Gut incretin hormones in identical twins discordant for non-insulin-dependent diabetes mellitus (NIDDM)—evidence for decreased glucagon-like peptide 1 secretion during oral glucose ingestion in NIDDM twins

Allan A Vaag, Jens J Holst, Aage Vølund and Henning Beck-Nielsen

Department of Endocrinology and Internal Medicine M, Odense University Hospital, Odense, Denmark; Department of Medical Physiology, Panum Institute, University of Copenhagen, Denmark; Novo Nordisk A/S, Novo Alle, Bagsværd, Denmark


The incremental glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) responses (areas under curves: AUCs) were determined during a standard 180-min 75-g oral glucose tolerance test in a group of 12 identical twin pairs discordant for non-insulin-dependent diabetes mellitus (NIDDM) and 13 matched controls without family history of diabetes using highly sensitive and specific radioimmunoassay hormone assays. Data were analysed using multifactor analysis of variance (ANOVA) to identify and correct for possible covariates and to correct for multiple comparisons. Fasting plasma GLP-1 and GIP concentrations were similar in all groups. The twins with frank NIDDM had a decreased incremental GLP-1 response during oral glucose ingestion compared with controls without family history of diabetes (AUC ± SEM: 0.55 ± 0.14 vs 1.17 ± 0.25 (mmol/l) × min, p < 0.05). The incremental GLP-1 secretion in the non-diabetic twins was not significantly different from neither their NIDDM co-twins nor the controls without family history of diabetes. The incremental GIP responses were similar in all study groups. Gender was identified as the major independent covariate for incremental glucose, insulin, GIP and GLP-1 responses, with higher values of all parameters in females. Waist-to-hip ratio and body mass index (BMI) were identified as independent but oppositely directed covariates for the incremental GLP-1 responses (waist-to-hip ratio: r = 0.43, p < 0.02; BMI: r = −0.34, p = 0.06). Incremental GLP-1 responses correlated with incremental insulin responses in the combined study population (N = 37; R = 0.42, p = 0.01). In conclusion, a decreased intestinal GLP-1 secretion may contribute to the abnormal insulin secretion during oral glucose ingestion in NIDDM twins. However, decreased secretion of gut incretin hormones (GLP-1 or GIP) does not explain all of the defects of pancreatic insulin secretion in NIDDM patients/twins or in non-diabetic individuals (identical twins) with a genetic predisposition to NIDDM.

Allan Vaag, Odense University Hospital, Department of Endocrinology and Internal Medicine M, Sdr. Boulevard, Odense, DK-5000, Denmark

The “incretin effect” denotes an enhanced pancreatic insulin secretion after oral compared with intravenous ingestion of similar glucose loads (1–3). The incretin effect is supposed to be mediated through release of one or more insulinotropic hormones from the gut after oral glucose ingestion (2, 3). Two gut incretin hormones have been identified: gastric inhibitory polypeptide (GIP) (4, 5) and glucagon-like peptide 1 (GLP-1) (6–8). Gastric inhibitory polypeptide is secreted in the proximal part and GLP-1 in the distal part of the small intestine in response to mixed meals, oral glucose and fat ingestion (4, 8). Both GIP and GLP-1 stimulate the secretion of insulin in the pancreatic β-cells (4, 8) and are, therefore, likely to play important roles in the regulation of post-prandial glucose homeostasis in man.

We recently identified a defective insulin secretion pattern to both oral and intravenous glucose in non-diabetic identical co-twins of NIDDM patients (9), indicating the contribution of a primary and possibly genetic insulin secretion defect to hyperglycaemia in NIDDM. However, the secretion of insulin as stimulated by both oral and intravenous glucose was further decreased in the twins with frank NIDDM compared with their non-diabetic identical co-twins, demonstrating that the insulin secretion defect in NIDDM has a quantitatively important secondary (non-genetic) component (9). Importantly, other studies have indicated a decreased incretin effect in NIDDM patients (10). Thus, the decreased or delayed insulin secretion in NIDDM and/or in genetically predisposed prediabetic subjects...
after oral glucose ingestion may, at least partly, be due to an impaired secretion (or perhaps action) of one or more gut incretin hormones.

Studies addressing the role(s) of GIP and GLP-1 in the pathophysiology of the defective insulin secretion in NIDDM have not been conclusive (4, 5, 8, 11). Furthermore, we are unaware of any published studies addressing the potential role(s) of gut incretin hormones on insulin secretion in non- (and possibly pre-) diabetic individuals with a genetic predisposition to NIDDM. In the present paper we report analyses of plasma GIP and GLP-1 concentrations in the fasting state and during oral glucose ingestion in identical twins discordant for NIDDM and matched controls without a family history of diabetes (9). For the GLP-1 determinations we used a highly sensitive RIA specifically measuring the fully processed and amidated GLP-1 (proglucagon(7–36) amide). Thus, previous GLP-1 measurements in NIDDM patients were less specific and included pancreatic GLP-1-containing peptide moieties (11) that are likely to be elevated in diabetics.

Subjects and methods

Subjects

A total of 12 patients (twins) with NIDDM and their genetically identical non-diabetic co-twins were included in the study. The twins were primarily traced through the Danish Twin Registry as described previously (9). Duration of diabetes in the NIDDM twins was 9 ± 3 years. The group of identical non-diabetic co-twins included five subjects with normal glucose tolerance (NGT) and seven subjects with impaired glucose tolerance (IGT) according to the WHO criteria. Thirteen healthy age- and sex-matched

Table 1. Clinical characteristics of study subjects.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>NGT</th>
<th>IGT</th>
<th>NIDDM twins</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (M/F)</td>
<td>13 (7/6)</td>
<td>5 (3/2)</td>
<td>7 (4/3)</td>
<td>12 (7/5)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60 ± 2</td>
<td>59 ± 5</td>
<td>67 ± 4</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>(M/F)</td>
<td>58 ± 3/10</td>
<td>59 ± 8/10</td>
<td>66 ± 1/10</td>
<td>63 ± 3/10</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.5 ± 0.8</td>
<td>27.2 ± 1.5</td>
<td>27.6 ± 2.0</td>
<td>30.1 ± 1.8</td>
</tr>
<tr>
<td>(M/F)</td>
<td>(25.1 ± 1.0)</td>
<td>(27.9 ± 2.5)</td>
<td>(25.9 ± 0.7)</td>
<td>(28.5 ± 1.9)</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.90 ± 0.03 d</td>
<td>0.93 ± 0.02</td>
<td>1.04 ± 0.03 g</td>
<td>1.00 ± 0.02 c</td>
</tr>
<tr>
<td>(M/F)</td>
<td>(0.93 ± 0.04)</td>
<td>(0.94 ± 0.02)</td>
<td>(1.02 ± 0.04)</td>
<td>(1.02 ± 0.02/</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.26 ± 0.22</td>
<td>1.34 ± 0.30</td>
<td>1.16 ± 0.14</td>
<td>1.83 ± 0.31</td>
</tr>
<tr>
<td>(M/F)</td>
<td>(1.47 ± 0.37)</td>
<td>(1.13 ± 0.30)</td>
<td>(0.95 ± 0.10)</td>
<td>(1.51 ± 0.30/</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.9 ± 0.3</td>
<td>5.6 ± 0.4</td>
<td>5.3 ± 0.4</td>
<td>5.5 ± 0.3</td>
</tr>
<tr>
<td>(M/F)</td>
<td>(6.1 ± 0.03)</td>
<td>(5.5 ± 0.4)</td>
<td>(5.0 ± 0.7)</td>
<td>(5.0 ± 0.4/)</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.4 ± 0.2 d</td>
<td>6.2 ± 0.1 a</td>
<td>5.8 ± 0.2</td>
<td>11.4 ± 1.1d</td>
</tr>
<tr>
<td>(M/F)</td>
<td>(5.5 ± 0.2)</td>
<td>(6.3 ± 0.1)</td>
<td>(6.1 ± 0.4)</td>
<td>(12.5 ± 1.8)</td>
</tr>
<tr>
<td>2-h Glucose (mmol/l)</td>
<td>5.7 ± 0.3</td>
<td>6.8 ± 0.3 a</td>
<td>9.1 ± 0.5 c</td>
<td>19.5 ± 1.7c</td>
</tr>
<tr>
<td>(M/F)</td>
<td>(5.3 ± 0.3)</td>
<td>(7.2 ± 0.3)</td>
<td>(8.7 ± 0.6)</td>
<td>(19.6 ± 2.6)</td>
</tr>
<tr>
<td>Fasting plasma insulin (µU/ml)</td>
<td>6.9 ± 0.9</td>
<td>7.1 ± 0.9</td>
<td>7.5 ± 0.9</td>
<td>12.7 ± 1.6b</td>
</tr>
<tr>
<td>(M/F)</td>
<td>(5.8 ± 0.6)</td>
<td>(6.1 ± 0.5)</td>
<td>(7.1 ± 0.8)</td>
<td>(10.7 ± 0.9)</td>
</tr>
<tr>
<td>Fasting plasma GIP (pmol/l)</td>
<td>14.2 ± 1.9</td>
<td>11.0 ± 2.5</td>
<td>12.4 ± 3.9</td>
<td>13.6 ± 2.8</td>
</tr>
<tr>
<td>(M/F)</td>
<td>(14.2 ± 2.6)</td>
<td>(13.2 ± 3.0)</td>
<td>(14.9 ± 4.2)</td>
<td>(22.8 ± 3.3)</td>
</tr>
<tr>
<td>Fasting plasma GLP-1 (pmol/l)</td>
<td>7.1 ± 0.7</td>
<td>7.1 ± 0.9</td>
<td>6.1 ± 0.9</td>
<td>7.9 ± 0.9</td>
</tr>
<tr>
<td>(M/F)</td>
<td>(8.6 ± 0.8)</td>
<td>(6.5 ± 1.3)</td>
<td>(7.2 ± 1.3)</td>
<td>(9.0 ± 1.2)</td>
</tr>
<tr>
<td></td>
<td>5.4 ± 0.5</td>
<td>7.8 ± 1.8</td>
<td>4.5 ± 0.2</td>
<td>6.5 ± 1.0</td>
</tr>
</tbody>
</table>

* p < 0.05 vs controls.
* p < 0.01 vs controls.
* p < 0.001 vs controls.
* p < 0.05 vs non-diabetic twins.
* p < 0.03 vs non-diabetic twins.
* p < 0.001 vs NGT twins.
* Females vs males: p < 0.05 (ANOVA).
subjects without any family history of diabetes were included as controls. The control group was selected primarily to match the non-diabetic twin group (normal and impaired glucose tolerant twins) according to weight. The clinical and biochemical characteristics of the study population were reported in detail previously (9) and are summarized in Table 1, with mean values given separately for males and females. As reported previously (9), the NIDDM twins were more obese (BMI) compared with both their identical non-diabetic co-twins and the controls without any family history of diabetes (Table 1). Furthermore, both non-diabetic and NIDDM twins had a higher abdominal obesity index of sex (waist-to-hip ratio) compared with the controls (Table 1). The BMI was higher in the females compared with the males in the total study population (p < 0.05). However, other subject characteristics such as weight, age or waist-to-hip ratios were not significantly different between females and males in the total study population. Informed consent was obtained from all subjects. The protocol was approved by the regional ethical committee and the procedures were performed according to the principles of the Helsinki Declaration.

The group of non-diabetic twins include both normal (NGTT) and impaired (IGTT) glucose tolerant twins according to the WHO criteria. However, as previously reported, all results relating to glucose metabolism and insulin secretion, including plasma glucose profiles during oral glucose tolerance tests (OGTTs), (except for the 120 min values) were qualitatively and quantitatively similar in the groups of NGTTs and IGTTs (9). This indicates that the common selection criterion (i.e. being an identical non-diabetic co-twin of a NIDDM twin), at least in the present connection, may provide a more appropriate separation towards the controls compared with the somewhat arbitrary defined WHO glucose tolerance criteria. Thus, in all comparisons made throughout the paper, results in the total group of non-diabetic twins (N = 12) are tested statistically against measurements made in the groups of NIDDM twins (N = 12) (to estimate the contribution of environmental factors on a given parameter) and controls without any family history of diabetes (in order to identify potential genetic components). However, the mean values are presented separately for both groups (i.e. NGTTs and IGTTs).

Oral glucose tolerance tests
A standard 180-min 75-g OGTT was performed in all subjects after an overnight fast as described previously (9). Medication, including antidiabetic drug therapy, was withdrawn 72 h prior to the tests (9). Blood samples were drawn at the following time points (min) during the OGTTs for the determination of plasma glucose, insulin, glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) concentrations: −20, −10, 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150 and 180 min. All samples for the plasma hormone concentrations were stored at −80°C.

**Assays**
Glucose in plasma was determined using an automated glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA, USA). Plasma insulin concentrations were measured using a double-antibody radioimmunological method (Pharmacia Diagnostics AB, Uppsala, Sweden). Cross-reactivity for proinsulin in the assay was 40%.

Plasma GLP-1 concentrations were measured using synthetic amidated GLP-1 (Peninsula, St Helens, England) as standard, 125I-labelled GLP-1 (12) and antiserum 89390. The antiserum 89390 has an absolute requirement for the amidated C-terminus of GLP-1 and neither reacts with glycine-extended nor C-terminally extended or truncated forms, but reacts fully with GLP-1(9–36)amide (12, 13). The experimental detection limit was 2 pmol/l. The recovery of synthetic GLP-1 added to plasma prior to extraction and assay was within 100 ± 15% of expected values. The intra-assay coefficient of variation was below 6% at a concentration of 30 pmol/l. For all assays, free and bound moieties were separated by plasma-coated charcoal (E Merck, Darmstadt, Germany).

Plasma GIP concentrations were measured using human synthetic GIP as standard (Peninsula), moniodinated 125I-labelled human GIP as tracer (prepared by the stoichiometric chloramine-T method and purified by HPLC (14)) and the antibody R65 (15). The antibody R65 reacts with the C-terminus of GIP and recognizes only a single molecular form in plasma (16–18). Thus, the 8-kD molecular form picked up by most other GIP assays does not interfere in this assay (18). The experimental detection limit was 1 pmol/l. The recovery of human GIP added to plasma prior to extraction and subsequent radioimmunoassay deviated less than 15% from expected values. The intra-assay coefficient of variation was 6% at 25 pmol/l.

**Calculations and statistical analysis**
The incremental glucose, insulin, GIP and GLP-1 areas under curves (AUCs) during the OGTTs were calculated using the trapezoidal method (with subtractions of individual baseline levels) from measurements obtained at the time points outlined above.

Non-parametric statistical methods (Kruskall–Wallis test for comparison of more than two groups; Mann–Whitney and Wilcoxon’s tests for comparison of unpaired and paired data) were employed for analysis of the data summarized in Table 1: Spearman’s rho (R) was used for assessment of correlations. The incremental AUCs summarized in Table 2 were analysed by means of a multifactor analysis of variance (ANOVA).
with patient groups and gender as qualitative factors and with quantitative factors of age, weight, BMI and waist-to-hip ratio as covariates using the Statgraphics Statistical Package. Results are presented as means ± SEM unless stated otherwise, and p < 0.05 was considered to be statistically significant.

Results

The incremental AUCs reflected the 2-h plasma glucose concentrations, with the highest AUC in the NIDDM twins, smaller AUCs in the non-diabetic twins and the smallest AUC in the controls without any family history of diabetes (Table 2).

The incremental insulin secretion (AUC) during the OGTTs was, as reported previously (9), significantly decreased in the NIDDM twins compared with both their non-diabetic co-twins (NGTTs and IGTTs) and controls without a family history of diabetes (Table 2 and Fig. 1). The non-diabetic twins (NGTTs and/or IGTTs) and controls had similar incremental insulin AUCs during the OGTTs (Table 2 and Fig. 1). However, as also reported previously (9), the plasma insulin concentration at the time point 30 min during OGTTs was significantly lower in the non-diabetic twins compared with controls (i.e. 28 ± 7 (NGTT) and 32 ± 5 (IGTT) vs 49 ± 8 (controls) µU/ml, p < 0.02). The 30-min plasma insulin concentrations were identical in NGTTs and IGTTs. The 30-min plasma insulin concentrations were decreased in the NIDDM twins compared with controls (19 ± 2 vs 49 ± 8 µU/ml, p < 0.0001) but similar in the groups of non-diabetic and NIDDM twins.

Fasting GLP-1 concentrations were similar in all study groups (Table 1). Secretion of GLP-1 was stimulated by the oral glucose loads in all groups (Fig. 1). However, the NIDDM patients had a significantly decreased incremental GLP-1 response (increase over basal values, AUC) to oral glucose compared with the control subjects without any family history of diabetes (Table 2 and Fig. 1). The incremental GLP-1 response in the non-diabetic twins was not significantly different from their identical NIDDM co-twins and nor was it different from the control subjects without any family history of diabetes (Table 2 and Fig. 1). Furthermore, plasma GLP-1 concentrations were not significantly different between the various study groups at the 30-min time points (NGTT: 15.0 ± 3.2; IGTT: 16.7 ± 2.8; NIDDM twins: 18.1 ± 1.7; controls: 22.4 ± 5.0 pmol/l; all NS).

Secretion of GIP was stimulated by the oral glucose loads in all groups (Fig. 1). Plasma GIP concentrations were similar in all groups in both the fasting state (Table 1) and during all time points during the OGTTs, including the 30-minute time points (i.e. NGTT: 93.2 ± 16.5; IGTT: 96.9 ± 12.0; NIDDM twins: 79.8 ± 10.8; controls: 78.0 ± 8.8 pmol/l; all NS). Accordingly, incremental GIP AUCs were similar in all study groups (Table 2 and Fig. 1).

When incremental incretin hormone responses (GLP-1 and GIP) were analysed separately for the groups of NGTTs and IGTTs, we were unable to detect any statistically significant differences between any of the two subgroups of non-diabetic twins compared with neither the controls nor the NIDDM twins.

Using simple non-parametric correlation analysis without correcting for covariates, the incremental plasma GLP-1 AUC correlated significantly with the incremental plasma insulin AUC in the total study group (N = 37; R = 0.42, p = 0.01). Furthermore, a weaker (but statistically significant) correlation between incremental plasma GIP AUC and incremental plasma insulin AUC concentrations was found in the total study group (N = 37; R = 0.37, p < 0.03). However, using ANOVA with or without correcting for covariates, no statistically significant relationships

Table 2. Incremental areas under curves (AUCs) during oral glucose tolerance tests of plasma glucose, insulin, GIP and GLP-1 concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Non-diabetic twins</th>
<th>NIDDM twins</th>
<th>ANOVA: difference between groups</th>
<th>ANOVA: impact of sex</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose AUC (nmol·l⁻¹·min⁻¹)</strong></td>
<td>21.2 ± 36.6d</td>
<td>55.8 ± 34.0b</td>
<td>239.4 ± 29.2b,d</td>
<td>&lt;0.0001</td>
<td>p &lt; 0.005</td>
</tr>
<tr>
<td>(M/F)</td>
<td>(64.9 ± 52.5, 41.7 ± 23.6)</td>
<td>(204.3 ± 24.5, 103.3 ± 54.2)</td>
<td>(302.3 ± 54.2, 109.8)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Insulin AUC (mU·ml⁻¹·min⁻¹)</strong></td>
<td>4.67 ± 0.76</td>
<td>3.51 ± 1.00</td>
<td>5.07 ± 1.09</td>
<td>0.0005</td>
<td>p &lt; 0.002</td>
</tr>
<tr>
<td>(M/F)</td>
<td>(2.99 ± 0.32, 6.63 ± 1.20)</td>
<td>(4.52 ± 0.88, 5.17 ± 1.83)</td>
<td>(4.97 ± 0.24, 5.81 ± 2.52)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>GIP AUC (nmol·l⁻¹·min⁻¹)</strong></td>
<td>8.15 ± 0.76</td>
<td>9.90 ± 1.73</td>
<td>10.15 ± 0.95</td>
<td>NS</td>
<td>p &lt; 0.005</td>
</tr>
<tr>
<td>(M/F)</td>
<td>(6.95 ± 0.85, 9.55 ± 1.12)</td>
<td>(7.34 ± 1.34, 13.73 ± 0.55)</td>
<td>(7.00 ± 0.95, 10.78 ± 0.89)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>GLP-1 AUC (nmol·l⁻¹·min⁻¹)</strong></td>
<td>1.17 ± 0.25</td>
<td>0.63 ± 0.28</td>
<td>1.09 ± 0.16</td>
<td>0.05 &lt; p &lt; 0.14</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>(M/F)</td>
<td>(0.97 ± 0.40, 1.40 ± 0.29)</td>
<td>(0.48 ± 0.47, 0.86 ± 0.24)</td>
<td>(0.44 ± 0.21, 0.94 ± 0.23)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

α Data are means ± SEM and are given for the total groups of NIDDM twins, non-diabetic twins and matched controls and for the female and male subgroups (F/M) in all of the different groups. Differences between groups and within sex were analysed by employing multifactor ANOVA with corrections for covariates (see text). Statistical significance (p < 0.05) compared with the groups of controls without any family history of diabetes, non-diabetic twins and NGTT twins are indicated by b, c and d, respectively.
between incremental plasma GLP-1 or plasma GIP AUCs and incremental plasma insulin AUCs were identified in the individual study subgroups. No correlations were found between the 30-min plasma insulin and GLP-1 concentrations or between the 30-min plasma insulin and GIP concentrations when tested in the total study group (N = 37) or in any of the study subgroups. Furthermore, incremental glucose AUCs did not correlate with either incremental GLP-1 or GIP AUCs in the total study group or in any of the study subgroups.

Multifactor ANOVA analysis identified gender as having a major independent impact on the incremental glucose, insulin, GLP-1 and GIP AUCs (Table 2). Thus, incremental glucose, insulin, GLP-1 and GIP AUCs were significantly higher in females compared with males (Table 2). Moreover, this major impact of sex was seen consistently and to a similar extent in all of the study subgroups (Table 2). Weight was identified as an additional significant covariate for the incremental glucose AUCs (r = −0.34, p < 0.05), and waist-to-hip ratio and BMI were both identified as independent significant (or near-significant) covariates for the incremental GLP-1 AUCs (waist-to-hip ratio: r = 0.43, p < 0.02; BMI: r = −0.34, p = 0.06). Besides gender, none of the patient characteristics tested, including age, weight, BMI and/or waist-to-hip ratios, were identified as significant covariates for incremental insulin and/or GIP AUCs in the present study material of twins and controls. Finally, the factors of age, BMI and/or waist-to-hip ratios were not significant covariates for the incremental glucose responses.

Discussion

The present study adds the new dimension of an “intestinal” component or “incretin defect” to the complicated and self-perpetuating process involved in the development of NIDDM. Specifically, the decreased GLP-1 secretion may provide a physiological explanation for the previous notion of an abnormal incretin effect in NIDDM patients (10). The decreased GLP-1 secretion, together with an intrinsic impaired pancreatic insulin secretion, a disproportionately elevated hepatic glucose production (HGP) and an impaired tissue (muscle) sensitivity to insulin (insulin resistance), may contribute to hyperglycaemia in NIDDM through a decreased GLP-1 effect on pancreatic insulin secretion during meals.

The aetiology of the decreased GLP-1 secretion in NIDDM is uncertain but deserves further attention. Firstly, the near-normal GLP-1 secretory pattern in the group of non-diabetic twins indicates that the abnormal GLP-1 secretion as seen in the NIDDM twins may be a consequence of the widespread metabolic abnormalities that follow the development of frank NIDDM, rather than a primary or genetic cause of the disease. Specifically, the factor “glucose toxicity” (19) is a possible candidate
to explain the defect. Thus, glucose toxicity may be involved in causing/aggravating both pancreatic insulin secretion and insulin resistance in NIDDM (19). Moreover, the slightly and non-significantly decreased GLP-1 secretion in the non-diabetic twins may reflect that the diabetic process, to some extent, is already initiated in this group (i.e. higher fasting and 2-h post-oral plasma glucose concentrations compared with controls). However, the early-phase plasma GLP-1 concentrations (i.e. 30-min values) were normal in the non-diabetic twins and did not correlate with the 30-min plasma insulin concentrations. Thus, an abnormal or delayed intestinal GLP-1 secretion is unlikely to explain the delayed insulin response (i.e. decreased 30-min plasma insulin values) in the group of non- and possibly pre-diabetic twins. In other words, the decreased intestinal GLP-1 secretion seen in the NIDDM twins probably reflects a non-genetic defect occurring late in the evolution process of NIDDM and may explain or contribute to the "secondary" component of the impaired pancreatic insulin secretion in NIDDM in response to an oral glucose load (9).

It should also be mentioned that a decreased intestinal GLP-1 secretion is not likely to be the full explanation for the non-genetic component of the impaired insulin secretion in NIDDM. For one thing, the non-genetic component of the defective insulin secretion in NIDDM also includes a decreased insulin secretion in response to an intravenous glucose infusion, which is unlikely to be explained by an abnormal gut incretin hormone secretion. On the other hand, GLP-1 has been claimed to have a "permissive" effect or role on the potency of glucose per se, to enhance insulin secretion in the pancreatic islet cells (i.e. GLP-1 may provide the pancreatic beta-cells with "glucose competence") (20). This means that, in theory, a relatively decreased concentration of GLP-1 at the level of pancreatic beta-cells could be associated with a decreased pancreatic insulin secretion in response to intravenous glucose. However, the normal fasting plasma GLP-1 concentrations in the NIDDM twins do not indicate such a hypothetical role of GLP-1 in causing the marked impairment of insulin secretion in response to intravenous glucose in patients with frank NIDDM.

One previous study from our laboratory reported increased plasma GLP-1 concentrations in patients with NIDDM in the fasting state and during OGTTs (11). However, this discrepancy with the present study may be explained by the use of different GLP-1 immunoassays, the previous study being a processing independent analysis that also measures pancreatic GLP-1-containing peptide moieties. Because the secretion of pancreatic proglucagon-derived peptides is often increased in diabetics, this may explain the increased levels. In the present study we used a GLP-1 radioimmunological assay with a very high specificity towards the C-terminus of proglucagon (78–107) amide of known intestinal origin (8), with no assay cross-reactivity with other proglucagon split products such as 78–108 amide or 78–106 amide. Other possible explanations could be the higher age in the NIDDM patients (twins) in the present study, degree of obesity, waist-to-hip ratio or glycaemia as discussed below, or simply the fact that we have studied identical twins discordant for NIDDM. Thus, we cannot totally exclude the theoretical possibility that this unique study population could represent a distinct subgroup of NIDDM not necessarily similar to other NIDDM patients who were not born as twins. However, the near-normal GLP-1 secretion in the non-diabetic twins indicates that the criterion of being an identical twin does not sufficiently explain the decreased GLP-1 secretion in the NIDDM twins.

Previous studies in both lean and obese NIDDM patients reported normal, augmented or diminished plasma GIP immunoreactivity responsiveness to oral glucose and mixed meals (for specific references, see recent reviews in Refs. 4 and 5). The present finding of a normal intestinal GIP secretion in the NIDDM twins is consistent with the previous balanced conclusion reached from several different studies, that a diminished intestinal GIP secretion may at most contribute to a decreased pancreatic insulin secretion in a minor subgroup of the total NIDDM population (4, 5). However, it should be mentioned that the effect of GIP to enhance the pancreatic secretion of insulin may be impaired in NIDDM (4, 21) whereas the stimulatory effect of GLP-1 on insulin secretion is preserved in NIDDM (8, 21). Therefore, the relative contribution of intestinal GIP secretion to the overall pancreatic insulin secretion in NIDDM may, in theory, be relatively diminished due to resistance towards the stimulatory effect of GIP to enhance pancreatic insulin secretion.

The study also demonstrated normal intestinal GIP secretion during oral glucose ingestion in the group of non-diabetic co-twins of the NIDDM twins. Therefore, a defective or delayed intestinal GIP secretion does not explain the delayed insulin secretion in the non-diabetic twins reported previously (9).

Previous studies reported higher incremental insulin (22–24) and GIP responses (25, 26) to oral glucose in females compared with males. In the present study we found a significant impact of sex on all incremental responses to oral glucose during the 180-min tests, including not only insulin and GIP but also glucose itself and plasma GLP-1 responses. The explanation for the consistently higher incremental areas in the females compared with males is uncertain and was not explained by differences in absolute weight, BMI, body composition (waist-to-hip ratios) or age. Therefore, the differences may not only be a result of different volumes of distributions in males and females, as previously suggested (25). However, the fact that all parameters, including incremental glucose AUCs, were higher in the females may indicate that some kind of gastrointestinal factor relating to either gastric emptying, intestinal
motility and/or glucose absorption may explain the results. In fact, it was demonstrated previously that gastric emptying after food/glucose ingestion is delayed in females compared with males (27, 28), which in the present context would explain a prolonged gut glucose exposure and possibly absorption in the females compared with males. In turn, a prolonged gut glucose exposure may prolong or enhance the glucose stimulus on intestinal hormone secretion, resulting in increased intestinal GLP-1 and GIP secretion rates and secondarily in an enhanced pancreatic insulin secretion. On the other hand, GLP-1 and GIP both inhibit gastric emptying (4, 29), making it impossible to determine which is cause and which is effect in the relationship between a possibly delayed gastric emptying and the increased incremental glucose and GLP-1 AUCs in the females in the present study. Future studies should address this important question.

In order to evaluate the impact of sex on the World Health Organization predefined glucose tolerance criteria, we also analysed the influence of sex on the absolute 120-min post-oral plasma glucose concentrations in the present study material. However, in contrast to the incremental AUCs, we were unable to demonstrate any significant impact of sex on this exact (and of course very important) time point of plasma glucose concentration (Table 1). The explanation for this was primarily that increased glucose AUCs as seen in the females were mostly due to increased areas during the last hour from 120 to 180 min after the oral glucose ingestion, which in turn is perfectly in agreement with the notion of a delayed and prolonged gastric emptying in females (27, 28). Finally, it should also be mentioned that the increased incremental glucose AUC in the females partly resulted from slightly lower baseline (fasting) plasma glucose concentrations (Table 1). The same was, to some extent, the case for the incremental GLP-1 AUC gender differences (Table 1). In contrast, both fasting insulin and GIP concentrations appeared higher in the females compared with males (Table 1). Therefore, the gender differences in incremental AUCs are not solely explained by different fasting levels.

In conclusion, a decreased intestinal GLP-1 secretion may contribute to the impaired pancreatic insulin release during oral glucose ingestion in NIDDM twins. In contrast, intestinal GIP secretion was normal in the NIDDM twins during oral glucose ingestion. The defective (delayed) insulin response to oral glucose in non-diabetic identical co-twins of NIDDM patients/ twins was not explained by an impaired or delayed intestinal GLP-1 or GIP secretion. The genetic component of the insulin secretion defect in NIDDM is, therefore, most likely to be due to an intrinsic defect at the level of the pancreatic beta-cells. Gender had a major impact on the incremental plasma glucose, insulin, GLP-1 and GIP AUCs, with higher values in females compared with males. The explanation for this is uncertain, but one possibility could be a delayed gastric emptying and/or intestinal motility in females compared with males.

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