dependent variable with placebo treatment were used as a covariate. If a significant difference between any of the three groups was documented by a P value < 0.05, or by an interaction of treatment and time (P < 0.05), values at individual time points were analyzed by ANCOVA. In the case of significant results (P < 0.05), Duncan post hoc test was used to determine the significance of differences between specific patient groups. A two-sided P value < 0.05 was taken to indicate significant differences. Linear regression analyses were performed using GraphPad Prism version 4.

RESULTS

Patients/Subjects

Detailed patient characteristics are summarized in Supplementary Tables 1 and 2. Patients with type 2 diabetes were older (64 ± 7 years) than patients with chronic pancreatitis (52 ± 12 years) and control subjects (45 ± 18 years; P = 0.004), and the proportion of female participants was greater in control subjects (60%) than in patients with type 2 diabetes (15.4%) and patients with chronic pancreatitis (0%; P = 0.005). Furthermore, patients with diabetes due to chronic pancreatitis had a lower BMI than patients with type 2 diabetes. Morphological changes indicating typical pathology of chronic pancreatitis were documented with various imaging procedures, listed in Supplementary Table 1. HbA1c levels were similarly
Hypoglycemia Counterregulation in Pancreatitis

Hypoglycemia Counterregulation

Hypoglycemic Clamp Tests

OGTTs

Fasting glucose concentrations were highest in patients with type 2 diabetes and lowest in the control subjects ($P < 0.05$) (Fig. 3). Oral glucose ingestion led to an increase in glucose levels in all groups, with the highest increments being observed in patients with type 2 diabetes. Fasting insulin and C-peptide levels were not significantly different between the groups. After glucose ingestion, insulin and C-peptide levels rose to higher levels in the control subjects compared with patients with type 2 diabetes and patients with diabetes and chronic pancreatitis ($P < 0.05$) (Fig. 3). In patients with diabetes and chronic pancreatitis, C-peptide levels were lower than in patients with type 2 diabetes at $t = 20$, $210$, and $240$ min ($P < 0.05$). Glucagon levels declined after oral glucose ingestion in control subjects but increased initially in patients with type 2 diabetes and diabetes and chronic pancreatitis ($P < 0.0001$), eventually reaching similarly suppressed levels as the control subjects. The two groups with diabetes also had elevated fasting levels.

Hyperinsulinemic, Stepwise Hypoglycemic Clamp Tests

Fasting glucose concentrations were higher in patients with type 2 diabetes and lower in control individuals ($P < 0.05$) (Fig. 1A). Exogenous insulin infusions reduced glucose concentrations, first into the normal fasting target range, and then toward the two hypoglycemic plateaus ($P < 0.0001$).

After reaching euglycemia, there were no significant differences in plasma glucose concentrations between the three groups. The reduction in plasma glucose concentrations toward euglycemia and later hypoglycemia was accompanied by a reduction in insulin and C-peptide concentrations (Fig. 1B and C), such that the concentrations of both peptides were not different between the three groups at hypoglycemia. Insulin infusion rates were highest in insulin-resistant patients with type 2 diabetes and lowest in healthy control subjects (Fig. 1E).

Hypoglycemic Counterregulation

Glucagon levels increased between steps two and three of the hypoglycemic clamp in all three groups, but only healthy control subjects showed a significant rise over basal values ($P < 0.05$). Thus, glucagon concentrations during hypoglycemia were significantly lower in both groups of patients with diabetes compared with control subjects, but there was no difference between patients with diabetes secondary to chronic pancreatitis and patients with type 2 diabetes (Fig. 2A).

Growth hormone concentrations increased with hypoglycemia ($P < 0.0001$). This increase was significantly reduced in patients with type 2 diabetes compared with healthy control subjects at the lowest glycemic plateau ($P < 0.05$), whereas growth hormone levels were not different between patients with diabetes secondary to chronic pancreatitis and control subjects.

Cortisol concentrations increased during hypoglycemia ($P < 0.0001$), but this was similar in all three groups ($P = 0.52$). The increase in adrenaline and noradrenaline concentrations with decreasing glucose concentrations was less obvious ($P = 0.003$ and $P = 0.10$, respectively), and there were no significant differences between groups.

Relationship Between Insulin and Glucagon Secretion

Insulin secretion, as judged by a C-peptide/glucose ratio determined 20 min after oral glucose ingestion, was inversely related to the changes in glucagon levels from $t = 20$ min to $t = 120$ min after oral glucose ingestion (all $P < 0.05$). The strongest correlation was found for the change in glucagon levels after 60 min ($r = 0.65; P < 0.0001$) (Fig. 4A). Likewise, the maximum glucagon levels reached at hypoglycemia (plateau C) during the clamp experiment were significantly related to insulin secretion ($r = 0.43; P = 0.014$) (Fig. 4B). Interestingly, there was also a significant inverse relationship between the changes in glucagon levels during the OGTT and during the hypoglycemic clamp ($r = 0.50; P = 0.0028$) (Supplementary Fig. 1), suggesting that individuals who fail to suppress their glucagon levels after oral glucose ingestion also exhibit an abnormally low increase in glucagon secretion in response to hypoglycemia.

CONCLUSIONS

The current study demonstrates higher glucagon levels after oral glucose ingestion in both patients with type 2 diabetes and with diabetes due to chronic pancreatitis, whereas the glucagon response to hypoglycemia was reduced. However, only patients with type 2 diabetes exhibited impairments in the growth hormone response to hypoglycemia.

In clinical practice, patients with diabetes due to chronic pancreatitis often exhibit high frequencies of hypoglycemia when treated with insulin or sulfonylureas (15). The present results suggest that this might partly be due to an inability of the $\alpha$-cells to respond to declining glucose concentrations. However, because the extent of this deficit is similar to that seen in patients with type 2 diabetes, a reduction in $\alpha$-cell mass alone (because of islet destruction) might not fully explain the phenomenon. It is therefore likely that the high prevalence of hypoglycemia in such patients is also caused by other factors, such as the lower body weight. In support of this, the BMI was significantly higher in patients with type 2 diabetes compared with patients with diabetes secondary to chronic pancreatitis in the current study. An alternative explanation for the $\alpha$-cell dysfunction observed in the patients with chronic pancreatitis would be impaired $\beta$-cell function, as suggested by the close correlations between insulin secretion after oral glucose ingestion and the glucagon response to hypoglycemia. Thus, it seems plausible that the functional integrity of glucagon secretion critically depends on the preserved regulation of intraislet insulin secretion (17).

Whereas a loss of glucagon secretion during hypoglycemia has uniformly been reported in patients with type 1 diabetes (24,25), the data in patients with type 2 diabetes are more controversial. Thus, Bolli et al. (26) initially reported a marked reduction in glucagon secretion as well as an impaired stimulation of hepatic...
glucose release in patients with type 2 diabetes. These findings were confirmed by some, but not all, studies (27). Taking together the evidence available, it appears that defects in glucagon counterregulation develop rather late during the course of type 2 diabetes, when the residual β-cell function is presumably rather low. In support of such reasoning, Segel et al. (27) have demonstrated a loss of glucagon response to hypoglycemia in patients with type 2 diabetes treated with insulin but not in those on oral glucose-lowering agents. In this context, it is important to emphasize that the mean duration of type 2 diabetes in this study was 17 years, and that 38.5% of patients were treated with insulin, suggesting a rather advanced stage of the disease. Therefore, the findings of this study might not necessarily be representative for patients with type 2 diabetes in earlier stages of the disease. Furthermore, the absence of chronic pancreatitis in the patients with type 2 diabetes was determined on the basis of the patient history only. Thus, it cannot fully be excluded that some subclinical degree of pancreatitis was present in these patients as well.

The current study also demonstrates that a failure to suppress glucagon secretion after oral glucose or meal ingestion not only affects patients with type 2 diabetes (17,28,29) but can also be observed in patients with diabetes secondary to chronic pancreatitis (30–32). Interestingly, both groups of patients with diabetes exhibited a small paradoxical rise in glucagon levels immediately after oral glucose ingestion.

The reasons underlying the abnormal α-cell function in patients with 2 diabetes and those with diabetes due to chronic pancreatitis cannot be completely explained with this study. However, there is little reason to assume a reduction in the number of pancreatic α-cells in the patients with type 2 diabetes. In fact, in histological studies of pancreatic tissue specimens from patients with type 2 diabetes, pancreatic α-cell mass was found to be either normal or even increased compared with individuals without diabetes (4). Furthermore, glucagon levels after meal ingestion were even higher in the patients with diabetes. It has been suggested that the impaired α-cell function in patients with type 2 diabetes is due to the reduction in endogenous insulin secretion, which might be necessary on the one hand to inhibit glucagon release after ingestion of carbohydrate-rich meals and on the other hand to allow for the rapid increase in glucagon release in response to hypoglycemia. In support of this reasoning, we have previously demonstrated that an ~50% loss of β-cells in pigs results in marked disturbances of the pulsatile interaction of intrasilet insulin and glucagon secretion (33), and that such loss of interaction between endogenous insulin and glucagon release can also be detected in patients with type 2 diabetes and even prediabetes (17,34). Furthermore, the current study demonstrates that both the glucose-induced suppression of glucagon levels and the hypoglycemia-induced rise in glucagon secretion are closely related to the endogenous insulin secretory capacity. In this regard, it is intriguing that patients with impaired α-cell function seem to exhibit defects in both the hypoglycemia response and the glucose-induced suppression at the same time, thereby suggesting a common etiology of the defects. This observation is consistent with the observation that augmentation of glucagon release in response to a fall in glucose concentration depends on functionally intact β-cells. According to this theory, a decline in endogenous insulin release in conjunction with low glucose concentrations may be required to allow for a rise in glucagon secretion (“switch-off hypothesis”) (35–37).

An alternative explanation would be a disturbance of amino acid metabolism in patients with diabetes secondary to chronic pancreatitis and those with type 2 diabetes (38). Indeed, recent evidence has suggested a feedback regulation between circulating amino acid levels and glucagon secretion, and alterations in amino acid concentrations have been described both in patients with type 2 diabetes and in patients with chronic pancreatitis (6,39). One may also argue
that differences in the circulating insulin levels and in the exogenous insulin infusion rates had an unequal impact on \( \alpha \)-cell function \((40)\). Indeed, because the insulin levels and exogenous infusion rates were higher in patients with type 2 diabetes and in patients with chronic pancreatitis, these levels, in the arterial circulation, might exert an inhibitory effect on \( \alpha \)-cell function, which could explain the lower response rates. Finally, it cannot be excluded that a substantial proportion of the circulating glucagon was derived from intestinal L cells rather than from pancreatic \( \alpha \)-cells \((41)\), and that the functional regulation of glucagon secretion might differ between these cell types.

It has also been suggested that defects in \( \alpha \)-cell function are a specific pathogenetic feature of patients with type 2 diabetes, which might be related to the secretion and action of incretin hormones \((42)\). The fact, however, that a similar impairment in the glucagon response to hypoglycemia occurs in patients with type 2 diabetes and in patients with secondary diabetes argues against such reasoning.

A potential limitation of this study is the unequal sex distribution among the groups. Thus, all patients with chronic pancreatitis were male, whereas both male and female participants were studied in the other groups. This higher prevalence of male patients is rather typical for chronic pancreatitis, whereas type 2 diabetes is usually more evenly distributed among both sexes. One might also argue that the group sizes in this study were still relatively small, even though the number of patients with chronic pancreatitis was still larger than in the previous studies on hypoglycemia counterregulation in this patient group \((12,13)\). Furthermore, glucagon levels were determined in response to oral glucose ingestion as well as in response to hypoglycemia. However, in order to determine the maximum glucagon secretory capacity, arginine stimulation might have been more suitable.

In the current study, the etiologies of chronic pancreatitis could not be fully resolved in the majority of patients. Thus, chronic alcohol consumption was reported by 20% of the patients, but the reliability of such self-assessments is typically very low. In the remaining patients, no detailed information on the etiology of pancreatitis could be obtained. Therefore, the current study cannot clarify whether the respective causes of pancreatitis might have an unequal impact on \( \alpha \)-cell function.

Another interesting finding from this study is the differential response of growth hormone to hypoglycemia in patients with type 2 diabetes and patients with diabetes secondary to chronic pancreatitis. The reduced growth hormone response in the patients with type 2 diabetes is consistent with some previous studies and is most likely due to the effects of obesity on growth hormone secretion \((25)\). Of note, the differences in growth hormone concentrations were found only under conditions of hypoglycemia, whereas insulin concentrations were different only in the euglycemic state. It is therefore unlikely that the unequal growth hormone responses were attributable to different insulin levels during the clamp.

Two previous reports have addressed the glucagon response to hypoglycemia in patients with chronic pancreatitis. One study from 1977 reported the complete absence of glucagon secretion during insulin-induced hypoglycemia in two patients with advanced pancreatitis \((12)\), and similar findings have been reported in 1990 by Larsen et al. \((13)\) in six patients with chronic pancreatitis. The present results are at variance with these reports by showing that the glucagon response in patients with chronic pancreatitis is diminished in comparison with healthy subjects but not completely abolished. Most likely, these discrepancies are due to the different disease stages of chronic pancreatitis. Thus, the patients in the previous studies were examined at very advanced stages of the disease. Indeed, whereas the diagnosis of chronic pancreatitis in the 1970s was primarily based on the loss of exocrine pancreatic secretion, which typically occurs very late in the course of the disease, modern imaging procedures, such as endoscopic ultrasound, high-resolution computed tomography, and magnetic resonance imaging nowadays allow for the diagnosis of chronic pancreatitis at much earlier stages. The patients studied herein already exhibited exocrine insufficiency in 50% of the cases, and pancreatic calcifications or atrophy were present in 60% of cases. Thus, the extent of chronic pancreatitis in this group might be considered moderate to severe, while probably still being less advanced than in the previous studies.

Notably, insulin resistance was greater in the patients with chronic pancreatitis compared with the other groups. This finding is surprising given the lower BMI in these patients and might be attributable to the systemic effects of the chronic inflammatory process in the pancreas. Alternatively, the chronic hyperglycemia in these patients or the exogenous insulin treatment might have caused the impairment in peripheral action.

The impairment in glucagon response to hypoglycemia in patients with diabetes secondary to chronic pancreatitis may suggest some caution regarding the use of insulin or insulinotropic agents in such patients. However, therapeutic alternatives to achieve glycemic control in such patients are still limited. Thus, approaches to reduce insulin resistance, e.g., via glitazones or metformin, do not address the insulin deficiency in such patients, and metformin or acarbose may further aggravate the malabsorption \((2)\). DPP-4 inhibitors and GLP-1 analogs may further increase the risk of pancreatitis \((14)\) and are formally contraindicated in patients with a history of pancreatitis, and the caloric loss induced by SGLT-2 inhibitors may not be desirable in insulinopenic patients with malabsorption either. Therefore, despite the increased susceptibility to hypoglycemia, insulin replacement still appears to be the rational treatment choice for patients with diabetes due to chronic pancreatitis.

In conclusion, the current study has shown reduced glucagon responses to hypoglycemia as well as impairments in glucose-induced suppression of glucagon in both patients with type 2 diabetes and in patients with diabetes secondary to chronic pancreatitis. The relationship with impaired insulin secretion suggests that both defects might be related to \( \beta \)-cell dysfunction. The impaired glucagon counterregulation should be born in mind when treating patients with chronic pancreatitis with insulinotropic agents or exogenous insulin replacement.

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Pharmaceuticals. He has consulted with Novo
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**Author Contributions.** L.M. researched and analyzed the data and wrote the manuscript. T.G.K.B., S.R., N.S., and J.J.H. researched and analyzed the data and wrote the manuscript. J.J.M. designed the study, researched and analyzed the data, and edited the manuscript. B.A.M. researched and analyzed the data and revised and edited the manuscript. M.A.N. analyzed data and wrote the manuscript. J.J.M. designed the study, researched and analyzed the data, and wrote the manuscript. J.J.M. 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.

**References**

19. Holst JJ. Evidence that entero glucagon (II) is identical with the C-terminal sequence (residues 33-69) of glicentin. Biochem J 1982;207:381–388