Altered Homeostatic Adaptation of First- and Second-Phase β-Cell Secretion in the Offspring of Patients With Type 2 Diabetes

Studies With a Minimal Model to Assess β-Cell Function

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We adapted a minimal model to assess β-cell function during a hyperglycemic glucose clamp and to uncover peculiar aspects of the relationship among β-cell function, plasma glucose, and insulin sensitivity (IS) in offspring of Caucasian patients with type 2 diabetes (OfT2D). We pooled two data sets of OfT2D (n = 69) and control subjects (n = 45) with normal glucose regulation. Plasma C-peptide was measured during a hyperglycemic clamp (~10 mmol/l) to quantify model-based first-phase secretion and glucose sensitivity of second-phase secretion (β). IS was quantified during the hyperglycemic clamp. In the pooled data, first-phase secretion was linearly and negatively related to fasting plasma glucose, but not IS; OfT2D lay on a distinct line shifted to the left of the control subjects. In contrast, β was negatively related to IS, and OfT2D lay on a distinct line shifted more and more to the left of the control subjects, as IS was worse. Thus, in OfT2D lower β-cell adaptive responses exist between ambient glycemia and first-phase insulin secretion and between IS and second-phase secretion. Under conditions leading to decreased insulin sensitivity, these disturbed relationships may lead to progression to diabetes in OfT2D.
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Type 2 diabetes is characterized by defects in both insulin secretion and insulin action (1). Both factors are important in the pathogenesis of the disease and both are influenced by environmental and genetic factors (1,2). The exact timing and relative importance with which these two factors appear and play a role in the natural history of the disease are still a matter of debate (3,4). In Pima Indians, insulin resistance, as assessed by insulin clamp, is an important risk factor for future development of diabetes, but the decline in the acute insulin response to intravenous glucose is the best pacemaker of disease progression (5). Thus, insulin resistance may be a better risk indicator, but insulin secretion appears to be the key pathogenetic factor in disease development (6). However, given the metabolic peculiarities of this ethnic group, it is unknown whether these findings are completely transferable to other ethnicities.

Because type 2 diabetes has a strong genetic basis (1–4), first-degree relatives (FDRs) of diabetic patients should be quite helpful in reconstructing the natural history of the disease (7–15). Nevertheless, the results of these studies are not uniform, with some emphasizing the presence of insulin resistance in FDRs (7,9,11), others strongly underscoring the early appearance of defects in insulin secretion (10,12,15), and yet others reporting evidence for both abnormalities (13,14).

Various methods, different ethnic backgrounds, heterogeneity of the disease, and different criteria to select populations of control individuals may account for these seemingly disparate findings. The latter factor is of special relevance because the β-cell adapts to the degree of insulin sensitivity (IS) (or insulin resistance) (4,5,16,17). If type 2 diabetes were the result of complex genetic defects in both insulin secretion and insulin action (1,2,18–25), a perfect matching between FDRs and control subjects for the indicators of insulin resistance might lead to select FDRs with only a demonstrable defect in insulin secretion, whereas a less stringent matching might lead to different conclusions.

Moreover, the stage at which the FDRs are studied may be relevant. As a late onset disease, the pathophysiological alterations leading to type 2 diabetes (5) might be detectable only beyond a threshold age and/or only when the body is required to cope with some "metabolic stressors."

In this article, we report observations from two separate data sets, each of which includes control subjects and FDRs, more specifically offspring of Caucasian patients with type 2 diabetes (OfT2D) with normal glucose regulation (26). The data sets differed as to age, thereby potentially reflecting two different temporal and/or metabolic stages of the disease in Caucasians. Subjects were studied with hyperglycemic clamps (both data sets) (27) and oral glucose tolerance tests (OGTTs) (one data set only), which were analyzed with ad hoc minimal models of

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BSA, body surface area; CV, coefficient of variation; FDR, first-degree relative; IS, insulin sensitivity; OfT2D, offspring of Caucasian patients with type 2 diabetes; OGTT, oral glucose tolerance test.

insulin secretion (28–30) in order to best characterize β-cell function with the use of plasma C-peptide levels. This was potentially important because many previous studies used insulin concentration after a secretory stimulus to assess β-cell function (5–13,15–17). However, insulin concentration is determined by both β-cell secretion and insulin clearance. Not only the former, but also the latter changes homeostatically in response to variations in IS (31). Furthermore, many IS indexes are derived from formulae in which ambient insulin concentration is included. Thus, the relationship between secretory indexes derived from insulin concentration and IS might be somewhat spurious, owing to the role of insulin clearance and collinearity problems. Because a good agreement exists between the IS index derived from the hyperglycemic clamp and the IS measured with the reference tool of the euglycemic clamp (10,32), we also used the hyperglycemic clamp to assess IS in these subjects.

RESEARCH DESIGN AND METHODS

Subjects. The studies were performed in two institutions with slightly different experimental maneuvers. On the whole, subjects studied in one institution were younger and somewhat leaner than subjects studied in the other institution and will be denoted henceforth as data set A. The others will be denoted as data set B. All subjects consumed a weight-maintaining diet containing 200–250 g carbohydrate/day for at least 3 days before study. Body weight was stable in all subjects for at least 3 months before study. All subjects had standard parameters of renal and liver function and complete blood cell count within normal limits. Each subject gave informed, written consent before participating in the study, which was approved by the Human Investigation Committees of Tübingen (data set A) and Utrecht (data set B) Universities.

Data set A. A total of 60 Caucasian subjects with normal glucose regulation (26), as ascertained by an OGTT, were recruited for study. Some data were reported in previous publications (22,33), but no model analysis of glucose-induced C-peptide secretion has ever been published. Further, 23 subjects had a negative family history for type 2 diabetes (control subjects), and 37 individuals had at least one FDR (father or mother) affected with type 2 diabetes.

Study design. In each subject, a hyperglycemic clamp was performed (27). The study started at 0830 after a 10- to 12-h overnight fast and lasted 150 min (−30 to 120 min). Catheters were introduced into an antecubital vein for the infusion of glucose and retrogradely into a wrist vein to sample arterialized venous blood. The hand was inserted into a heated box (60°C) during the study to ensure arterialization of the venous blood. Catheter patency was maintained by filling with mildly heparinized saline solution. At 0 min, a primed-variable glucose infusion (20% dextrose in water) was started to acutely raise and maintain glucose concentration at 10 mmol/l until the end of the study. Blood samples were collected between –30 and 0 min (baseline samples), every 2.5 min between 0 and 10 min and every 5 min thereafter to measure plasma glucose, and every 2.5 min between 0 and 10 min and at 20, 40, 60, 80, 100 and 120 min to measure plasma C-peptide concentrations.

Data set B. A total of 54 Caucasian subjects were recruited for study. Some data were reported in previous publications (12,15,34,35), but no model analysis of glucose-induced C-peptide secretion has ever been published. Further, 22 subjects had negative family history for type 2 diabetes (control subjects), and 32 individuals had at least one FDR (father or mother) affected with type 2 diabetes.

Study design. Each subject participated in two studies, which were performed randomly at 1- to 2-week intervals. Both studies started at 0830 after a 10- to 12-h overnight fast. On one occasion subjects were admitted to the clinical research center. A Teflon catheter was introduced into an antecubital vein to sample venous blood. Catheter patency was maintained by filling with mildly heparinized saline solution. The study lasted 130 min (−10 to 120 min). At 0 min, subjects ingested a 75-g glucose solution over 5 min. Blood samples were collected at −10, 0 (baseline samples), and at 30, 45, 60, 90, and 120 min to measure plasma glucose and C-peptide concentrations. On the other occasion, a hyperglycemic clamp was performed in the same fashion as in data set A. The study lasted 190 min (−10 to 180 min). Blood samples were collected between –10 and 0 min (baseline samples), every 2 min between 0 and 10 min, every 5 min thereafter to measure plasma glucose, and every 2 min between 0 and 10 min, and every 10 min thereafter to measure plasma C-peptide and insulin concentrations.

Analytical methods. Plasma insulin and C-peptide concentrations were measured by a microparticle enzyme immunoassay (Abbott, Wiesbaden, Germany) and a radioimmunoassay (Byk-Santec, Dietzenbach, Germany), respectively, in data set A. Plasma insulin was measured with a radioimmunoassay, using a polyclonal anti-insulin antibody (Caris 46) and ^{125}I-insulin (IM 166; Amersham, Buckinghamshire, U.K.) as tracer in data set B. Plasma C-peptide was measured with a standard radioimmunoassay (MD 315; Euro-Diagnostica, Malmo, Sweden) in data set B. Plasma glucose concentration was measured in duplicate on a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA).

Calculations. IS was computed as the M-to-I ratio (27), i.e., the ratio between the amount of glucose infused (after correction for glucose space of distribution) between 80 and 120 min of the clamp and the prevailing insulin concentration during the same time interval (micromoles per minute per meter^2 of body surface area [BSA] per picomole per liter of insulin) in both data sets, in order to ensure data comparability.

β-Cell secretion during the OGTT was estimated by applying an analogic minimal model of glucose-induced insulin secretion (28,29) to the glucose and C-peptide curves of each subject of data set B as previously described (30). OGTT β-index (in picomoles per minute^{-2} per meter^{-2} BSA), a global parameter of β-cell function during the OGTT, was computed as previously described (30). This index is fairly stable and reproducible on a day-to-day basis (30) and with different oral glucose loads (30), and is correlated to classic indexes of β-cell function, such as the acute insulin response to intravenous glucose (30).

β-Cell secretion during the hyperglycemic clamp was estimated by applying a minimal model of glucose induced insulin secretion to the glucose and C-peptide curves of each subject. This model was introduced by Toffolo and colleagues (28,29) to describe glucose-induced insulin secretion during the intravenous glucose tolerance test and has been modified by us for the hyperglycemic clamp as described in the APPENDIX. With this tool, we estimated first-phase insulin secretion (picomoles), the slope relating second-phase insulin secretion to the glucose stimulus (β: [picomole per minute] per [millimole per liter]), and the time constant, or delay (δ: units), of second-phase insulin secretion. Furthermore, we also computed the amount of insulin secreted during the second phase over the 120 min of the study in data set A and over the 180 min of study in data set B (second-phase secretion; units: picomoles over 120 or 180 min, as appropriate). All of these parameters, except δ, were normalized per meter^2 of BSA.

Parameters were estimated by implementing both (i.e., OGTT and hyperglycemic clamp) minimal models of C-peptide secretion in the SAAM II 1.1.2 software. Numerical values of the unknown parameters were estimated by using nonlinear least squares. Weights were chosen optimally, i.e., equal to the inverse of the variance of the measurement errors, which were assumed to be additive, uncorrelated, with zero mean, and a constant coefficient of variation (CV = 6%). In all cases the fit was acceptable, as shown by the C-peptide weighted residuals (Fig. 1), i.e., the differences between model-predicted and measured C-peptide concentrations divided by the standard deviation of C-peptide measurement.

In data set A, the coefficients of variation (mean ± SE) of first-phase insulin secretion, β, δ, and second phase insulin secretion were 6.62 ± 0.33, 8.81 ± 0.60, 26.3 ± 0.96, and 3.87 ± 0.05%, respectively. In data set B, the CVs (means ± SE) of first-phase insulin secretion, β, δ, second-phase insulin secretion, and OGTT β-index were 6.73 ± 0.46, 5.74 ± 0.59, 17.2 ± 0.9, 3.01 ± 0.05, and 9.6 ± 3.06%, respectively.

First- and second-phase insulin secretion were estimated also with conventional formulae (27), that is, by computing the area above basal under the curve of insulin concentration between 0 and 10 min (first-phase insulin concentration) and by averaging serum insulin concentration during the last 40 min of the hyperglycemic clamp (second-phase insulin concentration), respectively (27). The main variables measured in this study are listed in Table 1 with their definitions and units.

Statistical analyses. Weighted residuals, i.e., the differences between measured and model-predicted C-peptide concentrations divided by the standard deviation of C-peptide measurement are presented as means ± SD. All other data are presented as means ± SE. The Kolmogorov-Smirnov test was used to test whether any variable would significantly depart from a normal distribution. Statistically significant deviations from a gaussian distribution were detected only for first- and second-phase insulin concentration in the OfT2D of data set A. Accordingly, these comparisons were performed by Mann-Whitney's *U* test. All other comparisons between control subjects and OfT2D were run by ANOVA. Because some variables showed significant differences between control subjects and OfT2D and, in some cases, nonlinear relationships were plausible, simple correlations were sought by computing Spear-

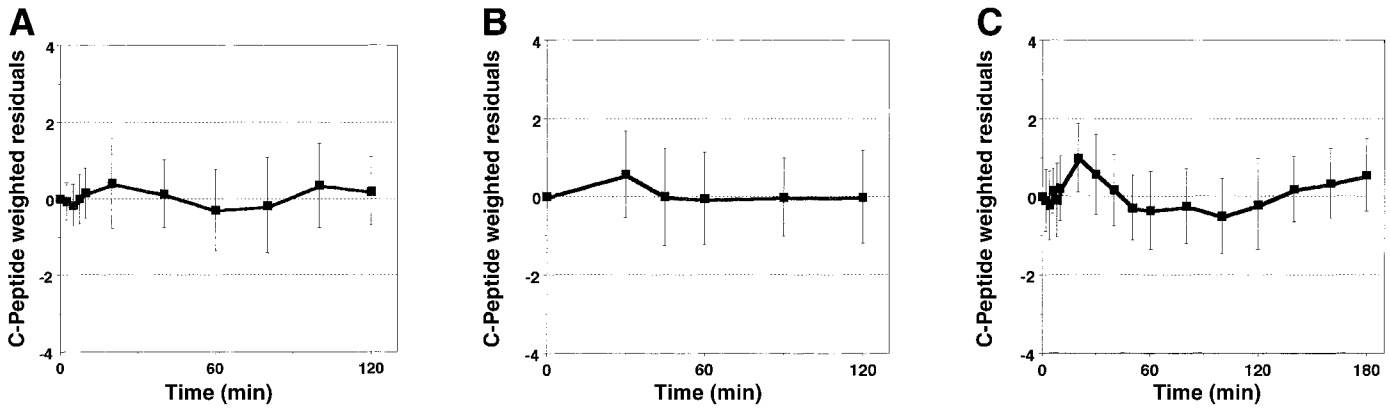

FIG. 1. Weighted residuals (i.e., difference between measured and model-predicted C-peptide concentration divided by the standard deviation of C-peptide measurement) of model fit to plasma C-peptide experimental data in the hyperglycemic clamps of data set A (*A*), in the OGTTs of data set B (*B*), and in the hyperglycemic clamps of data set B (*C*). The weighted residuals are a quantitative assessment of the goodness-of-fit of the models to the data: a theorically perfect fit should generate weighted residuals with mean 0 and SD 1.

man's nonparametric ρ correlation coefficient for the sake of consistency. Nonlinear least squares regression analyses in the pooled data sets were carried out by the sequential quadratic programming method with bootstrap estimates of the standard errors of the parameters. All statistics were computed with the SPSS 10.1 software. Statistical significance was declared at $P < 0.05$.

RESULTS

General analysis. Demographic, anthropometric, and clinical data of the participating control subjects and OfT2D are given in Tables 2 and 3. Age, sex distribution, and BMI were not different between the two groups in either data set. However, subjects in data set B were significantly older ($P < 0.01$) and had a somewhat higher BMI ($P < 0.07$) than subjects in data set A. Fasting and 2-h plasma glucose were higher in OfT2D than in control subjects in data set B. IS was similar in OfT2D and controls in both data sets, but subjects in data set B showed a trend for being less insulin sensitive than subjects in data set A ($P = 0.07$). The minimal models of both the OGTT and the hyperglycemic clamp provided a fairly good fit of the data as shown by the residual plots (Fig. 1).

Correlation analysis (Tables 4 and 5) showed a striking congruity between data set A and B. Model-derived measures of first- and second-phase insulin secretion (Tables 4 and 5) displayed strong correlations with first- and second-phase insulin concentration ($P < 0.001$–0.0001), respectively. In both data sets, IS was inversely related to model-derived and insulin concentration–derived measures of second-phase insulin secretion ($P < 0.001$–0.0001; Tables 4 and 5 and Figs. 2 and 3). In contrast, correlations with first-phase insulin concentration were much weaker and nonexisting with first-phase insulin secretion. In both data sets, fasting plasma glucose and 2-h plasma glucose were negatively correlated to model-derived first-phase insulin secretion (Tables 4 and 5 and Figs. 2 and 3).

In data set B, OGTT β-index was strongly correlated to first-phase insulin concentration and less strongly to second-phase insulin concentration. Furthermore, both fasting and 2-h glucose were negatively correlated to OGTT β-index (Table 5).

Comparisons between control subjects and OfT2D in the separate data sets. In data set A, control subjects

TABLE 1
Parameters derived from the analysis of the hyperglycemic clamp (HGC) and the OGTT

Parameter	Definition	Units
First-phase concentration	Area above basal under the curve of insulin concentration between 0 and 10 min of the HGC	pmol/l per 10 min
Second-phase concentration	Average insulin concentration during the last 40 min of the HGC	pmol/l
IS	Insulin sensitivity during the HGC	$\mu mol \cdot min^{-1} \cdot m^{-2}$ BSA per pmol/l
First-phase secretion	Amount of C-peptide secreted by the β-cell during the first-phase secretion of the HGC	$pmol \cdot m^{-2}$ BSA
Second-phase secretion	Amount of C-peptide secreted by the β-cell during the second-phase secretion of the HGC	pmol per 120 min (or 180 min) m^{-2} BSA
β	Sensitivity of β-cell second-phase secretion to glucose during the HGC	$(pmol \cdot min^{-1} \cdot m^{-2}$ BSA) per mmol
δ	Apparent delay (time constant) of β-cell second-phase secretion during the HGC	min
OGTT β-index (log units)	β-cell function during the OGTT	$\log(pmol \cdot min^{-2} \cdot m^{-2}$ BSA)

TABLE 2
Demographic, metabolic, and β-cell–related parameters of subjects of data set A

	Control subjects	OfT2D	*P*
n (M/F)	23 (12/11)	37 (19/18)	
Age (years)	33.1 ± 2.0	39.2 ± 2.0	0.049
BMI	25.1 ± 0.8	25.8 ± 0.9	0.60
FPG	4.77 ± 0.09	4.99 ± 0.10	0.14
2-h PG	5.09 ± 0.21	5.63 ± 0.21	0.09
IS	6.24 ± 0.80	6.11 ± 0.62	0.89
1st PH-CONC	2,482 ± 379	1707 ± 274	0.01
2nd PH-CONC	295 ± 60	261 ± 41	0.34
1st PH-SEC	3,360 ± 395	2,587 ± 244	0.07
β	61.6 ± 6.1	62.4 ± 3.4	0.90
2nd PH-SEC	31,208 ± 3,535	28,556 ± 1,484	0.43

Data are means ± SD. FPG, fasting plasma glucose; PG, plasma glucose; 1st PH-CONC, first-phase concentration; 2nd PH-CONC, second-phase concentration; 1st PH-SEC, first-phase secretion; 2nd PH-SEC, second-phase secretion.

and OfT2D were similar as to BMI, sex, fasting and 2-h plasma glucose, and IS. OfT2D were slightly older than control subjects ($P = 0.049$) (Table 2). There was a reduction in first-phase insulin concentration ($P < 0.01$) and a trend for a reduced first-phase insulin in OfT2D ($P = 0.07$), whereas both β and second-phase insulin secretion, as well as second-phase insulin concentration, were not different from that of control subjects (Table 2).

In data set B, control subjects were also well matched to OfT2D for age, BMI, sex, and IS (Table 3). However, different from data set A, fasting and 2-h plasma glucose was significantly higher in the OfT2D than in the control subjects (Table 3). First- and second-phase insulin concentrations were lower ($P < 0.10$ and $P < 0.05$) in OfT2D than in control subjects, but the former was short of statistical significance (Table 3). In apparent contrast to data set A, all secretory indexes derived from model analysis of the hyperglycemic clamp, with the exception of δ, were significantly lower in OfT2D than in control subjects (Table 3). OGTT β-index was also lower in OfT2D than in control subjects (Table 3).

Because both fasting and 2-h plasma glucose were significantly higher in OfT2D than in control subjects (Table 3), indicating that oral glucose tolerance, albeit normal, was worse in OfT2D, we selected in data set B only those control subjects ($n = 20$) and OfT2D ($n = 18$) with 2-h plasma glucose <6.0 mmol/l to improve matching for glucose and to include only those individuals whose glucose tolerance could be considered normal beyond any reasonable doubt (in our laboratory the between-day CV of 2-h plasma glucose was 11.1%, $n = 29$). These two subgroups were still similar for age, sex, BMI, and IS, but also for fasting ($P = 0.30$) and 2-h plasma glucose ($P = 0.22$) (Table 6). Neither first- nor second-phase insulin concentrations were significantly different between these two subgroups ($P = 0.09$ and $P = 0.17$, respectively). However, first- ($P = 0.01$) and second- ($P = 0.02$) phase insulin secretion and OGTT β-index ($P = 0.01$) were still lower in the OfT2D subgroup than in the control subgroup (Table 6).

Analysis of pooled data. To further elaborate on the influence that family history of type 2 diabetes exerts on the relationships between β-cell function and IS and plasma glucose, we ran a pooled analysis of first-phase insulin secretion, β, IS, and fasting plasma glucose. We used β instead of second-phase insulin secretion because the latter is the integral of β-cell second-phase secretion during the hyperglycemic clamp and changes according to the duration of the study (120 min in data set A vs. 180 min in data set B), whereas the former is a pure measure of β-cell sensitivity to intravenous glucose.

Even in the pooled data ($n = 114$), first phase was correlated to fasting plasma glucose ($\rho = -0.405$, $P < 0.0001$) but not to IS ($\rho = -0.11$, $P = 0.24$), whereas β was correlated to IS ($\rho = -0.412$, $P < 0.0001$), but not to fasting plasma glucose ($\rho = 0.044$, $P = 0.64$). The relation-

TABLE 3
Demographic, metabolic, and β-cell–related parameters of subjects of data set B

	Control subjects	OfT2D	*P*
n (M/F)	22 (6/16)	32 (6/26)	
Age (years)	47.9 ± 1.7	47.1 ± 1.1	0.68
BMI	26.4 ± 0.7	26.7 ± 0.7	0.80
FPG	4.50 ± 0.08	4.75 ± 0.09	0.046
2-h PG	4.83 ± 0.30	6.07 ± 0.32	0.01
IS	4.95 ± 0.44	4.59 ± 0.50	0.61
1st PH-CONC	1722 ± 112	1372 ± 154	0.096
2nd PH-CONC	568 ± 70	407 ± 42	0.04
1st PH-SEC	4230 ± 380	2900 ± 278	0.006
β	83.5 ± 7.9	66.8 ± 5.6	0.03
2nd PH-SEC	66189 ± 6651	47520 ± 2092	0.003
OGTT β-INDEX	5.62 ± 0.14	4.83 ± 0.16	0.001

Data are means ± SE. FPG, fasting plasma glucose; PG, plasma glucose; 1st PH-CONC, first-phase concentration; 2nd PH-CONC, second-phase concentration; 1st PH-SEC, first-phase secretion; 2nd PH-SEC, second-phase secretion.

TABLE 4
Nonparametric correlation matrix (Spearman's ρ) (n = 60) for data set A

	FPG	2-h PG	IS	1st PH-CONC	2nd PH-CONC	1st PH-SEC	β	δ
FPG	1							
2-h PG	0.53§	1						
IS	0.06	−0.19	1					
1st PH-CONC	−0.41§	−0.19	−0.41§	1				
2nd PH-CONC	−0.19	0.09	−0.77§	0.68§	1			
1st PH-SEC	−0.41§	−0.28*	−0.23	0.87§	0.54§	1		
β	0.05	0.13	−0.45§	0.37#	0.66§	0.45§	1	
δ	0.30*	0.26*	0.25*	−0.24	−0.22	−0.12	0.28*	1
2nd PH-SEC	−0.22	−0.07	−0.59§	0.59§	0.83§	0.60§	0.80§	−0.22

FPG, fasting plasma glucose; PG, plasma glucose; 1st PH-CONC, first-phase concentration; 2nd PH-CONC, second-phase concentration; 1st PH-SEC, first-phase secretion; 2nd PH-SEC, second-phase secretion. *$P < 0.05$; #$P < 0.01$; §$P < 0.001$.

ship between fasting plasma glucose and first-phase insulin secretion was not described better by nonlinear models (corrected r^2 = 0.12–0.18) than by a linear function (corrected r^2 = 0.19, $P < 0.001$), but the OfT2D lay on a line that was significantly ($P < 0.01$) shifted to the left, i.e., for any given plasma glucose level first phase was lower in OfT2D than in control subjects (Fig. 4). However, because this difference was rather constant across the entire range of glucose levels, OfT2D with higher fasting plasma glucose displayed a proportionally more severe defect in β-cell first-phase secretion. A superimposable relationship was found between 2-h plasma glucose level and first phase (data not shown).

The relationship between IS and β was described better with a power function with a negative exponent (corrected r^2 = 0.13, $P < 0.01$) than with a linear function (corrected r^2 = 0.09), but the OfT2D curve was significantly ($P < 0.05$) shifted to the left only in the lower half range of IS, i.e., the lower the IS the greater the difference in β between OfT2D and control subjects (Fig. 4). A linear relationship between plasma glucose and first phase and a power function relationship between IS and β were found also when the analyses were restricted to only the control subjects (data not shown).

Because different assays were used to measure insulin and C-peptide in the two data sets, all pooled analyses were repeated with an additional dummy variable to take into account possible center- and assay-related influences. In all cases this dummy variable did not achieve statistical significance, and all results were unchanged.

DISCUSSION

In this article, we applied minimal model analysis to both the OGTT (30) and the hyperglycemic glucose clamp (hyperglycemic clamp), aiming at gaining better insight in the secretory function of the β-cell in two distinct databases, which included individuals with no known diabetic

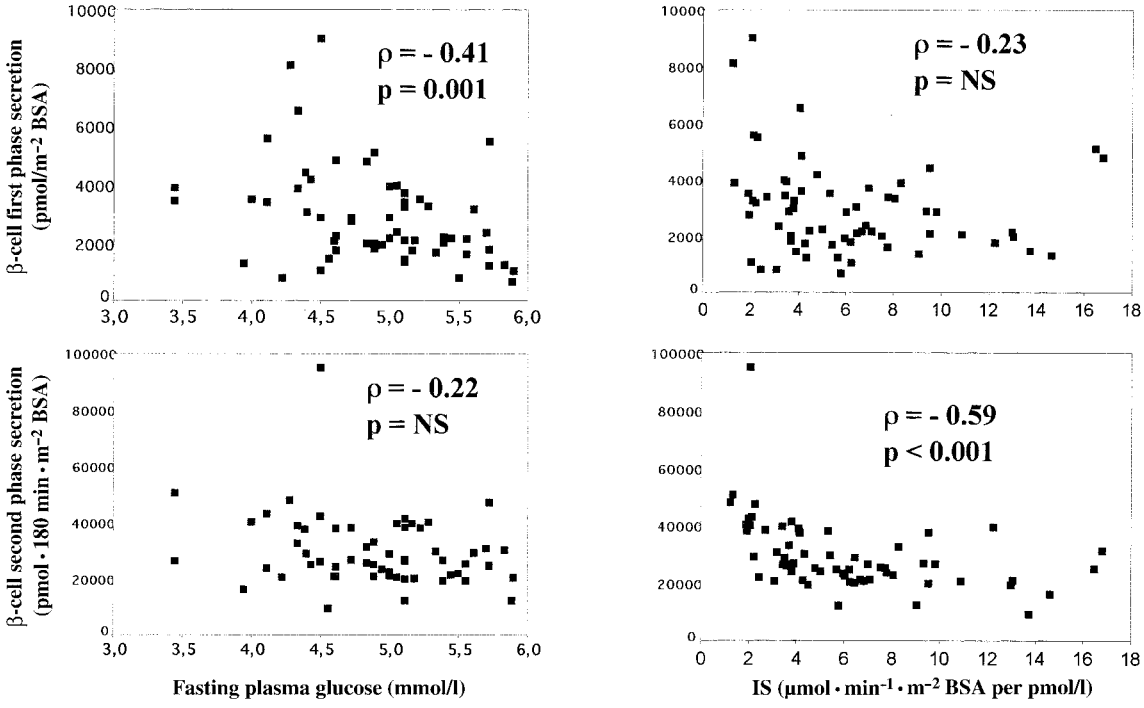

FIG. 2. Scatterplots of fasting plasma glucose (*x*-axes; *left panels*) and insulin sensitivity (*x*-axes; *right panels*) with β-cell first-phase secretion (*y*-axes; *upper panels*) and second-phase secretion (*y*-axes; *lower panels*) in data set A. ρ is Spearman's rank correlation coefficient.

TABLE 5
Nonparametric correlation matrix (Spearman's ρ) for data set B (n = 54)

	FPG	2-h PG	IS	1st PH-CONC	2nd PH-CONC	1st PH-SEC	β	δ	2nd PH-SEC
FPG	1								
2-h PG	0.50§	1							
IS	−0.33*	−0.29*	1						
1st PH-CONC	−0.17	−0.21	−0.34*	1					
2nd PH-CONC	0.09	−0.04	−0.68§	0.66§	1				
1st PH-SEC	−0.31*	−0.36#	0.05	0.70§	0.35#	1			
β	0.16	−0.07	−0.50§	0.42*	0.79§	0.43§	1		
δ	0.16	0.06	−0.19	−0.09	0.13	0.00	0.42#	1	
2nd PH-SEC	−0.02	−0.24	−0.39#	0.53§	0.78§	0.58§	0.88§	0.07	1
OGTT β-index	−0.28*	−0.39#	0.03	0.63§	0.27	0.57§	0.26	−0.04	0.32*

FPG, fasting plasma glucose; PG, plasma glucose; 1st PH-CONC, first-phase concentration; 2nd PH-CONC, second-phase concentration; 1st PH-SEC, first-phase secretion; 2nd PH-SEC, second-phase secretion. *$P < 0.05$; #$P < 0.01$; §$P < 0.001$.

relatives and individuals with one parent affected by type 2 diabetes. Importantly, OfT2D had normal glucose regulation. The average age was approximately one decade apart between the two databases, thereby possibly offering a cross-sectional view of two potentially distinct early stages in the natural history of type 2 diabetes.

Analogic minimal modeling of the OGTT was introduced and validated by us in a previous article (30). Minimal modeling of the hyperglycemic clamp, however, to the best of our knowledge, is unprecedented (see APPENDIX for details). In the present study, this approach was able to accommodate the entire course of insulin secretion during the hyperglycemic clamp (Fig. 1). Simpler (i.e., with less unknown parameters) models were unable to reasonably fit the data (data not shown). However, in both the case of the hyperglycemic clamp and the OGTT, as well as in the intravenous glucose tolerance test, the β-cell is modeled as if it were a relatively simple transducer (28–30). This kind of modeling exercise has its strength in being parsimonious and its limitation in being only an analogic model in which the relationship to the multiple physiological and molecular events underlying insulin secretion cannot be very close. Both in the OGTT and in the hyperglycemic clamp, the C-peptide kinetics was assumed on the basis of population-derived parameters (36), which are known to perform well also in the single individual (36,37). These analyses allowed us to compute first- and second-phase secretion, β and δ of β-cell second-phase secretion during the hyperglycemic clamp, as well as OGTT β-index (30). Thus, we could investigate some general physiological aspects of β-cell secretion.

OGTT β-index showed positive correlations with first-phase insulin concentration and secretion (Table 5), in line with our previous observation that it was correlated to acute insulin response during the intravenous glucose tolerance test (30). The reference indicators of glucose

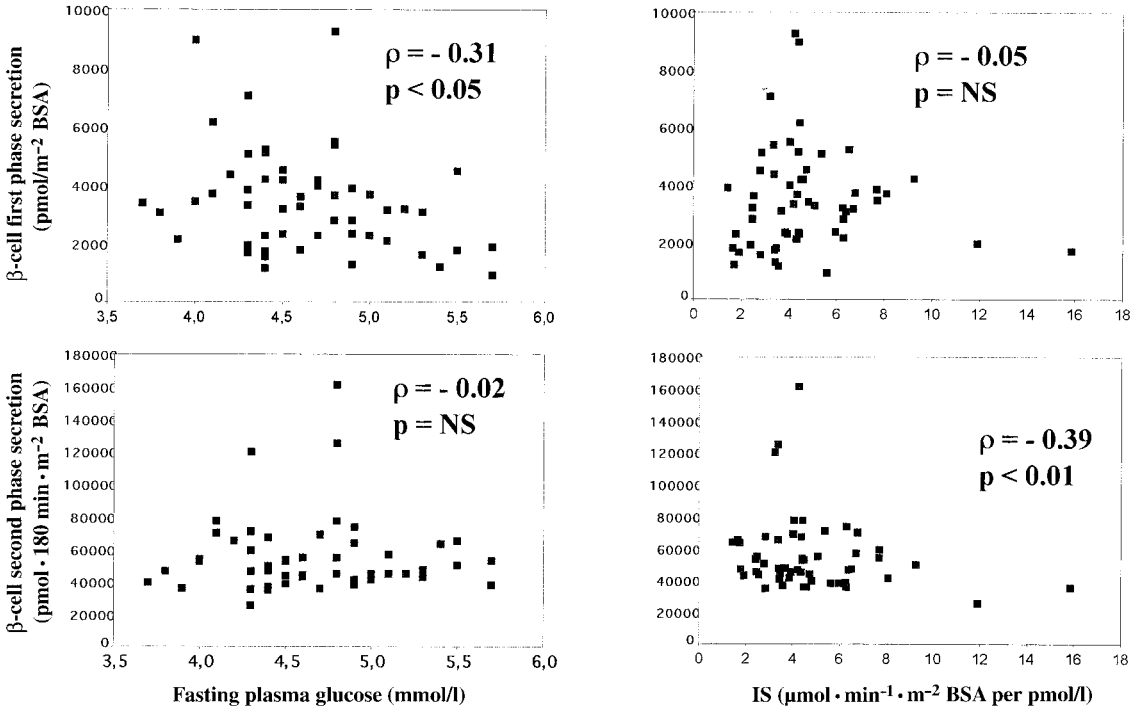

FIG. 3. Scatterplots of fasting plasma glucose (*x*-axes; *left panels*) and insulin sensitivity (*x*-axes; *right panels*) with β-cell first-phase secretion (*y*-axes; *upper panels*) and second-phase secretion (*y*-axes; *lower panels*) in data set B. ρ is Spearman's rank correlation coefficient.

TABLE 6
Subsets of control subjects and OfT2D with plasma glucose <6.0 mmol/l 2 h after the OGTT for data set B

	Control subjects (subgroup)	OfT2D (subgroup)	*P*
n (M/F)	20 (5/15)	18 (3/15)	
Age (years)	47.7 ± 1.7	44.6 ± 1.5	0.19
BMI	26.5 ± 0.8	26.5 ± 0.9	0.99
FPG	4.46 ± 0.08	4.61 ± 0.12	0.30
2-h PG	4.49 ± 0.16	4.83 ± 0.22	0.22
IS	4.55 ± 0.43	4.41 ± 0.52	0.91
1st PH-CONC	1,736 ± 119	1,505 ± 231	0.09
2nd PH-CONC	567 ± 77	449 ± 64	0.17
1st PH-SEC	4,459 ± 381	3174 ± 425	0.01
β	85.8 ± 8.6	69.7 ± 8.2	0.09
2nd PH-SEC	68,334 ± 7148	50,286 ± 2,956	0.02
OGTT β-INDEX	5.68 ± 0.15	5.11 ± 0.15	0.01

Data are means ± SD. FPG, fasting plasma glucose; PG, plasma glucose; 1st PH-CONC, first-phase concentration; 2nd PH-CONC, second-phase concentration; 1st PH-SEC, first-phase secretion; 2nd PH-SEC, second-phase secretion.

homeostasis, i.e., fasting and 2-h plasma glucose, showed negative correlations with first phase and OGTT β-index, but bore no relationship to second-phase insulin secretion (Figs. 2 and 3 and Tables 4 and 5). Conversely, in both databases IS was inversely related to the parameters of second-phase insulin secretion, but not to first phase or to OGTT β-index (Fig. 2 and 3 and Tables 4 and 5). Under the experimental conditions of prolonged hyperinsulinemia and hyperglycemia of the hyperglycemic clamp, IS is dependent only on peripheral IS, because endogenous glucose production is nil, or very close to it (38). At variance with findings from other laboratories (5,8,16), this negative correlation is present with second (or late)-phase insulin secretion, whereas it usually has been described with first-phase (or acute) insulin response, although some exception to this apparent rule exists (39). While the reasons for this discrepancy are not readily evident, it should be noted that we observed this relationship between IS and second- but not first-phase insulin secretion in two separate databases (Figs. 2 and 3). Thus, the finding of the present study may reflect a basic feature of the glucose/insulin system.

The negative relationship between plasma glucose and first-phase insulin secretion/OGTT β-index (Figs. 2 and 3 and Tables 4 and 5) may be a consequence of increasing glycemia, as a result of glucose toxicity (40), as well as a cause of increasing glycemia. Our data do not allow us to draw any firm conclusion. In any case, these indexes are the closest indicators of β-cell competence in glucose homeostasis.

These insights in glucose-regulated β-cell function were of special help in the analysis of the findings in OfT2D. In this regard, matching the groups for biographic and anthropometric data related to IS (Tables 2 and 3) is presumably important in revealing subtle differences in β-cell function between control subjects and OfT2D.

In data set A, composed of individuals in their thirties, a significant reduction in first-phase insulin concentration and a minor defect first-phase insulin secretion, which did not reach statistical significance (Table 2), were seen in the OfT2D. However, in data set B, which included subjects in their forties, OfT2D showed signs of impaired β-cell function during the OGTT and during both first- and

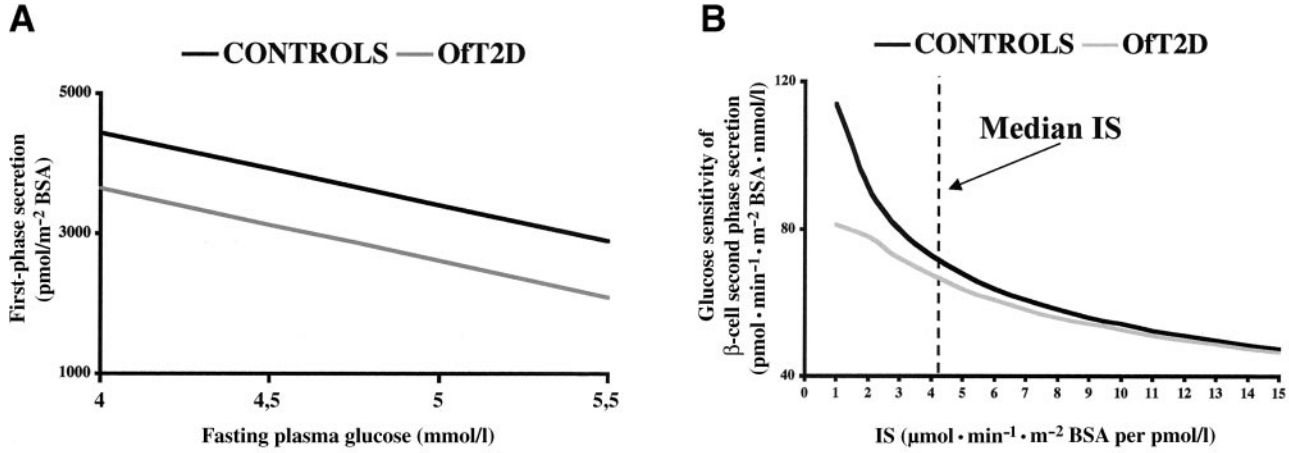

FIG. 4. *A*: Relationship between fasting plasma glucose (FPG; *x*-axis) and first-phase insulin secretion (1st PH-SEC; *y*-axis) in the pooled data (n = 114). The two lines are generated by the following equation (corrected r^2 = 0.19): 1st PS = A – fh · C – B · FPG, where fh = 0 in control subjects and fh = 1 in OfT2D, A = 8,566 (95% CI 5,923–11,209), B = 1,031 (470–1,593), and C = 801 (95% CI 184–1,418). *B*: Relationship between IS (*x*-axis) and glucose sensitivity of β-cell second-phase secretion (β; *y*-axis) in the pooled data (n = 114). The two curves are generated by the following equation (corrected r^2 = 0.13): $\beta = A \cdot (1 - fh \cdot C \cdot IS^{-1}) \cdot IS^{B}$, where fh = 0 in control subjects and fh = 1 in OfT2D, A = 114 (95% CI 81.7–199.6), B = −0.324 (−0.642 to −0.162), and C = 0.289 (0.00–0.71).

second-phase insulin secretion during the hyperglycemic clamp (Table 3).

Alterations in first-phase insulin secretion/β-index might reflect merely the difference in plasma glucose between control subjects and OfT2D in data set B for the following reasons: *1*) This finding was not replicated in data set A, where plasma glucose is similar in the two experimental groups (Table 2); *2*) in both databases first phase and plasma glucose are inversely related even within the range of normal glucose regulation (Figs. 2 and 3). Therefore, within database B, we selected a subgroup from the control subjects and a subgroup from the OfT2D with 2-h glucose <6 mmol/l with the aim to also match the plasma glucose levels and to be sure that all individuals had normal glucose tolerance, so that the so-called glucose toxicity could not be conceivably at work (Table 6). Even in this case OGTT β-index, first- and second-phase insulin secretion retained a statistically significant difference between control subjects and OfT2D (Table 6). Thus, we cannot simply conclude that the alterations in first-phase insulin secretion/OGTT β-index reflect a difference in plasma glucose.

The decrease in the second-phase secretion in OfT2D of data set B may be related to (older) age and being overweight. As a whole, subjects in data set B were older and somewhat fatter and more insulin-resistant than those in data set A (Tables 2 and 3). These aspects mirror the expected evolution of these parameters in the general population and point out that the individuals in data set B were more burdened with "metabolic stressors" than the individuals in data set A. These "metabolic stressors" may be responsible for unveiling alterations in β-cell function of OfT2D, even at a time when glucose regulation is still perfect. This is best appreciated by noting that, concomitantly with increasing age and BMI and decreasing IS, both first-phase secretion and β increase by ~30% between data set A and B (from 3,360 to 4,230, $P = 0.03$, and from 61.6 to 83.5, $P < 0.01$, respectively) in the control subjects but by only 10% in the OfT2D (from 2,587 to 2,900, $P = 0.31$, and from 62.4 to 67, $P = 0.85$, respectively).

These data have the limitation of being cross-sectional. The difference observed between the two data sets may be the result of a selection process, which has enriched the data set B with OfT2D least susceptible to develop diabetes, rather than of the natural history of glucose homeostasis in OfT2D. Furthermore, in the OfT2D subgroup matched for glucose levels, the impairment in β-cell function (Table 6) implies that some compensatory mechanisms are at work to maintain the same glucose homeostasis as in control subjects. Because peripheral IS is not increased, it may be speculated that IS of endogenous glucose production and/or glucose effectiveness at basal insulin may be higher in OfT2D than in control subjects, thereby counterbalancing decreased β-cell function. The experimental evidence on this issue still is controversial (41–43) and we did not assess either parameter in the present study. In either case, however, the resistance to develop diabetes cannot be attributed to β-cell function, which is less in the OfT2D than in the control subjects. Hence, the portrait of β-cell function herein outlined might still reflect its spontaneous evolution in OfT2D at risk of diabetes.

The analysis of the pooled data may further strengthen this interpretation. As a first step, no significant difference between the two data sets was detected in either the relationship between fasting plasma glucose and first phase or the relationship between IS and β (data not shown). Importantly, in both cases, OfT2D lay on a line significantly shifted to the left (Fig. 4). Thus, a FDR with low fasting plasma glucose and very high IS will have β-cell first- and second-secretion phases rather similar to his/her appropriate control subject, but as soon as plasma glucose increases, the relative difference in first-phase secretion widens, and, as soon as IS worsens, second-phase secretion departs more and more from the one seen in an individual with no family history of type 2 diabetes, both events favoring a further deterioration in glucose homeostasis. These two relationships (Fig. 4) unveil the existence of feed-forward mechanisms in the OfT2D, which, if the glucose/insulin system is put under stress, may be fundamental to start a relentless progression to diabetes. It is a legitimate concern that the two centers used different hormone assays, but both centers used external standards, which should help to minimize the potential differences between the assays. Moreover, use of a dummy variable for each center in the statistical analysis did not lead to different conclusions.

Our data are compatible with and expand the closed-loop hypothesis proposed by Bergman et al. (44), according to which type 2 diabetes is the result of a dysfunction of the closed-loop relationship between insulin resistance and insulin secretory response. OfT2D are characterized not only by a less steep power function linking IS and β-cell function (note that a hyperbola is a power function with exponent equal to −1), in close agreement with Bergman's tenet (44), but also by an apparently unfavorable relationship between plasma glucose and β-cell (Fig. 4).

A comparison between our findings and the findings in the Pima Indians progressing to diabetes may be useful. Also in that study, progressors to type 2 diabetes displayed an inappropriately low insulin secretion for their degree of IS, even when their glucose tolerance was preserved (5), thereby strengthening our assumption that our OfT2D can be considered prediabetic individuals. However, our OfT2D are to be considered very heterogeneous, at least as to their progression to diabetes, and, given the variability in other reports of FDR as to insulin secretion and sensitivity (7–15), they may not be necessarily representative of all FDR.

In summary, minimal model analysis of standard OGTTs and hyperglycemic clamps reveals that β-cell performance during the OGTT and first- and second-phase β-cell secretion during the hyperglycemic clamp bear distinct relationships to glucose levels and IS. Specifically, the relationship between β-cell first-phase secretion and fasting glucose is linear with a negative slope, whereas the relationship between β-cell second-phase secretion and IS is a power function. In both cases, OfT2D lie on distinct lines, significantly shifted to the left (Fig. 4). The stronger the "metabolic stressors" to which the OfT2D are exposed, the higher the likelihood of discovering defects in β-cell function, thereby allowing the identification of distinct stages in the natural history of glucose homeostasis in

OfT2D still within the class of normal glucose regulation. These distortions in the relationships between β-cell function and glucose/insulin action in OfT2D with still normal glucose regulation form a feed-forward mechanism, which in the presence of further, even mild, declines in IS and/or rises in glucose levels (Fig. 4) (be they due to acquired or genetic factors) (2–4,18–25) may lead to a further decline in the appropriateness of β-cell function and, hence, to a disruption in glucose homeostasis (5). Detection of distorted relationships between the glucose/insulin action system and β-cell function in OfT2D with still perfectly intact glucose regulation strongly suggests that at least some of their determinants are genetic and raises the possibility that prevention of diabetes should be targeted and tailored to distinct metabolic subphenotypes in this high-risk population.

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APPENDIX

Minimal model of insulin secretion. The analysis of the glucose and C-peptide curves during the hyperglycemic clamp follows the general strategy proposed by Toffolo et al. (28) for the intravenous glucose tolerance test with some slight changes. Briefly, C-peptide kinetics was assumed to be known in each subject, according to a two-compartmental model proposed by Polonsky et al. (45). Individual parameters are calculated from population data as indicated by Van Cauter et al. (36), according to sex, age, body surface area and presence/absence of obesity or type 2 diabetes.

C-peptide secretion is "appended" to this model of C-peptide kinetics and is assumed to consist of three components. The first component is constant throughout the experimental time and represents the basal C-peptide (insulin) secretion rate (CP-SR [0] in picomoles per minute):

$$\text{CP-SR (0)} = \text{basal C-peptide} \cdot K_{01} \cdot V_1$$

where K_{01} and V_1 are the rate constant and the volume of the accessible compartment of C-peptide, respectively.

The second component is the first-phase secretion, which is described as a square wave C-peptide (insulin) infusion, in which the value is CP-SR_1 (in picomoles per minute), starting at time = t_1 and ending at time = t_2, in response to the sudden hyperglycemic stimulus.

The third component is the second-phase secretion, which starts at time = 0 min and is described by the following equations:

$$\text{CP-SR}_2(t) = \delta^{-1} \cdot X(t)$$
$$dX(t)/dt = -\delta^{-1} \cdot X(t) + \beta \cdot [G(t) - Gb]$$
$$X(0) = 0$$

where $\text{CP-SR}_2(t)$ is the secretion rate (in picomoles per minute) of C-peptide in response to glucose, δ (in minutes) is the apparent time constant with which the amount of C-peptide made available for secretion is released into the circulation, X(t) is the amount of C-peptide made available for secretion by the glucose stimulus, β ([picomole per minute] per [millimole per liter]) is the slope of the straight line relating plasma glucose (in millimoles liter) to the provision of C-peptide (in picomoles per minute) to the secretory process, G(t) is the prevailing plasma glucose level (in millimoles per liter), and Gb is glucose level at baseline, which is assumed to be equal to the threshold (in millimoles per liter) above which glucose-stimulated insulin secretion starts.

The equations describing C-peptide mass in the accessible compartment (CP_1) during the hyperglycemic clamp, therefore, are as follows:

$$dCP_1(t)/dt = \text{CP-SR (0)} + \text{CP-SR}_1 + \text{CP-SR}_2(t) - [(k_{01} + k_{21}) \cdot CP_1(t)] + k_{12} \cdot CP_2(t)] \text{ for } t_1 \le t \le t_2$$

$$dCP_1(t)/dt = \text{CP-SR (0)} + \text{CP-SR}_2(t) - [(k_{01} + k_{21}) \cdot CP_1(t)] + k_{12} \cdot CP_2(t)] \text{ for } t < t_1 \text{ or } t > t_2$$

in which the k's are the rate constants of the C-peptide model and CP_2 is C-peptide mass in the remote compartment. Time t_1 and t_2 are found empirically by an iterative systematic search at discrete intervals of 0.1 min of the pair of values minimizing the Akaike information criterion (46) of the curve-fitting process performed with the SAAM II software in each study. The Akaike information criterion varies according to *1*) the number of the adjustable parameters, *2*) the total number of data points, and *3*) the objective function that is minimized during the optimization of fitting. Thus, in each study, the pair of t_1 and t_2 that minimizes the Akaike information criterion is the one associated with the lowest value of the objective function, i.e., with the best model fit of the data. The average interval $t_2 - t_1$ was 2.61 ± 0.17 min in the pooled data set (n = 114).

The parameters defining β-cell response during the hyperglycemic clamp are as follows:

1) First-phase secretion equals $\text{CP-SR}_1 \cdot (t_2 - t_1)$, in picomoles.

2) β, (in [picomoles per minute] per [millimoles per liter]), which is the sensitivity of β-cell to glucose during the second-phase secretion.

3) δ, the apparent delay or time constant (in minutes) of second-phase secretion.

4) The amount of C-peptide (insulin) secreted during the second phase, which, since the glucose stimulus is identical in all individuals, is a global measure of second-phase secretion (in picomoles per 120 or 180 min):

$$\text{second-phase secretion} = \text{CP-SR2}(t)$$

Except for δ, all parameters are then normalized per BSA.

REFERENCES

1. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM. In *International Textbook of Diabetes Mellitus*. 2nd ed. Alberti KGMM,

DeFronzo RA, Zimmet P, Honor KH, Ed. Chichester, UK, John Wiley & Sons, 1997, p. 635–712
2. Bell GI, Polonsky KS: Diabetes mellitus and genetically programmed defects in beta-cell function. *Nature* 414:788–791, 2001
3. Gerich JE: The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. *Endocr Rev* 19:491–503, 1998
4. Ferrannini E: Insulin resistance versus insulin deficiency in non-insulin-dependent diabetes mellitus: problems and prospects. *Endocr Rev* 19:477–490, 1998
5. Weyer C, Bogardus C, Mott DM, Pratley RE: The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* 104:787–794, 1999
6. Pratley RE, Weyer C: The role of impaired early insulin secretion in the pathogenesis of type 2 diabetes mellitus. *Diabetologia* 44:929–945, 2001
7. Ericksson J, Franssila-Kallunki A, Ekstrand A, Saloranta C, Widen E, Schalin C, Groop L: Early metabolic defects in persons at increased risk for non-insulin dependent diabetes mellitus. *N Engl J Med* 321:337–343, 1989
8. Johnston C, Ward WK, Beard JC, McKnight B, Porte D Jr: Islet function and insulin sensitivity in the non-diabetic offspring of conjugal type 2 diabetic patients. *Diabet Med* 7:119–125, 1990
9. 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* 41:1575–1586, 1992
10. Pimenta W, Korytkowski M, Mitrakou A, Jenssen T, Yki-Jarvinen H, Evron W, Dailey G, Gerich J: Pancreatic beta-cell dysfunction as the primary genetic lesion in NIDDM: evidence from studies in normal glucose tolerant individuals with a first degree relative NIDDM relative. *JAMA* 273:1855–1861, 1995
11. Perseghin G, Ghosh S, Gerow K, Shulman GI: Metabolic defects in lean nondiabetic offspring of NIDDM parents: a cross-sectional study. *Diabetes* 46:1001–1009, 1997
12. Van Haeften TW, Dubbeldam S, Zonderland ML, Erkelens DW: Insulin secretion in normal glucose-tolerant relatives of type 2 diabetic subjects: assessments using hyperglycemic glucose clamps and oral glucose tolerance tests. *Diabetes Care* 21:278–282, 1998
13. Laakso 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. *J Clin Invest* 101:86–96, 1998
14. Nyholm B, Walker M, Gravholt CH, Shearing PA, Sturis J, Alberti KG, Holst JJ, Schmitz O: Twenty-four-hour insulin secretion rates, circulating concentrations of fuel substrates and gut incretin hormones in healthy offspring of type II (non-insulin-dependent) diabetic parents: evidence of several aberrations. *Diabetologia* 42:1314–1323, 1999
15. Zonderland ML, Dubbeldam S, Erkelens DW, van Haeften TW: Lower beta-cell secretion in physically active first-degree relatives of type 2 diabetes patients. *Metabolism* 49:833–838, 2000
16. Bergman RN, Phillips LS, Cobelli C: Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *J Clin Invest* 68:1456–1467, 1981
17. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP, Porte D Jr: Quantification of the relationship between insulin sensitivity and β-cell function in human subjects. *Diabetes* 42:1663–1672, 1993
18. Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE, del Bosque-Plata L, Horikawa Y, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, Bell GI: Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 26:163–175, 2000
19. Mahtani MM, Widen E, Lehto M, Thomas J, McCarthy M, Brayer J, Bryant B, Chan G, Daly M, Forsblom C, Kanninen T, Kirby A, Kruglyak L, Munnelly K, Parkkonen M, Reeve-Daly MP, Weaver A, Brettin T, Duyk G, Lander ES, Groop LC: Mapping of a gene for type 2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. *Nat Genet* 14:90–94, 1996
20. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES: The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76–80, 2000
21. Baier LJ, Permana PA, Yang X, Pratley RE, Hanson RL, Shen GQ, Mott D, Knowler WC, Cox NJ, Horikawa Y, Oda N, Bell GI, Bogardus C: A calpain-10 gene polymorphism is associated with reduced muscle mRNA levels and insulin resistance. *J Clin Invest* 106:R69–R73, 2000
22. Stumvoll M, Fritsche A, Volk A, Stefan N, Madaus A, Maerker E, Teigeler A, Koch M, Machicao F, Haring H: The Gly972Arg polymorphism in the insulin receptor substrate-1 gene contributes to the variation in insulin secretion in normal glucose-tolerant humans. *Diabetes* 50:882–885, 2001
23. Fritsche A, Madaus A, Renn W, Tschritter O, Teigeler A, Weisser M, Maerker E, Machicao F, Haring H, Stumvoll M: The prevalent Gly1057Asp polymorphism in the insulin receptor substrate-2 gene is not associated with impaired insulin secretion. *J Clin Endocrinol Metab* 86:4822–4825, 2001
24. Jacob S, Stumvoll M, Becker R, Koch M, Nielsen M, Loblein K, Maerker E, Volk A, Renn W, Balletshofer B, Machicao F, Rett K, Haring HU: The PPARgamma2 polymorphism Pro12Ala is associated with better insulin sensitivity in the offspring of type 2 diabetic patients. *Horm Metab Res* 32:413–416, 2000
25. Stumvoll M, Fritsche A, Madaus A, Stefan N, Weisser M, Machicao F, Haring H: Functional significance of the UCSNP-43 polymorphism in the CAPN10 gene for proinsulin processing and insulin secretion in nondiabetic Germans. *Diabetes* 50:2161–2163, 2001
26. World Health Organization Consultation Group: *Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications: Report of a WHO Consultation*. Part 1. Geneva, World Health Org., 1999 (WHO/NCD/NCS/99.2)
27. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and insulin resistance. *Am J Physiol* 237: E214–E223, 1979
28. Toffolo G, De Grandi F, Cobelli C: Estimation of β-cell sensitivity from intravenous glucose tolerance test C-peptide data: knowledge of kinetics avoids errors in modeling the secretion. *Diabetes* 44:845–854, 1995
29. Monauni T, Zenti MG, Cretti A, Daniels MC, Targher G, Caruso B, Caputo M, McClain D, Del Prato S, Giaccari A, Muggeo M, Bonora E, Bonadonna RC: Effects of glucosamine infusion on insulin secretion and insulin action in humans. *Diabetes* 49:926–935, 2000
30. Cretti A, Lehtovirta M, Bonora E, Brunato B, Zenti MG, Tosi F, Caputo M, Caruso B, Groop LC, Muggeo M, Bonadonna RC: Assessment of β-cell function during the oral glucose tolerance test by a minimal model of insulin secretion. *Eur J Clin Invest* 31:405–416, 2001
31. Mittelman SD, Van Citters GW, Kim SP, Davis DA, Dea MK, Hamilton-Wessler M, Bergman RN: Longitudinal compensation for fat-induced insulin resistance includes reduced insulin clearance and enhanced beta-cell response. *Diabetes* 49:2116–2125, 2000
32. Bonadonna R, Groop L, Kraemer N, Ferrannini E, Del Prato S, DeFronzo RA: Obesity and insulin resistance in man: a dose response study. *Metabolism* 39:452–458, 1990
33. Fritsche A, Stefan N, Hardt E, Häring H, Stumvoll M: Characterisation of beta-cell dysfunction of impaired glucose tolerance: evidence for impairment of incretin-induced insulin secretion. *Diabetologia* 43:852–858, 2000
34. Hart LM, Dekker JM, van Haeften TW, Ruige J, Stehouwer CDA, Erkelens DW, Heine RJ, Maassen JA: Reduced second phase insulin secretion in carriers of a sulphonylurea receptor gene variant associating with type 2 diabetes mellitus. *Diabetologia* 43:515–519, 2000
35. Van Haeften TW, W Pimenta, A Mitrakou, M Korytkowski, T Jenssen, H Yki-Jarvinen, JE Gerich: Relative contributions of beta-cell function and tissue insulin sensitivity to fasting and post-glucose-load glycemia. *Metabolism* 49:1318–1325, 2000
36. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368–377, 1992
37. Hovorka R, Koukkou E, Southerden D, Powrie JK, Young MA: Measuring pre-hepatic insulin secretion using a population model of C-peptide kinetics: accuracy and required sampling schedule. *Diabetologia* 41:548–554, 1998
38. DeFronzo RA, Ferrannini E, Hendler, R. Felig P, Wahren J: Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia. *Diabetes* 32:35–45, 1983
39. Lehtovirta M, Kaprio J, Forsblom C, Ericksson J, Tuomilehto J, Groop L: Insulin sensitivity and secretion in monozygotic and dizygotic twins. *Diabetologia* 43:285–293, 2000
40. Rossetti L, Giaccari A, DeFronzo RA: Glucose toxicity. *Diabetes Care* 13:610–630, 1990
41. Henriksen JE, Alford F, Handberg A, Vaag A, Ward GM, Kalfas A, Beck-Nielsen H: Increased glucose effectiveness in normoglycemic but insulin-resistant relatives of patients with non-insulin-dependent diabetes mellitus: a novel compensatory mechanism. *J Clin Invest* 94:1196–1204, 1994
42. Henriksen JE, Levin K, Thye-Ronn P, Alford F, Hother-Nielsen O, Holst JJ,

Beck-Nielsen H: Glucose-mediated glucose disposal in insulin-resistant normoglycemic relatives of type 2 diabetic patients. *Diabetes* 49:1209–1218, 2000
43. Nielsen MF, Nyholm B, Caumo A, Chandramouli V, Schumann WC, Cobelli C, Landau BR, Rizza RA, Schmitz O: Prandial glucose effectiveness and fasting gluconeogenesis in insulin-resistant first-degree relatives of patients with type 2 diabetes. *Diabetes* 49:2135–2141, 2000
44. Bergman RN, Ader M, Huecking K, Van Citters G: Accurate assessment of β-cell function: the hyperbolic correction. *Diabetes* 51 (Suppl. 1):S212–S220, 2002
45. Polonsky KS, Licinio-Paixao J, Given BD, Pugh W, Rue P, Galloway J, Karrison T, Frank B: Use of biosynthetic human C-peptide in the measurement of insulin secretion rates in normal volunteers and type I diabetic patients. *J Clin Invest* 77:98–105, 1986
46. Akaike H: A new look at the statistical model identification. *IEEE Trans Autom Control* 19:716–723, 1974