In middle-aged siblings of patients with Type 2 diabetes mellitus normal glucose tolerance is associated with insulin resistance and with increased insulin secretion. The SPIDER study

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Abstract

Objectives: To evaluate the frequency of impaired glucose tolerance (IGT) and of Type 2 diabetes mellitus (Type 2 DM) in siblings of patients with Type 2 DM, and to assess insulin release and insulin sensitivity in siblings with normal glucose tolerance (NGT), compared with NGT spouses of probands without family history of Type 2 DM.

Design and Methods: We evaluated 87 families including 103 Type 2 DM patients (87 probands), and we carried out an oral glucose tolerance test (OGTT) in 130 siblings and in 60 spouses. Among NGT subjects, 12 siblings and 16 spouses underwent a low-dose insulin-glucose infusion test (LDIGIT) to evaluate C-peptide release and insulin sensitivity.

Results: After the OGTT, 24 siblings were classified as having Type 2 DM, 31 as IGT, and only 14 spouses as IGT (P = 0.0012 vs siblings). NGT siblings (n = 75) showed higher insulin levels at 120 min than NGT spouses (n = 46) at OGTT, in spite of identical blood glucose levels; at LDIGIT, NGT siblings secreted more C-peptide and showed a lower insulin sensitivity than NGT spouses.

Conclusions: These data indicate that middle-aged siblings of probands with Type 2 DM have a high frequency of IGT and Type 2 DM, and that NGT siblings have increased insulin resistance and increased insulin secretion when compared with adequate controls.

European Journal of Endocrinology 143 681–686

Introduction

Obesity and body fat distribution (1), lifestyle (2), impaired glucose tolerance (IGT), and family history of Type 2 DM (3, 4) represent risk factors for Type 2 DM. First degree relatives (FDRs) of patients with Type 2 DM (offspring and relatives) frequently show abnormal glucose tolerance, and share several metabolic abnormalities of the full blown disease (5–12), and have a 30–40% risk of developing Type 2 DM themselves (4). These considerations make FDRs a good model for the study of the pathogenesis of Type 2 DM in the absence of hyperglycaemia, which impairs insulin action (13) and insulin release (14) making it hard to understand which condition precedes the other in the development of the disease (15).

A number of studies on the offspring of patients with Type 2 DM have consistently shown the presence of insulin resistance (6, 16–22), a major risk factor for Type 2 DM in Pima Indians, in Mexican Americans and in white Caucasians (7, 23–25); a defective insulin release in the presence of normal insulin sensitivity has been shown in a few studies, and these studies were not different from the others in terms of age or body mass index of either offspring or controls (26–28). Two criticisms can be made to these studies. First, controls were from the general population and not from the same cohorts; it is well known that members of the same family usually share the same lifestyle, in terms of physical activity and diet, while controls chosen from the general population may have a different lifestyle. Second, studies on offspring are limited in terms of the age of the subjects, especially if they are studied before the age of 40 years. A preferable alternative might be the study of siblings; they are FDR, are generally of older age than offspring, and one can be more confident that, if the subject is not diabetic when evaluated, the chance that they will develop Type 2 DM in the following years is reduced. This approach has been used so far in only three studies, and has yielded evidence of frequent IGT (8, 9, 11); in glucose tolerant (NGT) siblings, high fasting insulin levels, insulin resistance and reduced hepatic insulin clearance were shown; in these studies,
however, controls were again from the general population, not from the same cohort. The Botnia study (29) was the only study evaluating FDRs of patients with Type 2 DM (parents, siblings, and children together), with their spouses as controls; this study revealed increased insulin resistance as the main feature of FDRs compared with controls.

The aims of the current study were: (1) to evaluate the frequency of IGT and of unknown Type 2 DM in middle-aged siblings of patients with Type 2 DM; (2) to evaluate insulin release and insulin resistance in NGT subjects, using as controls only NGT spouses of probands without family history of diabetes.

**Patients and methods**

The study, called SPIDER (an acronym for Studio di Prevenzione dell’Insorgenza del Diabete ad Estensione Regionale, i.e. regional study of prevention of onset of diabetes), is an on-going family study aimed at prediction and prevention of Type 2 DM in families with at least one patient affected by Type 2 DM.

Each family evaluated consists of at least one Type 2 DM patient (the proband), their siblings, and the spouse of the proband provided they have no family history of diabetes mellitus. Spouses were chosen as controls because they are generally of similar age and share the same environment. Only those Type 2 DM patients treated with diet and/or hypoglycemic agents were considered. Families in which Type 2 DM onset was below the age of 40 years were not considered. The protocol of the study was approved by the Ethics Committee of Istituto San Raffaele, and the aims and nature of the study are displayed in the out-patients clinics of the participating centers. All subjects, i.e. probands, siblings, and spouses, after giving informed consent, are evaluated for anthropometric measures (body mass index, BMI in kg/m²), waist/hip ratio, arterial blood pressure; all subjects fill in a questionnaire about physical activity (30), and are asked to note their dietary intake over the previous 3 days (31); an i.v. antecubital (15 ml) blood sample is withdrawn after overnight fasting for evaluation of metabolic and endocrine variables including blood glucose, serum insulin, pro-insulin, C-peptide, triglycerides, cholesterol, HDL cholesterol, creatinine levels, plasma free fatty acids. Siblings and spouses undergo an oral glucose tolerance test (OGTT, 75 g) with a second evaluation of blood glucose, insulin, pro-insulin, C-peptide at 120 min, and evaluation of GAD antibodies. On the basis of OGTT results (32), siblings and spouses are classified as normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or Type 2 DM.

**Low dose insulin–glucose infusion test**

To evaluate insulin release and insulin sensitivity, the low dose insulin–glucose infusion test (LDIGIT) was performed in 12 NGT siblings and 16 NGT spouses. In brief, LDIGIT consists of a constant infusion of glucose (4 mg/kg per min) and insulin (25 mU/kg per h) lasting 150 min (33). Steady-state (SS) blood glucose and serum insulin are calculated during the last 30 min. The insulin sensitivity index (ISI) of LDIGIT is calculated as glucose infusion divided by SS insulin and normalizing the ratio to SS glucose, i.e. ml of glucose/kg per min per pmol/l insulin; in our hands the ISI of LDIGIT has an r value of 0.91 compared with the ISI of the euglycemic clamp (33). C-peptide release is expressed as the incremental area (ΔAUC 0–15 min), calculated by the trapezoidal method; LDIGIT and hyperglycemic clamp show a r = 0.82 for C-peptide release (33).

**Assays**

Blood glucose levels were measured by a glucose-oxidase method (YSI, Yellow Springs, OH, USA). Free fatty acids (FFA) and triglyceride levels were assayed by an enzymatic technique on Cobas Fara II Centrifugal Analyser (Cobas Fara II, Roche, Basel, Switzerland, 34). In our laboratory, FFA and triglyceride intra-assay coefficients of variation (CV) are 2.3 and 1.7%, and the interassay CVs are 3.0 and 3.4%, respectively. Total cholesterol and HDL cholesterol levels were assayed with Cobas Fara II by enzymatic automated spectrophotometric methods, with an intra-assay CV of 4.3% and an interassay CV of 8.8%. Insulin was assayed using a microparticle enzyme immunoassay (MEIA, IMX, Abbott Laboratories, Abbott Park, II, USA) with a monoclonal antibody without cross-reactivity to human pro-insulin: sensitivity was 6.0 pmol/l; intra-assay CV 3.0%, interassay CV 5.0%. C-peptide was assayed by a commercial radioimmunoassay kit (DPC, Euro/DPC, Llanberis, UK), with a sensitivity 0.005 ng/ml; intra-assay CV was 3.0%, and interassay CV was 5.0%. Pro-insulin was assayed using a commercial kit (Total Proinsulin, Dako Diagnostics, Ely, UK), without cross-reactivity with human insulin: sensitivity was 2.0 pmol/l, intra-assay CV 7.0%, interassay CV 10.0%. GAD antibodies were assessed, in 183 subjects (23 newly diagnosed Type 2 DM, 45 IGT, 115 NGT), by a combined radiobinding assay (35).

**Calculations and statistical analysis**

Values are expressed as mean plus 95% confidence intervals, as means ± s.e. or as absolute numbers. Comparisons between absolute frequencies were performed using the χ² test; comparisons between groups were performed using Student’s t-test for unpaired data. P values < 0.05 were considered significant.

**Results**

Eighty-seven families were evaluated for a total of 87 probands and 16 siblings with known Type 2 DM, 130
siblings and 60 spouses — only those spouses without a family history of Type 2 DM could be included.

After OGTT, 24 siblings were classified as having Type 2 DM, 31 as IGT, 75 as NGT; 14 spouses were classified as IGT, 46 as NGT.

Table 1 shows clinical and metabolic details of all siblings and spouses divided according to glucose tolerance; statistical comparisons were performed between NGT siblings and NGT spouses, and revealed significant differences only for waist/hip ratio and for HDL cholesterol. GAD antibodies were absent in all subjects tested. During OGTT, serum insulin increased more in NGT siblings than in NGT spouses: 37.8 ± 24.3 μU/ml, P = 0.0205, in spite of similar blood glucose levels at 0 and at 120 min (Fig. 1). Table 2 shows baseline metabolic values of NGT siblings and NGT spouses undergoing LDIGIT. During LDIGIT, NGT siblings showed significantly higher C-peptide and lower insulin sensitivity index than NGT spouses (Fig. 2).

Discussion

In this study we confirmed a high frequency of IGT and of unsuspected Type 2 DM among siblings of patients with Type 2 DM. This high frequency of IGT/Type 2 DM is in agreement with previous studies evaluating offspring of patients with Type 2 DM (5–12), slightly higher probably due to the older age of our siblings and spouses.

In this study, the only differences observed were a

**Table 1** Clinical, endocrine and metabolic details of subjects in the study, divided according to the results of OGTT. Mean values are shown with 95% confidence intervals in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Siblings</th>
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<th>Spouses</th>
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<tr>
<td></td>
<td>Type 2 diabetes</td>
<td>IGT</td>
<td>NGT</td>
<td>NGT</td>
</tr>
<tr>
<td>Number (men/women)</td>
<td>24 (13/11)</td>
<td>31 (8/23)</td>
<td>75 (22/53)</td>
<td>46 (18/28)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.2 (53.8–60.7)</td>
<td>53.9 (51.2–56.7)</td>
<td>50.0 (47.7–52.3)</td>
<td>52.8 (50.1–55.5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.6 (27.4–31.8)</td>
<td>28.7 (26.9–30.5)</td>
<td>25.9 (25.0–26.8)</td>
<td>24.9 (23.8–26.1)</td>
</tr>
<tr>
<td>W/H ratio</td>
<td>0.92 (0.88–0.95)</td>
<td>0.90 (0.87–0.94)</td>
<td>0.89 (0.87–0.91)</td>
<td>0.85 (0.83–0.87)</td>
</tr>
<tr>
<td>Physical activity</td>
<td>7.9 (5.2–10.6)</td>
<td>9.3 (6.9–11.6)</td>
<td>7.6 (6.5–8.6)</td>
<td>8.2 (6.4–9.9)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>141 (134–148)</td>
<td>135 (130–139)</td>
<td>128 (124–131)</td>
<td>127 (122–132)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>84 (81–87)</td>
<td>84 (80–88)</td>
<td>79 (77–85)</td>
<td>80 (77–82)</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>72.5 (64.5–81.3)</td>
<td>67.2 (59.3–75.1)</td>
<td>74.3 (70.7–77.8)</td>
<td>79.6 (75.1–84.0)</td>
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<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.89 (1.52–2.26)</td>
<td>1.70 (1.39–2.01)</td>
<td>1.29 (1.14–1.46)</td>
<td>1.30 (1.05–1.48)</td>
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<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.87 (5.21–6.52)</td>
<td>5.87 (5.41–6.31)</td>
<td>5.72 (5.42–6.01)</td>
<td>5.81 (5.41–6.20)</td>
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<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.48 (1.15–1.81)</td>
<td>1.36 (1.15–1.57)</td>
<td>1.51 (1.37–1.65)</td>
<td>1.71 (1.54–1.86)</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>0.71 (0.58–0.82)</td>
<td>0.68 (0.61–0.76)</td>
<td>0.56 (0.51–0.62)</td>
<td>0.54 (0.48–0.60)</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>8.0 (7.1–8.9)</td>
<td>6.6 (6.3–6.9)</td>
<td>5.7 (5.5–5.9)</td>
<td>5.8 (5.6–6.0)</td>
</tr>
<tr>
<td>120 min blood glucose (mmol/l)</td>
<td>14.2 (12.7–15.8)</td>
<td>9.6 (9.2–9.9)</td>
<td>5.8 (5.5–6.1)</td>
<td>5.8 (5.5–6.2)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.2 (5.8–6.6)</td>
<td>6.0 (5.6–6.4)</td>
<td>5.5 (5.3–5.7)</td>
<td>5.3 (5.1–5.6)</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>75.6 (61.8–89.4)</td>
<td>87.0 (49.8–123.6)</td>
<td>55.2 (44.4–66.0)</td>
<td>43.2 (37.3–49.2)</td>
</tr>
<tr>
<td>Pro-insulin (pmol/l)</td>
<td>9.7 (5.7–13.7)</td>
<td>7.6 (5.4–9.8)</td>
<td>4.7 (3.8–5.6)</td>
<td>4.6 (3.4–5.7)</td>
</tr>
<tr>
<td>C-peptide (nmol/l)</td>
<td>2.5 (2.0–3.0)</td>
<td>3.0 (2.4–3.6)</td>
<td>2.0 (1.8–2.3)</td>
<td>1.9 (1.6–2.1)</td>
</tr>
</tbody>
</table>

§P < 0.05 vs NGT siblings.

**Figure 1** Serum insulin levels as a function of blood glucose levels at 120 min during OGTT in spouses (left panel, n = 46) and in siblings (right panel, n = 75) with normal glucose tolerance.

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lower HDL cholesterol and a higher waist/hip ratio in NGT siblings versus NGT spouses, in spite of identical age, BMI, and physical activity; at OGTT the only difference was the 120 min insulin level. Even though insulin levels were evaluated only at 0 and at 120 min, this finding is more compatible with enhanced insulin release and increased insulin resistance than with reduced insulin release; this finding was substantiated by the fact that, at LDIGIT, performed in a subgroup of NGT subjects, NGT siblings showed greater C-peptide and lower insulin sensitivity than NGT spouses. This finding is in agreement with previous studies carried out on the offspring of patients with Type 2 DM (6, 16–22); the only study evaluating siblings and offspring of patients with Type 2 DM, with controls from the same cohort (Botnia study, (29)), indicated insulin resistance as the main feature of siblings and offspring.

In contrast to the Botnia study (29, 36) we found no evidence of GAD antibodies in subjects with or without a family history of Type 2 DM; since the value of GAD antibodies predicts insulin requirement in Type 2 DM (35, 36), this finding was expected due to our choice to evaluate only siblings of probands with onset of Type 2 DM above the age of 40 years and under treatment with diet and/or hypoglycaemic agents, and agrees with the data of a population study carried out in northern Italy (35) showing a very low prevalence of GAD antibodies in normal subjects, and in subjects with either IGT or Type 2 DM. Therefore it seems that, in general, GAD antibodies are quite rare among patients with Type 2 DM in northern Italy, as well as in the general population (35), as opposed to the population in northern Europe (36).

It is tempting to put forward an interpretation of the different results reported so far in studies dealing with FDRs of Type 2 DM patients. Most of the papers, including the present study, have emphasized insulin resistance (6, 16–22, 29), while a few other studies have underlined defective insulin release (26–28); Martin et al. (37) have shown that insulin resistance clusters in the offspring of parents with Type 2 DM and therefore varies in different families. Vauhkonen et al.

### Table 2

<table>
<thead>
<tr>
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<th>NGT siblings (n = 12)</th>
<th>NGT spouses (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>6/6</td>
<td>7/9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51.0±2.0</td>
<td>50.8±1.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6±0.9</td>
<td>23.0±0.6</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>4.7±0.1</td>
<td>4.6±0.1</td>
</tr>
<tr>
<td>Fasting IRI (µU/ml)</td>
<td>8.1±0.9</td>
<td>6.0±0.7</td>
</tr>
<tr>
<td>Fasting C-peptide (nmol/l)</td>
<td>1.6±0.1</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>Steady-state blood glucose (mmol/l)</td>
<td>5.1±0.3</td>
<td>4.3±0.2</td>
</tr>
<tr>
<td>Steady-state IRI (µU/ml)</td>
<td>39.1±4.0</td>
<td>28.1±2.0</td>
</tr>
</tbody>
</table>

**Figure 2** (A) Integrated C-peptide release (ΔAUC) and (B) insulin sensitivity index (ISI) during low dose insulin–glucose infusion (LDIGIT) in NGT siblings (solid columns, n = 12) and in NGT spouses (open columns, n = 16). Means ± S.E.
(12) have indicated that offspring of patients resemble their parents as to insulin resistance or defective insulin release. Finally, one-quarter of normal subjects from the general population is reported to show insulin resistance (38). All these data together help us to understand why some authors have indicated insulin resistance, while others have emphasized defective insulin release as the main feature of the pre-diabetic state. Candidate gene studies are required to help elucidate the reasons for these discrepancies.

In conclusion, this study has revealed an abnormal frequency of IGT and of unsuspected Type 2 DM in siblings of patients with Type 2 DM, compared with adequate controls. The first abnormality to develop among siblings seems to be an increased insulin resistance, compensated by enhanced insulin release.

Acknowledgements

This study was partly supported by a grant from the Lombardia branch of the Società Italiana di Diabetologia (SID), from Consiglio Nazionale Ricerche (CNR), contract 95 01682.CT04, from Ministero dell’Università e della Ricerca Scientifica e Tecnologica (MURST, grant 9806409093). Novo Nordisk Farmaceutici Italia generously donated an educational grant; we wish to thank all physicians who collaborated in recruiting families of Type 2 DM patients and in encouraging patients, siblings and spouses to participate in the study.

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Received 9 May 2000
Accepted 31 July 2000