CLINICAL STUDY

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

A E Pontiroli, L D Monti, S Costa, P E Sandoli, A Pizzini, S B Solerte, E Mantovani and P M Piatti

Università degli Studi di Milano, Cattedra di Medicina Interna, Istituto San Raffaele, Unità di Malattie Metaboliche, Milano, Italy, Dipartimento di Medicina Interna, Ospedale S. Margherita, Università degli Studi di Pavia, Pavia, Italy and Ospedale di Bozzolo, Mantova, Italy

(Correspondence should be addressed to A E Pontiroli, Istituto San Raffaele, Via Olgettina 60, 20132 Milano, Italy; Email: antonio.pontiroli@unimi.it)

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 pmolar 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 (Δ AUC 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, IL, 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 was 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 $\pm$ s.e. or as absolute numbers. Comparisons between absolute frequencies were performed using the χ^2 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

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.

	Siblings			Spouses	
	Type 2 diabetes	IGT	NGT	NGT	IGT
Number (men/women)	24 (13/11)	31 (8/23)	75 (22/53)	46 (18/28)	14 (7/7)
Age (years)	57.2 (53.8–60.7)	53.9 (51.2–56.7)	50.0 (47.7–52.3)	52.8 (50.1–55.5)	57.9 (52.1–63.7)
BMI (kg/m ²)	29.6 (27.4–31.8)	28.7 (26.9–30.5)	25.9 (25.0–26.8)	24.9 (23.8–26.1)	29.5 (26.8–32.1)
W/H ratio	0.92 (0.88–0.95)	0.90 (0.87–0.94)	0.89 (0.87–0.91)	0.85 (0.83–0.87) [§]	0.91 (0.86–0.96)
Physical activity	7.9 (5.2–10.6)	9.3 (6.9–11.6)	7.6 (6.5–8.6)	8.2 (6.4–9.9)	6.1 (4.2–8.0)
Dietary intake (kcal)	2020 (1500–2500)	2103 (1600–2600)	2233 (1950–2450)	2075 (1850–2300)	2211 (1950–2400)
Systolic BP (mmHg)	141 (134–148)	135 (130–139)	128 (124–131)	127 (122–132)	140 (132–149)
Diastolic BP (mmHg)	84 (81–87)	84 (80–88)	79 (77–85)	80 (77–82)	85 (80–90)
Creatinine (μmol/l)	72.5 (64.5–81.3)	67.2 (59.3–75.1)	74.3 (70.7–77.8)	79.6 (75.1–84.0)	85.7 (76.9–94.6)
Triglycerides (mmol/l)	1.89 (1.52–2.26)	1.70 (1.39–2.01)	1.29 (1.14–1.46)	1.30 (1.05–1.48)	1.66 (0.92–2.39)
Cholesterol (mmol/l)	5.87 (5.21–6.52)	5.87 (5.41–6.31)	5.72 (5.42–6.01)	5.81 (5.41–6.20)	5.74 (4.82–6.65)
HDL cholesterol (mmol/l)	1.48 (1.15–1.81)	1.36 (1.15–1.57)	1.51 (1.37–1.65)	1.71 (1.54–1.86) [§]	1.36 (1.08–1.63)
FFA (mmol/l)	0.71 (0.58–0.82)	0.68 (0.61–0.76)	0.56 (0.51–0.62)	0.54 (0.48–0.60)	0.70 (0.54–0.86)
Fasting blood glucose (mmol/l)	8.0 (7.1–8.9)	6.6 (6.3–6.9)	5.7 (5.5–5.9)	5.8 (5.6–6.0)	6.4 (5.9–6.9)
120 min blood glucose (mmol/l)	14.2 (12.7–15.8)	9.6 (9.2–9.9)	5.8 (5.5–6.1)	5.8 (5.5–6.2)	9.1 (8.4–9.8)
HbA1c (%)	6.2 (5.8–6.6)	6.0 (5.6–6.4)	5.5 (5.3–5.7)	5.3 (5.1–5.6)	5.8 (4.9–6.7)
Insulin (pmol/l)	75.6 (61.8–89.4)	87.0 (49.8–123.6)	55.2 (44.4–66.0)	43.2 (37.3–49.2)	64.2 (33.0–95.4)
Pro-insulin (pmol/l)	9.7 (5.7–13.7)	7.6 (5.4–9.8)	4.7 (3.8–5.6)	4.6 (3.4–5.7)	4.7 (1.7–7.8)
C-peptide (nmol/l)	2.5 (2.0–3.0)	3.0 (2.4–3.6)	2.0 (1.8–2.3)	1.9 (1.6–2.1)	2.8 (2.0–3.7)

[§]*P* < 0.05 vs NGT siblings.

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 (*P* = 0.0012 vs siblings). 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 ± 4.21 vs 24.3 ± 2.13 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

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.

Table 2 Basal and steady-state levels during low dose insulin–glucose infusion test (LDIGIT) in NGT siblings of Type 2 DM patients, and in NGT spouses without family history of Type 2 DM. Means $\pm$ S.E.

	NGT siblings ($n = 12$)	NGT spouses ($n = 16$)
Men/women	6/6	7/9
Age (years)	51.0 $\pm$ 2.0	50.8 $\pm$ 1.4
BMI (kg/m ²)	24.6 $\pm$ 0.9	23.0 $\pm$ 0.6
Fasting blood glucose (mmol/l)	4.7 $\pm$ 0.1	4.6 $\pm$ 0.1
Fasting IRI (μ U/ml)	8.1 $\pm$ 0.9	6.0 $\pm$ 0.7
Fasting C-peptide (nmol/l)	1.6 $\pm$ 0.1	1.4 $\pm$ 0.1
Steady-state blood glucose (mmol/l)	5.1 $\pm$ 0.3	4.3 $\pm$ 0.2
Steady-state IRI (μ U/ml)	39.1 $\pm$ 4.0	28.1 $\pm$ 2.0

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.*

Figure 2 (A) Integrated C-peptide release (Δ AUC) 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 $\pm$ 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.

References

- Ohlson LO, Larsson B, Svardsudd K, Welin L, Eriksson H, Wilhelmsen L *et al.* The influence of body fat distribution on the incidence of diabetes mellitus. 13.5 years of follow-up in the participants in the study of men born in 1913. *Diabetes* 1985 **34** 1055–1058.
- Helmrich SP, Ragland DR, Leung RW & Paffenbarger RS Jr. Physical activity and reduced occurrence of non-insulin dependent diabetes mellitus. *New England Journal of Medicine* 1991 **325** 147–152.
- Barnett AH, Eff C, Leslie RD & Pyke DA. Diabetes in identical twins. A study of 200 pairs. *Diabetologia* 1981 **20** 87–93.
- Pierce M, Keen H & Bradley C. Risk of diabetes in offspring of parents with non-insulin dependent diabetes. *Diabetic Medicine* 1995 **12** 6–13.
- Leslie RD, Volkmann HP, Poncher M, Hanning I, Orskov H & Alberti KG. Metabolic abnormalities in children of non-insulin dependent diabetics. *British Medical Journal* 1986 **293** 840–842.
- Laws A, Stefanick ML & Reaven GM. Insulin resistance and hypertriglyceridemia in nondiabetic relatives of patients with noninsulin-dependent diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism* 1989 **69** 343–347.
- Warram JH, Martin BC, Krolewski AS, Soeldner JS & Kahn CR. Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. *Annals of Internal Medicine* 1990 **113** 909–915.
- Pontirol AE, Piatti PM, Perfetti MG & Pozza G. Impaired glucose tolerance, endocrine and metabolic abnormalities in families of patients with type 2 diabetes mellitus. *Archives of Gerontology and Geriatrics suppl* 1991 **2** 227–230.
- Stewart MW, Humphriss DB, Berrish TS, Barriocanal LA, Trajano LR, Alberti KG *et al.* Features of syndrome X in first-degree relatives of NIDDM patients. *Diabetes Care* 1995 **18** 1020–1022.
- Vestbo E, Damsgaard EM, Froland A & Mogensen CE. Clinical features in persons with a family history of diabetes compared with controls (The Second Generation Fredericia Study). *Journal of Internal Medicine* 1996 **240** 381–387.
- Humphriss DB, Stewart MW, Berrish TS, Barriocanal LA, Trajano LR, Ashworth LA *et al.* Multiple metabolic abnormalities in normal glucose tolerant relatives of NIDDM families. *Diabetologia* 1997 **40** 1185–1190.
- Vauhkonen I, Niskanen L, Vanninen E, Kainulainen S, Uusitupa M & Laakso M. Defects in insulin secretion and insulin action in non-insulin-dependent diabetes mellitus are inherited. Metabolic studies on offspring of diabetic probands. *Journal of Clinical Investigation* 1998 **101** 86–96.
- Yki-Jarvinen H. Glucose-toxicity. *Endocrine Reviews* 1992 **13** 415–431.
- Davalli AM, Ricordi C, Socci C, Braghi S, Bertuzzi F, Fattor B *et al.* Abnormal sensitivity to glucose of human islets cultured in a high glucose medium: partial reversibility after an additional culture in a normal glucose medium. *Journal of Clinical Endocrinology and Metabolism* 1991 **72** 202–208.
- De Fronzo RA. The triumvirate: β -cell, muscle, liver: a collusion responsible for NIDDM. *Diabetes* 1988 **37** 667–687.
- Eriksson J, Franssila-Kallunki A, Ekstrand A, Saloranta C, Widen E, Schalin C *et al.* Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus. *New England Journal of Medicine* 1989 **321** 337–343.
- Ho LT, Chang ZY, Wang JT, Li SH, Liu YF, Chen YD *et al.* Insulin insensitivity in offspring of parents with type 2 diabetes mellitus. *Diabetic Medicine* 1990 **7** 31–34.
- Osei K, Cottrell DA & Orabella MM. Insulin sensitivity, glucose effectiveness, and body fat distribution pattern in nondiabetic offspring of patients with NIDDM. *Diabetes Care* 1991 **14** 890–896.
- Vaag A, Henriksen JE & Beck-Nielsen H. Decreased insulin activation of glycogen synthase in skeletal muscles in young nonobese caucasian first-degree relatives of patients with non-insulin-dependent diabetes mellitus. *Journal of Clinical Investigation* 1992 **89** 782–788.
- Henriksen JE, Alford F, Handberg A, Vaag A, Ward GM, Kalfas A *et al.* Increased glucose effectiveness in normoglycemic but insulin-resistant relatives of patients with non-insulin-dependent diabetes mellitus. A novel compensatory mechanism. *Journal of Clinical Investigation* 1994 **94** 1196–1204.
- Perseghin G, Ghosh S, Gerow K & Shulman GI. Metabolic defects in lean nondiabetic offspring of NIDDM parents: a cross-sectional study. *Diabetes* 1997 **46** 1001–1009.
- Ishikawa M, Pruneda ML, Adams-Huet B & Raskin P. Obesity-independent hyperinsulinemia in nondiabetic first-degree relatives of individuals with type 2 diabetes. *Diabetes* 1998 **47** 788–792.
- Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E *et al.* Insulin resistance and insulin secretory dysfunction as precursors of non-insulin dependent diabetes mellitus. *New England Journal of Medicine* 1993 **329** 1988–1992.
- Haffner SM, Stern MP, Hazuda HP, Pugh JA & Patterson JK. Hyperinsulinemia in a population at high risk for non-insulin-dependent diabetes mellitus. *New England Journal of Medicine* 1986 **315** 220–224.
- Gulli G, Ferrannini E, Stern M, Haffner S & DeFronzo RA. The metabolic profile of NIDDM is fully established in glucose-tolerant offspring of two mexican-american NIDDM parents. *Diabetes* 1992 **41** 1575–1586.
- O'Rahilly SP, Nugent Z, Rudenski AS, Hosker JP, Burnett MA, Darling P *et al.* Beta-cell dysfunction, rather than insulin insensitivity, is the primary defect in familial type 2 diabetes. *Lancet* 1986 **2** 360–364.

- 27 Pimenta W, Korytkowski M, Mitrakou A, Jenssen T, Yki-Jarvinen H, Evron W *et al.* Pancreatic beta-cell dysfunction as the primary genetic lesion in NIDDM. Evidence from studies in normal glucose-tolerant individuals with a first-degree NIDDM relative. *Journal of the American Medical Association* 1995 **273** 1855–1861.
- 28 Van Haeften TW, Dubbeldam S, Zonderland ML & Erkelens DW. Insulin secretion in normal glucose-tolerant relatives of type 2 diabetic patients. Assessments using hyperglycemic glucose clamps and oral glucose tolerance tests. *Diabetes Care* 1998 **21** 278–282.
- 29 Groop L, Forsblom C, Lehtovirta M, Tuomi T, Karanko S, Nissen M *et al.* Metabolic consequences of a family history of NIDDM (the Botnia study). Evidence for sex-specific parental effects. *Diabetes* 1996 **45** 1585–1593.
- 30 Voorrips LE, Ravelli ACJ & Dongelmans PCA. A physical activity questionnaire for the elderly. *Medicine Science and Sports Exercise* 1991 **23** 974–979.
- 31 Toeller M. Diet and diabetes. *Diabetes-Metabolism Reviews* 1993 **9** 93–108.
- 32 World Health Organization. In *Diabetes Mellitus Report of a WHO Study Group Technical Report 727*. Geneva: World Health Organization, 1985.
- 33 Piatti PM, Monti LD, Caumo A, Santambrogio G, Magni F, Galli-Kienle M *et al.* The continuous low dose insulin and glucose infusion test: a simplified and accurate method for the evaluation of insulin sensitivity and insulin secretion in population studies. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 34–40.
- 34 Knox DP & Jones DG. Automated enzymatic determination of plasma free fatty acids by centrifugal analysis. *Journal of Automated Chemistry* 1984 **6** 152–154.
- 35 Bosi E, Garancini MP, Poggiali E, Bonifacio E & Gallus G. Low prevalence of islet autoimmunity in adult diabetes and low predictive value of islet autoantibodies in the general population of northern Italy. *Diabetologia* 1999 **42** 840–844.
- 36 Tuomi T, Carlsson AL, Li H, Isomaa B, Miettinen A, Nilsson A *et al.* Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. *Diabetes* 1999 **48** 150–157.
- 37 Martin BC, Warram JH, Rosner B, Rich SS, Soeldner JS & Krolewski AS. Familial clustering of insulin sensitivity. *Diabetes* 1992 **41** 850–854.
- 38 Hollenbeck C & Reaven GM. Variations in insulin-stimulated glucose uptake in healthy individuals with normal glucose tolerance. *Journal of Clinical Endocrinology and Metabolism* 1987 **64** 1169–1173.

Received 9 May 2000

Accepted 31 July 2000