Similar Insulin Secretory Response to a Gastric Inhibitory Polypeptide Bolus Injection at Euglycemia in First-Degree Relatives of Patients With Type 2 Diabetes and Control Subjects

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Insulin secretion following the intravenous infusion of gastric inhibitory polypeptide (GIP) is diminished in patients with type 2 diabetes and at least a subgroup of their first-degree relatives at hyperglycemic clamp conditions. Therefore, we studied the effects of an intravenous bolus administration of GIP at normoglycemic conditions in the fasting state. Ten healthy control subjects were studied with an intravenous bolus administration of placebo, and of 7, 20, and 60 pmol GIP/kg body weight (BW), respectively. Forty-five first-degree relatives of patients with type 2 diabetes and 33 matched control subjects were studied with (1) a 75-g oral glucose tolerance test (OGTT) and (2) an intravenous bolus injection of 20 pmol GIP/kg BW with blood samples drawn over 30 minutes for determination of plasma glucose, insulin, C-peptide, and GIP. Statistical analysis applied repeated-measures analysis of variance (ANOVA) and Duncan's *post hoc* tests. Insulin secretion was stimulated after the administration of 20 and of 60 pmol GIP/kg BW in the dose-response experiments ($P < .0001$). GIP administration (20 pmol/kg BW) led to a significant rise of insulin and C-peptide concentrations in the first-degree relatives and control subjects ($P < .0001$), but there was difference between groups ($P = .64$ and $P = .87$, respectively). Also expressed as increments over baseline, no differences were apparent ($\Delta_{insulin}$, 7.6 ± 1.2 and 7.6 ± 1.6 mU/L, $P = .99$; $\Delta_{C\text{-}peptide}$, 0.35 ± 0.06 and 0.38 ± 0.08 ng/mL, $P = .75$). Integrated insulin and C-peptide responses after GIP administration significantly correlated with the respective insulin and C-peptide responses after glucose ingestion (insulin, $r = 0.78$, $P < .0001$; C-peptide, $r = 0.35$, $P = .0015$). We conclude that a reduced insulinotropic effect of GIP in first-degree relatives of patients with type 2 diabetes cannot be observed at euglycemia. Therefore, a reduced GIP-induced insulin secretion in patients with type 2 diabetes and their first-degree relatives at hyperglycemia is more likely due to a general defect of B-cell function than to a specific defect of the GIP action.

INSULIN SECRETION following an oral glucose or fat load is stimulated not only by rise of glucose concentrations, but also by the release of incretin hormones, namely, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), from the gut.[1,2] A diminished GIP effect on insulin secretion, resulting in a reduced incretin effect, is characteristic of the type 2 diabetic phenotype,[3-5] but the mechanisms underlying the reduced GIP effect have not yet been identified.[6,7]

To clarify whether the defective GIP-responsiveness in patients with type 2 diabetes reflects a primary, possibly genetically determined, defect, we have recently studied the insulinotropic effect of GIP in first-degree relatives of patients with type 2 diabetes, healthy control subjects, and patients with type 2 diabetes under hyperglycemic clamp conditions.[8] Following the infusion of GIP, insulin secretion was diminished in at least 50% of the first-degree relatives, similar to the patterns observed in the patients with type 2 diabetes. These results led to the conclusion that a reduced insulinotropic effect of GIP might predispose to the development of type 2 diabetes. However, since those experiments had been performed under hyperglycemic clamp conditions, it was difficult to distinguish between a defective glucose-induced insulin secretion on the one hand, and specific defects of the B-cell response towards exogenous GIP on the other hand. We therefore aimed to study the effects of a bolus administration of GIP at fasting glucose levels in first-degree relatives of patients with type 2 diabetes and healthy controls. Preliminary data have been communicated in abstract form.[9]

MATERIALS AND METHODS

Study Protocol

The study protocol was approved by the Ethics Committee of the Medical Faculty of the Ruhr-University, Bochum, on December 12, 1999 (registration no. 1420) prior to study commencement. Written informed consent was obtained from all participants.

Study Design

All subjects participated in a screening visit. Ten healthy control subjects participated in 4 dose-finding experiments with the intravenous administration of 7, 20, and 60 pmol GIP/kg body weight (BW) or placebo. Thirty-three healthy control subjects without a family history of type 2 diabetes and 45 first-degree relatives of patients with type 2 diabetes were studied with (1) the intravenous administration 20 pmol GIP/kg BW and (2) an oral glucose tolerance test (OGTT; 75 g).

Screening visit. Venous blood samples were drawn in the fasting state for standard hematological and clinical chemistry parameters and a clinical examination was performed. Patients with anemia (hemoglobin < 12 g/dL), an elevation in liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyltransferase) to higher than double the respective normal value, or elevated creatinine concentrations (>1.5 mg/dL), were excluded. If subjects met the inclusion criteria, they were recruited for the following tests.

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Submitted February 18, 2003; accepted June 16, 2003.

Supported by Deutsche Forschungsgemeinschaft (DFG) Grants No. Na 203/6-1 and Ga 386/8-1, FoRUM Grant-No. F277/01, and the Deutsche Diabetes-Getellschaft (DDG).

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0026-0495/03/5212-0051$30.00/0

doi:10.1016/S0026-0495(03)00327-5

Table 1. Characteristics of the Participants

Parameter	Healthy Control Subjects	First-Degree Relatives of Patients With Type 2 Diabetes	Significance (*P* value)
Sex (female/male)	16/17	35/10	.009
Age (yr)	42 ± 2	44 ± 2	.42
Body mass index (kg/m^2)	24.2 ± 0.5	25.0 ± 0.6	.33
Waist-to-hip ratio (cm/cm)	0.89 ± 0.02	0.82 ± 0.0	.00082
HbA_{1c} (%)	5.4 ± 0.1	5.2 ± 0.1	.07
Oral glucose tolerance			
Fasting plasma glucose (mg/dL)	102 ± 2	98 ± 2	.038
120-min plasma glucose (mg/dL)	125 ± 4	128 ± 4	.53
First-degree relatives with type 2 diabetes			
Father	—	27	—
Mother	—	25	—
Siblings	—	3	—
Blood pressure			
Systolic (mm Hg)	119 ± 2	117 ± 3	.48
Diastolic (mm Hg)	77 ± 1	75 ± 1	.17
Total cholesterol (mg/dL)	210 ± 7	211 ± 6	.89
HDL-cholesterol (mg/dL)	56 ± 4	59 ± 3	.24
LDL-cholesterol (mg/dL)	128 ± 9	128 ± 7	.92
Triglycerides (mg/dL)	110 ± 56	125 ± 13	.35
Creatinine (mg/dL)	0.78 ± 0.02	0.76 ± 0.02	.40

NOTE. Values are means ± SE. Statistics: ANOVA or χ^2 test.

Experimental Procedures

All tests were performed in the morning after an overnight fast in a sedentary position throughout the experiments. A large forearm vein was punctured with a Teflon cannula (Moskito 123, 18 gauge, Vygon, Aachen, Germany), and kept patent for blood sampling using 0.9% NaCl. A contralateral vein was punctured in case of injections of GIP or placebo. Both ear lobes were made hyperemic using Finalgon (Nonivamid 4 mg/g, Nicoboxil 25 mg/g; Boehringer Ingelheim, Ingelheim, Germany). Plasma glucose was determined in 100-μL capillary samples drawn from an ear lobe, and venous blood samples were drawn for hormone measurements.

Dose-response study. Subjects participated in 4 dose-finding experiments with the intravenous administration of placebo (0.9% NaCl with 1% human serum albumin), and of 7, 20, and 60 pmol GIP/kg BW. Capillary and venous blood samples were obtained twice in the basal state and 1, 3, 5, 10, 15, 20, and 30 minutes after GIP/placebo injections. At least 1 day had to pass between the experiments. The mean interval between between the tests was 29 ± 14 days.

GIP bolus injection test. After drawing basal venous and capillary blood samples (−5 and 0 minutes), 20 pmol GIP/kg BW was injected intravenously into a large forearm vein. Blood samples were obtained as described.

Oral glucose tolerance test. After drawing 2 basal (−15 and 0 minutes) capillary and venous blood samples, an oral glucose drink (75 g; O.G.T., Roche Diagnostics, Mannheim, Germany) was ingested within 5 minutes. Capillary and venous blood samples were obtained after 30, 60, 90, and 120 minutes. The interval between the OGTT and the GIP bolus test was 28 ± 8 days in the control subjects and 21 ± 6 days in the first-degree relatives ($P = .46$).

Subjects

Dose-response study. Ten healthy subjects were studied: 9 men and 1 woman, age 33 ± 11 years, hemoglobin A_{1c} (HbA_{1c}) 4.9% ± 1.2% (mean ± SD; normal range, 4.8% to 6.0%), body mass index (BMI) 26.8 ± 2.2 kg/m^2, OGTT fasting glucose concentration 5.6 ± 0.3 mmol/L, OGTT 120-minute glucose concentration 5.7 ± 1.5 mmol/L, total cholesterol 190 ± 74 mg/dL, high-density lipoprotein (HDL)-cholesterol 40 ± 25 mg/dL, low-density lipoprotein (LDL)-cholesterol 131 ± 52 mg/dL, and triglycerides124 ± 62 mg/dL.

Comparison of first-degree relatives of patients with type 2 diabetes and control subjects. Subject characteristics are listed in Table 1. The groups were matched for age, BMI, and blood pressure, as well as other features of the metabolic syndrome.

Peptides

Synthetic human GIP was purchased from PolyPeptide Laboratories GmbH, Wolfenbüttel, Germany. The lot number (pharmaceutical grade) was E-0517, and net peptide content was 80.3%. The peptide was dissolved in 0.9% NaCl/1% human serum albumin (HSA Behring, salt poor, Marburg, Germany), filtered through 0.2-μm nitrocellulose filters (Sartorius, Göttingen, Germany) and stored frozen at −28 °C as previously described.[8] High-performance liquid chromatography (HPLC) profiles (provided by the manufacturer) showed that the preparation was greater than 99% pure (single peak coeluting with appropriate standards). Samples were analyzed for bacterial growth (standard culture techniques) and for pyrogens (laboratory of Dr Balfanz, Münster, Germany). No bacterial contamination was detected. Endotoxin concentrations in samples from the GIP stock solution were 0.08 IU/mL.

Blood Specimens

Blood was drawn into chilled tubes containing EDTA and aprotinin (Trasylol; 20,000 KIU/mL, 200 μL per 10 mL blood; Bayer AG, Leverkusen, Germany) and kept on ice. After centrifugation at 4°C, plasma for hormone analyses was kept frozen at −28°C.

Capillary blood samples (~100 μL) were stored in NaF (Microvette CB 300; Sarstedt, Nümbrecht, Germany) for the immediate measurement of glucose.

Laboratory Determinations

Glucose was measured using a glucose oxidase method with a Glucose Analyzer 2 (Beckman Instruments, Munich, Germany). Insulin was measured using an insulin microparticle enzyme immu-

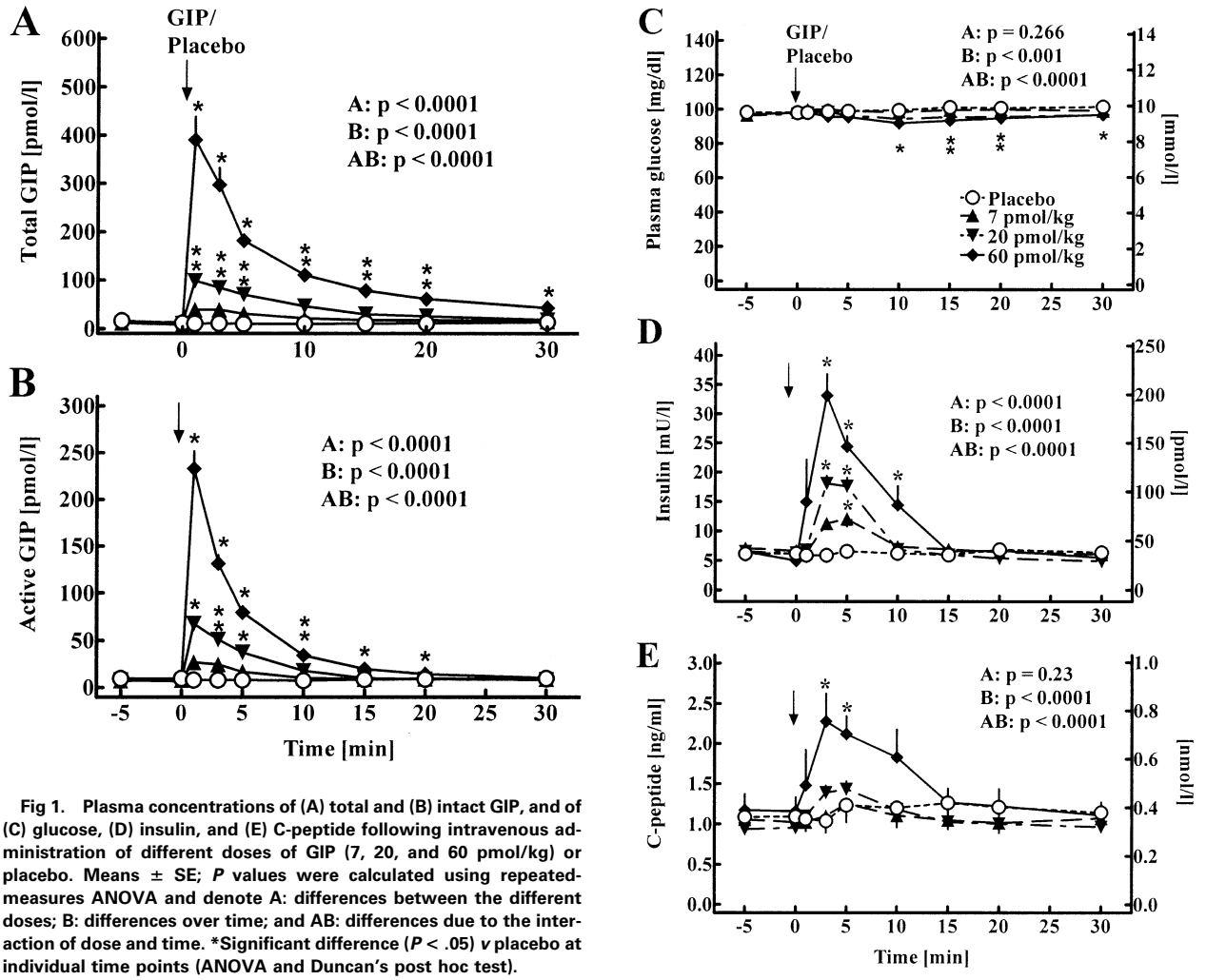

Fig 1. Plasma concentrations of (A) total and (B) intact GIP, and of (C) glucose, (D) insulin, and (E) C-peptide following intravenous administration of different doses of GIP (7, 20, and 60 pmol/kg) or placebo. Means ± SE; *P* values were calculated using repeated-measures ANOVA and denote A: differences between the different doses; B: differences over time; and AB: differences due to the interaction of dose and time. *Significant difference (*P* < .05) *v* placebo at individual time points (ANOVA and Duncan's post hoc test).

noassay (MEIA), IMx Insulin (Abbott Laboratories, Wiesbaden, Germany). Intra-assay coefficients of variation were approximately 4%. C-peptide was measured using C-peptide antibody-coated microtiter wells (C-peptide MTPL EIA) from DRG Instruments GmbH, Marburg, Germany. Intra-assay coefficients of variation were approximately 6%. Human insulin and C-peptide were used as standards. Total immunoreactive (IR)-GIP (1-42 amide plus split products) was determined as previously described,[10] using antiserum R 65 (final dilution 1:150 000) and synthetic human GIP (Peninsula Laboratories, St Helens, Merseyside, UK) for tracer preparation and as standard. The experimental detection limit was less than 1 pmol/L. Antiserum R 65 binds to the midportion of the GIP molecule. Intra-assay coefficients of variation were approximately 8%, and interassay coefficients of variation were less than 6%. Intact GIP (1-42 amide) was determined as described[10] using antiserum 98171 (final dilution 1:40 000) and synthetic human GIP (Peninsula Laboratories, Europe Ltd) for tracer preparation and as standard. The detection limit was less than 5 pmol/L. Cross-reactions with GIP (3-42 amide), GLP-1 (7-36 amide), GLP-1 (9-36 amide), GLP-2 (1-33 amide), GLP-2 (3-33 amide), or glucagon were less than 0.1%. Intraassay variation was less than 6%, and interassay coefficients of variation were 8%.

Calculations

Increments (Δ) in insulin and C-peptide concentrations were calculated as differences between values determined 3 minutes after the administration of GIP of placebo (time of maximal stimulation) and basal levels (−5 and 0 minutes). Insulin resistance and B-cell function were calculated according to the homeostasis model assessment.[11]

Integrated insulin and C-peptide responses to the administration of exogenous GIP were correlated to the integrated insulin and C-peptide responses after the ingestion of oral glucose.

Statistical Analysis

Results are reported as the mean ± SEM. All statistical calculations were performed by repeated-measures analysis of variance (ANOVA) using Statistica version 5.0 (Statsoft Europe, Hamburg, Germany). This analysis provides *P* values for differences between groups/experiments (A), differences over time (B), and for the interaction of group/experiment with time (C). If a significant interaction of treatment and time was documented ($P < .05$), values at single time points were compared by 1-way ANOVA. A 2-sided *P* value $< .05$ was taken to indicate significant differences.

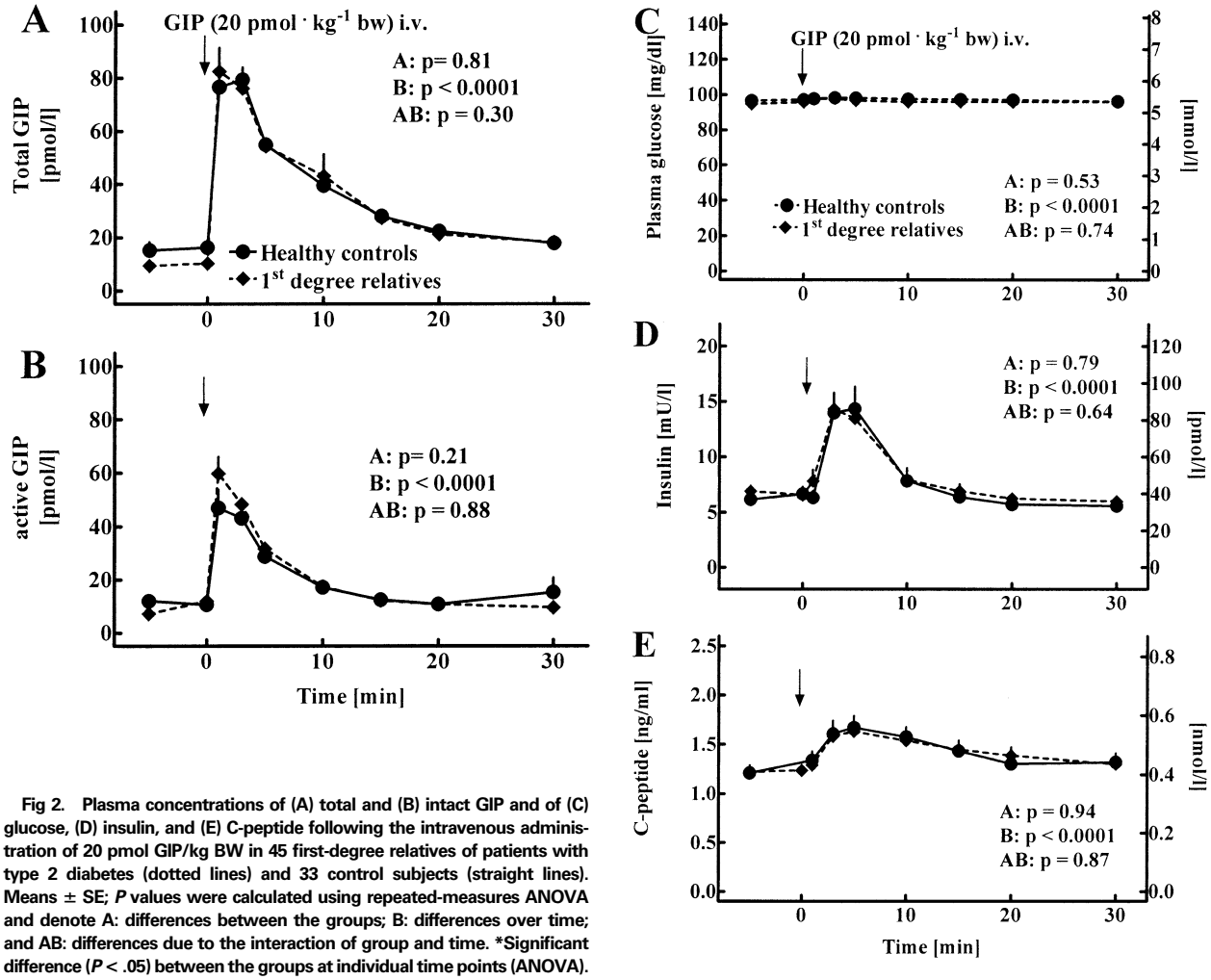

Fig 2. Plasma concentrations of (A) total and (B) intact GIP and of (C) glucose, (D) insulin, and (E) C-peptide following the intravenous administration of 20 pmol GIP/kg BW in 45 first-degree relatives of patients with type 2 diabetes (dotted lines) and 33 control subjects (straight lines). Means ± SE; *P* values were calculated using repeated-measures ANOVA and denote A: differences between the groups; B: differences over time; and AB: differences due to the interaction of group and time. *Significant difference (*P* < .05) between the groups at individual time points (ANOVA).

RESULTS

Dose-Response Study

Following the intravenous administration of placebo, and of 7, 20, and 60 pmol GIP per kg BW, the concentrations of total GIP (1-42 plus split products) rose to peak levels of 11 ± 3, 39 ± 6, 99 ± 12, and 390 ± 48 pmol/L, respectively ($P < 0.001$), the peak concentrations of intact GIP (1-42) were 8 ± 1, 27 ± 4, 68 ± 8, and 233 ± 19 pmol/L ($P < .001$; Fig 1). Insulin and C-peptide concentrations dose-dependently increased after GIP administration ($P < .001$), whereas they remained almost unchanged after placebo. Glucose concentrations were only slightly lowered after GIP administration (Fig 1). A significant stimulation of insulin and C-peptide concentrations versus placebo administration was found after the injection of 20 and 60 pmol GIP/kg, but not after 7 pmol GIP (Δinsulin: −0.29 ± 0.64, 4.39 ± 0.61, 11.68 ± 1.3, 27.4 ± 3.45 mU/L for placebo and for 7, 20, and 60 pmol GIP/kg, respectively; $P < .0001$, ΔC-peptide: −0.05 ± 0.08, 0.06 ± 0.06, 0.44 ± 0.07, 1.11 ± 0.19 ng/mL, $P < .0001$). Based on these dose-finding studies, an intermediate dose of 20 pmol GIP/kg was chosen for the following experiments.

Comparison of First-Degree Relatives of Patients With Type 2 Diabetes and Control Subjects

GIP bolus injection test. Following the bolus injection of 20 pmol GIP/kg BW, comparable plasma concentrations of GIP were reached in the first-degree relatives of patients with type 2 diabetes and the control subjects ($P = .3$ and .88 for the total [GIP 1-42 plus split products] and for the active GIP [1-42], respectively; Fig 2). Glucose concentrations were only slightly lowered by GIP. No significant differences regarding glucose, insulin, or C-peptide concentrations were found between the first-degree relatives of patients with type 2 diabetes and the control subjects after GIP administration ($P = .74$, .64, and .87, respectively; Fig 2). Also expressed as increments over baseline, no differences were apparent between both groups (Δinsulin: 7.6 ± 1.6 and 7.6 ± 1.2 for healthy control subjects and for first degree relatives of patients with type 2 diabetes, respectively, $P = .99$; ΔC-peptide: 0.38 ± 0.08 and 0.35 ± 0.06, $P = .75$).

Oral glucose tolerance test. The first-degree relatives of type 2 diabetic patients were characterized by slightly, but significantly lower plasma glucose concentrations in the fasting

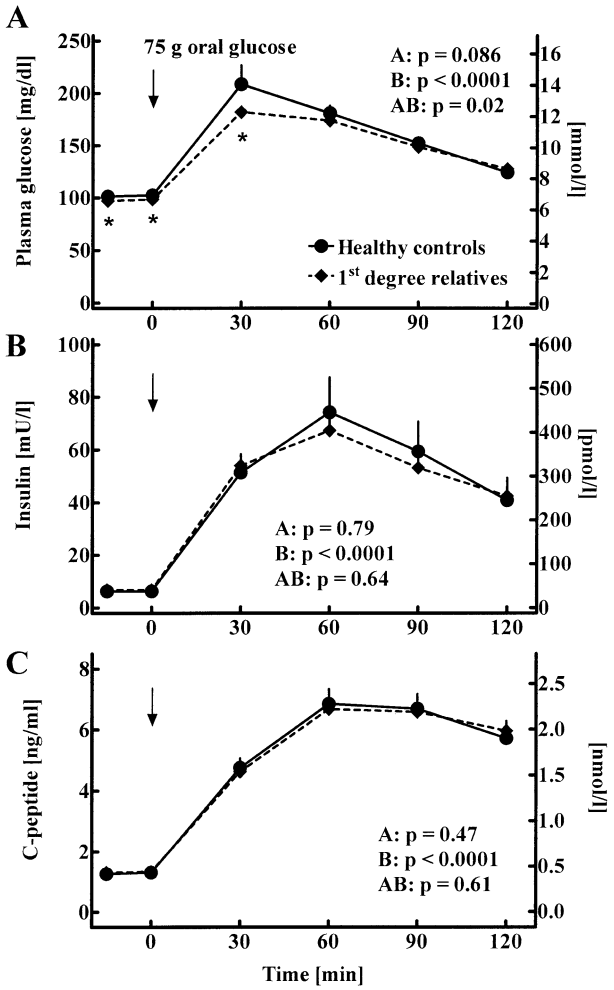

Fig 3. Plasma concentrations of (A) glucose, (B) insulin, and (C) C-peptide after 75 g oral glucose in 45 first-degree relatives of patients with type 2 diabetes (dotted lines) and 33 control subjects (straight lines). Means ± SE, *P* values were calculated using repeated-measures ANOVA and denote A: differences between both groups; B: differences over time; and AB: differences due to the interaction of group and time. *Significant difference (*P* < .05) between both groups at individual time points (ANOVA and Duncan's post hoc test).

state, as well as 30 minutes after the oral glucose load, compared to the control subjects ($P = .04$ and .028 for 0 and 30 minutes, respectively; Fig 3). From 60 minutes after the oral glucose load on, glucose concentrations were comparable between both groups. Insulin and C-peptide concentrations increased after glucose ingestion in both groups ($P < .0001$), but no significant differences occurred between the first-degree relatives and the healthy controls ($P = .64$ and .61 for insulin and C-peptide, respectively; Fig 3).

HOMA B-cell function index (according to Matthews et al[11]) was 59% ± 4% in the healthy controls and 74% ± 6% in the first-degree relatives ($P = .043$). HOMA insulin resistance indices were 1.62 ± 0.21 and 1.64 ± 0.13 ($P = .92$).

Correlation analysis. Integrated insulin and C-peptide responses after the intravenous administration of exogenous GIP closely correlated to the respective integrated insulin and C-peptide responses after oral glucose ingestion (Fig 4; insulin: $r = 0.78$, $P < .0001$; C-peptide: $r = 0.35$, $P = .0015$).

DISCUSSION

Since we have previously described a reduced insulinotropic effect of GIP in first-degree relatives of patients with type 2 diabetes under hyperglycemic clamp conditions,[8] it was tempting to evaluate whether a simple GIP bolus injection test at normal fasting glucose conditions would duplicate these previous results. The GIP dose of 20 pmol/kg BW was chosen on the basis of a dose-response study (Fig 1). This dose raised the GIP plasma concentrations to similar levels as in our previous

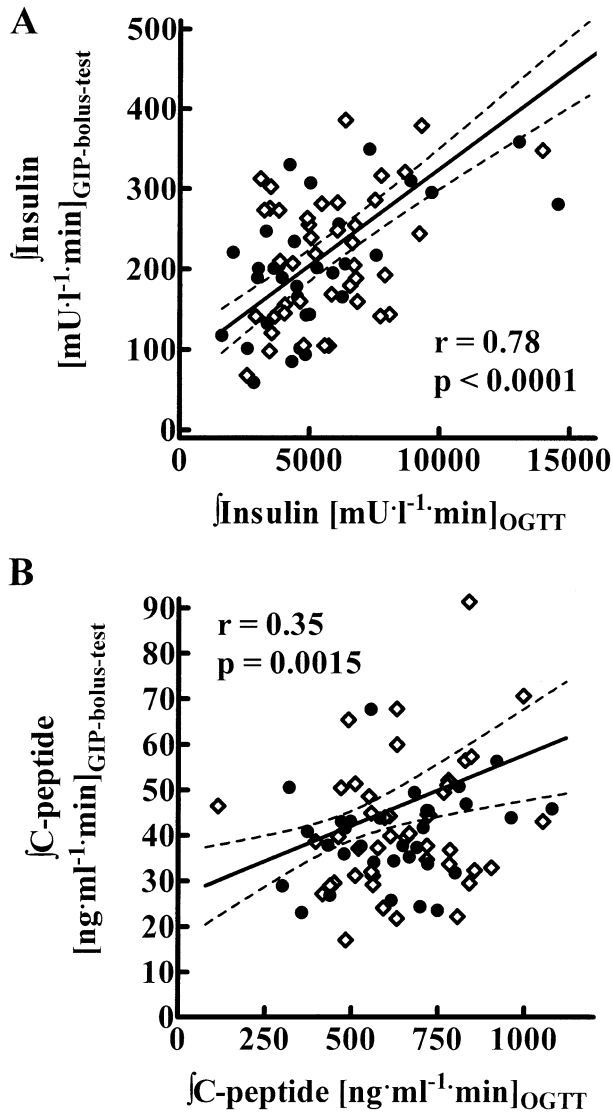

Fig 4. Correlation analysis of the integrated plasma responses of (A) insulin and (B) C-peptide (B) after intravenous administration of 20 pmol GIP/kg BW and after the ingestion of 75 g oral glucose in 45 first-degree relatives of patients with type 2 diabetes (◇) and 33 control subjects (●). The dotted lines indicate the upper and lower 95% confidence intervals. *r*: correlation coefficient.

clamp study.[8] These levels are comparable to the ones reached by endogenous secretion after meal ingestion.[12]

The subject characteristics were similar in the present study and in our previous experiments (Table 1).[8] However, with the GIP bolus administration, no differences were apparent in either insulin or C-peptide concentrations between the first-degree relatives and the control subjects (Fig 2). Therefore, the question arises, why the insulinotropic effect of GIP is reduced in the first-degree relatives of patients with type 2 diabetes under hyperglycemic clamp conditions, but not after a bolus administration at euglycemia. There are different ways to interpret these results. First, it is possible that only the late-phase insulin response to GIP, as detected with the hyperglycemic clamp experiments, is defective in patients with type 2 diabetes, while the first-phase secretion, as assessed with a GIP bolus, is intact. This is supported by a recent study comparing patients with type 2 diabetes and control subjects.[13]

Second, since an important difference between our previous clamp study and the present GIP bolus injection test lies in the different glucose concentrations, it is also possible that differences in GIP actions only become apparent under hyperglycemic conditions.[8] This is supported by the fact that the insulinotropic activity of GIP is strictly glucose-dependent.[14-16]

Third, the reduced insulin secretion in response to the combination of GIP and hyperglycemia might reflect a diminished insulin response to glucose rather than GIP. Accordingly, diminished insulin secretion after intravenous glucose has been described in first-degree relatives of patients with type 2 diabetes.[17] Furthermore, each stimulus superimposed to a glucose stimulus can potentiate defects in insulin secretion.[18-20]

Although a reduced insulin secretory response to GIP stimulation in type 2 diabetes was described years ago,[5,21] and a number of groups have attempted to characterize structural or defects of the GIP receptor[22,23] as well as its quantitative expression on B cells,[24] it was yet impossible to identify the molecular and, possibly, genetic defects underlying this phenomenon. Based on our data showing normal insulin secretion after GIP administration in first-degree relatives of patients with type 2 diabetes at least at normal glucose concentrations, and on the preserved early-phase insulin secretion in response to GIP in patients with type 2 diabetes,[13] it may be questioned whether such a specific defect in the GIP signaling cascade really exists. The close association between the insulin secretory responses to the intravenous GIP administration and to oral glucose ingestion (Fig 4) also points to a general B-cell defect in patients with type 2 diabetes rather than to a specific defect in GIP action. This would also explain the normal percentage contribution of the incretin effect to the overall insulin secretion after an oral glucose load in first-degree relatives of patients with type 2 diabetes.[25]

It would be of interest to compare healthy subjects and patients with type 2 diabetes with a GIP bolus injection test. However, due to the different fasting glucose levels in patients with and without diabetes, this might require prior normalization of fasting glycemia in patients with diabetes or identical hyperglycemic conditions.

A drawback may be seen in the possibility of carryover effects between the GIP bolus injections and the OGTTs. However, since the interval between the experiments was similar in both groups, such carryover effects appear to be negligible.

In conclusion, GIP dose-dependently stimulates insulin secretion at basal glucose concentrations. Whereas under hyperglycemic clamp conditions the insulinotropic effect of GIP is diminished in first-degree relatives of patients with type 2 diabetes compared to control subjects,[8] these differences are absent at normoglycemic fasting conditions. Therefore, the most likely explanation of the reduced GIP-induced insulin secretion in patients with type 2 diabetes and their first-degree relatives at hyperglycemia is a more general defect of B-cell function rather than a specific defect of the GIP action.

ACKNOWLEDGMENT

The excellent technical assistance of Kirsten Mros, Birgit Baller, and Lona Albaek is greatly appreciated.

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