No Hypoglycemia After Subcutaneous Administration of Glucagon-Like Peptide-1 in Lean Type 2 Diabetic Patients and in Patients With Diabetes Secondary to Chronic Pancreatitis

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OBJECTIVE — Glucagon-like peptide 1 (GLP-1) is a proglucagon derivative secreted primarily from the L-cells of the small intestinal mucosa in response to the ingestion of meals. GLP-1 stimulates insulin secretion and inhibits glucagon secretion. It has previously been shown that intravenous or subcutaneous administration of GLP-1 concomitant with intravenous glucose results in hypoglycemia in healthy subjects. Because GLP-1 is also effective in type 2 diabetic patients and is currently being evaluated as a therapeutic agent, it is important to investigate whether GLP-1 may cause hypoglycemia in such patients. We have previously shown that GLP-1 does not cause hypoglycemia in obese type 2 diabetic patients with insulin resistance amounting to $5.4 \pm 1.1$ according to homeostasis model assessment (HOMA). In this study, we investigated diabetic patients with normal or close to normal insulin sensitivity.

RESEARCH DESIGN AND METHODS — Eight lean type 2 diabetic patients (group 1) aged 60 years (range 50–72) with BMI 23.1 kg/m² (20.3–25.5) and HbA1c 8.0% (6.9–11.4) and eight patients with type 2 diabetes secondary to chronic pancreatitis (group 2) aged 52 years (41–62) with BMI 21.9 kg/m² (17.6–27.3) and HbA1c 7.8% (6.2–12.4) were given a subcutaneous injection of 1.5 nmol GLP-1/kg body wt. Then, 15 min later, at the time of peak GLP-1 concentration, plasma glucose (PG) was raised to 15 mmol/l with an intravenous glucose bolus. HOMA (mean ± SEM) showed insulin resistance amounting to 1.9 ± 0.3 and 1.7 ± 0.5 in the two groups, respectively.

RESULTS — In both groups, PG decreased rapidly and stabilized at 7.5 mmol/l (range 3.9–10.1) and 7.2 mmol/l (3.1–10.9) in groups 1 and 2, respectively, after 90 min. Neither symptoms of hypoglycemia nor biochemical hypoglycemia were observed in any patient.

CONCLUSIONS — We conclude that a GLP-1–based therapy would not be expected to be associated with an increased risk of hypoglycemia in insulin-sensitive type 2 diabetic patients.

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diabetic patients and patients with diabetes secondary to chronic pancreatitis.

The aim of the present study was to investigate whether hypoglycemia, as seen in healthy subjects after administration of high doses of GLP-1 and glucose, can also be elicited in subgroups of type 2 diabetic patients with close to normal insulin sensitivity.

RESEARCH DESIGN AND METHODS

Patients
We studied two groups of patients. One group (group 1) included eight lean type 2 diabetic patients (seven men and one woman) with a mean age 60 years (range 50–72), BMI 23.1 kg/m² (20.3–25.3), HbA1c 8.0% (6.9–11.4), fasting plasma glucose (FPG) 10.2 mmol/l (7.6–12.0), and duration of diabetes 52 months (7–114). One of the patients in this group was treated with diet alone, whereas seven were treated with diet and oral antidiabetic drugs (sulfonylureas and/or biguanides). Three patients had a history of hypertension and were treated with ACE inhibitors. The remaining five patients in the group were without cardiovascular disease. All of the lean type 2 diabetic patients were diagnosed according to the criteria of the World Health Organization (WHO) (8,9). The second group (group 2) consisted of eight patients with type 2 diabetes, diagnosed according to WHO criteria (8,9), secondary to chronic pancreatitis. The eight patients in group 2 included five men and three women with a mean age of 52 years (range 38–62), BMI 21.9 kg/m² (17.6–27.3), HbA1c 7.8% (6.2–12.4), FPG 9.2 mmol/l (6.0–13.8), and duration of diabetes 21.5 months (8–40). Diabetes was developed after the diagnosis of chronic pancreatitis was established, and none of the participants in this group had first-degree relatives with type 1 or type 2 diabetes. Four of the patients were treated with diet alone, whereas the remaining four were treated with diet and oral antidiabetic drugs (sulfonylureas and/or biguanides). All were without cardiovascular disease, except for one who had a history of hypertension and was treated with furosemide and diltiazem. All were without clinical or biochemical signs of acute inflammatory activity in the pancreas. Two of the patients had elevated levels of serum alkaline phosphatase, whereas serum albumin, prothrombin, and bilirubin were within normal limits. The etiology of chronic pancreatitis was judged to be alcoholism in six patients and idiopathic in two patients. None of the patients drank alcohol on a daily basis. The diagnostic criteria of chronic pancreatitis were according to Linder et al. (10), and all patients had reduced meal-stimulated duodenal concentration of lipase and amylase or reduced concentration of elastase in stool plus unequivocal morphologic changes of the pancreas shown at ultrasonography, computed tomography scan, or endoscopic retrograde cholangiopancreatography according to the Cambridge classification (11). Two patients were regularly treated with oral pancreatic enzyme supplementation because of steatorrhea.

None of the patients in the two groups had impaired renal function (normal serum creatinine levels [<130 μmol/l] and no albuminuria) or proliferative retinopathy, and all patients were negative with regards to islet cell autoantibodies. None of the participants had more than two times the upper reference range for biochemical liver parameters (alanine and aspartate transaminases, alkaline phosphatase, bilirubin, albumin, and coagulation factor II, VII, and X).

All subjects agreed to participate after oral and written information. The study was approved by the Copenhagen County ethical committee in July 2000 (journal number in the committee: KA00087 m), and the study was conducted according to the principles of the Helsinki Declaration.

Methods
All oral antidiabetic drugs were discontinued 3 days before the study, except for metformin, which was discontinued 7 days before the study. After an overnight fast (including water, coffee, and cigarettes) from 10:00 P.M., the subjects were studied in the recumbent position, with two cannulas, one inserted into the cubital vein for glucose infusion, and one inserted in the retrograde direction in the opposite dorsal hand vein for the collection of arterialized blood samples. The hand with the retrograde cannula was kept in a heating box (42°C) throughout the experiment. At time zero, GLP-1 was injected subcutaneously into the periumbilical region (GLP-1 at 1.5 nmol/kg body wt). The injected volume was 1.3 ml (range 1.26–1.40). We have previously shown (12) that peak GLP-1(7–36)amide concentrations occur ~15 min after subcutaneous injection. Therefore, at 15 min, plasma glucose (PG) was elevated to 15 mmol/l by an intravenous glucose (50% wt/vol) bolus administered within 1 min, calculated as follows: (15 mmol/l – FPG) × 35 mg glucose × body weight in kilograms. Arterialized blood was sampled 15, 10, and 0 min before and 10, 18, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 105, 110, 120, and 135 min after GLP-1 administration. Recombinant human GLP-1(7–36)amide, supplied as a 3–ml 1.0 mg/ml liquid sterile formulation, with a peptide purity >99% by reverse-phase high-performance liquid chromatography, was a generous gift from BioNebraska (Now Restoragen, Lincoln, NE). Before injection, the dissolved peptide was mixed with 0.5 ml of human serum albumin (5% wt/vol human albumin, guaranteed to be free of hepatitis-B surface antigen, hepatitis-C virus antibodies, and human immunodeficiency virus antibodies; Statens Serum Institute, Copenhagen) and 0.5 ml sterile saline. Blood was distributed into fluoride tubes for bedside PG measurements. The tubes were centrifuged for 3 min at 10,000 rpm (room temperature) immediately, and PG was measured. Blood was distributed into lithium heparin and EDTA (6 mmol/l) tubes with aprotinin (Trasylo, 500 KIU/ml blood; Bayer, Leverkusen, Germany) and a specific dipetidyl peptidase IV (DPP-IV) inhibitor (valine-pyrrolidide, 0.01 mmol/l final concentration; a gift from Drs. R.D. Carr and L.B. Christiansen, Novo Nordisk, Bagsvaerd, Denmark) for peptide and hormone analyses. Tubes were chilled immediately on ice and centrifuged for 20 min at 3,000 rpm and 4°C. Plasma for GLP-1 and glucagon analyses was stored at –20°C and plasma for insulin and C-peptide analyses was stored at –80°C until analysis.

During the experiments, the participating patients were observed continuously for possible side effects of GLP-1 and asked about their state of well-being and other subjective parameters such as dizziness, nausea, sweats, and the urge to defecate every 10 min the first 60 min after administration of GLP-1.
Analyses

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

Plasma insulin and C-peptide concentrations were measured using commercial AutoDELFIA time-resolved fluoroimmunoassay (Wallac Oy, Turku, Finland). The detection limits of the assays are 3 pmol/l for insulin and 17 pmol/l for C-peptide. The intra- and interassay coefficients of variation are 4.0–5.0% at 37–319 pmol/l and 4.8–5.4% at 78–718 pmol/l, respectively, for insulin and 2.0–8.0% at 423–2,726 pmol/l and 5.5–6.6% at 366–3,646 pmol/l for C-peptide. The cross-reactivities with intact and COOH-terminally truncated fragments of proinsulin in the C-peptide assay is therefore retracted (i.e., the sum of the intact peptide plus the primary metabolite), whereas NH$_2$-terminal assays measure the resulting concentration of intact surviving GLP-1. COOH-terminal immunoreactivity of GLP-1 was measured as described previously by Orskov et al. (15) against standards of synthetic GLP-1(7–36) amide [proglucagon(78–107) amide], using antiserum no. 89390, the cross-reaction of which is <0.01% with COOH-terminally truncated fragments and 83% with GLP-1(9–36) amide. The detection limit is 1 pmol/l. NH$_2$-terminal immunoreactivity was measured using antiserum no. 93242 (16), which cross-reacts ~10% with GLP-1(1–36) amide and <0.1% with both GLP-1(8–36) amide and GLP-1(9–36) amide. The assay has a detection limit of 2 pmol/l. For both assays, intra- and interassay coefficients of variation were <6% and <15%, respectively, at 40 pmol/l.

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. The detection limit of the assay is ~1 pmol/l, and the intra-assay coefficient of variation is <6% in the range between 10 and 25 pmol/l (17).

A quantitative assessment of insulin resistance was made by comparing the subjects’ fasting insulin and FPG using homeostasis model assessment (HOMA) (18). The formula is as follows: insulin resistance = (FI X FPG)/22.5, where FI is fasting insulin (μU/ml) and FPG is in mmol/l.

Results

All results are presented as the mean ± SEM. Time of GLP-1 injection = 0 min, and time of intravenous glucose bolus = 15 min.

Statistical analysis and calculations

All results are presented as the mean ± SEM or followed by the range in parentheses. The significance of difference between glucose and glucagon concentrations within the groups was evaluated using Wilcoxon’s test for pair differences. Significance of differences for insulin and C-peptide concentrations and insulin–to–C-peptide ratios between the groups were evaluated using two-factor ANOVA and for insulin resistance, they were evaluated using the Mann-Whitney rank-sum test for unpaired data. The level of statistical significance was set at P < 0.05.
19,125) and 68,831 min × pmol/l (42,690–104,758), respectively, in group 1 and 10,035 min × pmol/l (2,745–20,845) and 53,415 min × pmol/l (25,520–105,008) in group 2. No statistically significant differences of total or intact GLP-1 concentrations could be shown between the two groups.

Subjective side effects of GLP-1 were described by 7 (4 patients in group 1 and 3 patients in group 2) of the 16 patients, who reported an impaired state of well-being, light to moderate nausea, sweating, and/or the urge to defecate 10–40 min after the subcutaneous GLP-1 injection. All side effects diminished rapidly, and after 40 min all of the patients felt well, with no side effects at all.

Time courses of insulin and C-peptide concentrations are presented in Fig. 2A and B. After injection of GLP-1, an increase in insulin and C-peptide concentrations was seen, and injection of the glucose bolus 15 min later augmented the insulin and C-peptide responses further. Peak insulin and C-peptide concentrations occurred on the average 10 and 30 min, respectively, after intravenous glucose in group 1 and 20 min after intravenous glucose in group 2. Mean peak concentrations of insulin and C-peptide were 132 ± 21 and 1,509 ± 148 pmol/l, respectively, in group 1 and 203 ± 62 and 1,697 ± 353 pmol/l, respectively, in group 2. Insulin concentrations reached basal levels in both groups and stayed there for the last 15 min of the experiment, whereas C-peptide concentrations were still supra-basal but approaching basal levels in both groups at the end of the experiment. The iAUCs for insulin and C-peptide constituted 6,184 min × pmol/l (range 11,767–1,630) and 94,125 min × pmol/l (45,034–184,388), respectively, in group 1 and 7,029 min × pmol/l (812–13,587) and 87,410 min × pmol/l (16,590–141,909) in group 2. No statistical signifi-
cant differences between the two groups were found.

As illustrated in Fig. 2C, plasma glucagon concentrations decreased after subcutaneous administration of GLP-1 to sub-basal values in both groups. In group 1, we observed a statistically significant fall from a basal value of 17.3 pmol/l (range 15–19.3) in the fasting state to a nadir of 12.5 pmol/l (10–15) (P = 0.01) at 29 min (20–60) after the subcutaneous GLP-1 injection. In group 2, we observed a statistically significant fall from 16.2 pmol/l (range 11.7–21.3) to a nadir of 12.1 pmol/l (10–15) (P = 0.01) at 45 min (20–80) after the injection. From the nadir levels and for the remainder of the experiment, the concentrations of glucagon slowly, but statistically signifi-
cantly, increased to the end values of 15.5 pmol/l (range 10.7–21.3) in group 1 and 16.4 pmol/l (13–19) (P = 0.02) in group 2. We observed no statistically significant difference in basal values, nadir values, or end values between the two groups.

FPG was 10.2 mmol/l (range 9.0–11.7) and 9.2 mmol/l (6.0–13.8) in groups 1 and 2, respectively (Fig. 3). At 3 min after the intravenous glucose bolus, PG had increased to 15.2 mmol/l (range
12.3–17.3) mmol/l in group 1 and 15.3 mmol/l (14.0–16.1) in group 2. During the initial 17 min after the PG peak (at 18 min), the PG decreased rapidly to 11.5 mmol/l (14.0–12.3) in groups 1 and 2, respectively. At 50 min after subcutaneous administration of GLP-1, the PG returned to the starting point in both groups. PG administration of GLP-1 caused biochemical hypoglycemia in overweight patients with type 2 diabetes (7). The lack of hypoglycemic reactions in the patients was, therefore, not due to inadequate levels of GLP-1 in the investigational period. GLP-1 injected alone in the fasting state is not likely to elicit hypoglycemia because of the glucose dependency of the insulin response to GLP-1 (20). Even in diabetic subjects with high FPG, only a very small reduction in blood glucose can be elicited after subcutaneous administration of GLP-1 in the fasting state (21). Therefore, experimentally, we brought up blood glucose to high levels at the time for maximal concentration of GLP-1, mimicking the injection of a large therapeutic dose of GLP-1 followed by ingestion of carbohydrates. The earlier reported hypoglycemic reaction in healthy subjects (7) was interpreted to reflect the greatly enhanced insulin secretion induced by GLP-1 and the relatively long inactivation time of insulin’s effect on glucose disposal, even after normoglycemia had been reached.

As mentioned above, no hypoglycemic reaction was reported among overweight type 2 diabetic patients (7). The possible contributors to the absence of hypoglycemia in obese insulin-resistant type 2 diabetic patients was judged to be a combination of impaired insulin response and increased insulin resistance. One could imagine, therefore, that groups of patients characterized predominantly by impaired insulin secretory capacity but with near-normal insulin sensitivity, including lean type 2 diabetic patients and patients with pancreatogenic diabetes,

**CONCLUSIONS** — The present study reveals that subcutaneous administration of the highest therapeutically relevant dose of GLP-1 in combination with intravenous glucose to lean type 2 diabetic patients and patients with diabetes secondary to chronic pancreatitis (patients with close to normal insulin sensitivity) does not provoke hypoglycemia.
still would run the risk of hypoglycemia in a GLP-1–based antidiabetic therapy, in view of the powerful insulinotropic and hypoglucagonotropic action of GLP-1. Both groups in the present study responded with a marked increase in insulin secretion in response to GLP-1 plus glucose, but the rate of secretion, as judged by the iAUCs for C-peptide, amounted to only ~40% of that reported earlier for healthy subjects (7). Thus, it seems that an impaired insulin secretory capacity is more important than insulin resistance with respect to the proneness to develop hypoglycemia after subcutaneous administration of GLP-1 in combination with intravenous glucose. In some studies, GLP-1 has been found to enhance insulin-mediated glucose transport in adipose and muscle tissues and to reduce glucose production in the liver (i.e., to enhance the insulin sensitivity) (22–25), and a recent study by Egan et al. (26) concluded that GLP-1 has insulinomimetic properties per se in insulin-resistant states. Our study suggests that regained insulin sensitivity (e.g., from weight loss or GLP-1 therapy) does not render the patient sensitive to the hypoglycemic effects of GLP-1.

We observed a significant increase in glucagon concentration at the end of the experiment in both groups (P = 0.02), but it is unlikely that elevated glucagon levels protect against GLP-1–induced hypoglycemia in the present experiment because GLP-1 caused a marked and apparently normal suppression of the secretion of glucagon (7).

Our results suggest that a GLP-1–based therapy for type 2 diabetes will not be associated with an increased risk of hypoglycemia, even in patients with preserved or restored insulin sensitivity, because the present groups of patients were characterized by a predominantly impaired insulin secretory capacity and close to normal insulin sensitivity. Nevertheless, GLP-1 has trophic actions on pancreatic β-cells and also promotes differentiation of β-cells from progenitor duct cells (27). Therefore, prolonged GLP-1 therapy may improve the insulin secretory capacity, and such patients might then run the risk of GLP-1–induced hypoglycemia, which, on the other hand, should be amendable by reducing the dose of GLP-1. On the basis of several studies, we conclude that treatment with GLP-1 is not likely to elicit hypoglycemia in type 2 diabetic patients. However, the new long-acting GLP-1 analogs still need to be studied with the risk of hypoglycemia in mind.

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References