Reduced Insulinotropic Effect of Gastric Inhibitory Polypeptide in First-Degree Relatives of Patients With Type 2 Diabetes

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In patients with type 2 diabetes, gastric inhibitory polypeptide (GIP) has lost much of its insulinotropic activity. Whether this is similar in first-degree relatives of patients with type 2 diabetes is unknown. A total of 21 first-degree relatives, 10 patients with type 2 diabetes, and 10 control subjects (normal oral glucose tolerance) were examined. During a hyperglycemic "clamp" (140 mg/dl for 120 min), synthetic human GIP (2 pmol · kg^{-1} · min^{-1}) was infused intravenously (30–90 min). With exogenous GIP, patients with type 2 diabetes responded with a lower increment (Δ) in insulin (P = 0.0003) and C-peptide concentrations (P < 0.0001) than control subjects. The GIP effects in first-degree relatives were diminished compared with control subjects (Δ insulin: P = 0.04; Δ C-peptide: P = 0.016) but significantly higher than in patients with type 2 diabetes ($P \leq 0.05$). The responses over the time course were below the 95% CI derived from control subjects in 7 (insulin) and 11 (C-peptide) of 21 first-degree relatives of patients with type 2 diabetes. In conclusion, a reduced insulinotropic activity of GIP is typical for a substantial subgroup of normoglycemic first-degree relatives of patients with type 2 diabetes, pointing to an early, possibly genetic defect. *Diabetes* 50:2497–2504, 2001

Insulin secretion after meals is stimulated not only by the rise in glycemia after glucose absorption but also by the secretion and insulinotropic action of gut hormones with "incretin" activity (1,2). The main candidates for the incretin role are gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) (3,4). Together, they are responsible for approximately half of the insulin increment after oral glucose (5).

It is characteristic of the type 2 diabetic phenotype that GIP has lost most of its insulinotropic activity (6), whereas GLP-1 remains active. Indeed, GLP-1 is being developed as a new treatment for patients with type 2 diabetes (7). The molecular basis for this differential responsiveness remains obscure, especially as the signal transduction of both GIP and GLP-1 involves many common steps, with the exception of similar but clearly separate receptor species that cannot be activated by the other hormone (8,9). This has led to the hypothesis that type 2 diabetes involves a defective expression of GIP receptors on pancreatic B-cells and that this may be responsible for the overall reduced incretin effect in such patients (10,11).

Many type 2 diabetes traits such as insulin resistance and defective first-phase insulin response after acutely raising glycemia can be found in at least a subgroup of first-degree relatives of patients with type 2 diabetes (12–14). These individuals carry a lifetime risk of developing type 2 diabetes of ~40–50% (15). A similar proportion of first-degree relatives should be carriers of genetic traits that predetermine type 2 diabetes. First-degree relatives have been studied to differentiate primary and secondary phenotypic abnormalities of type 2 diabetes, i.e., those that are genetically determined and precede the onset of diabetes and those that are caused or worsened by hyperglycemia itself (16).

The aim of the present study was to test the hypothesis that the insulinotropic effect of GIP might be reduced in (at least) a subgroup of first-degree relatives of patients with type 2 diabetes, as has been previously shown in patients with type 2 diabetes (6,17,18). For this purpose, GIP was infused intravenously under hyperglycemic clamp conditions in healthy control subjects, patients with type 2 diabetes, and first-degree relatives of patients with type 2 diabetes. Preliminary data have been communicated in abstract form (19).

RESEARCH DESIGN AND METHODS

Study protocol. The study protocol was approved before the study by the ethics committee of the medical faculty of the Ruhr-University, Bochum, in April 1998 (registration number 1114). Written informed consent was obtained from all participants.

Participants. A total of 10 healthy control subjects, 10 patients with type 2 diabetes, and 21 first-degree relatives of patients with type 2 diabetes were studied. Participant characteristics are presented in Table 1. The groups were matched for sex, obesity, and age. Nondiabetic participants were subjected to an oral glucose tolerance test (75 g) (Boehringer O.G.T.; Roche Diagnostics, Mannheim, Germany) with the determination of capillary glucose in the fasting state and 120 min after the ingestion of glucose. In healthy control subjects, any first- or second-degree relatives with type 2 diabetes were excluded by history-taking. One first-degree relative, who was initially screened but had a diabetic oral glucose tolerance, was excluded from the study.

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ANOVA, analysis of variance; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; HOMA, homeostasis model assessment.

TABLE 1
Characteristics of the participants in hyperglycemic clamp experiments with GIP infusion

Parameter	Healthy control subjects	First-degree relatives of type 2 diabetic patients	Type 2 diabetic patients	Significance (P value)*
Sex (female/male)	4/6	15/6	3/7	0.059
Age (years)	49 ± 17	49 ± 12	52 ± 9	0.83
BMI (kg/m^2)	25.7 ± 3.6	26.0 ± 4.2	28.6 ± 5.1	0.23
Waist-to-hip ratio (cm/cm)	0.89 ± 0.1	0.84 ± 0.1§	0.93 ± 0.07	0.028
Participants with first-degree relatives (type 2 diabetes)	0/10§	21/21§	4/10‡	<0.0001
Father	—	8	2	
Mother	—	15	2	
Siblings	—	3	3	
HbA_{1c} (%)†	5.0 ± 0.5§	5.1 ± 0.3§	6.2 ± 0.7‡	<0.0001
Oral glucose tolerance				
Fasting plasma glucose (mg/dl)	94 ± 7§	88 ± 8§	117 ± 15‡	<0.0001
120-min plasma glucose (mg/dl)	101 ± 16	112 ± 15	NE	0.81
Blood pressure				
Systolic (mmHg)	128 ± 9	130 ± 18	131 ± 14	0.93
Diastolic (mmHg)	81 ± 4	80 ± 9	77 ± 5	0.36
Triglycerides (mg/dl)	113 ± 65	117 ± 75	189 ± 107	0.06
HDL cholesterol (mg/dl)	60 ± 26	71 ± 24§	43 ± 19	0.013
LDL cholesterol (mg/dl)	140 ± 30	120 ± 35	146 ± 25	0.70
Creatinine (mg/dl)	1.1 ± 0.1§	1.0 ± 0.1§	1.0 ± 0.1‡	0.017

Data are n and means ± SE. NE, not examined. *ANOVA or χ^2 tests; †normal range, 4.0–6.2%; ‡significant difference ($P < 0.05$) versus healthy control subjects (Duncan's post hoc test); §significant difference ($P < 0.05$) versus patients with type 2 diabetes (Duncan's post hoc test).

From all participants, blood was drawn in the fasting state for measurement of standard hematological and clinical chemistry parameters. Spot urine was sampled for the determination of albumin, protein, and creatinine by standard methods. Participants with anemia (hemoglobin <12 g/dl), elevation in liver enzymes (ALAT, ASAT, AP, and γ-GT) to higher activities that were more than double the respective normal value, or elevated creatinine concentrations (>1.5 mg/dl) were excluded. One female first-degree relative had an elevated γ-GT activity (90 units/l, normal <28 units/l), which most likely was caused by cholelithiasis. Body height and weight were determined, and waist and hip circumferences were measured to calculate BMI and the waist-to-hip ratio, respectively (Table 1). Blood pressure was determined according to the Riva-Rocci method.

Five patients with type 2 diabetes had been treated with diet alone, and five patients received oral antidiabetic treatment (3.5 mg/day glibenclamide in one case, 150 mg/day acarbose in three cases, and 1,700 mg/day metformin in one case). None of the patients had been treated with insulin. In these patients, the usual antidiabetic medication was withdrawn the day before the study.

Study design. All participants were studied on two or three occasions:

1) At a screening visit, an oral glucose tolerance test was performed in all participants with unknown oral glucose tolerance in the fasting state, and laboratory parameters were screened. If individuals met the inclusion criteria, they were recruited for the second test.

2) A hyperglycemic clamp test aiming at a steady capillary plasma glucose concentration of 140 mg/dl (7.8 mmol/l) was started by injecting 40% glucose as a bolus and maintained by infusing glucose (20% in water, wt/vol) as appropriate, based on glucose determinations performed every 5 min. From 30 to 90 min, GIP (glucose-dependent insulinotropic peptide) was administered intravenously at an infusion rate of 2.0 pmol · kg^{-1} · min^{-1}.

3) A subgroup of six participants (five healthy control subjects and one first-degree relative) participated in a third experiment (hyperglycemic clamp experiment with the administration of placebo instead of GIP) to judge the insulin secretory response to prolonged hyperglycemia alone.

Peptides. Synthetic GIP was purchased from PolyPeptide Laboratories (Wolfenbüttel, Germany) and processed for intravenous infusions as previously described (20).

Experimental procedures. The tests were performed in the morning after an overnight fast with participants in a supine position throughout the experiments and the upper body lifted by ~30°. Two forearm veins were punctured with a Teflon cannula (Moskito 123, 18 gauge; Vygon, Aachen, Germany) and kept patent using 0.9% NaCl (for blood sampling and for glucose and GIP administrations, respectively). Both earlobes were made hyperemic using Finalgon (Nonivamid 4 mg/g and Nicoboxil 25 mg/g).

After drawing basal blood specimens at −15 and 0 min, a bolus of 40% glucose (in water, wt/vol) was administered at 0 min to elevate capillary glucose concentrations to 140 mg/dl (7.8 mmol/l). The dose was based on the fasting plasma glucose concentrations and body weight. Then, an intravenous infusion of glucose 20% (in water, wt/vol) was started and maintained at a rate that adjusted capillary plasma glucose concentrations to ~140 mg/dl (7.8 mmol/l) (Fig. 1*A*). Thirty minutes later, an infusion of human synthetic GIP (2.0 pmol · kg^{-1} · min^{-1}) was begun and maintained for 60 min (rate, 20 ml/h [Perfusor secura; Braun, Melsungen, Germany]; diluted in 0.9% NaCl with 1% human serum albumin). At 5-min intervals, plasma glucose was determined in 100-μl capillary samples drawn from an earlobe. The glucose infusion rates and time points at which rates were changed were recorded to allow a calculation of the amount of glucose infused.

Laboratory determinations. Blood was drawn and processed as previously described (3,21). Glucose was measured using a glucose oxidase method with a Glucose Analyser 2 (Beckman Instruments, Munich, Germany). Insulin, C-peptide, GIP, and glucagon were determined by immunoassays as described (3,6,20,21). Proinsulin was measured using a commercially available enzyme-linked immunosorbent assay (DAKO Diagnostics, Cambridgeshire, U.K.). This assay also cross-reacts with split (65-66) proinsulin (100%), and split (31-32) proinsulin (100%). Detection limit was <0.2 pmol. Intra-assay coefficient of variation was 3.2–5.7% and interassay coefficients of variation were 3.6–6.0%.

Calculations. Increments (Δ) in insulin and C-peptide concentrations were calculated as differences between values determined at the end of (90 min) and in the last samples before (30 min) GIP infusions. Integrated incremental responses to exogenous GIP were calculated by the trapezoidal rule using the mean value at 15 and 30 min as baseline. Insulin resistance and B-cell function were calculated according to various methods summarized by Albareda et al. (22).

Statistical analysis. Results are means ± SE. All statistical calculations were carried out using repeated measures analysis of variance (ANOVA) using Statistica Version 5.0 (Statsoft Europe, Hamburg, Germany). If a significant interaction of treatment and time was documented ($P < 0.05$), values at single time points were compared by one-way ANOVA and Duncan's post hoc test. A two-sided P value of <0.05 was taken to indicate significant differences.

RESULTS

In comparison with healthy control subjects and first-degree relatives, patients with type 2 diabetes had higher fasting plasma glucose and HbA_{1c} concentrations but lower HDL cholesterol and creatinine concentrations (Table 1). There were no significant differences in any other parameter between healthy control subjects and first-degree relatives (Table 1).

Patients with type 2 diabetes were hyperglycemic in the

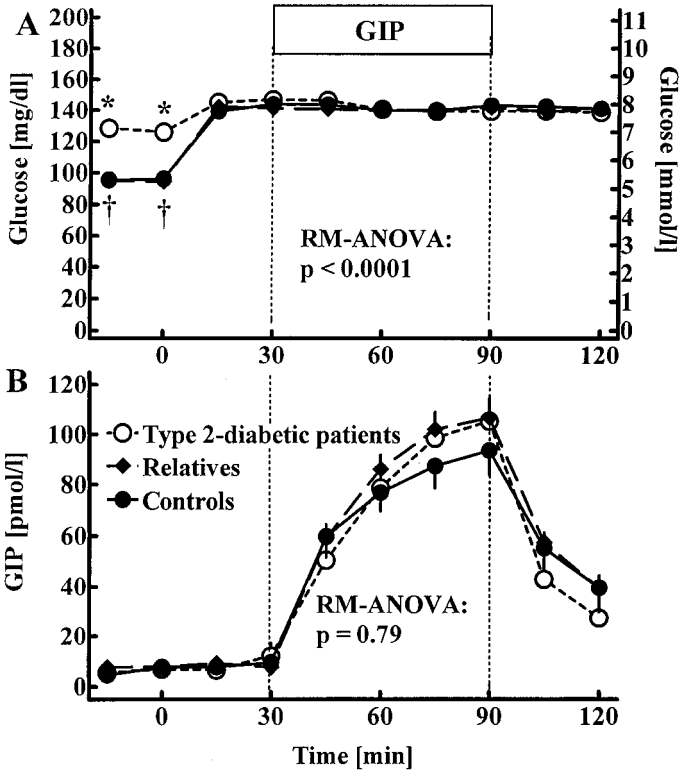

FIG. 1. Plasma concentrations of glucose (capillary measurement; *A*) and immunoreactive GIP (*B*) in 21 first-degree relatives of patients with type 2 diabetes (◆), 10 patients with type 2 diabetes (○), and 10 healthy control subjects (●) participating in hyperglycemic clamp experiments with intravenous infusions of GIP (2 pmol · kg^{-1} · min^{-1}). Mean ± SE *P* values were calculated using repeated measures by ANOVA for the interaction of group assignment and time. Significant difference ($P < 0.05$) at individual time points: *to patients with type 2 diabetes; †to normal subjects (Duncan's post hoc test).

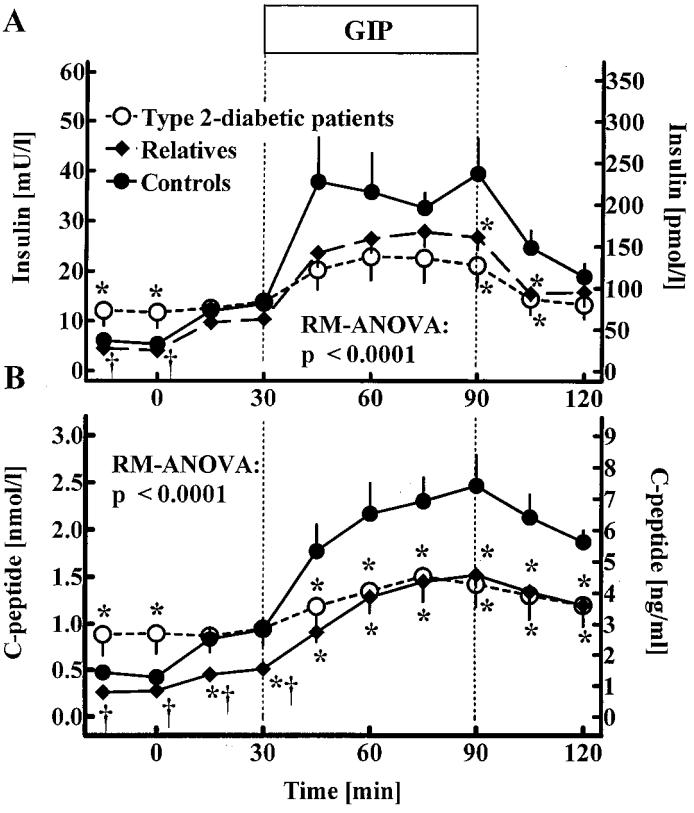

FIG. 2. Plasma concentrations of insulin (*A*) and C-peptide (*B*) in 21 first-degree relatives of patients with type 2 diabetes (◆), 10 patients with type 2 diabetes (○), and 10 healthy control subjects (●) participating in hyperglycemic clamp experiments with intravenous infusions of GIP (2 pmol · kg^{-1} · min^{-1}). Mean ± SE *P* values were calculated using repeated measures by ANOVA for the interaction of group assignment and time. Significant difference ($P < 0.05$) at individual time points: *to patients with type 2 diabetes; †to normal subjects (Duncan's post hoc test).

basal state (Fig. 1*A*). Steady-state glucose concentrations did not differ between the groups (Fig. 1*A*). During the infusion of GIP, similar plasma levels were achieved in healthy control subjects, first-degree relatives, and patients with type 2 diabetes (Fig. 1*B*). Glucose infusion rates necessary to maintain hyperglycemia were higher in healthy control subjects and first-degree relatives than in patients with type 2 diabetes ($P \leq 0.0001$) but did not differ between control subjects and relatives ($P = 0.99$, repeated measures ANOVA/Duncan's post hoc test) throughout the experiments (Table 2).

Basal plasma insulin concentrations were significantly lower in normoglycemic relatives than in hyperglycemic patients with type 2 diabetes (Fig. 2*A*). Raising plasma glucose concentrations to 140 mg/dl (7.8 mmol/l, 30 min) (Fig. 2*A*) increased plasma insulin to similar values in healthy control subjects, first-degree relatives, and patients with type 2 diabetes ($P = 0.29$) (Fig. 2*A*). In response to exogenous GIP, plasma insulin increased further by 26.0 ± 5.3, 16.8 ± 1.8, and 7.3 ± 2.8 mU/l in healthy control

TABLE 2
Glucose infusion rates (mg · kg^{-1} · min^{-1}) during hyperglycemic clamp experiments with the exogenous administration of GIP (30–90 min: 2 pmol · kg^{-1} · min^{-1})

Experimental period	Healthy control subjects	First-degree relatives of type 2 diabetic patients	Type 2 diabetic patients	Significance (*P* value, ANOVA)
0–15 min	4.0 ± 0.4	3.9 ± 0.3	1.3 ± 0.4*	<0.0001
15–30 min	4.4 ± 0.6	4.0 ± 0.4	1.7 ± 0.7*	0.0025
30–45 min	4.2 ± 0.6	4.4 ± 0.4	1.3 ± 0.5*	0.0004
45–60 min	4.5 ± 0.5	5.3 ± 0.5	1.2 ± 0.5*	<0.0001
60–75 min	6.9 ± 0.9	6.9 ± 0.8	1.5 ± 0.5*	<0.0001
75–90 min	8.2 ± 1.0	7.8 ± 0.6	2.3 ± 0.6*	<0.0001
90–105 min	8.5 ± 1.5	8.2 ± 0.8	2.3 ± 0.6*	0.0003
105–120 min	8.2 ± 1.3	8.4 ± 0.9	1.7 ± 0.5*	<0.0001

Data are means ± SE. *P* values were calculated by ANOVA with Duncan's post hoc test to describe differences between the three groups of patients. *Significant difference ($P < 0.05$) versus healthy control subjects.

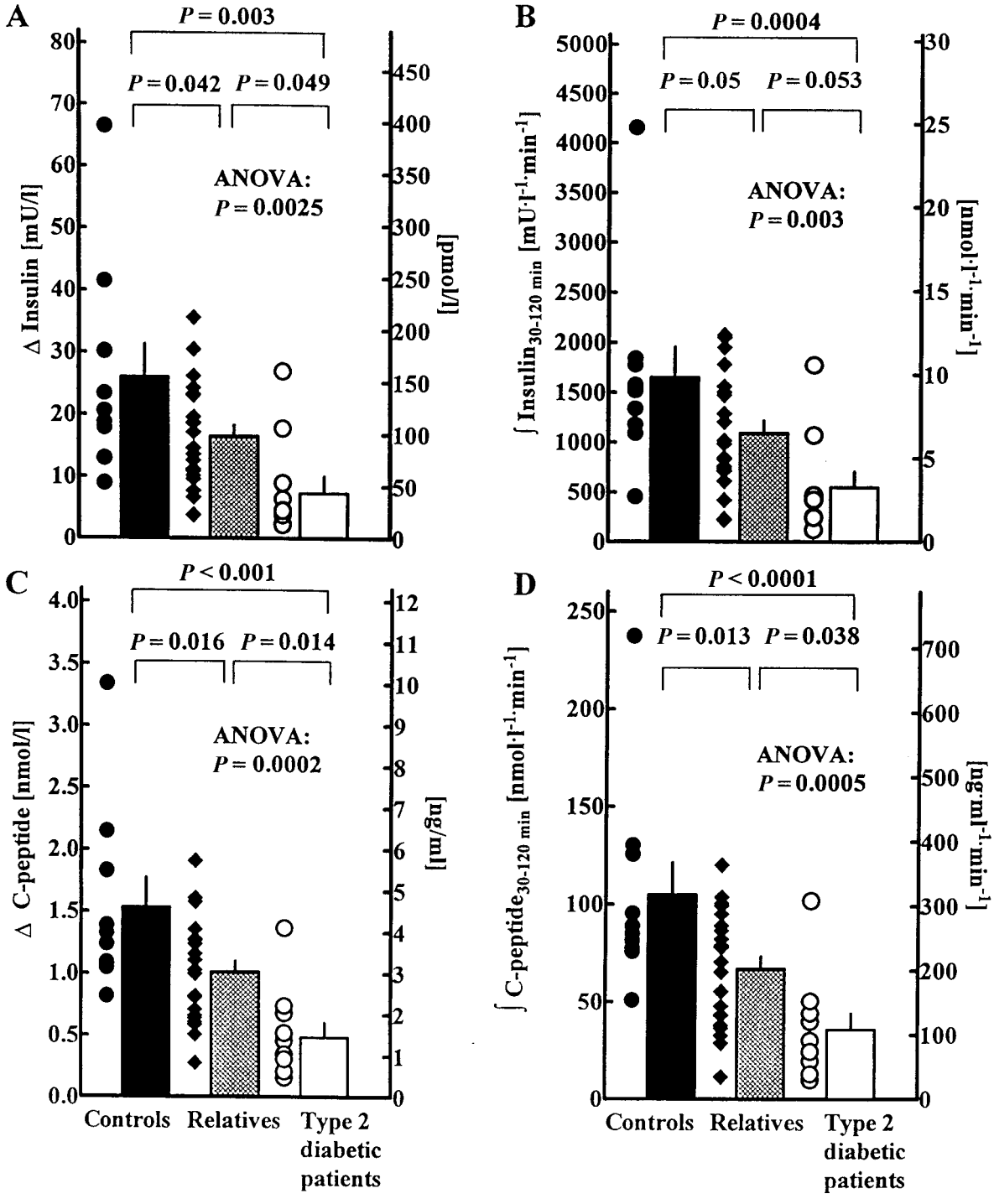

FIG. 3. Differences (Δ) between the values at 30 min (hyperglycemia) and 90 min (hyperglycemia plus exogenous GIP) for insulin (*A*) and C-peptide (*B*) concentrations in 21 first-degree relatives of patients with type 2 diabetes (◆), 10 patients with type 2 diabetes (○), and 10 healthy control subjects (●). *P* values: ANOVA (overall comparison) and Duncan's post hoc test (comparison of individual groups).

subjects, first-degree relatives, and patients with type 2 diabetes, respectively ($P = 0.0025$) (Fig. 3*A*). The corresponding numbers for C-peptide increments were 1.53 ± 0.24, 1.01 ± 0.09, and 0.48 ± 0.12 nmol/l in healthy control subjects, first-degree relatives, and patients with type 2 diabetes, respectively ($P = 0.0002$) (Fig. 3*B*). Regarding both insulin and C-peptide increments, all three groups differed significantly from each other.

Preliminary experiments comparing hyperglycemic clamp experiments with and without exogenous GIP in six participants had shown that insulin and C-peptide increased to higher concentrations with GIP than with placebo (all $P < 0.0001$) and that the differences in integrated incremental responses between experiments with and without exogenous GIP correlated significantly with the increments between the values at 30 min (hyperglycemia alone) and at 90 min (hyperglycemia plus GIP), determined during the experiments with exogenous GIP (insulin: $r^2 = 0.721$, $P = 0.032$; C-peptide: $r^2 = 0.945$, $P = 0.0011$).

Judging individual responses in relation to 95% CIs

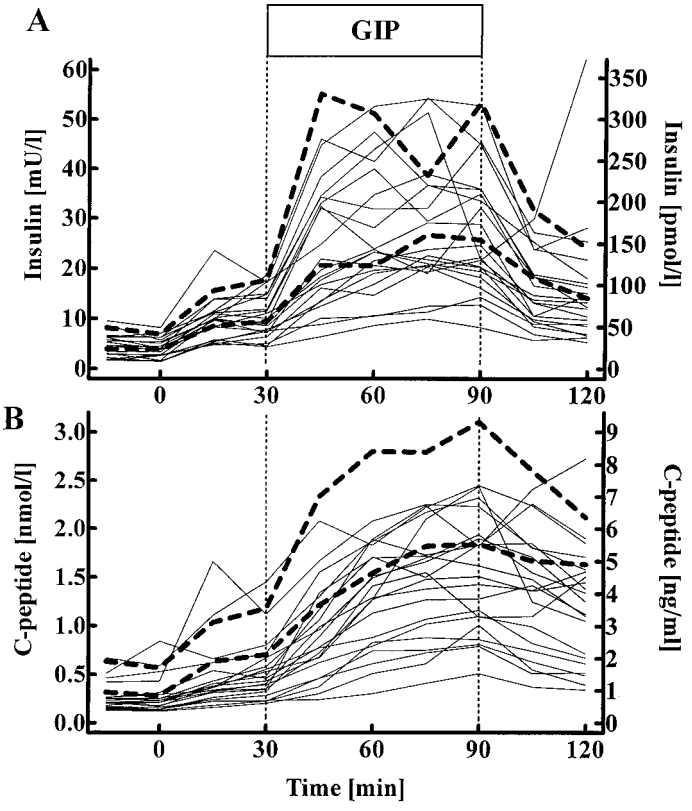

FIG. 4. Individual plasma concentrations (thin lines) of insulin (*A*) and C-peptide (*B*) in 21 first-degree relatives shown in relation to the upper and lower 95% CI for normal subjects (thick dashed lines).

based on the results in healthy participants, 7 of 21 relatives had insulin values below the lower normal limits (Fig. 4*A*), and 11 relatives had C-peptide concentrations below the 95% CI of normal control subjects (Fig. 4*B*).

When expressing B-cell secretory responses as a percentage value of the mean concentrations observed in the control subjects, a reduced rate was present in first-degree relatives in the fasting state (insulin, 75 ± 8% and C-peptide, 60 ± 8%), under hyperglycemic conditions (mean 15/30 min) (insulin, 79 ± 7% and C-peptide, 55 ± 8%), and in response to exogenous GIP (mean 75/90 min) (insulin, 77 ± 7% and C-peptide, 62 ± 6%).

Basal proinsulin concentrations were significantly higher in the patients with type 2 diabetes compared with control subjects ($P = 0.049$) and first-degree relatives of patients with type 2 diabetes ($P = 0.012$). The difference between control subjects and the first-degree relatives was not significant ($P = 0.16$). When expressing proinsulin as its relative proportion of insulin-like immunoreactivity, significantly higher values were found in patients with type 2 diabetes (26 ± 12%) than in the first-degree relatives (12 ± 5%; $P = 0.0067$) or control subjects (16 ± 8%).

Glucagon concentrations in the fasting state did not show any significant differences among the groups ($P = 0.26$). Hyperglycemia induced a reduction in glucagon concentrations in control subjects and the first-degree relatives, whereas in patients with type 2 diabetes, the values did not change significantly. With exogenous GIP, glucagon concentrations continued to decline in control subjects and first-degree relatives but did not change in patients with type 2 diabetes (data not shown).

B-cell function was assessed by calculating various indexes (Table 3) (22). Neither by homeostasis model assessment (HOMA) nor by insulin/glucose ratios determined in the basal state and under conditions of hyperglycemia (before GIP administration) were any significant differences seen, especially between healthy control subjects and first-degree relatives of patients with type 2 diabetes.

DISCUSSION

GIP has lost part of its insulinotropic effect, at least in a subgroup of first-degree relatives of patients with type 2 diabetes (Figs. 2–4). This is similar to a well-recognized phenotypic abnormality in patients with type 2 diabetes (6,17,18). According to the present study, this reduced insulinotropic effect of GIP precedes any clinically relevant disturbance of glucose tolerance, because the first-degree relatives all had a normal or (in one participant) an impaired oral glucose tolerance.

The distribution of insulin secretory responses to the exogenous administration of GIP suggests that ~50% of first-degree relatives show a normal response, whereas at least half of them respond very much like patients with type 2 diabetes, i.e., with a markedly reduced insulin secretory response toward GIP (Fig. 4). This proportion is

TABLE 3
Insulin sensitivity and B-cell function according to various models of calculation

Parameter [reference]	Healthy control subjects	First-degree relatives of type 2 diabetic patients	Type 2 diabetic patients	Significance (*P* value, ANOVA)	Reference
B-cell function					
HOMA B-cell function index (% of normal)	67 ± 7	49 ± 5	63 ± 15	0.27	42
Insulin/glucose [basal] ([mU/l]/[mg/dl])	0.06 ± 0.01	0.05 ± 0.0	0.09 ± 0.01	0.19	55
Insulin/glucose [hyperglycemia‡] ([mU/l]/[mg/dl])	0.09 ± 0.01	0.07 ± 0.01	0.10 ± 0.02	0.34	no reference
Insulin sensitivity					
HOMA insulin resistance index (fold normal)	1.31 ± 0.95*	1.03 ± 0.12*	3.56 ± 1.01†	0.0002	42
Glucose infusion/insulin ([mg · kg^{-1} · min^{-1}]/[mU/l])§	0.43 ± 0.09	0.46 ± 0.05	0.25 ± 0.13	0.19	no reference
Fasting Belfiore index ([l/mU] · [dl/mg])	0.078 ± 0.008	0.107 ± 0.012*	0.045 ± 0.014†	0.0006	56
FIRI ([l/mU] · [dl/mg])	1.11 ± 0.2*	0.88 ± 0.1*	3.55 ± 0.98†	0.0003	57

Data are means ± SE. *P* values were calculated by ANOVA with Duncan's post hoc test to describe differences among the three groups of patients. *Significant difference ($P < 0.05$) versus type 2 diabetic patients; †significant difference ($P < 0.05$) versus healthy control subjects; ‡hyperglycemia refers to the time point 30 min (see Fig. 1), i.e., before start of the GIP infusion; §glucose infusion was calculated for the period 15–30 min (see Fig. 1) and related to the mean insulin concentrations at 15 and 30 min.

similar to the percentage of first-degree relatives of patients with type 2 diabetes who ultimately will develop diabetes themselves (15). Therefore, it is tempting to speculate that a reduced insulinotropic response after GIP is an early marker of a predisposition to develop type 2 diabetes. It might also precede other metabolic disturbances that are characteristic of type 2 diabetes, such as insulin resistance (23), hyperproinsulinemia (24,25), and diminished B-cell secretory capacity (12,13), as none of these factors were present in the first-degree relatives in the present study. Along these lines, we favor the interpretation that a reduced insulinotropic effectiveness of GIP is an early marker that characterizes an abnormality of B-cell function that might predispose to type 2 diabetes. The first-degree relatives presented in our analysis will eventually be followed-up to clarify this point. However, some parameters were assessed using rather insensitive methods (e.g., HOMA to estimate insulin resistance), and parameters of insulin secretion (insulin and C-peptide) were lower already in the fasting state and during stimulation by hyperglycemia alone (Fig. 2). On the basis of the results presented in Table 3, no general impairment of B-cell function can be demonstrated in our group of first-degree relatives, at least under the conditions studied. It would be of importance to study similar subjects with different insulinotropic stimuli to allow a firmer conclusion regarding the specificity of the defective response to GIP in relation to other indexes of B-cell stimulation. One possible method to approach this question was published recently by Fritsche et al. (26), using hyperglycemia, exogenous GLP-1, and arginine.

The nature of the reduced insulinotropic effectiveness of GIP in patients with type 2 diabetes and in our first-degree relatives is not known. It could be a specific defect, for example, concerning the level of expression of GIP receptors on pancreatic B-cells in patients with type 2 diabetes (11). One obvious interpretation is to suspect mutations in the GIP receptor leading to an impaired interaction with its ligand, GIP, or a reduced expression of the GIP receptor as a result of reduced mRNA transcription, translation, or post-translational modifications that affect its biological activity. Polymorphisms in the GIP receptor coding or promoter region in humans (27,28), however, have not been found to be associated with type 2 diabetes. It is not very likely that other components of the GIP signal transduction pathway are defective, because even in patients with type 2 diabetes, GLP-1 is still very effective in augmenting insulin secretory responses (6,29). As already mentioned, GIP and GLP-1 share most of the components of intracellular signal transduction apart from their receptor molecules, which are different and do not cross-react with the other ligand (30–32). This also would point to a GIP-specific rather than a general impairment of B-cell function in patients with type 2 diabetes and, with all likelihood, in their first-degree relatives. It may be worthwhile to exclude mutations in the noncoding sequence of the GIP receptor gene, but clearly studies of the postreceptor activation of the B-cell are also warranted. The hypothesis of a reduced expression of the GIP receptor in patients with type 2 diabetes (and possibly their first-degree relatives) is supported by recent findings in Zucker diabetic fatty rats (33).

Nonetheless, it cannot be firmly excluded that the impairment in GIP function found in the present study is one of several aspects of reduced B-cell function in more general terms, including a reduced responsiveness to glucose, arginine, and possibly other secretagogues (24,34). Such a reduced B-cell function has also been found in first-degree relatives of patients with type 2 diabetes with different stimuli (13,14,35,36). A reduced B-cell secretory function relative to normal subjects who are in the fasting state and under hyperglycemic conditions, as found in the present examination, could be interpreted in favor of this hypothesis. Likewise, with the use of HOMA and other indexes of insulin resistance, patients with type 2 diabetes tended to be more insulin resistant compared with both nondiabetic groups, but with none of the indexes was there any significant difference between healthy control subjects and first-degree relatives. Other authors have found impaired insulin action in first-degree relatives of patients with type 2 diabetes in populations from Sweden (13), California (37), Arizona (Pima Indians) (38), the U.K. (39), and Finland (14). Conversely, other groups from Europe (12,40) and the U.S. (41) have found no difference, as in our study. Our results may apply only to first-degree relatives without insulin resistance. It might be worthwhile to study insulin-resistant first-degree relatives as well to illustrate the mutual interdependence of insulin sensitivity and GIP-stimulated insulin secretion. By correlation analysis, increasing insulin resistance (calculated by the HOMA insulin resistance index [42]) was associated with a reduced GIP-stimulated insulin secretory response (data not shown). This suggests that GIP-stimulated insulin secretion does not compensate for a greater insulin requirement in the presence of insulin resistance, which could have obscured differences between healthy control subjects and insulin-resistant first-degree relatives of patients with type 2 diabetes.

Another interpretation is that GIP and glucose might act in a synergistic way in stimulating B-cells in the fasting state as well as under hyperglycemic conditions. Holz et al. (43) showed 100 pmol/l of the other incretin hormone, GLP-1, to be necessary to make B-cells responsive to glucose. They named this phenomenon induction of "glucose competence." However, fasting concentrations of 30–100 pmol/l are more typical for GIP (6,44) than for GLP-1, for which concentrations of ~2–10 pmol/l typically are measured in fasting humans (3,45,46). However, antagonizing GLP-1 effects using exendin(9-36)amide in the basal state increased glucagon, pointing to an effect on islets at these low, fasting concentrations (47). Considering the almost equivalent dose-response relationships for both incretin hormones in the perfused pancreas (48), it may be hypothesized that basal GIP is necessary for the induction of glucose competence as well. Therefore, even the reduced effect of hyperglycemia (under clamp conditions) (Fig. 2) on insulin secretion in first-degree relatives may be viewed as the consequence of reduced GIP activity in these individuals. This hypothesis, which has in a similar way been put forward by Almind et al. (28), clearly needs to be substantiated by additional experiments, possibly by using a peptide GIP receptor antagonist (49), which so far has not been used in humans.

Would a reduced insulinotropic effect of exogenous GIP

lead to an impaired insulin secretory pattern? If one believes that a significant proportion of the total insulin secretory response after oral glucose and mixed nutrient intake is mediated by incretin hormones (1,5,10), then one would expect a smaller rise in postprandial insulin levels if GIP, the most important incretin hormone in healthy subjects (3), is no longer fully effective. This most likely would lead to a higher glycemic rise. Along this line, oral glucose tolerance was worse (but within normal limits) in our group of first-degree relatives of patients with type 2 diabetes (Table 1) and impaired in one such participant. Very likely, more marked differences would appear during the earlier postprandial period, when insulin concentrations (and the secretory activity) peak, i.e., ~30–45 min after nutrient intake (5,10). Therefore, it would be of interest to estimate insulin secretion after a standardized nutrient stimulus (e.g., oral glucose) and to quantify, in such first-degree relatives, the incretin effect to determine whether a reduced insulinotropic effectiveness in response to GIP predicts a reduced incretin effect in particular and a reduced postprandial insulin secretion in general. Such a study would also reveal whether a reduced insulinotropic effectiveness of exogenous GIP correlates with an enhanced GIP response after nutrients, which was recently demonstrated in first-degree relatives of patients with type 2 diabetes (50), most likely as a compensatory mechanism. In patients with type 2 diabetes, a reduced insulinotropic effectiveness of GIP and a reduced contribution of incretin hormones to insulin secretory responses after oral glucose are well established (6,10,11), and GIP receptor knockout mice are characterized by glucose intolerance (51).

Insulin resistance is another phenotypic peculiarity of patients with type 2 diabetes, and it also has been recognized in first-degree relatives (13,14,36,52,53). Because it is generally accepted that both secretion defects and a reduced insulin sensitivity have to be present to explain all facets of type 2 diabetes, it was of interest to determine whether our first-degree relatives displayed features of both insulin resistance and impaired insulin secretion. The use of HOMA may have limitations (54), but insulin resistance was not a characteristic feature of the same participants who displayed a reduced insulinotropic effectiveness of exogenous GIP. This is further supported by the similar glucose infusion rates (Table 2) for control subjects and first-degree relatives, despite even lower insulin concentrations (Figs. 2 and 4) in the latter.

In conclusion, we demonstrated a reduced insulinotropic effectiveness of GIP in normal glucose-tolerant first-degree relatives of patients with type 2 diabetes in comparison with healthy control subjects. This is a new phenotypic abnormality in such individuals, which may be genetically determined. It seems worthwhile to study in more detail the secretion and function of incretin hormones in patients with type 2 diabetes and their first-degree relatives.

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