Insulin Secretion in Normal Glucose-Tolerant Relatives of Type 2 Diabetic Subjects

Assessments using hyperglycemic glucose clamps and oral glucose tolerance tests

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OBJECTIVE — To assess insulin secretion in normal glucose-tolerant Caucasian first-degree relatives of type 2 diabetes subjects and in matched normal glucose-tolerant control subjects and to compare insulin secretion as assessed using a hyperglycemic glucose clamp with insulin secretion as assessed using an oral glucose tolerance test (OGTT).

RESEARCH DESIGN AND METHODS — Twenty-one first-degree relatives of type 2 diabetic subjects and 21 control subjects without a family history of type 2 diabetes, who were matched for sex, age, BMI, waist-to-hip ratio, and aerobic capacity, underwent a hyperglycemic glucose clamp (10 mmol/l, 180 min). An OGTT (75 g glucose in 300 ml water) was also performed.

RESULTS — First-phase insulin release (plasma insulin, 0–10 min) was not different (multiple analysis of variance [MANOVA]: $F = 2.63$, $P = 0.11$). Second-phase insulin release was lower (MANOVA: $F = 4.18$, $P = 0.047$). Separate analyses of variance showed decreased plasma insulin levels from 120 min onward (all $P < 0.05$), decreasing to geometric mean (95% CI) levels of 330 (270–402) and 462 (366–582) pmol/l at 180 min in relatives and control subjects, respectively. The insulin sensitivity index (ISI) as assessed using a hyperglycemic clamp was not different between the two groups. Mean ± SE ISI during the 3rd hour was 27.5 ± 2.2 and 30.5 ± 3.0 $\mu g \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1} \cdot l^{-1}$ in relatives and control subjects, respectively ($P > 0.20$). At 90 min after the OGTT, log plasma insulin levels correlated significantly with second-phase insulin release as assessed using the hyperglycemic glucose clamp.

CONCLUSIONS — Normal glucose-tolerant first-degree relatives of type 2 diabetic subjects have a decreased second-phase insulin release, compared with matched control subjects. After an OGTT, 90-min values of log plasma insulin and 90-min values of the ratio of log plasma insulin to blood glucose may be good indicators of insulin secretory properties in normal glucose-tolerant family members of type 2 diabetic subjects.

Patients with clinically manifest type 2 (noninsulin-dependent) diabetes mellitus are characterized by both insulin resistance and an impairment in insulin secretion (1). The defects in insulin secretion include a markedly impaired and often absent first-phase insulin release, while second-phase insulin release is impaired, often by >50% (2). Since hyperglycemia itself can impair both insulin sensitivity and secretion, the so-called glucose toxicity (3), studies in manifest type 2 diabetic patients do not reveal if one of these conditions precedes the other.

Since genetic factors provide an important contribution in the pathogenesis of type 2 diabetes, studies of family members of type 2 diabetic patients have the potential to identify the primary defects responsible for type 2 diabetes. It has been estimated that 30–40% of the first-degree relatives of patients with type 2 diabetes will develop diabetes themselves (4,5). Insulin sensitivity and insulin secretion are both under genetic control (6,7).

An impaired tissue sensitivity to insulin has been described in first-degree relatives of patients with type 2 diabetes, and insulin resistance has been shown to be a major risk factor for the development of type 2 diabetes in Pima and Mexican Indians (8–10) and in others (11). Subtle defects in pancreatic β-cell function can also be detected before the development of overt diabetes (8,11,12). There is one caveat to these studies. Various factors, such as obesity (or, more specifically, the distribution of body fat in the central rather than the peripheral regions) and physical inactivity, influence insulin action and, thereby, insulin secretion (13). Aerobic capacity (Vo_{2max}) is a further main determinant of insulin action (14). In some of the above cited studies, these factors may have influenced the conclusions about whether an impaired insulin sensitivity or a decreased β-cell function is the primary defect in type 2 diabetes. To avoid potential bias in the study outcome, we studied insulin secretion during a hyperglycemic glucose clamp (at a moderately elevated glucose level of 10 mmol/l) in a group of strictly normal glucose-tolerant first-degree relatives (offspring) of type 2 diabetic subjects and in a group of control subjects who were matched individually for sex, age, BMI, waist-to-hip ratio (WHR), and aerobic capacity.

Although oral glucose tolerance tests (OGTT) are often used as an assessment of insulin release, there is scarce data regarding the relationship between the two assessments of insulin release. We therefore

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Abbreviations: GIR, glucose infusion rate; ISI, insulin sensitivity index; MANOVA, multiple analysis of variance; OGTT, oral glucose tolerance test; WHR, waist-to-hip ratio.

sought to study the relationships between insulin secretion in the first-degree relatives as assessed using an OGTT and as assessed using a hyperglycemic glucose clamp, since knowledge of the relationships of insulin secretion at each time point of the OGTT may provide a simpler method to study insulin secretion than the use of hyperglycemic glucose clamps.

RESEARCH DESIGN AND METHODS

Subjects

Twenty-one healthy first-degree offspring (17 women and 4 men) of type 2 diabetic subjects took part in this study. They all had a parent who had developed type 2 diabetes after 50 years of age. Maturity-onset diabetes of the young was excluded on the basis of the late onset of diabetes; they were a part of a group of 26 healthy first-degree relatives. The 21 subjects were matched to 21 subjects with no family history for diabetes who took part in the studies as control subjects. The control subjects were individually matched with the 21 first-degree relatives for each of the following variables: sex, age, BMI, WHR, and aerobic capacity (Vo_{2max}) (Table 1). The study had been approved by the local ethical committee, and after the nature of the study had been explained to each participant, informed written consent was obtained. All subjects had normal values for routine laboratory measurements for hematology, HbA_{1c} (upper limit of normal, 6.1%), lipids, cortisol, and kidney, liver, and thyroid function.

Aerobic capacity

Aerobic capacity was determined by a maximal multi-stage exercise test performed on a cycle ergometer (Lode, Groningen, The Netherlands). Subjects started cycling with a 30-W external resistance, which was increased every 3 min by 30 W until exhaustion. Ventilatory measurements were made with an Oxycon-beta (Mijnhardt, Bunnik, The Netherlands).

OGTT

Blood samples for glucose and insulin determinations were taken at specified time-points before and after the oral administration of 75 g glucose (in 300 ml water) and put on ice.

Hyperglycemic glucose clamp

Intravenous lines were placed in both forearms. One line was used for intravenous glucose infusion; the other was used for sampling of arterialized blood with the use of a heated box (55°C). A hyperglycemic glucose clamp was performed during 180 min, aiming at a glucose level of 10 mmol/l, starting with an intravenous bolus of 35 mg glucose/kg per mmol/l intended glucose increase. Blood samples for insulin determination were determined at all points specified in Fig. 2. The glucose infusion rate (GIR) was assessed during the clamp.

Table 1—*Characteristics of 21 first-degree relatives of type 2 diabetic patients and 21 healthy matched control subjects*

	Relatives	Control subjects
Sex (women/men)	17/4	17/4
Age (years)	45.4 ± 1.3	45.6 ± 1.4
BMI (kg/m^2)	26.4 ± 0.8	26.1 ± 0.8
Vo_{2max} (ml · kg^{-1} · min^{-1})	29.1 ± 1.4	29.4 ± 1.7
WHR	0.803 ± 0.015	0.816 ± 0.015
HbA_{1c} (%)	5.2 ± 0.1	5.2 ± 0.1
Total cholesterol (mmol/l)	4.8 ± 0.2	5.2 ± 0.2
HDL cholesterol (mmol/l)	1.4 ± 0.1	1.5 ± 0.1
Triglycerides (mmol/l)	1.1 ± 0.1	1.2 ± 0.1

Data are means ± SE.

Laboratory measurements

Blood glucose was determined immediately with a glucose analyzer (YSI, Yellow Springs, Ohio). Plasma insulin was determined by radioimmunoassay with ^{125}I-labeled insulin (IM 166, Amersham, U.K.).

Calculations

OGTT. Both (log-transformed) plasma insulin levels and the ratio between (log-transformed) plasma insulin level and blood glucose level (log insulin/glucose) were used to assess insulin secretion after the OGTT.

Hyperglycemic clamp. The inaccuracy of the clamp was assessed as a coefficient of variance (the standard deviation divided by the mean) of blood glucose levels.

Log-transformed plasma insulin levels from 0 to 10 min were used (in ANOVA for repeated measures; see below) for first-phase insulin release and log-transformed plasma insulin levels from 60 to 180 min for second-phase release. Analysis of the second phase during the last hour of the clamp gave identical results.

The GIRs of the 2nd and 3rd hours were assessed. The Insulin Sensitivity Index (ISI) was assessed as the GIR divided by the averaged plasma insulin levels of 60, 80, and 100 min (2nd hour) and of 120, 140, and 160 min (3rd hour). Plasma insulin levels at the end of each hour (i.e., 120 min and 180 min) were left out of this calculation since they do not influence insulin action in the preceding 20 min.

Statistical analysis

Data are presented as mean ± SE. Plasma insulin levels are presented as geometric means with 95% CI since they were not normally distributed (15). Matching was assessed by mean difference between the paired matches with 95% CI for age, BMI, aerobic capacity, and WHR (15). For the OGTTs and hyperglycemic clamps, MANOVA for repeated measures was used for insulin (after log-transformation) and glucose levels and for log insulin/glucose ratios; a separate ANOVA was performed if MANOVA showed statistically significant differences. Mean (95% CI) ratios of plasma insulin levels between the relatives and control subjects were calculated (15). GIR and ISI were assessed with ANOVA.

Linear correlations between (log-transformed) plasma insulin levels after the OGTT and plasma insulin levels at 4 min (first phase) and at 120 min (second phase) during the clamp were investigated. Correlations of log insulin/glucose (OGTT) and 4- and 120-min insulin levels during the hyperglycemic clamp were also investigated.

RESULTS

Matching

Table 1 gives the baseline characteristics of both relatives and control subjects. First-degree relatives and control subjects were all matched for sex. Mean (95% CI) differences between the two groups were −0.19 years (1.32 to −1.70) for age, 0.31 kg/m^2 (1.69 to −1.08) for BMI, −0.36 ml · kg^{-1} · min^{-1}

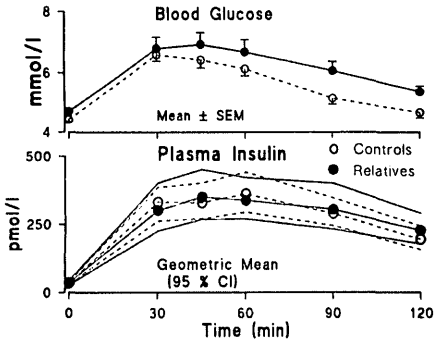

Figure 1—*Mean ± SE blood glucose levels and geometric mean (95% CI) plasma insulin levels after an OGTT in 21 first-degree relatives of type 2 diabetic subjects (●) and 21 matched control subjects (○).*

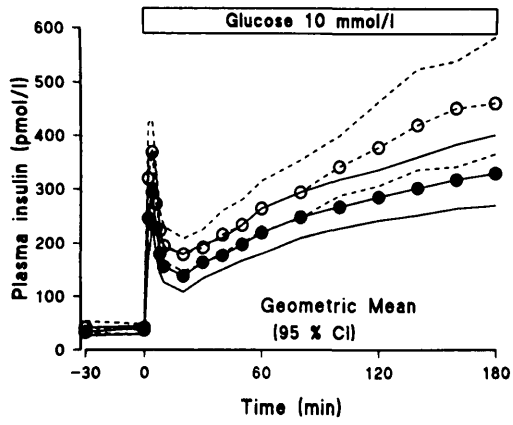

Figure 2—*Geometric mean (95% CI) plasma insulin levels during a 180-min hyperglycemic glucose clamp, aiming at glucose levels of 10 mmol/l in 21 first-degree relatives (●) and 21 matched control subjects (○).*

(2.00 to −2.71) for aerobic capacity, and −0.013 (0.013 to −0.040) for WHR.

OGTT

The mean blood glucose and geometric mean plasma insulin levels are depicted in Fig. 1. Basal blood glucose levels were slightly higher in relatives than in control subjects (4.7 ± 0.1 vs. 4.4 ± 0.1 mmol/l; P = 0.04). After the OGTT, blood glucose levels showed a trend toward higher levels in the relatives, according to ANOVA for repeated measures (P = 0.052). At 120 min, the mean blood glucose level was higher: 5.4 ± 0.2 vs. 4.7 ± 0.2 mmol/l (P = 0.013). No differences in plasma insulin were found either at baseline or after the OGTT (MANOVA, P = 0.66).

Since log insulin/glucose showed significant correlations with first-phase insulin release for the first 60 min, log insulin/glucose was assessed with separate MANOVAs for 0–60 min and for 90–120 min. MANOVA for 0–60 min showed no differences (F = 0.766, P = 0.39), while MANOVA for 90–120 min showed lower log insulin/glucose ratios in the relatives than in the control subjects (F = 5.29, P = 0.025). Separate analysis showed that log insulin/glucose at 90 min was lower in the relatives (0.424 ± 0.017 vs. 0.491 ± 0.019; P = 0.013).

Hyperglycemic glucose clamp

Blood glucose concentrations rose from mean basal levels of 4.7 ± 0.09 and 4.6 ± 0.08 to 9.99 ± 0.23 and 10.1 ± 0.15 mmol/l at 4 min in relatives and control subjects, respectively, and remained at mean averaged glucose levels of 10.00 ± 0.02 and 9.99 ± 0.04 mmol/l with CVs of 3.6 ± 0.4 and 5.0 ± 0.6%, respectively.

Insulin release

First-phase insulin secretion (0–10 min) was not different (F = 2.63, P = 0.11) (Fig. 2). Geometric plasma insulin levels increased to 294 (234–372) and 366 (318–432) pmol/l at 4 min in relatives and control subjects, respectively, and declined thereafter. The mean (95% CI) ratio of plasma insulin levels at 4 min was 0.77 (0.49–1.21). Second-phase insulin release (2nd and 3rd hours) was lower in the relatives than in control subjects (MANOVA: F = 4.18, P = 0.047). Geometric mean plasma insulin levels increased to 330 (270–402) and 462 (366–582) pmol/l at 180 min in relatives and control subjects, respectively. A separate ANOVA showed significantly lower plasma insulin levels from 120 min onwards (all P < 0.05), with mean (95% CI) ratios of plasma insulin levels decreasing from 0.79 (0.62–0.998) at 120 min, to 0.76 (0.59–0.94) at 140 min, 0.72 (0.55–0.95) at 160 min, and 0.74 (0.55–0.98) at 180 min.

GIR

The GIRs were lower in relatives than in control subjects (5.6 ± 0.5 vs. 8.1 ± 0.7 ml · kg^{-1} · min^{-1} [P = 0.01] and 8.2 ± 0.8 vs. 11.9 ± 0.8 ml · kg^{-1} · min^{-1} [P = 0.004] for the 2nd and 3rd hours, respectively). However, when the GIR was divided by the averaged plasma insulin level to obtain the ISI, no differences were observed (both P > 0.20; Fig. 3).

Relationships between insulin release after the OGTT and during the hyperglycemic clamp

In the relatives, basal plasma insulin (OGTT) correlated with second-phase release (log plasma insulin levels at 120 min: r = 0.497, P = 0.021), while this relationship failed to achieve statistical significance in the control group (P = 0.095). After the OGTT, log postload plasma insulin at 60, 90, and 120 min correlated with log plasma insulin of second-phase release (at 120 min during the clamp) in the relatives (r = 0.468, P = 0.031; r = 0.466, P = 0.032; and r = 0.460, P = 0.034). For the control subjects, such a relationship was only found for 90-min postload plasma insulin with the second phase (r = 0.503, P = 0.019). When the ratio of log plasma insulin and blood glucose level (log insulin/glucose) was taken as an index of insulin release, postload 30-, 45-, and 60-min log insulin/glucose correlated strongly with first-phase insulin release (4 min) in the relatives (r = 0.555, P = 0.0089; r = 0.66, P = 0.0013; and r = 0.542, P = 0.011, respectively). Similarly, in the control subjects, 30 and 45 min postload log insulin/glucose correlated with first-phase (4 min) plasma insulin levels

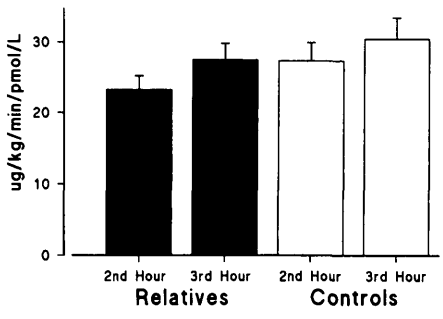

Figure 3—*Mean ± SE ISI, assessed as GIR/I, for the 2nd and 3rd hours of the hyperglycemic glucose clamps (10 mmol/l) in 21 first-degree relatives and 21 matched control subjects. ISI was calculated as the GIR divided by the mean plasma insulin level to give GIR/I. Black bars represent first-degree relatives; white bars represent control subjects.*

(r = 0.437, P = 0.045, and r = 0.453, P = 0.037).

CONCLUSIONS — The present studies indicate that first-degree family members (offspring) of type 2 diabetes subjects have lower insulin secretion than control subjects strictly matched for various parameters known to influence glucose homeostasis. This finding points to the impairment of insulin secretion as a major genetic factor involved in the development of type 2 diabetes. The relatives had a glucose tolerance well within normal limits, although baseline and 120-min blood glucose levels were slightly higher than in the control subjects. After the OGTT, plasma insulin levels in relatives were indistinguishable from those in the control subjects. In the early studies of Eriksson et al. (16), plasma insulin levels after an OGTT were slightly (but statistically not significantly) elevated in glucose-tolerant relatives; however, the relatives were more obese than control subjects.

In the present study, hyperglycemic glucose clamps were used for the assessment of insulin secretion. Although a difference in first-phase insulin release did not attain statistical significance (P = 0.11), this may be due to a lack of statistical power due to the large variation in first-phase secretion. However, the second-phase insulin secretion was clearly lower in the relatives than in the control subjects.

So far, only two relatively large studies have reported insulin secretion assessed with hyperglycemic glucose clamps in normal glucose-tolerant relatives. Gulli et al. (10) performed euglycemic and hyperglycemic clamps in 11 nondiabetic nonobese Mexican-American subjects of whom both parents had type 2 diabetes. They found a decreased insulin sensitivity and an increased insulin release. The differences with the present study may be due to differences in ethnic background. It has still to be shown that findings in specific ethnic groups, such as the Pima Indians in whom insulin sensitivity is genetically determined within families (17), can be extrapolated to Caucasians.

Pimenta et al. (18) used hyperglycemic glucose clamps in 50 Caucasian relatives (both obese and nonobese) of type 2 diabetic subjects. Both first- and second-phase insulin secretion were decreased in comparison with 50 control subjects who were matched for sex, BMI, and WHR. Moreover, euglycemic clamps in subsets of the subjects did not show differences in insulin sensitivity.

Since aerobic capacity has a major influence on insulin action (14), the subjects in the present study were also matched for aerobic capacity. The present findings show that even normal glucose-tolerant first-degree relatives of Caucasian type 2 diabetic subjects have diminished insulin release rather than an increased insulin release.

During the clamp, lower glucose infusion rates were necessary to obtain the desired blood glucose level of 10 mmol/l in the relatives than in the control subjects. Glucose infusion rates were corrected for averaged plasma insulin levels to obtain a more precise assessment of insulin sensitivity. In previous studies in which subjects had undergone both hyperglycemic clamps (at 10 mmol/l during 180 min) and euglycemic-hyperinsulinemic clamps, insulin sensitivity as assessed using the hyperglycemic clamp correlated nicely with insulin action as assessed using the euglycemic-hyperinsulinemic clamp. The slope of the correlation between the two assessments of insulin sensitivity was 1.03, and the Y-intercept was 0 (19), which validates the use of hyperglycemic glucose clamps for the assessment of the ISI. It is noteworthy that in the present studies, insulin action assessed in this manner (using the hyperglycemic clamp) was not markedly decreased in the relatives. Even if one would assume that some of our subjects were insulin resistant (those with elevated BMI), our studies indicate that they do not hypersecrete insulin but have a diminished insulin secretion, in sharp contrast to the findings of Gulli et al. (10) in (nonobese) Mexican-Americans.

Plasma insulin levels obtained after an OGTT are often used for the evaluation of insulin release (20,21). We assessed the relationship of postload plasma insulin levels with first- and second-phase insulin release (hyperglycemic glucose clamp). First-phase insulin release only correlates with the ratio of (log) plasma insulin and blood glucose level at 30 and 45 min in both groups and also with 60-min postload ratio of (log) plasma insulin and blood glucose level in the relatives. Although one could argue that some statistical correction for multiple testing should be performed, it is clear that the combined findings of these correlations of early insulin release after an OGTT with first-phase secretion point to a physiologically plausible relationship; one would actually be amazed if they were not correlated. Moreover, even the application of the most severe statistical correction (i.e., the Bonferroni method [22], multiplying the P value of the strongest correlation (P = 0.0013) with the total number of correlations performed [25]) would still yield a statistically significant figure ($P = 25 \times 0.0013 = 0.0325$, 45-min log insulin/glucose with first-phase secretion). As expected, it appears that 90-min postload insulin levels correlate with second-phase (120 min) insulin release. Interestingly, the ratio of (log) plasma insulin and blood glucose level (log insulin/glucose) at 90 min was lower in the relatives. Since the postload plasma insulin levels at 90 min were not different between the groups, the finding of the lower ratio of postload log plasma insulin and blood glucose level points already to a decreased insulin release (and not to insulin resistance, which would have resulted in increased plasma insulin levels). Plasma insulin levels at 90 min after an OGTT (and the ratio of log-insulin and glucose) may be useful in evaluating insulin secretion in relatives of type 2 diabetic subjects, although the correlation coefficient of r = 0.466 indicates that only 22% of the variability of the plasma insulin levels after the OGTT can be explained on the basis of variation in β-cell function. Since many of these subjects will sooner or later develop type 2 diabetes themselves, such evaluation may be of importance for eventual preventive management.

In conclusion, we have shown that normal glucose-tolerant Caucasian subjects with a family history of type 2 diabetes have lower insulin secretion than control subjects, while their insulin sensitivity is not disturbed. It therefore now appears that a disturbance in insulin release is a primary event in the development of type 2 diabetes in those individuals who are genetically at risk for type 2 diabetes. This study also suggests that log plasma insulin levels and the ratio of plasma insulin and blood glucose at 90 min after an OGTT may be of use in assessing insulin secretion in relatives of type 2 diabetic subjects.

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