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Secretion of incretin hormones and the insulinotropic effect of gastric inhibitory polypeptide in women with a history of gestational diabetes

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Abstract *Aims/hypothesis:* The insulinotropic effect of gastric inhibitory polypeptide (GIP) is reduced in patients with type 2 diabetes and around 50% of their first-degree relatives under hyperglycaemic conditions. It is unknown whether this is a result of a specific defect in GIP action or of a general reduction in beta cell function. Moreover, impaired secretion of glucagon-like peptide 1 (GLP-1) has been described in patients with type 2 diabetes. Therefore, we studied the insulinotropic effect of GIP in women with previous gestational diabetes (pGDM) under euglycaemic fasting conditions and during a hyperglycaemic clamp experiment. The secretion of GIP and GLP-1 was assessed following oral glucose ingestion. *Materials and methods:* On separate occasions we performed an OGTT and administered an i.v. bolus of 20 pmol GIP/kg body weight in 20 women with pGDM and 20 control women. An additional hyperglycaemic clamp experiment (140 mg/dl [7.8 mmol/l] over 120 min) with i.v. infusion of GIP (2 pmol kg^{-1} min^{-1}; 30–90 min) was performed in 14 women in each group. Capillary and venous blood samples were drawn for the measurement of glucose (glucose oxidase), insulin, C-peptide, GIP and GLP-1 (specific immunoassays). Indices of insulin sensitivity and beta cell function were calculated. Statistical analyses were carried out using repeated measures ANOVA. *Results:* Following oral glucose ingestion, plasma glucose, insulin and C-peptide concentrations increased to higher levels in the women with pGDM than in the control women ($p<0.05$). The women with pGDM were characterised by a higher degree of insulin resistance than the control women ($p=0.007$ for the Matsuda index), but showed no overt defects in glucose-stimulated insulin secretion ($p=0.40$ for the insulinogenic index following i.v. glucose). The secretion of GLP-1 and GIP was not different between the groups ($p=0.87$ and $p=0.57$, respectively). The insulin secretory response to GIP administration was similar in the two groups both after GIP bolus administration and during the hyperglycaemic clamp experiment ($p=0.99$ and $p=0.88$, respectively). A hyperbola-like relationship was found between the degree of insulin sensitivity (Matsuda index) and the insulin secretory response to GIP and i.v. glucose administration. *Conclusions/interpretation:* These results do not support the hypothesis of an early defect in GIP action as a risk factor for subsequent development of diabetes in women with previous gestational diabetes. The inverse relationship between insulin resistance and the insulin secretory response to glucose or GIP suggests that beta cell secretory function in response to different stimuli increases adaptively when insulin sensitivity is diminished.

Keywords Gastric inhibitory polypeptide · Gestational diabetes · Glucose-dependent insulinotropic polypeptide · Incretin hormones · Insulin secretion · Type 2 diabetes

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Abbreviations GIP: gastric inhibitory polypeptide · GLP-1: glucagon-like peptide 1 · HOMA: homeostasis model assessment · pGDM: previous gestational diabetes

Introduction

The incretin effect describes the phenomenon that oral ingestion of glucose elicits greater increases in insulin secretion than comparable amounts of glucose administered via the intravenous route [1, 2]. This effect is mediated by the secretion of peptide hormones from the gut, which stimulate insulin secretion [2, 3]. The two known incretin hormones are gastric inhibitory polypeptide (GIP; also referred to as glucose-dependent insulinotropic polypeptide) and glucagon-like peptide 1 (GLP-1) [4, 5].

A reduced incretin effect is characteristic of patients with type 2 diabetes [6], and two different defects in the entero-insular axis have been described in these patients. Firstly, the insulinotropic effect of GIP is markedly reduced in patients with type 2 diabetes, while the effectiveness of GLP-1, although decreased, is preserved to a much higher degree [7, 8]. Secondly, the secretion of GLP-1 is impaired in patients with type 2 diabetes [9, 10], while GIP secretion appears to be more or less normal [10]. To clarify whether the loss of GIP effect in type 2 diabetes is a result of a primary, possibly genetically determined, defect or whether it develops later in the pathogenesis of type 2 diabetes, we previously studied the insulinotropic effects of GIP in a cohort of first-degree relatives of type 2 diabetes patients during a hyperglycaemic clamp [11]. Under these conditions, around 50% of the relatives showed defects in GIP-induced insulin secretion, reminiscent of the secretion pattern observed in patients with type 2 diabetes. These results led to the assumption of a primary, possibly genetically determined, defect in GIP action that confers the risk of developing type 2 diabetes [11, 12]. However, the first-degree relatives included in the study were also characterised by an impairment in glucose-induced insulin secretion [11], meaning that it was difficult to distinguish between an impairment in glucose-stimulated insulin secretion and a specific defect in GIP action.

Women with previous gestational diabetes (pGDM) represent another group at high risk of developing type 2 diabetes [13–15], and defects in insulin secretion and insulin action have been described in pGDM women from different geographic and ethnic backgrounds [15]. In addition, it has been suggested that type 2 diabetes in these women develops with a different genetic basis than in other high-risk cohorts, such as in first-degree relatives [15]. We saw this as an opportunity to investigate the insulinotropic effect of GIP as well as the secretion of incretin hormones in this pre-diabetic cohort. To distinguish between defects in beta cell function in response to intravenous glucose and those in response to intravenous GIP, the insulinotropic effect of GIP was examined both under euglycaemic fasting conditions and during a hyperglycaemic clamp.

Subjects and methods

Study protocol

Prior to the study, the study protocol was approved by the ethics committee of the medical faculty of the Ruhr-University of Bochum on 1 January 2001 (registration number 1615). Written informed consent was obtained from all participants.

Subjects

Twenty women with pGDM and 20 control women with no history of diabetes in themselves or their first-degree relatives were studied. Participants were randomly recruited by local advertisements as well as by contacting local obstetrics departments. In the women with pGDM, the disease had been diagnosed by their respective gynaecologists. Since uniform guidelines for the diagnosis of gestational diabetes had not been established in all centres at that time, the diagnosis of gestational diabetes was based on an OGTT (15 cases) or on elevated fasting glucose levels (five cases). The diagnosis was made during gestational week 26±6 (mean±SD). The average time interval between pregnancy and the experiments was 4.1±6.5 years. Four women required insulin treatment during pregnancy, whereas hyperglycaemia was controlled by a dietary regimen in the other cases. None of the women required hypoglycaemic treatment between delivery and the time of study commencement. The number of previous gestations (prior to the occurrence of gestational diabetes) was 1.8±1.1. Eight women with pGDM had a first-degree relative with type 2 diabetes, and an additional three women in this group had a second-degree relative with type 2 diabetes. Two women with pGDM, but no control women, had had spontaneous abortions during previous pregnancies. Hypertension was present in one woman with pGDM, but was absent in all control subjects. No subject had overt hyperlipidaemia. None of the women received any medication with a known effect on glucose homeostasis.

Blood was drawn in the fasting state from all women for measurement of standard haematological and clinical chemistry parameters. None of the women had anaemia (haemoglobin <11 g/dl [110 g/l]), an elevation in liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase or γ-glutaryltransferase) to higher activities than double the respective normal value, or elevated creatinine concentrations (>1.5 mg/dl [132.6 μmol/l]). Body height, weight and waist and hip circumference were measured to calculate body mass index and waist-to-hip

Table 1 Characteristics of the women with previous gestational diabetes (pGDM) and of control subjects

Parameter	Women with pGDM	Control women	*p* value (ANOVA)
Age (years)	36.2±5.1	37.5±7.9	0.57
Weight (kg)	71.8±13.7	63.1±9.7	0.03
Height (cm)	167±6	169±4	0.25
BMI (kg/m^2)	25.9±5.1	22.2±3.2	0.01
WHR	0.82±0.08	0.78±0.07	0.081
Birthweight of infants (g)	3,615±661	3,165±289	0.046
Systolic BP (mmHg)	114±15	110±10	0.40
Diastolic BP (mmHg)	72±12	71±11	0.68
Total cholesterol (mmol/l)	5.02±0.68	5.05±0.80	0.92
HDL-cholesterol (mmol/l)	1.21±0.49	1.53±0.43	0.041
LDL-cholesterol (mmol/l)	3.28±0.97	3.16±0.82	0.69
Triglycerides (mmol/l)	1.29±0.56	0.95±0.57	0.07
HbA_1c (%)	5.5±0.4	5.6±0.6	0.71

Data are means±SD

ratio (Table 1). Blood pressure was taken according to the Riva–Rocci method.

Study design

All women first participated in a screening visit. Patient history was recorded, a clinical examination was performed, and haematological and clinical chemistry parameters were screened in the fasting state. Urine was tested for β-human chorionic gonadotrophin to exclude the possibility of pregnancy. If subjects met the inclusion criteria, they were recruited for the following tests:

(1) an oral glucose tolerance test. After drawing two basal (−15 and 0 min) capillary and venous blood samples, a glucose drink (75 g; Boehringer OGT, Mannheim, Germany) was ingested within 5 min. Capillary and venous blood samples were obtained after 30, 60, 90 and 120 min;
(2) intravenous bolus administration of GIP. After drawing basal venous and capillary blood samples (−5 and 0 min), synthetic human GIP (20 pmol/kg body weight) was injected intravenously into a large forearm vein, as described previously [16]. Venous blood samples from a cannula placed into a contralateral forearm vein, and capillary samples, were obtained at 1, 3, 5, 10, 15, 20 and 30 min. Both experiments were carried out in random order with an average interval of 16±23 days (mean±SD) separating the tests. All women were contacted again 12±3 months after these initial experiments and asked to participate in an additional test to study the insulinotropic effect of GIP under hyperglycaemic conditions. Fourteen of the originally studied 20 women in each group participated in this additional experiment. A hyperglycaemic clamp test designed to achieve a steady capillary plasma glucose concentration of 7.8 mmol/l (140 mg/dl) was performed in 28 women (14 in each group). The clamp procedure was started by injecting 40% glucose as a bolus and maintained for 120 min by infusing glucose (20% in water, w/v) as appropriate, based on glucose measurements performed every 5 min. Glucose was infused alone for the first 30 min to estimate glucose-stimulated insulin secretion. From 30 to 90 min, GIP was administered intravenously at an infusion rate of 2.0 pmol kg^{-1} min^{-1}, as previously described [11].

Peptides

Synthetic GIP was purchased from PolyPeptide Laboratories (Wolfenbüttel, Germany) and processed for infusion as described previously [11].

Experimental procedures

The tests were performed in the morning after an overnight fast. The subjects were in a supine position with the upper body lifted approximately 30° throughout the experiments. Two forearm veins were punctured using 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 administration, respectively). Both ear lobes were made hyperaemic using Finalgon (Nonivamid 4 mg/g, Nicoboxil 25 mg/g).

Blood specimens

Venous blood was drawn into chilled tubes containing EDTA and aprotinin (Trasylol; 20,000 Kallikrein Inhibitor Units/ml, 200 μl per 10 ml blood; Bayer, Leverkusen, Germany) and kept on ice. After centrifugation at 4°C, plasma for hormone analyses was kept frozen at −28°C. This procedure has previously been shown to prevent in vitro degradation of incretin hormones in human plasma samples [17]. Capillary blood samples (approximately 100 μl) were added to NaF (Microvette CB 300; Sarstedt, Nümbrecht, Germany) for the immediate measurement of glucose.

Laboratory measurements

Glucose was measured by the glucose oxidase method using a Glucose Analyser 2 (Beckman Instruments, Munich, Germany).

Insulin was measured as described previously [11] using an insulin microparticle enzyme immunoassay (IMx Insulin; Abbott Laboratories, Wiesbaden, Germany). Intra-assay coefficients of variation were approximately 4%.

C-peptide was measured as described previously [11] using an ELISA from Dako (Cambridge, UK). Intra-assay coefficients of variation were 3.3 to 5.7%; inter-assay variation was 4.6 to 5.7%. Human insulin and C-peptide were used as standards.

GIP immunoreactivity was measured using two different assays specific for the C terminus or the N terminus of the peptide, as previously described [18]. The C-terminal assay involving antiserum R65 reacted fully with intact GIP (1-42) and the truncated metabolite (3–42) but not with the so-called 8-kDa GIP, of which the chemical nature and molecular relation to GIP is uncertain. The assay had a detection limit of less than 2 pmol/l and an intra-assay variation of approximately 6%. The N-terminal assay measures the concentration of intact GIP (1–42) using antiserum 98171. The cross-reactivity with GIP (3–42) was less than 0.1%. The lower detection limit of the assay was approximately 5 pmol/l. Intra-assay variation was less than 6%, and inter-assay variation was approximately 8 and 12% for 20 and 80 pmol/l standards, respectively. For both assays, human GIP (Peninsula Laboratories Europe, St Helens, UK) was used as standard, and radiolabelled GIP was obtained from Amersham Pharmacia Biotech (Aylesbury, UK).

GLP-1 immunoreactivity was determined using a C-terminal radioimmunoassay that measures the sum of the intact peptide and the primary metabolite GLP-1 (9–36 amide), using the antiserum 89390 and synthetic GLP-1 (7–36 amide) as standard. This assay is particularly suitable for estimating the rate of GLP-1 secretion and cross-reacts less than 0.01% with C-terminally truncated fragments and 83% with GLP-1 (9–36 amide). The detection limit was 3 pmol/l. Intra-assay and inter-assay coefficients of variation were less than 6 and 15%, respectively, at 40 pmol/l [17].

Valine pyrrolidide (0.01 mmol/l, final concentration) was added to the assay buffers to prevent N-terminal degradation of GIP and GLP-1 during the assay incubation periods.

Calculations

Insulin resistance/sensitivity was calculated using homeostasis model assessment (HOMA), which is based on fasting insulin and glucose concentrations [19], and the Matsuda insulin sensitivity index, which takes into account mean insulin and glucose levels following oral glucose stimulation [20]. The latter index has recently been specifically validated in women with gestational diabetes [21] and was therefore used for subsequent analyses.

Beta cell function was estimated using three different approaches. The HOMA beta cell function index estimates beta cell function using fasting insulin and glucose levels [19]. The insulinogenic index used 30 min after oral glucose ingestion provides a measure of early insulin secretion in response to oral glucose stimulation [22]. To assess the insulin secretory response to intravenous glucose, a similar index was calculated from the glucose and insulin plasma concentrations measured after the administration of intravenous glucose during the clamp experiment. The following equation was used: insulinogenic index clamp $15'=(\text{insulin}_{\text{clamp }15'}-\text{insulin}_{\text{clamp }0'})/(\text{glucose}_{\text{clamp }15'}-\text{glucose}_{\text{clamp }0'})$.

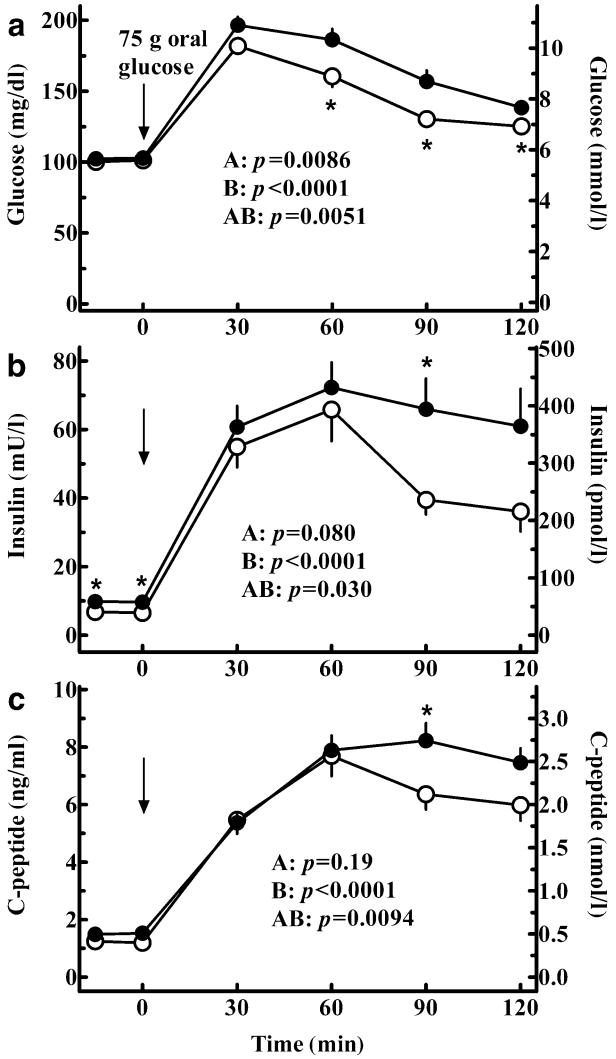

Fig. 1 Plasma concentrations of glucose (**a**), insulin (**b**) and C-peptide (**c**) after the ingestion of 75 g oral glucose in 20 women with a history of gestational diabetes (*filled symbols*) and 20 control women (*open symbols*). Data are presented as means±SEM; *p* values were calculated using repeated measures ANOVA and denote *A* differences between the groups, *B* differences over time, and *AB* differences owing to the interaction of group and time. *Asterisks* indicate significant differences at individual time points ($p<0.05$ by one-way ANOVA)

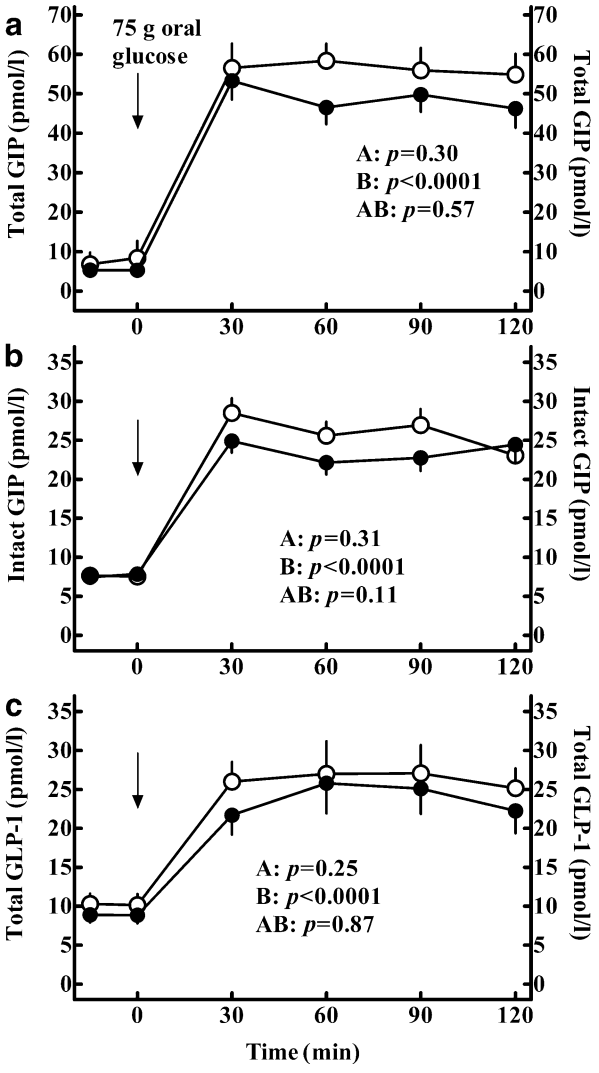

Fig. 2 Plasma concentrations of total GIP (1–42 plus split products; **a**), intact GIP (1–42; **b**) and total GLP-1 (7–36 amide plus split products; **c**) after the ingestion of 75 g oral glucose in 20 women with a history of gestational diabetes (*filled symbols*) and 20 control women (*open symbols*). Data are presented as means±SEM; *p* values were calculated using repeated measures ANOVA and denote *A* differences between the groups, *B* differences over time, and *AB* differences owing to the interaction of group and time

Plasma insulin levels at 5 min were chosen to assess the insulin secretory response to the GIP bolus administration, because at this time point the stimulation of insulin secretion was maximal. Plasma insulin concentrations measured after 15 min of the hyperglycaemic clamp experiment were chosen as a marker of the acute insulin response to intravenous glucose administration. Since GIP plasma concentrations reached peak levels after 90 min, plasma insulin concentrations at this time point were used to assess the beta cell response to the combined stimulation with GIP and glucose. For integrated incremental responses of glucose and insulin, the AUC was calculated using the trapezoidal method (baseline subtracted).

Statistical analysis

Results are reported as means±SEM. The following parameters were defined as main outcome variables: (1) total GLP-1 plasma concentrations after 120 min of the OGTT; (2) total GIP plasma concentrations after 120 min of the OGTT; (3) plasma insulin concentrations 5 min after GIP bolus administration; and (4) plasma insulin concentrations after 90 min of the hyperglycaemic clamp experiment. All variables were normally distributed according to the non-parametric Kolmogorov–Smirnov test. Continuous variables were compared using repeated measures ANOVA and Statistica version 5.0 software (Statsoft Europe, Hamburg, Germany). This analysis provides *p* values for differences between groups/experiments (A), differences over time (B) and the interaction of group/experiment with time (AB). If a significant interaction of treatment and time was documented ($p<0.05$), values at single time points were compared by one-way ANOVA. A *p* value of less than 0.05 was taken to indicate a significant difference. Regression analyses were performed using GraphPad Prism, version 3.0 (San Diego, CA, USA).

Results

Subject characteristics

Both groups were matched for age and HbA_1c levels (Table 1). The women with pGDM had a higher body mass index than the control women ($p=0.01$). The birthweight of the infants was higher in the women with pGDM ($p=0.046$). No differences in the prevalence of hypertension or hyperlipidaemia were found between the groups, but the women with pGDM were characterised by some-

Table 2 Insulin sensitivity/resistance and beta cell function calculated according to different indices based on fasting measurements or an oral glucose tolerance test

Index	Women with pGDM	Control women	*p* value (ANOVA)	Reference
Insulin sensitivity/resistance				
HOMA	2.47±0.29	1.67±0.18	0.029	[19]
Matsuda index	3.64±0.29	5.62±0.61	0.0070	[20]
Insulin secretion				
HOMA beta cell function	92.1±12.6	66.1±7.3	0.09	[19]
$\text{Insulinogenic index}_{\text{OGTT (30′)}}$	60.1±6.7	65.6±7.5	0.64	[22]
$\text{Insulinogenic index}_{\text{clamp (15′)}}$	23.3±4.5	17.6±4.5	0.40	–

Data are means±SEM

what lower HDL cholesterol concentrations (p=0.041) and tended to have higher triglyceride levels (p=0.07; Table 1).

Oral glucose tolerance test

Fasting glucose concentrations were similar in the two groups (Fig. 1a). After the ingestion of 75 g glucose, plasma glucose concentrations reached significantly higher levels in the women with pGDM from 60 to 120 min (p=0.0051, Fig. 1a). Among the women with pGDM, 13 had normal oral glucose tolerance, four had impaired glucose tolerance and three had impaired fasting glucose. In contrast, oral glucose tolerance was normal in all control women according to American Diabetes Association guidelines [23].

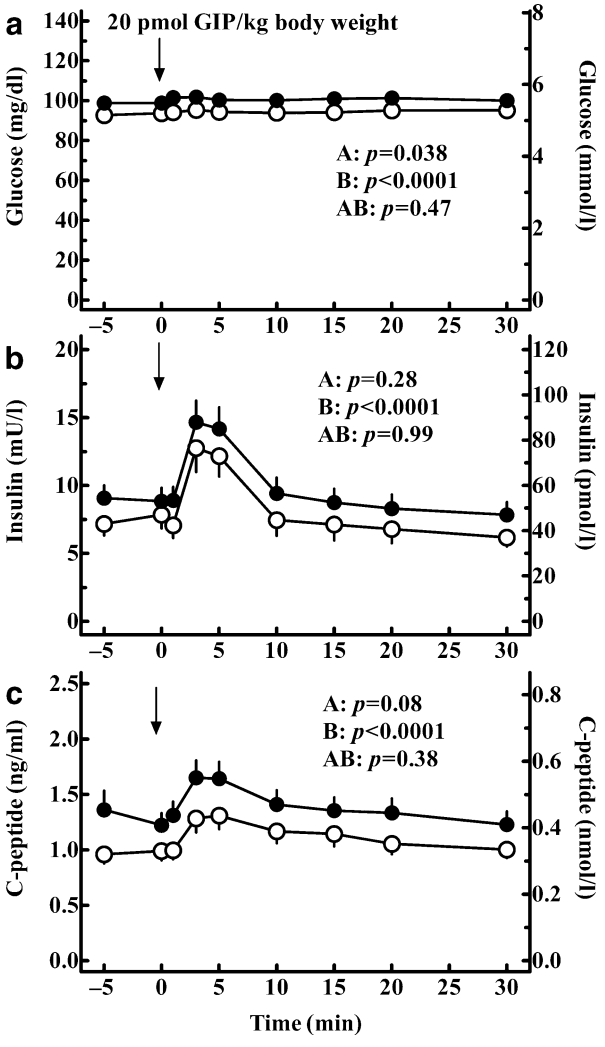

Fig. 3 Plasma concentrations of glucose (**a**), insulin (**b**) and C-peptide (**c**) after the intravenous bolus administration of 20 pmol GIP/kg body weight in 20 women with a history of gestational diabetes (*filled symbols*) and 20 control women (*open symbols*). Data are presented as means±SEM; p values were calculated using repeated measures ANOVA and denote *A* differences between the groups; *B* differences over time, and *AB* differences owing to the interaction of group and time

Fasting insulin concentrations were already somewhat elevated in the women with pGDM compared with the control women (p<0.05), and these differences became more apparent between 90 and 120 min after glucose ingestion (p=0.03; Fig. 1b). Likewise, plasma C-peptide levels were significantly higher at these time points (but not in the fasting state) in the women with pGDM (p=0.0094; Fig. 1c).

Fasting concentrations of both incretin hormones were not different between the groups (Fig. 2). The glucose load led to a significant increase in the secretion of GIP and GLP-1 (p<0.001; Fig. 2). Peak concentrations were 60.4±4.7 and 65.5±5.7 pmol/l for total GIP (p=0.51), 29.8±1.7 and 31.2±1.8 pmol/l for intact GIP (p=0.57) and 30.7±4.0 and 33.0±4.1 pmol/l for total GLP-1 (p=0.70), for women with pGDM and control women, respectively. There was no difference in the time course of incretin secretion between the groups (Fig. 2).

Indices of insulin resistance and beta cell function

The degree of insulin resistance was significantly higher in the women with pGDM than in the control women, according to HOMA and the Matsuda index (p<0.05;

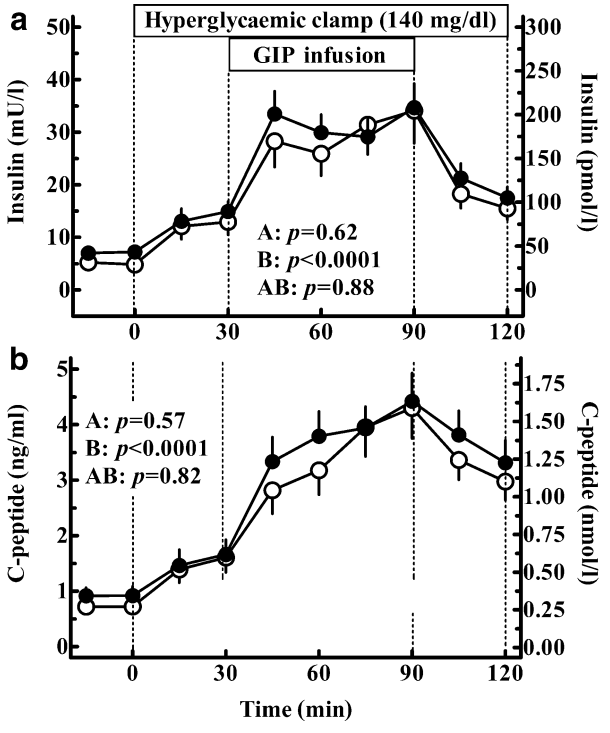

Fig. 4 Plasma concentrations of insulin (**a**) and C-peptide (**b**) during a hyperglycaemic clamp experiment with the administration of GIP in 14 women with a history of gestational diabetes (*filled symbols*) and 14 control women (*open symbols*). The hyperglycaemic clamp experiment was maintained from 0 to 120 min. From 30 to 90 min, synthetic human GIP was infused intravenously at an infusion rate of 2 pmol kg^{-1} min^{-1}. Data are presented as means±SEM; p values were calculated using repeated measures ANOVA and denote *A* differences between the groups, *B* differences over time; and *AB* differences owing to the interaction of group and time

Table 2). These differences remained valid when the women who had not participated in the hyperglycaemic clamp experiment were excluded from the analyses (details not shown). In contrast, beta cell function was comparable in the two groups by all measures applied (Table 2).

GIP bolus administration

The intravenous bolus administration of GIP in the fasting state led to a similar rise in GIP plasma concentrations in the two groups (p=0.97 for total GIP and p=0.20 for intact GIP; details not shown). Plasma glucose levels slightly increased after GIP bolus administration (p< 0.0001; Fig. 3a), while insulin and C-peptide levels increased significantly (p<0.0001). There were no differences between the groups in the plasma concentrations of glucose, insulin or C-peptide following GIP administration (p=0.47, p=0.99 and p=0.38, respectively; Fig. 3). However, plasma glucose concentrations as well as insulin and C-peptide levels were higher by trend in the women with pGDM during the entire experimental period, compatible with a higher degree of insulin resistance.

Hyperglycaemic clamp experiment with GIP infusion

During the hyperglycaemic clamp experiment, similar plasma glucose levels were reached in the two groups. Mean plasma glucose concentrations were 140.1±0.7 and 139.0± 0.8 mg/dl [7.8±0.04 and 7.7±0.04 mmol/l] in women with pGDM and control women, respectively (p=0.12). The mean glucose infusion rates required to maintain hyperglycaemia were 4.16±0.38 and 4.76±0.45 mg kg^{-1} min^{-1} in women with pGDM and control women, respectively (p=0.32). The intravenous infusion of GIP led to similar plasma levels for total and intact GIP in the two groups (p=0.13 and p=0.40, respectively; details not shown).

Plasma concentrations of insulin and C-peptide increased to a similar extent in the two groups during the hyperglycaemic clamp period without GIP infusion (15–30 min; Fig. 4). Exogenous GIP administration further augmented insulin secretion (p<0.0001). However, insulin secretory responses to GIP administration during the hyperglycaemic clamp were not different between the groups (p=0.88 and p=0.82 for insulin and C-peptide, respectively; Fig. 4). Integrated AUCs of insulin concentra-

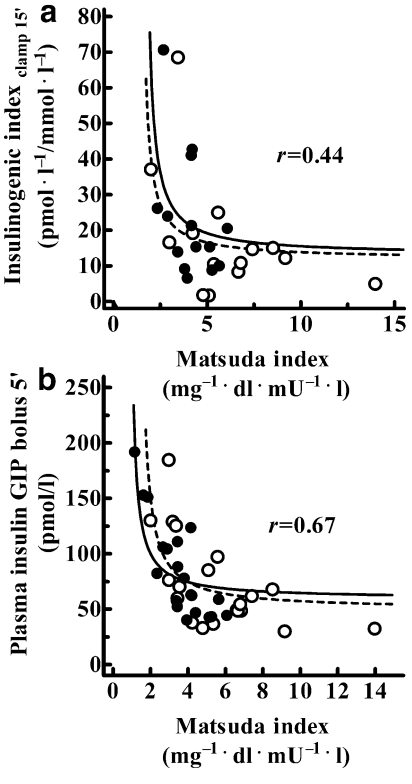

Fig. 5 The relationship between the individual degree of insulin sensitivity, as derived from the Matsuda index, and the insulinogenic index calculated during the intravenous administration of glucose alone (after 15 min of the clamp experiment; (**a**) and during the combined stimulation with intravenous glucose and GIP (after 90 min of the clamp experiment; (**b**) in 14 women with a history of gestational diabetes (*filled symbols*) and 14 control women (*open symbols*). *r*, correlation coefficient, calculated by non-linear regression analysis. *Straight lines*, women with pGDM; *dashed lines*, control women

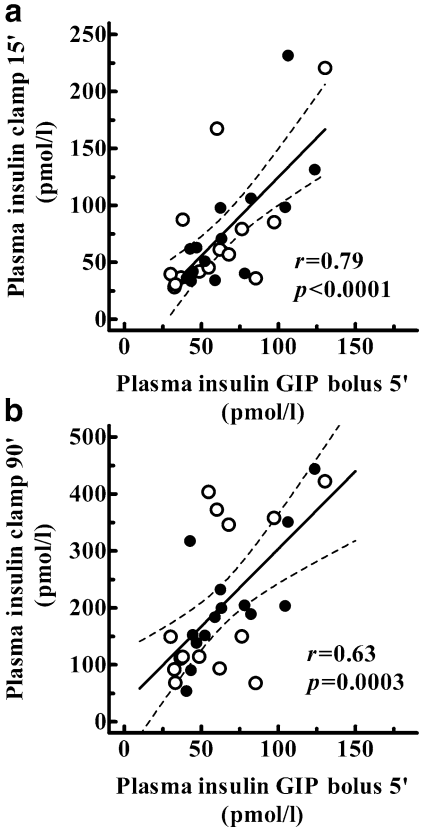

Fig. 6 Correlation analyses between the plasma insulin concentrations measured 5 min after the bolus administration of GIP (20 pmol/kg body weight) and the plasma insulin concentrations measured during the intravenous administration of glucose alone (after 15 min of the clamp experiment; **a**) and the combined stimulation with intravenous glucose and GIP (after 90 min of the clamp experiment; **b**) in 14 women with a history of gestational diabetes (*filled symbols*) and 14 control women (*open symbols*). *r*, correlation coefficient, calculated by linear regression analysis

tions during the infusion of GIP (30–90 min) were 863.5± 114.2 mU l^{-1} min in the women with pGDM and 860.6± 168.9 mU l^{-1} min in the control women (p=0.99). For C-peptide, the integrated AUCs during this period were 112.1±12.0 and 99.0±16.9 ng ml^{-1} min, respectively (p=0.53).

Regression analyses

The insulinogenic index calculated 15 min after starting the hyperglycaemic clamp was used to indicate the acute insulin response to glucose stimulation. A hyperbola-like relationship was found between this index and the degree of insulin sensitivity, as derived from the Matsuda index (r=0.44; Fig. 5a). This association was not different between the women with pGDM and the control women (Fig. 5a). A similar non-linear inverse relationship was apparent between the insulin concentrations measured 5 min after the GIP bolus administration and the values of the Matsuda index (r=0.67). Again, the slope of the regression line was not different between the groups (Fig. 5b).

The insulin concentrations measured 5 min after the GIP bolus administration were closely related to those measured during intravenous glucose administration (clamp 15′; r=0.79; p<0.0001) or during the combined administration of glucose and GIP (clamp 90′; r=0.63; p=0.0003). These relations were similar in the two groups (Fig. 6).

There was also a strong linear relationship between the insulin secretory response to GIP bolus administration and the body mass index (details not shown). Interestingly, this association was much stronger in the women with pGDM than in the control women (r=0.90, p<0.0001 and r=0.50, p=0.025, respectively).

Discussion

Although a reduced insulinotropic effect of GIP in patients with type 2 diabetes has uniformly been reported by different investigators [7, 8, 11, 24–26], the underlying defects are as yet unknown. Indeed, if the action of GIP in type 2 diabetes was impaired as a result of a specific defect, e.g. expression or action of the GIP receptor [27], this may be an interesting target for the development of novel hypoglycaemic agents. Assuming a primary defect in GIP action, we would expect the insulinotropic effect of GIP to be impaired in subjects with a genetic background of type 2 diabetes as well. Consistent with this hypothesis, we recently described a reduced insulinotropic effect of GIP, not only in patients with manifest type 2 diabetes but also in around 50% of their first-degree relatives, when studied under hyperglycaemic clamp conditions [11]. However, since glucose-stimulated insulin secretion is also impaired in first-degree relatives [15, 28], it was difficult to distinguish between defects in insulin secretion that are specific for the action of GIP and those that are a result of a general impairment in the beta cell function. Therefore, in the present study, a cohort of women with a history of gestational diabetes was first studied under euglycaemic fasting conditions. To compare the responsiveness of insulin secretion to GIP at basal and elevated glucose levels, 14 women in each group were re-studied under hyperglycaemic clamp conditions. We report that the insulinotropic effect of GIP is preserved in these women, both under basal euglycaemic conditions and hyperglycaemic conditions, suggesting that a diminished effect of GIP on pancreatic beta cells is unlikely to confer the risk of developing type 2 diabetes in these individuals.

One question arising from the present data is why the insulinotropic effect of GIP was impaired in the first-degree relatives of patients with type 2 diabetes [11] but not in the women with pGDM in the present experiments. The most likely explanation is that diminished GIP-induced insulin secretion in the first-degree relatives was a result of a general impairment in beta cell function. Indeed, these subjects had impaired insulin secretion, not only in response to GIP but also during the intravenous administration of glucose alone [11]. In contrast, all measures of beta cell function in the women with pGDM revealed normal values. Thus, a reduced insulinotropic effect of GIP appears to coincide with a general reduction in beta cell function. This hypothesis is also consistent with our recent observation that the dose-response relationship for the insulinotropic effect of GIP is almost preserved in patients with type 2 diabetes, although this is at generally lower levels of beta cell function [29]. Moreover, the fact that impairments in GIP-induced insulin secretion not only occur in type 2 diabetes but also in other types of diabetes, including MODY 3, latent autoimmune diabetes in adults, early type 1 diabetes and diabetes secondary to chronic pancreatitis, strongly argues against a primary defect in GIP action in the pathogenesis of type 2 diabetes [30].

One intriguing observation from the present study is that insulin secretion in response to GIP increases with higher levels of insulin resistance and obesity. This indicates that defects in insulin action can be compensated for by increased insulin secretion, not only in response to glucose but also in response to GIP and, potentially, other insulin secretagogues. This would also explain the increased insulin responses to GIP administration recently reported in another group of first-degree relatives, who, unlike our previous cohort of subjects, showed a marked impairment in insulin action [31].

A second characteristic defect of the entero-insular axis in patients with type 2 diabetes is a diminished secretion of GLP-1 [9, 10]. In the present study, based on the same radioimmunoassays as in the previous studies [10], no differences in the secretion of incretin hormones after oral glucose ingestion were found in women with a history of gestational diabetes (Fig. 2). These findings are consistent with previous data from our group and others, demonstrating preserved or even increased (in the case of GIP) secretion of incretin hormones in first-degree relatives of patients with type 2 diabetes [32, 33] as well as in subjects with impaired glucose tolerance [34]. However, the present data are at variance with one recent study that reported an impairment of around 20% in early (0–30 min) GLP-1

response to oral glucose but no alteration in overall (0–120 min) GLP-1 secretion in a group of women with pGDM [35]. The differences between this and the present study may be a result of the different degrees of metabolic alteration in the pGDM subjects included. Indeed, glycaemic excursions following ingestion of oral glucose in these women were much higher than those in the women included in this study. Therefore, the minor reduction in GLP-1 secretion observed in this study may well be secondary to other metabolic disturbances in the pathogenesis of type 2 diabetes.

Some caution should be taken regarding the number of subjects included in this study: while the invasive character and the high experimental effort associated with these studies did not justify testing a larger cohort, it is theoretically possible that minor differences in GIP-induced insulin secretion were overlooked owing to the limited number of participants.

One prominent difference between the women with pGDM and the control subjects in this study was the degree of obesity. This suggests that in this specific group of women with pGDM, obesity was, at least in part, responsible for the observed disturbances in glucose homeostasis. Moreover, the responsiveness of insulin secretion to GIP administration significantly increased with body mass index, particularly in the women with pGDM. Therefore, increased insulin secretion in response to GIP may contribute to the compensation of beta cell function for obesity in women with a history of gestational diabetes.

The modest increase in blood glucose levels (~2 mg/dl [0.1 mmol/l]), despite an increase in insulin secretion observed after GIP bolus administration, is surprising. Probably this was a result of the glucagonotropic effect of GIP, which could influence hepatic glucose release [36]. Alternatively, other glucoregulatory hormones, e.g. cortisole or epinephrine, may also be influenced by GIP administration.

Abnormalities in beta cell function as well as in insulin action have been described in other cohorts of women with a history of gestational diabetes [15, 37–42]. The heterogeneity between these studies is probably a result of the different ethnic and geographic origins of the populations studied. Recently, Buchanan proposed the concept of a failure in beta cell compensation for increasing insulin resistance in a large cohort of women with a Hispanic background [42]. It was suggested that the ambient degree of insulin sensitivity should be taken into account for the accurate assessment of beta cell function [42, 43]. The group of women with pGDM included in the present study was characterised by higher levels of insulin resistance at normal levels of beta cell function. The similar inverse negative relation between glucose-stimulated insulin secretion and the Matsuda index for insulin sensitivity in both groups confirmed the absence of a major defect in beta cell function in the women with pGDM (Fig. 5). Interestingly, a similar hyperbola-like relationship was found between the Matsuda index and the insulin secretory response to the GIP bolus administration. This indicates that insulin secretion in response to GIP and glucose increases to compensate for higher levels of insulin resistance.

In conclusion, the secretion of incretin hormones and the insulinotropic effect of GIP are normal in women with a history of gestational diabetes. Therefore, an early defect in GIP action does not appear to be a risk factor for subsequent development of diabetes in these individuals. An inverse relationship between insulin resistance and the insulin secretory response to glucose or GIP suggests that beta cell secretory function in response to different stimuli compensates for changes in insulin sensitivity.

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