Assessment of Insulin Secretion in Relatives of Patients With Type 2 (non–insulin-dependent) Diabetes Mellitus: Evidence of Early β-Cell Dysfunction

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To examine β-cell function in glucose-tolerant offspring of type 2 diabetic families, 41 insulin-resistant (hyperinsulinemic-euglycemic clamp, $P < .001$) first-degree relatives and 32 controls underwent oral (OGTT) and intravenous (IVGTT) glucose tolerance tests and a constant intravenous glucose infusion (4.0 or 4.5 mg/kg/min) with blood sampling every minute for insulin determinations. Insulin concentration time-series were analyzed with complementary mathematical models (deconvolution and autocorrelation analysis, approximate entropy [ApEn], and coefficient of variation [CV] for a 6-point moving average, together with a combined index for regularity and stationarity [RaS] based on the last 2 measures). During the OGTT, the area under the curve (AUC) for plasma glucose was moderately (11%) but significantly ($P < .01$) elevated in the relatives despite a trend for increased serum insulin (AUC, $P = .14$). The acute-phase serum insulin response (IVGTT) did not differ between groups (2,055 ± 330 *v* 1,766 ± 229 pmol/L · 10 min, $P = .84$) but was inappropriately low (individually, $P < .05$ *v* control group) for the degree of insulin resistance in 16 relatives. Deconvolution analysis of the insulin time-series did not uncover differences in either the intersecretory pulse interval (5.8 ± 0.2 *v* 5.7 ± 0.2 min/pulse) or the fractional secretory burst amplitude (133% ± 10% *v* 116% ± 7% over basal) between the 2 groups. Similarly, significant autocorrelation coefficients were observed in a comparable number of relatives and control subjects ($P = .74$). In contrast, the RaS index was significantly higher (ie, insulin time-series was more irregular and nonstationary) in the relatives (0.221 ± 0.194) than in the controls (−0.318 ± 0.176, $P < .05$), primarily attributed to the pattern of insulin secretion in relatives with a strong genetic burden. In conclusion, nonstationary and disorderly insulin secretion patterns during glucose stimulation and a low acute-phase serum insulin response associated with significant insulin resistance suggest early β-cell regulatory dysfunction in individuals genetically predisposed to type 2 diabetes mellitus prior to any evident alterations in insulin secretory burst frequency or mass.

TYPE 2 (non–insulin-dependent) diabetes mellitus is characterized by defects in both insulin action and insulin secretion.[1,2] The latter has been established primarily through an absent or blunted acute-phase serum insulin response following an intravenous glucose challenge.[2] In addition, more complex abnormalities in insulin secretion have been detected, such as irregular and impaired pulsatile insulin delivery.[3,4] In individuals with overt type 2 diabetes mellitus, one obviously cannot clarify whether these abnormalities are of primary pathophysiological origin or secondary to hyperglycemia.[5] To circumvent this impasse, investigators have examined insulin action and secretion in healthy individuals selected for a considerably increased risk to develop type 2 diabetes mellitus, such as first-degree relatives of type 2 diabetic patients, in whom plasma glucose concentrations are normal.

Insulin resistance is a common finding in healthy first-degree relatives of type 2 diabetic patients.[6-13] In contrast, many studies investigating first-degree relatives of type 2 diabetics in whom glucose tolerance is still normal have failed to demonstrate consistent abnormalities in insulin secretion. The methods used to evaluate insulin secretion have mainly included oral (OGTT) and intravenous (IVGTT) glucose tolerance tests or the hyperglycemic clamp technique, which are less refined compared with methods used to evaluate insulin sensitivity.[14] It is possible that early and subtle abnormalities in β-cell function may emerge by assessment of more complex regulatory mechanisms requiring glucose sensing and signaling pathways of the β cell,[15,16] eg, as indicated by the occurrence of irregular rapid insulin oscillations assessed by autocorrelation analysis of insulin concentration time-series[17] and/or the lack of ultradian insulin oscillations[18] in individuals with impaired glucose tolerance. Applying the scale- and model-independent statistic, approximate entropy (ApEn), and the coefficient of variation (CV) for a 6-point moving average (MA) to insulin time-series obtained during constant intravenous glucose infusion in a small number of individuals, we recently found evidence of disorderly and nonstationary insulin secretion in normal glucose-tolerant relatives of type 2 diabetic patients.[19]

The present study was performed to further examine in vivo β-cell secretory function in individuals genetically predisposed to type 2 diabetes mellitus, by comparing patterns of insulin secretion quantitatively in normal glucose-tolerant relatives of type 2 diabetic patients and an age-, gender-, and body mass index (BMI)-matched control group and assessing the discriminative abilities of several analytical strategies to quantify in vivo β-cell secretory differences in these 2 populations. Moreover, we sought to evaluate both insulin action and secretion in the same individuals. For this purpose, (1) we performed an OGTT and an IVGTT; (2) we applied different mathematical approaches to time-series analysis: ApEn, CV for a 6-point MA, a combined insulin secretion index based on these 2 measures (index of regularity and stationarity [RaS]), as well as deconvolution and autocorrelation analysis of glucose-stimulated insu-

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lin concentration time-series; and finally, (3) we estimated insulin sensitivity using the hyperinsulinemic-euglycemic clamp technique.

SUBJECTS AND METHODS

Subjects

Forty-one first-degree relatives of type 2 diabetic patients and 32 control subjects without a family history of diabetes participated in the study. The relatives and control subjects were matched in groups (but not in pairs) with respect to age, gender, and the BMI. Thirteen relatives had 1 known family member (first-degree) with type 2 diabetes mellitus (1 parent), 21 had 1 first-degree relative and 1 or more second-degree relatives with the disease, and 7 had 2 first-degree relatives (both parents) with type 2 diabetes mellitus. All subjects were healthy and on no medications, and all were of caucasian origin. The relatives were recruited via their type 2 diabetic parents attending the outpatient clinic in Medical Department M, Aarhus University Hospital. When more than 1 offspring was available in a family, 1 was randomly selected to participate. The control group was recruited as healthy subjects with no family history of diabetes mellitus. None of the subjects in the control group were related. Exclusion criteria were a BMI more than 30 kg/m^2 and age more than 54 years. These exclusion criteria were used to minimize a possible confounding effect of heterogeneity in the study populations and to exclude subjects with overt obesity, the latter of which may have the confounding effect of obesity-induced insulin resistance. The pertinent clinical data for the 2 groups are summarized in Table 1. All subjects were instructed to consume a weight-maintaining diet containing at least 250 g carbohydrate for 3 days prior to all examinations, and none of the subjects engaged in heavy physical exercise during the same period. All females were examined in the follicular phase of the menstrual cycle (1 in each group was postmenopausal). The protocol was approved by the Ethics Committee of the County of Aarhus, and all subjects provided written informed consent.

Study Design

Subjects were examined on 4 separate occasions to determine the patterns of insulin secretion and insulin sensitivity. The OGTT (1) was always performed as the first test, whereas the IVGTT (2), constant intravenous glucose infusion (3), and hyperinsulinemic-euglycemic clamp (4) were performed in random order. All subjects underwent tests 1, 3, and 4. Thirty-two relatives and 22 control subjects also were examined using an IVGTT (2). These latter individuals (relatives *v* control subjects) were also closely matched in groups with respect to age (36.8 ± 1.5 *v* 36.0 ± 1.8 years), gender (ratio of males to females, 18/14 *v* 13/9), and BMI (25.9 ± 0.5 *v* 25.8 ± 0.5 kg/m^2). The interval between each examination was approximately 4 weeks. All examinations were performed following a 10- to 12-hour overnight fast.

Test 1: OGTT. Before the OGTT (ie, in the fasting state), subjects underwent a physical examination including a determination of the BMI, waist to hip ratio (WHR), and lean body weight (LBW) using bioelectric impedance analysis (Animeter; HTS-Engineering, Odense, Denmark). At 8:00 AM, an OGTT (75 g glucose) was performed. Blood samples for immediate determination of plasma glucose and later measurement of serum insulin and C-peptide were obtained at 0, 30, 60, 90, and 120 minutes. Blood for determination of hemoglobin A_{1C} (HbA_{1c}) was collected at time 0.

Test 2: IVGTT. One catheter was inserted into a deep antecubital vein of one arm (for glucose infusion), and another catheter was placed in the contralateral arm in a heated dorsal hand vein for sampling arterialized blood. At 8:00 AM, a bolus dose of 50% glucose (0.5 g/kg with a maximum of 25 g) was injected over a period of 2 minutes. Blood was collected at −15, −5, 0, 2, 4, 6, 8, 10, 12, 19, 22, 24, 25, 30, 35, 40, 50, 70, 90, and 180 minutes for determination of plasma glucose and serum insulin and C-peptide.

Test 3: constant intravenous glucose infusion. The total duration of the constant glucose infusion test was 135 minutes, with a stabilization period of 60 minutes (time 0 to 60 minutes) followed by a sampling period of 75 minutes (time 60 to 135 minutes). At 8:00 AM (time 0), 1 catheter was inserted in an antecubital vein and infusion of glucose 5% was initiated and continued throughout the study at a constant rate (4.0 and 4.5 mg/kg/min in relatives and control subjects, respectively). This difference in the glucose infusion rate was chosen through pilot experiments to match plasma glucose in the 2 groups to compensate for the assumed insulin resistance in the relatives. In the contralateral arm, another cannula (Venflon 17G/45 mm; BOC Ohmeda, Helsingborg, Sweden) was placed in an antecubital vein for blood sampling. The sampling procedure has been described in detail elsewhere.[19] Blood for measurement of serum insulin was collected every minute during the 75-minute sampling period, while blood samples at times 0, 75, 85, 110, and 135 minutes also were analyzed for serum C-peptide. Furthermore, plasma glucose was monitored every 10 minutes throughout the study.

Test 4: hyperinsulinemic-euglycemic clamp. One catheter was inserted into a deep antecubital vein of one arm for all infusions, and another catheter was placed in a heated dorsal hand vein of the same arm for sampling arterialized blood. The subjects underwent a hyperinsulinemic (insulin infusion 0.6 mU/kg/min)-euglycemic (plasma glucose ~5 mmol/L) clamp for 150 minutes (time 0 to 150 minutes) starting at 10:30 AM (time 0). The serum insulin level was measured at times 0, 60, 90, 120, 135, and 150 minutes, and plasma glucose was determined every 5 to 10 minutes during the clamp. The mean glucose infusion rate between 120 and 150 minutes was defined as steady-state insulin-stimulated glucose uptake (ISGU).

Table 1. Clinical Attributes of the Study Groups

Variable	Relatives (n = 41)		Controls (n = 32)	
	Mean	Range	Mean	Range
Sex ratio (male/female)	22/19		18/14	
Age (yr)	35.8	21-53	35.1	21-53
BMI (kg/m^2)	25.4	16.8-30.0	24.9	18.7-29.5
WHR	0.90	0.77-1.04	0.88	0.73-1.06
LBW (% of body weight)	75.9	60-87	77.1	61-92
HbA_{1c} (%)	5.3	4.3-6.5	4.9	4.4-5.5*
ISGU (mg/kg LBW/min)	5.87	3.19-11.35	7.68	3.92-11.61†

*$P < .01$ and †$P < .001$. For all other comparisons, $P > .20$.

Assays

Plasma glucose was determined in duplicate immediately after sampling (Beckman Instruments, Palo Alto, CA). HbA_{1c} was determined by high-performance liquid chromatography (reference range with 95% confidence limits, 4.4% to 6.4%). Circulating insulin concentrations were measured by enzyme-linked immunosorbent assay using a 2-site immunoassay (Dako Diagnostics, Cambridgeshire, UK) that does not detect proinsulin and split(32-33)- and des(31-32)-proinsulin, whereas split(65-66)- and des(64-65)-proinsulin cross-react 30% and 63%, respectively.[20] During the OGTT and IVGTT, serum insulin was measured in duplicate, whereas serum insulin measurements during the constant glucose infusion test were performed in triplicate. The intraassay CV was 2% (n = 75) at a serum level of 200 pmol/L, and the interassay CV on measurements in triplicate was 3% to 4%. Serum C-peptide was determined using a commercial kit (Dako Diagnostics).

Analytical Strategy

The ability to detect and quantify pathophysiological insulin release in a group of glucose-tolerant first-degree relatives of patients with type 2 diabetes mellitus (*v* a matched control group) was assessed by several

complementary analytical methods addressing different aspects of the insulin release process.

The overall insulin secretory response to oral glucose was assessed by a standard OGTT. The IVGTT, measuring the immediate insulin secretory response to an abrupt increase in circulating glucose (from ~5 to 25 mmol/L), was used to evaluate the acute-phase serum insulin response (incremental area under the curve [AUC] from 0 to 10 minutes). This test likely reflects the maximal islet secretory capacity from immediately releasable insulin pools.

Insulin secretion is known to occur as a series of punctuated secretory bursts, and the functional state of this pulsatile mode of insulin release was assessed through estimates of the frequency, mass, amplitude, and relative contribution of the secretory bursts versus basal release, as detected and quantified by a validated deconvolution technique as previously described.[16,21] Briefly, this method assumes that insulin release consists of serial discrete insulin secretory bursts, characterized by a common half-duration and distinct time locations with individual quantifiable secretory burst amplitude and a time-invariable basal insulin release rate. The insulin secretory rate is estimated by deconvolving the insulin concentration time-series, using previously estimated biexponential insulin decay kinetics (2.8 and 5.0 minutes, with the slow fractional component representing 0.28 of the total decay amplitude[16]).

Autocorrelation analysis was applied to the insulin time-series of all individuals in the study. Triplicate serum insulin measurements were smoothed by a 3-point MA to reduce noise. To compensate for a long-term trend due to stimulation, data were made stationary by sequential first-differencing. The stationary data were analyzed by autocorrelation analysis in which data from the observed time-series are correlated pointwise to an exact copy moved to the right by 1-minute intervals. An autocorrelation coefficient was considered significant if the first positive peak after the first trough exceeded the 95% confidence interval or if the autocorrelation had a graph sinusoidal shape and one of the subsequent peaks exceeded the confidence limit.

The regularity of the process of insulin release from the β-cell population into the systemic circulation was quantified for serial orderliness by the application of ApEn to detrended (first-differenced) insulin concentration time-series.[22,23] Briefly, this method measures the logarithmic likelihood that the runs of patterns that are similar remains similar on the next incremental comparisons. The method has been previously described in detail,[19,22,23] and can separate more disorderly or irregular (patho)physiological hormonal release processes in disease states from more orderly release patterns in healthy humans, as well as distinguish secretory profiles between the sexes.[23-25] In the present study, as in virtually all other applications of ApEn to endocrinologic data, values for r and m were 0.2 SD and 1, respectively, where r denotes the threshold or tolerance level for pattern recurrence and m defines the length of the run. The stationarity of the insulin time-series was measured by the CV for a 6-point MA. This method and the choice of 6 points for the MA, have been previously described in detail.[19] The latter 2 measures were combined into a joint index for regularity and stationarity (RaS), where the individual values for the ApEn and CV for a 6-point MA are centralized, normalized, and averaged, producing an index with equal weighting of these 2 analytical inputs. The procedure has been described in detail elsewhere.[19]

Statistical Analyses

Data in the text and figures are presented as the mean ± SEM unless otherwise indicated. Student's 2-tailed t test for unpaired data was used to compare data between the relatives and control subjects. When the data were not normally distributed (as for circulating insulin concentrations and the derived insulin data), the Mann-Whitney rank-sum test for unpaired data was used. When comparing more than 2 groups, we used 1-way ANOVA or Kruskal-Wallis 1-way ANOVA on ranks when appropriate. The AUC was calculated as the total AUC during the OGTT and the sampling period of the glucose infusion test (time 60 to 135 minutes) using the trapezoidal rule. The acute-phase serum insulin response was calculated as the AUC above baseline from time 0 to 10 minutes during the IVGTT by subtracting baseline values from the corresponding total AUC. The dynamics of the OGTT and glucose infusion were analyzed with a 2-way ANOVA for repeated measures. Relationships between measures were tested by correlation analyses (Pearson's product-moment correlation or Spearman rank-order correlation, respectively) and/or linearlized (hyperbolic) regression analyses. All relationships involving insulin sensitivity were computed using results from the hyperinsulinemic-euglycemic clamp (ISGU). Chi-square analysis was used to compare subjects with and without significant autocorrelation coefficients and to calculate the probability of particular outcomes on the basis of chance.

RESULTS

Insulin Sensitivity

ISGU during the hyperinsulinemic clamp was diminished by approximately 25% in the relatives compared with the control subjects (5.87 ± 0.33 *v* 7.68 ± 0.33 mg/kg LBW/min, $P < .001$; Table 1).

OGTT

All subjects had a normal OGTT using criteria recently defined by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.[26] Absolute fasting plasma glucose, the 120-minute value, and the AUC for plasma glucose were significantly higher in the relatives compared with the control subjects (Table 2). Similar results were obtained when analyzing circulating C-peptide levels, while differences in serum insulin reached marginal statistical significance between the 2 groups. The dynamic changes in plasma glucose, serum insulin, and C-peptide over time as calculated by 2-way ANOVA for repeated measures (over 120-minute blocks) were comparable in relatives and control subjects (Fig 1).

IVGTT

Acute-phase serum insulin responses (2,055 ± 330 *v* 1,766 ± 229 pmol/L · 10 min, $P = .84$) did not differ between the relatives and the control subjects (Fig 2). As expected, the acute-phase serum insulin response correlated inversely with ISGU in both relatives ($r = -.43$, $P < .05$) and control subjects ($r = -.46$, $P < .05$). However, this relationship was not linear, but could be best described by a hyperbolic function in both groups (Fig 3). The respective equations were as follows:

Table 2. OGTT Results in the Study Groups

Parameter	Relatives	Controls	*P*
Plasma glucose			
Fasting (mmol/L)	5.3 ± 0.1	5.0 ± 0.1	<.01
120 min (mmol/L)	5.5 ± 0.2	4.8 ± 0.2	<.01
AUC (mmol/L · 120 min)	822 ± 19	734 ± 23	<.01
Serum insulin			
Fasting (pmol/L)	42 ± 5	35 ± 5	.13
120 min (pmol/L)	179 ± 22	119 ± 14	.09
AUC (pmol/L · 120 min)	32,500 ± 2,600	26,600 ± 1,900	.14
Serum C-peptide			
Fasting (nmol/L)	0.52 ± 0.03	0.42 ± 0.02	<.01
120 min (nmol/L)	1.53 ± 0.10	1.19 ± 0.10	<.05
AUC (nmol/L · 120 min)	199 ± 12	166 ± 10	<.05

NOTE. Data are the mean ± SEM.

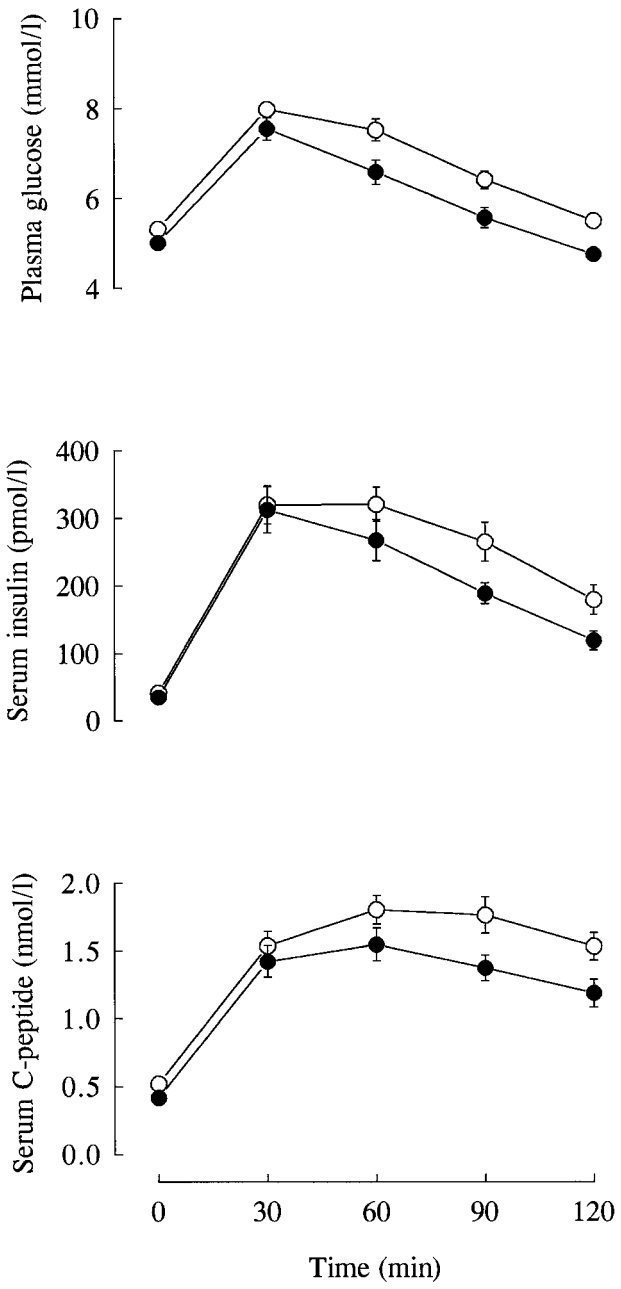

Fig 1. Plasma glucose and serum insulin and C-peptide during a standard OGTT in first-degree relatives of patients with type 2 diabetes mellitus (○) and control subjects (●) matched by age, gender, and BMI.

acute-phase serum insulin response = (−2,739 + 37,258 [1/ISGU]) in control subjects and (−880 + 18,199 [1/ISGU]) in relatives. A comparison of regression lines from the inverse-transformed ISGU data versus the acute-phase serum insulin responses showed nearly significant differences ($P = .08$) between the 2 groups. The hyperbolic function between the acute-phase serum insulin response and ISGU in the control subjects suggested that this difference was mainly ascribable to a subgroup of 16 relatives with low ISGU and acute-phase serum insulin values, below the lower 95% confidence limit (of the mean) of the control regression line (Fig 3). However, these 16 relatives did not differ from the rest of the relative group with respect to the AUC for plasma glucose during the OGTT (822 ± 35 *v* 823 ± 23 mmol/L · 120 min), but both groups had significantly higher plasma glucose compared with the control subjects both ($P < .05$). Circulating insulin during the OGTT was comparable in the 2 relative subgroups (36,031 ± 4,731 *v* 30,303 ± 3,059 pmol/L · 120 min, $P = .20$, relatives with low and adequate acute-phase serum insulin response to IVGTT, respectively), but increased in the 16 relatives with a low acute-phase serum insulin response for the degree of insulin resistance compared with the control subjects ($P < .05$).

Constant Intravenous Glucose Infusion

The mean plasma glucose concentrations did not differ between relatives and control subjects during the stabilization period (time 0 to 60 minutes, 6.8 ± 0.10 *v* 6.7 ± 0.14 mmol/L, $P = .60$) or the test period (time 60 to 135 minutes, 8.1 ± 0.2 *v* 7.8 ± 0.2 mmol/L, $P = .39$). However, the total AUC for circulating insulin (11,362 ± 1,122 *v* 9,060 ± 894 pmol/L · 75 min, $P = .17$) and C-peptide (104.7 ± 9.1 *v* 86.1 ± 7.8 nmol/L · 75 min, $P = .13$) tended to be higher in the relatives. Furthermore, while plasma glucose remained nearly constant during the sampling period in both groups, serum insulin and C-peptide dynamics differed between the relatives and control subjects (Fig 4). In the relatives, sluggish and continued increases in both circulating insulin and C-peptide were observed, in contrast to the almost constant levels present in the control subjects ($P < .01$ by ANOVA).

Measurements of Insulin Release Dynamics

Deconvolution analysis and autocorrelation analysis. The detected mean insulin intersecretory pulse interval (5.8 ± 0.2 *v* 5.7 ± 0.2 min/pulse, $P = .73$) was almost identical in the relatives and control subjects. In contrast, the mean basal insulin secretion (23.3 ± 2.5 *v* 19.1 ± 1.9, pmol/L/min, $P = .19$), mean pulse amplitude (29.3 ± 3.4 *v* 21.1 ± 2.1 pmol/L/min, $P = .13$), and mean insulin burst mass (70.2 ± 7.6 *v* 51.3 ± 4.9 pmol/L/min, $P = .13$) tended to be higher in the relatives. This reflected overall augmented insulin secretion, since the relative pulse amplitude (calculated as pulse amplitude/basal secretion expressed as a percentage) did not differ between the relatives and control subjects (133% ± 10% *v* 116% ± 7%, $P = .31$) (Fig 5).

Finally, the CVs for pulse frequency (34% ± 2% *v* 34% ± 1%, $P = .89$), pulse amplitude (33% ± 2% *v* 32% ± 2%, $P = .71$), and pulse burst mass (33% ± 2% *v* 32% ± 2%, $P = .69$) were comparable in the 2 groups, indicating similar dispersion of individual values for these measures.

There were no correlations between the ISGU and pulse frequency, CV for pulse frequency, CV for pulse amplitude, or relative amplitude in either relatives or control subjects (all $P > .30$). A highly significant correlation was found between the pulse burst mass and AUC for circulating insulin during constant glucose infusion in both groups (relatives, $r = .92$, $P < .005$; control subjects, $r = .86$, $P < .005$), reflecting the fact that secretory bursts are the main source of the circulating insulin concentration, consistent with our prior reports.[16,27] Figure 5 presents the peripheral plasma insulin concentration

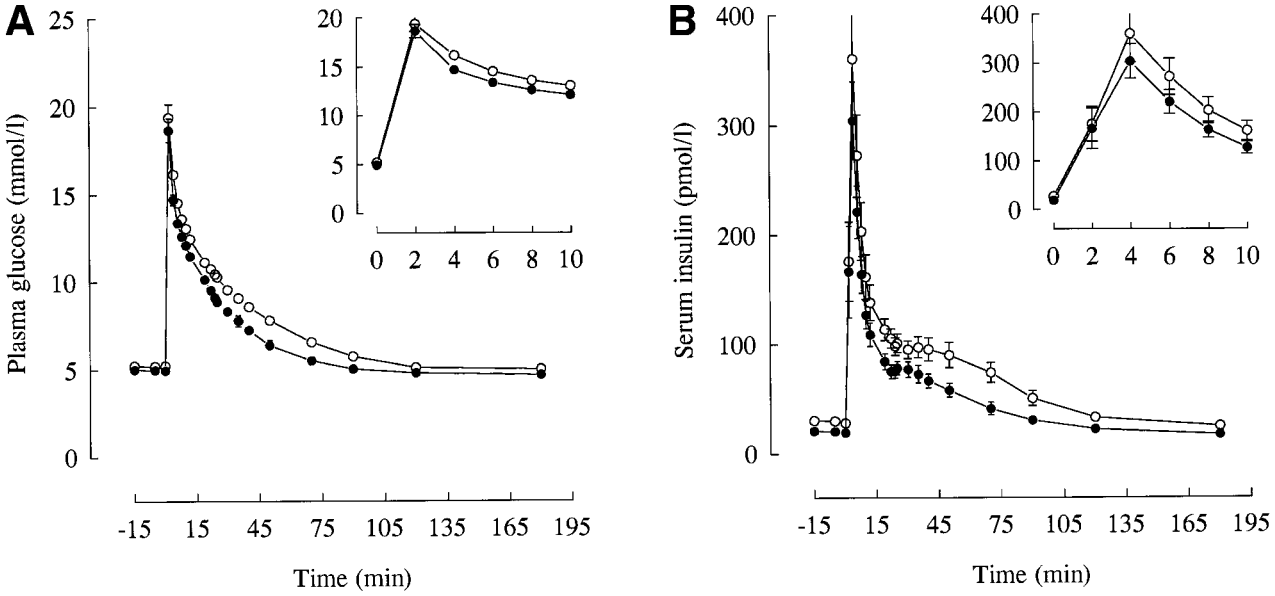

Fig 2. (A) Plasma glucose and (B) serum insulin during an IVGTT (0.5 g glucose/kg, with a maximum dose of 25 g) in first-degree relatives of patients with type 2 diabetes mellitus (○) and control subjects (●) matched by age, gender, and BMI. Insets, acute-phase responses (time 0-10 minutes).

and the corresponding calculated insulin secretion rate by deconvolution analysis in an offspring of a type 2 diabetic patient and in a control subject.

Only 9 of 41 and 9 of 32 relatives and control subjects, respectively, exhibited significant autocorrelation coefficients ($P = .74$, relatives *v* control subjects).

ApEn, CV for a 6-point MA, and RaS. Although neither ApEn (1.491 ± 0.014 *v* 1.466 ± 0.016, $P = .26$) nor nonstationarity of insulin secretion as assessed by the CV for a 6-point MA (0.157 ± 0.014 *v* 0.129 ± 0.009, $P = .37$) significantly discriminated between the relatives and control subjects, the joint index, RaS, provided an effective contrast for statistical differences between these groups. The RaS index was significantly higher in the relatives compared with the control subjects (0.221 ± 0.194 *v* 0.318 ± 0.176, $P < .05$), indicating a higher degree of continued disorderly and nonstationary insulin secretion in the relatives. Figure 6 shows that 10 of the 12 highest values for the RaS index (≥ + 1) were observed in the relatives. Conversely, 8 of the 12 lowest values (≤ − 1) were found in control subjects. The probability for this particular outcome by chance is less than .05. There was no correlation between the ApEn, CV for a 6-point MA, or RaS index and ISGU, HbA_{1c}, acute-phase serum insulin response (IVGTT), and fasting, 120-minute, and AUC values during the OGTT for serum insulin and plasma glucose in either of the 2 groups. With respect to the 16 relatives with low-acute phase insulin secretion (IVGTT) relative to the degree of insulin resistance (described earlier), we did not observe any differences from the remainder of the relatives with respect to the more subtle indices of (regular) insulin secretion (intersecretory pulse intervals, 5.8 ± 0.37 *v* 5.8 ± 0.28 min/pulse; pulse burst mass, 69.7 ± 9.6 *v* 70.4 ± 10.9 pmol/L/min; relative pulse amplitude, 120% ± 9% *v* 140% ± 15%; ApEn, 1.49 ± 0.02 *v* 1.49 ± 0.02; 6-point MA, 0.14 ± 0.01 *v* 0.16 ± 0.02; RaS index, 0.06 ± 0.25 *v* 0.33 ± 0.28; all $P > .30$, relatives with low and adequate acute-phase serum insulin response to IVGTT, respectively).

Insulin Action and Secretion Versus Family History of Type 2 Diabetes Mellitus

To examine a possible genetic dose-effect on the pattern of insulin secretion, the relative were divided into 3 groups by their family history of type 2 diabetes mellitus. These 3 groups were as follows: FDR1 (n = 13), subjects with 1 known family member with type 2 diabetes mellitus (1 parent); FDR2 (n = 21), subjects with 1 parent and 1 or more second-degree relatives with the disease; and FDR3 (n = 7), subjects with 2 first-degree relatives (both parents) with type 2 diabetes mellitus. All groups were insulin-resistant compared with the control subjects (ISGU: FDR1, FDR2, and FDR3, 5.76 ± 0.51, 5.86 ± 0.52, and 5.58 ± 0.60 mg/kg LBW/min, *v* controls, 5.87 ± 0.33, all $P < .01$), whereas insulin sensitivity was comparable in the 3 relative groups (P by ANOVA = .59). The mean plasma glucose during constant intravenous glucose infusion was almost identical in the relative groups (FDR1, FDR2, and FDR3, 8.0 ± 0.3, 8.1 ± 0.2, and 8.1 ± 0.2 mmol/L, P by ANOVA = .60). However, the more complex measurements of insulin release dynamics revealed a clear genetic dose-effect, ie, irregularity and nonstationarity of insulin release were primarily observed in relatives with a strong family history of type 2 diabetes mellitus. Thus, ApEn was significantly higher in FDR3 compared with controls (1.563 ± 0.046 *v* 1.466 ± 0.016, $P < .05$) but did not differ between controls and FDR1 (1.501 ± 0.027) or FDR2 (1.489 ± 0.021, both $P > .20$). Likewise, FDR3 had increased ApEn compared with FDR2 and FDR1, although it was barely statistically significant (P by ANOVA = .09). With respect to the (non)stationarity of insulin release calculated as the CV for the 6-point MA, an even more pronounced genetic dose-effect was observed (FDR1,

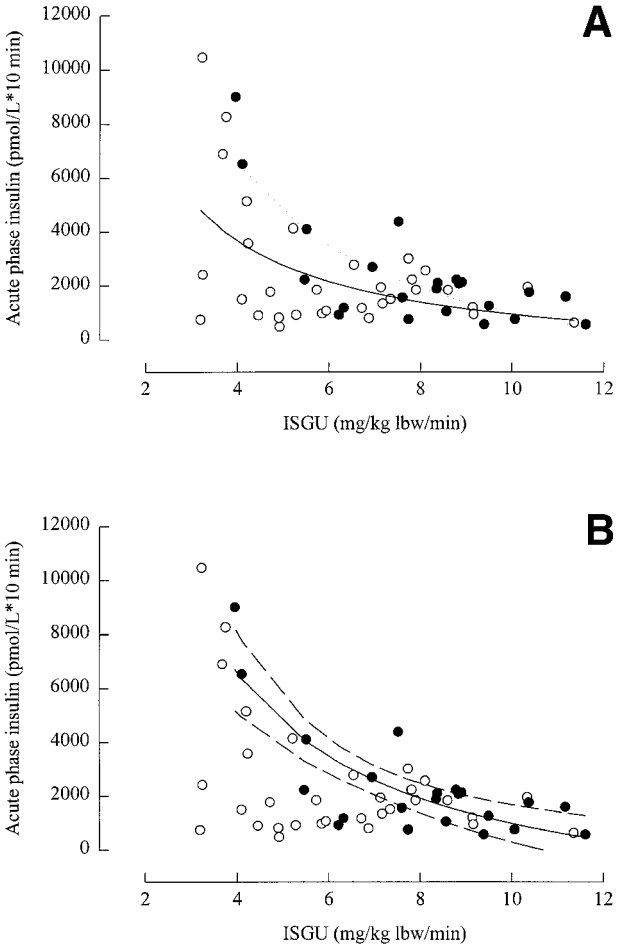

Fig 3. (A) Hyperbolic relationship (best fit) between acute-phase serum insulin response and ISGU in relatives (○) and controls (●). (B) Hyperbolic relationship between acute-phase serum insulin response and ISGU plotted for the control subjects (—) with 95% confidence limits of the mean (- - -).

FDR2, and FDR3, 0.129 ± 0.017, 0.167 ± 0.022, and 0.175 ± 0.014, *P* by ANOVA = .06). Compared with controls (0.129 ± 0.009), the CV for the 6-point MA was significantly increased in both FDR2 and FDR3 (both $P < .05$). Accordingly, the combined RaS index was higher (indicating increased disorderliness) and nonstationarity of insulin release) for a stronger family history of type 2 diabetes mellitus (FDR1, FDR2, and FDR3, −0.024 ± 0.293, 0.445 ± 0.315, and 0.743 ± 0.437, *P* by ANOVA < .05; FDR2 and FDR3 *v* controls [−0.318 ± 0.176], $P < .05$ and $P < .01$, respectively). We did not observe a genetic dose-effect with respect to other measurements of insulin secretion either within the 3 relative groups or between any of the groups compared with the controls (data not shown).

DISCUSSION

The present study examines glucose-regulated β-cell secretory function in individuals genetically predisposed to type 2 diabetes mellitus (healthy first-degree relatives of type 2 diabetic patients) using classic measures (OGTT and IVGTT) together with dynamic analytical strategies (deconvolution and

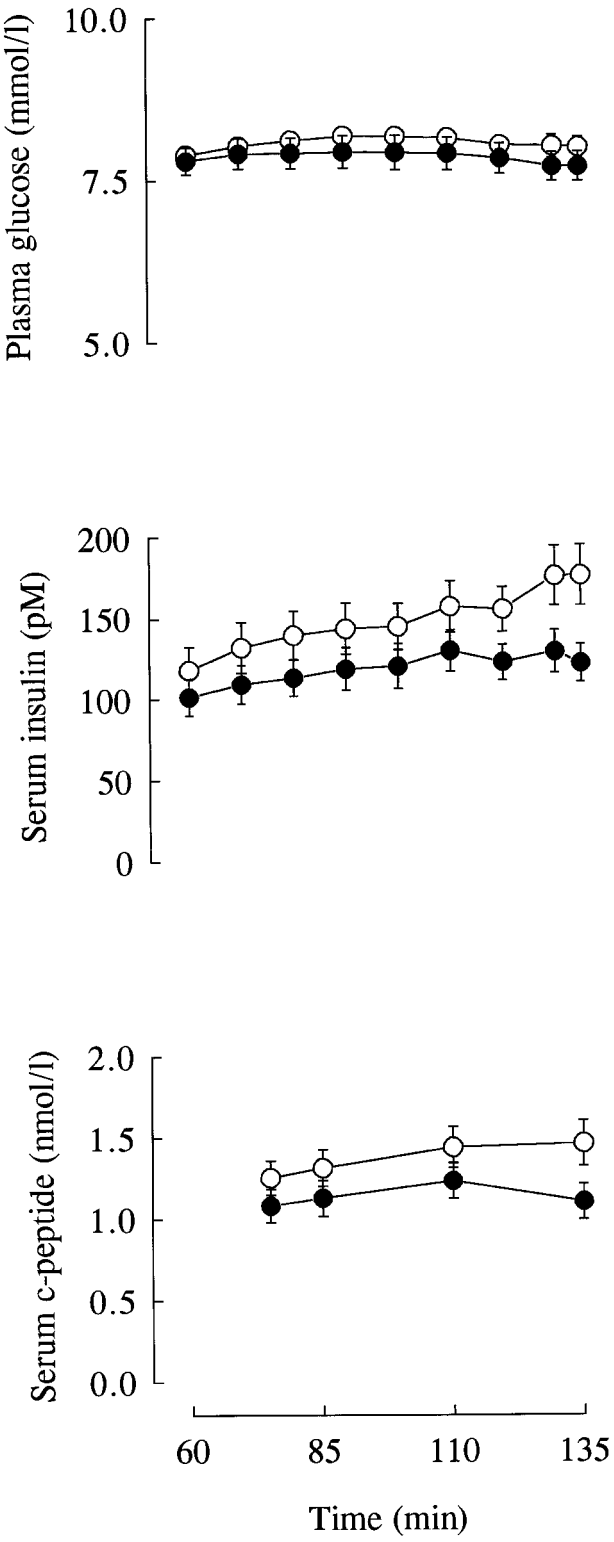

Fig 4. Mean plasma glucose and serum insulin and C-peptide during intravenous glucose infusion (4.0 and 4.5 mg/kg/min, time 60-135 minutes) in relatives (○) and control subjects (●).

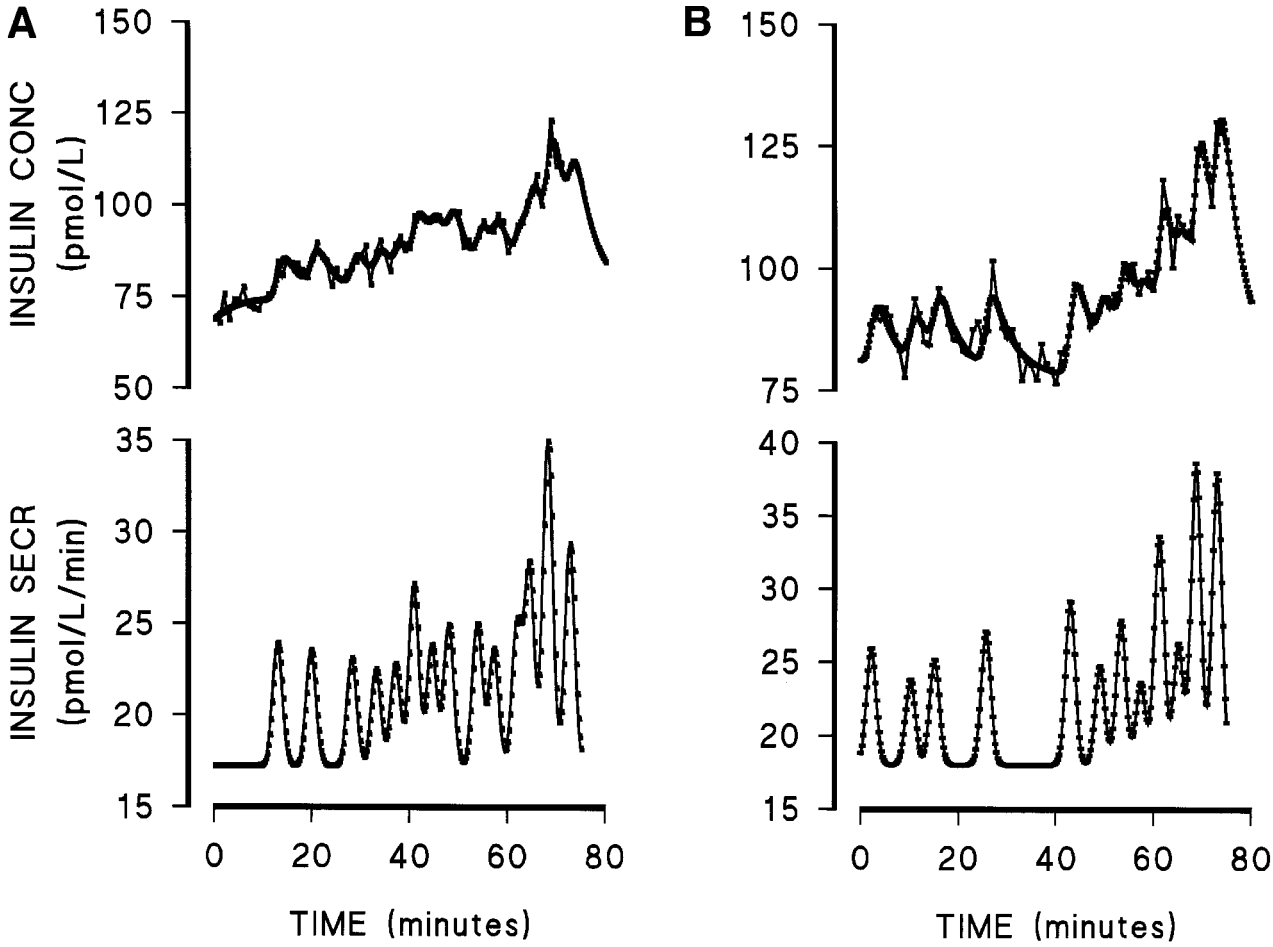

Fig 5. Peripheral serum insulin concentration and the corresponding calculated insulin secretion rate assessed by deconvolution analysis in (A) an offspring of a type 2 diabetic patient and (B) a matched control subject.

autocorrelation analysis, ApEn, CV for a 6-point MA, and a combined index of the last 2). To this end, relatives of type 2 diabetic patients and control subjects without a known family history of diabetes were matched in groups in terms of age, gender, and BMI. Offspring of type 2 diabetic patients have an a priori likelihood of diabetes of approximately 40% to 80%, depending on the magnitude of the family history.[13,28] The relatives in our study were insulin-resistant as determined by the hyperinsulinemic-euglycemic clamp technique. This metabolic feature is a common finding in healthy first-degree relatives of type 2 diabetic families[6-13] and is considered a major risk factor for the development of type 2 diabetes mellitus.[12,13,28-30]

Following the OGTT, the dynamic responses in plasma glucose, serum insulin, and C-peptide were comparable in the relatives and control subjects, although the AUCs for plasma glucose, serum C-peptide, and insulin were augmented in the relatives. Because the compensatory increase in insulin secretion failed to superimpose the glucose profile of the relatives on that of the control group, one could argue that there was relative hypoinsulinemia due to a subtle β-cell defect. On the other hand, because the glycemic and insulin secretory responses during an OGTT are influenced by numerous factors, eg, insulin sensitivity of the peripheral tissues and the liver, glucose effectiveness, release of (gut) incretins, suppression of glucagon, and gastric emptying, the glucose and insulin excursions should be interpreted with caution. Among studies dealing with normal glucose-tolerant relatives of type 2 diabetic subjects, data on insulin, C-peptide, and glucose profiles during an OGTT are not frequently reported in detail and/or are somewhat conflicting. The latter probably is ascribable primarily to heterogeneity among the relatives (eg, age, BMI, and ethnic origin), but differences in the sampling paradigm, sample processing, and/or insulin assays may also contribute. In the present study, the 120-minute plasma glucose concentration after glucose ingestion was significantly increased in the relatives, suggesting that a significant proportion of these relatives are prediabetic subjects.[31,32]

Acute-phase serum insulin release in response to the IVGTT was similar in the 2 groups. This observation is in agreement with most studies in normal glucose-tolerant first-degree relatives of type 2 diabetic patients,[6,8,11-13] although both increased[7] and diminished[33,34] acute-phase serum insulin responses have been reported. Moreover, a low acute-phase serum insulin response has been shown to be an independent risk factor for the development of type 2 diabetes mellitus.[29,35,36] In agreement

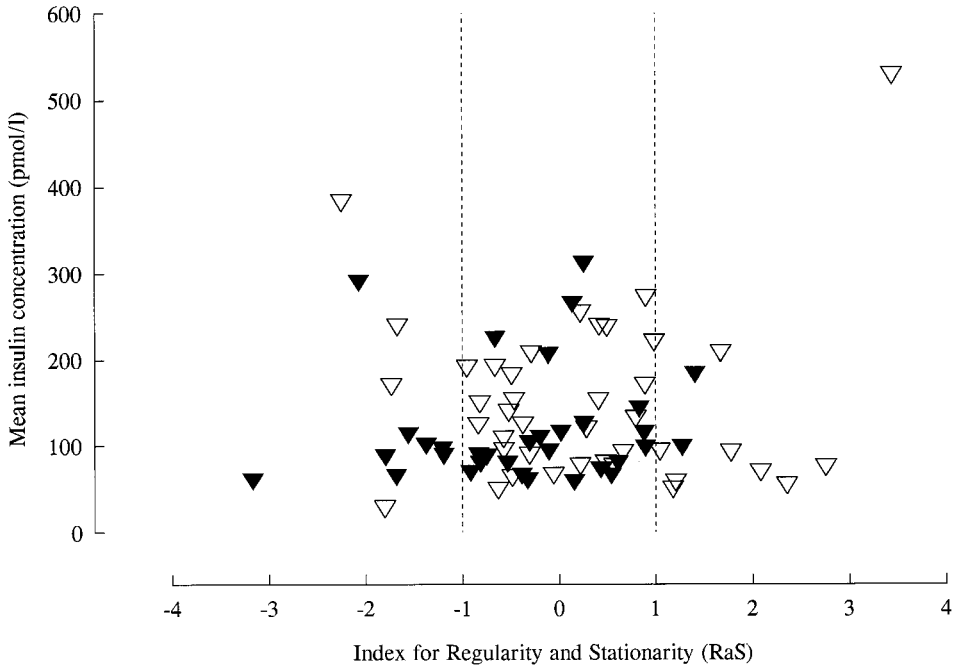

Fig 6. Individual subject values for the RaS index versus the mean serum insulin concentration (pmol/L) in first-degree relatives of type 2 diabetic patients (▽) and control subjects (▼). Vertical lines indicate subjects with the highest (ie, most irregular) and lowest (ie, most regular) values in the 2 groups.

with the observations of Bergman et al[37] and Kahn et al,[38] we found a hyperbolic relationship between the acute-phase serum insulin response and ISGU in both relatives and control subjects. However, this relationship almost differed between the 2 groups, mainly ascribable to a subgroup of 16 relatives who individually exhibited decreased ISGU and a diminished acute-phase serum insulin response compared with 95% of control values. It seems reasonable to conjecture that the acute-phase insulin response was inappropriately low for the degree of insulin resistance in these particular subjects. Similar results have been reported by other investigators.[2,8,11] Moreover, a recent study—examining the heritability of β-cell function in the offspring of type 2 diabetic families—has underlined the importance of considering insulin secretion in the context of insulin sensitivity in these individuals genetically predisposed to type 2 diabetes mellitus.[39] Nevertheless, in the present study, the subset of relatives with an individually low acute-phase insulin response given their degree of insulin resistance did not differ from the other relatives with respect to the more complex measurements of insulin secretion calculated from the insulin time-series. The lack of correlation of the impaired acute-phase serum insulin response with the RaS could suggest (but does not prove) that 2 separate abnormalities of β-cell function operate in normal glucose-tolerant offspring of type 2 diabetic patients.

During recent years, it has become evident that β-cell secretory activity is complex, with insulin release occurring as pulses with an interval of 10 to 13 minutes in the fasting state[15] and 5 to 8 minutes during slight hyperglycemia in healthy humans,[16] superimposed on slower oscillations with a periodicity of approximately 120 minutes.[40] Although it is only poorly understood mechanistically, this common oscillatory behavior requires coordination among individual β cells and islets. Consequently, to improve the sensitivity for uncovering β-cell defects, we used several analytical models (deconvolution and autocorrelation analysis, ApEn, CV for a 6-point MA, and a combined insulin secretion index) that are strongly complementary in their analytical basis and implications.

In the present study, we have used deconvolution and autocorrelation analysis for the first time to characterize glucose-stimulated oscillatory insulin secretion in normal glucose-tolerant first-degree relatives of type 2 diabetic patients. Deconvolution analysis has been established as a reliable method for the detection and quantification of (pulsatile) hormone secretion[16,21] in humans. This study implements a multiparameter deconvolution technique to assess insulin secretory activity based on the serum insulin concentration time-series in individuals genetically predisposed to type 2 diabetes mellitus. We observed a tendency for an increased insulin secretory burst amplitude and mass in the relatives compared with the control subjects, thereby explicating hyperinsulinemia in the relatives, but there were no abnormalities in insulin pulse frequency between the 2 groups. Our data do not support the concept that high-frequency pulsatile insulin secretion is disrupted in normoglycemic individuals genetically predisposed to type 2 diabetes mellitus as assessed either by deconvolution analysis or by autocorrelation analysis. This observation may seem in contrast to the prior report by O'Rahilly et al,[17] where the latter mathematical model showed perturbed oscillatory insulin secretory activity in the offspring of type 2 diabetic patients. However, of note, the relatives in the study by O'Rahilly et al[17] were examined at baseline, and all exhibited impaired glucose tolerance. This means that their β-cell function may have been influenced by glucose toxicity, a suggestion supported in a recent study by Gumbiner et al[41] demonstrating less disturbed insulin pulsatility when metabolic control improved in obese type 2 diabetic patients following weight loss.

In a recent study[19] measuring the orderliness and stationarity of insulin release by the ApEn, CV for a 6-point MA, and combined RaS index on insulin time-series in a small group of healthy relatives, significant differences were observed in all 3

variables between the relatives and a matched control group. These data were interpreted to suggest disorderly and nonstationary insulin secretion in the relatives. The present study in a much larger group of relatives supports the former observation. Even though the 2 individual indices, ApEn and CV for a 6-point MA, considered alone could not significantly discriminate between the 2 groups, the joint RaS index was significantly higher in the relatives independently of serum insulin and insulin sensitivity. This supports the foregoing conclusion and suggests that the combined index is a sensitive parameter for evaluating disrupted β-cell behavior. Indeed, in the current study, this method was the only single measure of insulin release that distinguished between relatives and control subjects. Of importance, a significant genetic dose-effect was observed with respect to the orderliness and stationarity of insulin release in the relatives, ie, disorderly and nonstationary insulin secretion was primarily observed in relatives with a strong family history of type 2 diabetes mellitus. This finding further supports the idea of a primary and possibly genetic mechanism for the disruptions in oscillatory insulin release in genetically predisposed individuals.

In exploring the mechanisms responsible for the irregular and nonstationary insulin release in the relatives, one could argue that since the relatives were slightly hyperglycemic compared with the control subjects, this finding reflects glucose toxicity of the β cell. Whereas this consideration cannot be completely ruled out, it is notable that the combined RaS index was statistically independent of insulin sensitivity, fasting plasma glucose, 120-minute plasma glucose, and the AUC during the OGTT in both glucose-tolerant relatives of type 2 diabetic parents and healthy subjects without any family history of diabetes. Nonetheless, further study is required to define the relative contribution of mild subclinical hyperglycemia to the impact of glucose toxicity in these otherwise healthy relatives.

It is still under debate as to whether insulin resistance or impaired insulin secretion represents the primary defect in type 2 diabetes mellitus.[14,34,42] A recent study including middle-aged Swedish men found that a family history of diabetes was associated with decreased insulin secretion rather than insulin resistance in subjects with newly diagnosed type 2 diabetes mellitus.[43] Vauhkonen et al[44] studied type 2 diabetic patients and their offspring. The parents were grouped by insulin sensitivity and insulin secretion, and it was concluded that defects in both insulin secretion and insulin action seem to be inherited. These latter results are in accordance with the findings of the present study demonstrating both insulin resistance and β-cell dysfunction in glucose-tolerant relatives of type 2 diabetic parents compared with a matched control group, although the results do not allow us to clarify whether insulin resistance antedates β-cell dysfunction or vice versa.

In conclusion, the present study evaluates β-cell secretory function in normal glucose-tolerant but insulin-resistant relatives of patients with type 2 diabetes mellitus using an array of analytical techniques. IVGTT allowed the separation of a group of relatives with a low acute-phase serum insulin response for their degree of insulin resistance. Moreover, ApEn combined with the CV for a 6-point MA provided evidence of a disorderly and nonstationary pattern of insulin release in relatives, strongly suggesting a disruption of the orderliness of the coordinated insulin release process as one of the earliest definable β-cell alterations in these individuals genetically predisposed to type 2 diabetes mellitus. Of note, disorderly and nonstationary insulin release was primarily observed in relatives with a strong genetic burden, in support of a primary and possibly genetic background for these disruptions in oscillatory insulin secretion. In contrast, both deconvolution and autocorrelation analysis failed to discriminate between the relatives and control subjects, suggesting preserved macroscopic insulin pulse frequency and relative amplitude during modest hyperglycemia. A disorderly insulin release pattern and attenuated acute-phase serum insulin response for the degree of insulin resistance suggest that (at least) 2 β-cell abnormalities may occur in individuals predisposed to type 2 diabetes mellitus. However, additional studies will be needed to delineate the long-term pathophysiological consequences of the dynamic disruptions and to establish the robustness of these parameters in predicting type 2 diabetes mellitus.

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