

# $\beta$ -Cell Function in Subjects Spanning the Range from Normal Glucose Tolerance to Overt Diabetes: A New Analysis

Ele Ferrannini, Amalia Gastaldelli, Yoshinori Miyazaki, Masafumi Matsuda, Andrea Mari, and Ralph A. DeFronzo

Diabetes Division (E.F., Y.M., M.M., R.A.D.), Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78229-3900; Metabolism Unit (E.F., A.G.), Department of Internal Medicine and Consiglio Nazionale delle Ricerche Institute of Clinical Physiology, University of Pisa School of Medicine, 56126 Pisa, Italy; and Consiglio Nazionale delle Ricerche Institute of Biomedical Engineering (A.M.), 35127 Padova, Italy

The nature of the progressive  $\beta$ -cell failure occurring as normal glucose tolerant (NGT) individuals progress to type 2 diabetes (T2DM) is incompletely understood. We measured insulin sensitivity (by a euglycemic insulin clamp) and insulin secretion rate (by deconvolution of plasma C-peptide levels during an oral glucose tolerance test) in 188 subjects [19 lean NGT (body mass index [BMI]  $\leq 25$  kg/m<sup>2</sup>), 42 obese NGT, 22 BMI-matched impaired glucose tolerance [IGT], and 105 BMI-matched T2DM]. Main determinants of  $\beta$ -cell function on the oral glucose tolerance test were derived from a mathematical model featuring the following: 1) glucose concentration-insulin secretion dose response (glucose sensitivity), 2) a secretion component proportional to the derivative of plasma glucose concentration (rate sensitivity); and 3) a potentiation factor. When NGT and T2DM were subgrouped by 2-h plasma glucose concentrations, insulin secretion rate revealed an inverted U-shaped pattern, rising through NGT up to IGT and falling off thereafter. In contrast,  $\beta$ -cell glucose sensitivity dropped in a monophasic, curvilinear fash-

ion throughout the range of 2-h plasma glucose. Within the NGT range (2-h glucose of 4.1–7.7 mmol/liter),  $\beta$ -cell glucose sensitivity declined by 50–70% ( $P < 0.02$ ). Insulin sensitivity decreased sharply in the transition from lean to obese NGT and then declined further in IGT and mild T2DM to level off in the higher three quartiles of diabetic hyperglycemia. In T2DM, defective  $\beta$ -cell potentiation and rate sensitivity also emerged ( $P \leq 0.05$ ). In the whole data set, insulin sensitivity and the dynamic parameters of  $\beta$ -cell function explained 89% of the variability of 2-h plasma glucose levels. The following conclusions were reached: 1)  $\beta$ -cell glucose sensitivity falls already within the NGT range in association with rising 2-h plasma glucose concentrations, although absolute insulin secretion rates increase; and 2) throughout the glucose tolerance range, dynamic parameters of  $\beta$ -cell function (glucose sensitivity, rate sensitivity, and potentiation) and insulin sensitivity are independent determinants of 2-h plasma glucose levels. (*J Clin Endocrinol Metab* 90: 493–500, 2005)

MAINTENANCE OF NORMAL glucose tolerance is dependent on the finely tuned balance between insulin secretion and insulin action (1, 2). Among subjects with normal glucose tolerance (NGT), insulin sensitivity varies over a 6- to 7-fold range (3). Nonetheless, glucose tolerance remains normal because the  $\beta$ -cell is able to adjust its secretion of insulin to compensate for the existing level of tissue insensitivity to the hormone. Numerous studies, using a variety of techniques, have demonstrated that individuals with impaired glucose tolerance (IGT) and overt type 2 diabetes (T2DM) are characterized by moderate to severe insulin resistance (1, 4–7). However, a thorough evaluation of  $\beta$ -cell function during the transition from NGT to IGT to T2DM has yet to be undertaken. In an early study by Brunzell *et al.* (8), the acute (3–5 min) incremental plasma insulin response to iv glucose tended to be reduced in subjects ( $n = 7$ ) with

fasting glucose levels between 5.6 and 6.3 mmol/liter in comparison with subjects whose fasting glycemia was less than 5.56 mmol/liter. In that study, the small sample size precluded appropriate statistical analysis, and insulin sensitivity was not measured. On the other hand, Tripathy *et al.* (9) found insulin resistance (by the homeostasis model assessment index) but no evidence of  $\beta$ -cell dysfunction in subjects with impaired fasting glycemia (or a fasting glucose between 6.1 and 6.9 mmol/liter). In contrast, another study using homeostasis model assessment reported normal insulin sensitivity but defective insulin secretion in impaired fasting glycemia (10). Polonsky *et al.* (11) used graded glucose infusions with C-peptide deconvolution to study insulin secretion in IGT and T2DM subjects. Compared with controls, both IGT and diabetic subjects manifested a slower rise in insulin secretion as a function of rising plasma glucose concentration, with a greater defect in the diabetic compared with IGT subjects. Because insulin sensitivity was not measured in these studies, the insulin secretory rate could not be related to the severity of underlying insulin resistance. In prospective studies, insulin resistance has been shown to be a strong predictor of incident diabetes in both monkeys (12) and humans (13, 14). Later analyses of prospective data from Pima Indians indicated that both insulin resistance and in-

First Published Online October 13, 2004

Abbreviations: BMI, Body mass index; FFA, free fatty acid; FFM, fat-free mass; GLP-1, glucagon-like peptide-1-(7–36)-amide; IGT, impaired glucose tolerance; ISR, insulin secretion rate; NGT, normal glucose tolerant; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes.

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

sulin secretory dysfunction are independent predictors of worsening glucose tolerance (15–17).

The relevance of acute insulin response to iv glucose to the  $\beta$ -cell response to an oral glucose load is, however, unclear. Recent studies documented the importance of decreased circulating levels and/or resistance to the stimulatory effect of incretins, glucagon-like peptide-1-(7–36)-amide (GLP-1) and glucose-dependent insulinotropic peptide, in the impairment in insulin secretion in diabetic individuals (18–25). Thus, the phenomenon of  $\beta$ -cell potentiation, which primarily is dependent on incretins, cannot be evaluated during iv glucose administration. Moreover, the precise level of glucose intolerance at which  $\beta$ -cell function begins to decline has not been established. The diagnostic criteria (26) for IGT are not defined on the basis of any pathophysiologic abnormalities, *i.e.* decreased insulin sensitivity and impaired insulin secretion. Thus, it is possible that the decline in  $\beta$ -cell function occurs at an earlier stage than IGT, *i.e.* in individuals who are considered to have NGT according to current diagnostic criteria.

*In vitro* studies have contributed greatly to our current understanding of the factors that regulate the  $\beta$ -cell's secretory response to a glucose stimulus (27). Four factors have been shown to play a major role: 1) glucose sensitivity, which reflects the ability of the  $\beta$ -cell to respond to changes in the plasma glucose concentration (in absolute terms); 2) rate sensitivity, which reflects the ability to respond to the rate of change in plasma glucose concentration; 3) potentiation, which explains the well-documented observation that the insulin secretory response to a glucose challenge is greater in the presence of potentiating factors; and 4) in an *in vivo* context, insulin resistance and aspects of the insulin secretory response are influenced by the severity of the underlying insulin resistance (28). No previous study has simultaneously quantitated, in individuals spanning the entire range of glucose tolerance, tissue sensitivity to insulin and the principal determinants of  $\beta$ -cell function. In the present study, we employed the euglycemic clamp technique (29) and the oral glucose tolerance analyzed with a validated  $\beta$ -cell model of insulin secretion (30, 31) to provide a detailed quantitative analysis of  $\beta$ -cell function in subjects with a wide range of glucose tolerance.

## Subjects and Methods

### Subjects

The study group included 188 subjects recruited at the Clinical Research Center of the University of Texas Health Science Center (San Antonio, TX) through advertising within the medical center and in local newspapers. Subjects responding to the advertisement were screened by a 75-g oral glucose tolerance test (OGTT) and were invited to participate in the insulin clamp study. The studies were conducted over a period of 6 yr, during which nondiabetic and diabetic subjects, Mexican-American or Caucasian, were studied in no particular order. Based on the OGTT, subjects were classified as having NGT (*i.e.* fasting glucose < 6.1 mmol/liter and 2-h glucose < 7.8 mmol/liter,  $n = 61$ ), IGT (*i.e.* fasting glucose < 7 mmol/liter and 2-h glucose between 7.8 and 11.1 mmol/liter,  $n = 22$ ), or T2DM (*i.e.* fasting glucose > 7 mmol/liter or 2-h glucose  $\geq$  11.1 mmol/liter,  $n = 105$ ) according to the American Diabetes Association criteria (26). Seven of the IGT subjects also had impaired fasting glucose (*i.e.* fasting glucose between 6.1 and 7.0 mmol/liter). The NGT group was further subdivided into lean [*i.e.* a body mass index (BMI)  $\leq$  25 kg/m<sup>2</sup>,  $n = 19$ ] and overweight/obese controls (BMI > 25

kg/m<sup>2</sup>,  $n = 42$ ). Of the study population, 76% were Mexican-American, and the remainder were of Caucasian descent. This ethnic distribution reflects the population in the San Antonio area. Model-derived data from 43 NGT and 22 IGT subjects have been included in a previous paper (7).

All subjects had normal liver, cardiopulmonary, and kidney function as determined by medical history, physical examination, screening blood tests, electrocardiogram, and urinalysis. No NGT or IGT subject was taking any medication known to affect glucose tolerance. T2DM subjects taking sulfonylureas or metformin (35%) had their oral hypoglycemic agent discontinued 3 d before the study. No diabetic subject had received treatment with a thiazolidinedione or insulin. None of the subjects participated in any regular physical activity program. Body weight was stable ( $\pm 2$  kg) for at least 3 months before study in all subjects. The study protocol was approved by the Institutional Review Board of the University of Texas Health Science Center, San Antonio, and informed written consent was obtained from all subjects before their participation.

### Anthropometric measurements

The waist circumference was determined by measuring the circumference at the narrowest part of the torso.

### Metabolic measurements

All metabolic tests were performed at the clinical research center in the morning (0700–0800 h) after a 10- to 12-h, overnight fast. For the OGTT, blood samples were collected at –30, –15, 0, 30, 60, 90, and 120 min for the measurement of plasma glucose, C-peptide, insulin, and free fatty acid (FFA) concentrations. At time 0, subjects also received 100  $\mu$ Ci of <sup>3</sup>H<sub>2</sub>O, and plasma samples for <sup>3</sup>H<sub>2</sub>O radioactivity were obtained at 100, 110, and 120 min for determination of fat-free mass (FFM) (33). Before the start of the insulin clamp, all subjects received a primed (20–25  $\mu$ Ci) continuous (0.20–0.25  $\mu$ Ci/min) infusion of 3-[<sup>3</sup>H]glucose (DuPont NEN Life Science Products, Boston, MA), which was continued for 2 h (3 h in diabetics). In diabetic subjects the tracer prime was increased in proportion to the increase in fasting plasma glucose as previously described (7). After the basal tracer equilibration period, subjects received a primed-continuous insulin infusion at the rate of 240 pmol·min<sup>–1</sup>·m<sup>–2</sup> for 120 min. During the last 30 min of the basal equilibration period, plasma samples were taken at 5- to 10-min intervals for the determination of plasma glucose and insulin concentrations and tritiated glucose radioactivity. During insulin infusion, plasma glucose concentration was measured every 5 min, and a variable infusion of 20% glucose was adjusted, based on the negative feedback principle, to maintain the plasma glucose concentration at each subject's fasting plasma glucose level with a coefficient of variation less than 5%. In the diabetic group, the plasma glucose concentration was allowed to decline to 5.6 mmol/liter, at which level it was clamped. Plasma samples were collected every 15 min from 0 to 90 min and every 5–10 min from 90 to 120 min for the determination of plasma glucose and insulin concentrations.

### Analytical techniques

Plasma glucose was measured by the glucose oxidase reaction (glucose analyzer, Beckman, Fullerton, CA). Plasma insulin and C-peptide concentrations were measured by RIA using specific kits (Linco Research, St. Louis, MO). Plasma 3-[<sup>3</sup>H]glucose radioactivity was measured in Somogyi precipitates as previously described (7).

### Data analysis

The rate of glucose disappearance (M value) during the insulin clamp was determined by adding the rate of residual endogenous glucose production (as calculated from the tracer glucose data) (34) to the exogenous glucose infusion rate during the last 30 min of the insulin clamp and was expressed per kilogram of FFM (35).

Insulin secretion rates were calculated from plasma C-peptide concentrations by deconvolution, as previously described (36). Parameters of  $\beta$ -cell function were derived from mathematical analysis of plasma glucose and C-peptide concentrations during the OGTT, according to a previously developed model (30, 31). According to this approach, glu-

cose-stimulated insulin secretion is the sum of two components, according to the equation:

$$S(t) = S_g(t) + S_d(t)$$

The first component,  $S_g(t)$ , represents the dependence of insulin secretion on the absolute glucose concentration ( $G$ ) at any time point and is characterized by a dose-response function,  $f(G)$ , relating these variables. A characteristic parameter of the dose response is its mean slope in the observed glucose concentration range, denoted as  $\beta$ -cell glucose sensitivity. The dose response is modulated by a potentiation factor,  $P(t)$ , which incorporates glucose-mediated and non-glucose-mediated potentiation (*i.e.* by nonglucose substrates, gastrointestinal hormones, and neurotransmitters):

$$S_g(t) = P(t)f(G)$$

Potentiation is a time-dependent phenomenon (20, 21). The potentiation factor is therefore modeled as a positive function of time and averages 1 during the experiment. The potentiation parameter used for the present analysis is the ratio of the potentiation factor at the end of the OGTT (100–120 min) to the one at the beginning of the OGTT (0–20 min).

The second insulin secretion component (denoted as rate sensitivity and represented as a function of the glucose concentration derivative,  $S_d(t) = kd \cdot dG(t)/dt$ ) is proportional to the rate of change of plasma glucose concentration during the OGTT and accounts for the observation that rapid changes in glucose concentration enhance insulin release.

Areas under glucose and insulin concentration curves were calculated by the trapezoidal rule. The insulinogenic index was calculated as the ratio of the insulin concentration increment to the glucose concentration increment at 30 min into the OGTT. The ability of the  $\beta$ -cell to compensate for the extant degree of insulin resistance was estimated by calculating the product of the  $M$  value by the insulinogenic index, analogous to the disposition index proposed by Kahn *et al.* (37).

### Statistical analysis

Anthropometric data are given as the mean  $\pm$  SEM. Plasma glucose concentrations and all insulin parameters were nonnormally distributed in this group of subjects; they are therefore given as the median [interquartile range] in tables and text and plotted as the median and SEM in the figures. Categorical variables were compared by the  $\chi^2$  test. Univariate associations were tested with the use of Spearman rank correlation. Group values were compared by ANOVA, with between-group values then compared by Bonferroni-Dunn *post hoc* analysis. The contribution of multiple factors to fasting or 2-h plasma glucose levels was assessed by multiple regression using general linear models with both continuous and categorical variables as independent variables. For both ANOVA and multiple regression, variables with nonnormal distribution were log transformed.

## Results

### Clinical characteristics

Genders were equally represented across diagnostic groups (Table 1). Age was progressively higher from NGT through IGT to diabetes ( $P < 0.0001$ ), whereas BMI was not significantly different among obese NGT, IGT, and T2DM, in all of whom it was significantly higher than in lean controls. Waist circumference tended to be higher in IGT and T2DM than in obese NGT and lowest in lean NGT subjects. Fasting plasma glucose concentrations were higher in T2DM than in all other groups, in whom they did not differ significantly from one another. At 2 h after glucose ingestion, plasma glucose levels were higher in T2DM, IGT, and obese NGT subjects, compared with the lean NGT group.

### Insulin secretion, insulin sensitivity, and $\beta$ -cell function

Fasting plasma insulin concentrations and insulin secretion rates (ISRs) rose through lean NGT, obese NGT, and IGT to plateau in T2DM (Table 2). In contrast, insulin area under the curve, the insulinogenic index, and total insulin output increased from lean NGT to obese NGT, plateaued in IGT, and decreased in T2DM. Insulin sensitivity declined across the four groups, whereas the product insulin sensitivity  $\times$  insulinogenic index [an equivalent of the disposition index according to Kahn *et al.* (37)] was lower in T2DM than in any other group.  $\beta$ -Cell glucose sensitivity did not differ significantly between lean and obese NGT but was significantly lower in both IGT and T2DM. Rate sensitivity was significantly depressed in only T2DM, whereas the potentiation factor decreased across the four groups.

To enhance discrimination among degrees of glucose tolerance, the obese NGT group was subdivided into tertiles, and the T2DM group into quartiles, of fasting or 2-h plasma glucose concentrations. As displayed in Fig. 1, both fasting ISRs and total insulin output changed in an irregular fashion as a function of the corresponding plasma glucose concentrations, the former rising to a plateau at approximately 8 mmol/liter, the latter changing in an approximately inverted U manner (with a maximum at  $\sim 10$  mmol/liter). In contrast,  $\beta$ -cell glucose sensitivity dropped in a monotonic curvilinear fashion throughout the range of 2-h plasma glucose levels ( $P < 0.02$  for changes within the obese NGT group alone)

**TABLE 1.** Clinical characteristics

	NGT		IGT	T2DM
	Lean	Obese		
n	19	42	22	105
Gender (F/M)	9/10	26/16	13/9	52/53
Age (yr)	35 $\pm$ 2	37 $\pm$ 2	42 $\pm$ 3	53 $\pm$ 1 <sup>a</sup>
Diabetes duration (yr)				3.1 $\pm$ 0.4
BMI (kg·m <sup>-2</sup> )	23.4 $\pm$ 0.4	30.6 $\pm$ 0.9 <sup>b</sup>	31.7 $\pm$ 0.9 <sup>b</sup>	31.8 $\pm$ 0.6 <sup>b</sup>
Fat-free mass (kg)	48 $\pm$ 1	51 $\pm$ 2	51 $\pm$ 2	53 $\pm$ 1 <sup>b</sup>
Fat mass (%)	28 $\pm$ 2	38 $\pm$ 1 <sup>b</sup>	39 $\pm$ 1 <sup>b</sup>	38 $\pm$ 1 <sup>b</sup>
Waist (cm)	76 $\pm$ 2	94 $\pm$ 2 <sup>b</sup>	104 $\pm$ 3 <sup>b</sup>	104 $\pm$ 1 <sup>b</sup>
Fasting plasma G (mmol/liter)	5.00 [0.34]	5.25 [0.50]	5.58 [0.65]	9.81 [4.04] <sup>a</sup>
2-h plasma G (mmol/liter)	5.08 [1.90]	6.52 [1.44] <sup>a</sup>	8.52 [1.44] <sup>a</sup>	18.26 [5.29] <sup>a</sup>

Data in brackets represent interquartile range.

<sup>a</sup>  $P < 0.05$  or less vs. every other group.

<sup>b</sup>  $P < 0.0001$  vs. lean NGT.

**TABLE 2.** Insulin parameters

	NGT		IGT	T2DM
	Lean	Obese		
Fasting plasma insulin (pmol/liter)	20 [16] <sup>a</sup>	48 [32] <sup>b</sup>	74 [47] <sup>b</sup>	94 [58] <sup>c</sup>
Insulin AUC (nmol/liter-h)	22 [15]	45 [38] <sup>b</sup>	65 [33] <sup>b</sup>	24 [23] <sup>d</sup>
$\Delta I/\Delta G$ (pmol/mmol)	73 [84]	163 [172] <sup>b</sup>	122 [140]	19 [33] <sup>a</sup>
Fasting ISR (pmol·min <sup>-1</sup> ·m <sup>-2</sup> )	53 [34] <sup>a</sup>	85 [53]	116 [39]	112 [71] <sup>c</sup>
Total IS (nmol·m <sup>-2</sup> )	30 [18]	43 [19]	47 [17] <sup>b</sup>	24 [19] <sup>d</sup>
Insulin sensitivity (M) ( $\mu$ mol/min·kg <sub>FFM</sub> <sup>-1</sup> )	54 [23] <sup>a</sup>	32 [15] <sup>a</sup>	23 [10] <sup>a</sup>	14 [8] <sup>a</sup>
M· $\Delta I/\Delta G$ (mmol/min·kg <sub>FFM</sub> <sup>-1</sup> ·[pmol/mmol])	3.8 [3.0]	4.6 [3.7]	3.7 [2.5]	0.2 [0.5] <sup>a</sup>
$\beta$ -cell glucose sensitivity (pmol·min <sup>-1</sup> ·m <sup>-2</sup> ·mM <sup>-1</sup> )	147 [97]	92 [98]	55 [34] <sup>b</sup>	14 [15] <sup>a</sup>
Rate sensitivity (nmol·m <sup>-2</sup> ·mM <sup>-1</sup> )	0.89 [1.69]	1.37 [1.39]	1.10 [1.23]	0.17 [0.45] <sup>a</sup>
Potential (fold)	1.9 [2.3] <sup>a</sup>	1.6 [1.1]	1.2 [0.8]	1.0 [0.3]

Data in *brackets* represent interquartile range. AUC, Area under curve;  $\Delta I/\Delta G$ , ratio of insulin increment to glucose increment at 30 min (insulinogenic index); IS, insulin output.

<sup>a</sup>  $P < 0.05$  or less vs. every other group.

<sup>b</sup>  $P < 0.05$  or less vs. lean NGT.

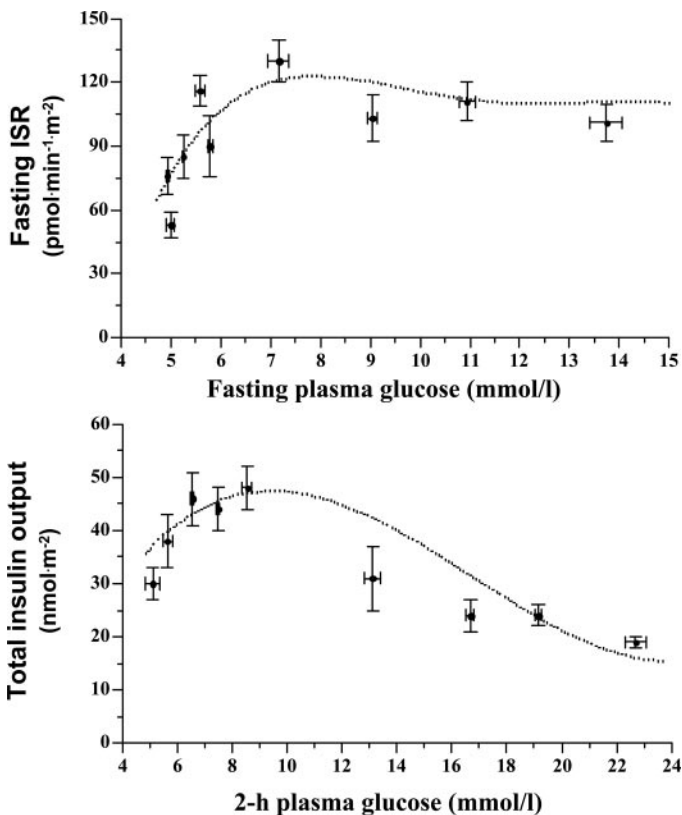
<sup>c</sup>  $P < 0.05$  or less vs. obese NGT.

<sup>d</sup>  $P < 0.05$  or less vs. obese NGT and IGT.

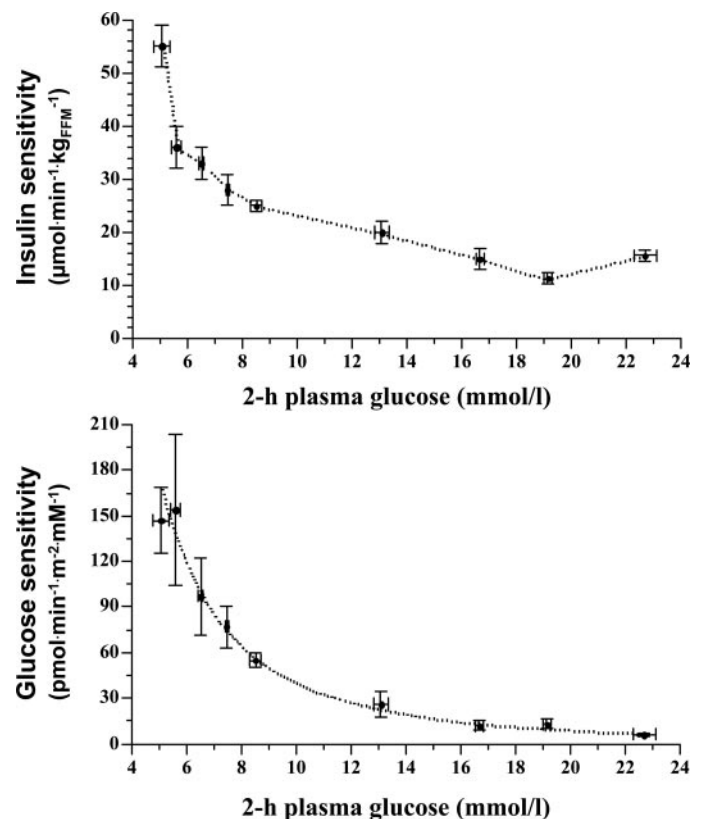
(Fig. 2). Whole-body insulin sensitivity decreased sharply in the transition from lean to obese NGT, showed a trend (which fell short of statistical significance) to decrease across obese NGT tertiles, and then declined further through IGT and mild T2DM to level off in the higher three quartiles of diabetic hyperglycemia.

The dose-response curves for glucose-stimulated insulin

secretion (whose mean slope is  $\beta$ -cell glucose sensitivity) show a progressive shift to the right and downward across groups of progressively worse glucose tolerance (Fig. 3). Of note is that the dose-response curve for the lean NGT group falls between those of the first two glucose tertiles of obese NGT (from which it does not differ significantly) and is significantly better ( $P = 0.0005$ ) than the worst glucose tertile



**FIG. 1.** Plots of fasting insulin secretion rate against fasting plasma glucose concentration (*top*) and total insulin secretion against 2-h plasma glucose concentration (*bottom*) in nine subgroups of subjects: from left to right, lean NGT, obese NGT by tertile of 2-h plasma glucose, IGT, and T2DM by quartile of 2-h plasma glucose. Points are median  $\pm$  SEM for both variables.



**FIG. 2.** Plots of whole-body insulin sensitivity (on the euglycemic clamp) (*top*) and  $\beta$ -cell glucose sensitivity (*bottom*) against 2-h plasma glucose concentration in the same nine subgroups as in Fig. 1. Points are median  $\pm$  SEM for both variables.

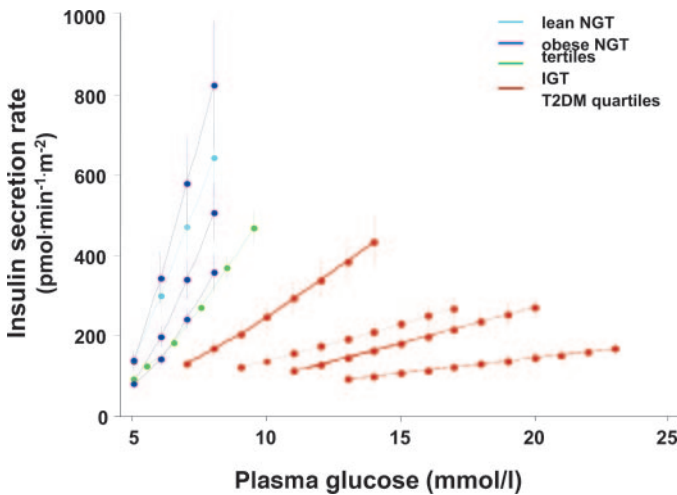


FIG. 3. Dose-response curves for glucose-stimulated insulin secretion in the same nine subgroups as in Fig. 1. Points are mean  $\pm$  SEM.

of obese NGT, which in turn is very close to the IGT curve ( $P = 0.39$ ).

*Relationships*

When regressing  $\beta$ -cell glucose sensitivity on 2-h plasma glucose concentration within each category of glucose tolerance,  $\beta$ -cell glucose sensitivity was inversely related to 2-h plasma glucose in NGT ( $\rho = -0.38, P < 0.004$ ), IGT ( $\rho = -0.42, P < 0.05$ ), and T2DM ( $\rho = -0.65, P < 0.001$ ). More importantly, in a log-log plot, the slope of the regression of  $\beta$ -cell glucose sensitivity on 2-h glucose levels was similar across diagnostic groups [ $-1.5 \pm 0.49$  (mean  $\pm$  SE) in NGT,  $-2.2 \pm 0.83$  in IGT, and  $-2.8 \pm 0.31$  in T2DM,  $P = 0.054$ ], giving rise to a powerful overall relationship ( $\rho = -0.83, P < 0.0001$ ) (Fig. 4). Of note, this relationship was essentially unchanged after adjusting for gender, age, BMI, or waist circumference. In contrast, insulin sensitivity was related to 2-h plasma glucose levels among NGT subjects ( $\rho = -0.60, P < 0.0001$ ), weakly related in T2DM ( $\rho = -0.23, P = 0.02$ ), and unrelated among IGT subjects ( $\rho = 0.01, P = \text{ns}$ ) (Fig. 4). Insulin sensitivity was also reciprocally related to BMI ( $P < 0.0001$ ) independently of 2-h plasma glucose concentrations.

Fasting insulin secretion was inversely related to insulin sensitivity ( $\rho = 0.54, P < 0.0001$ ), with all subjects falling on the same regression line. In contrast, insulin sensitivity and  $\beta$ -cell glucose sensitivity were essentially independent of one another when tested within each diagnostic group. In the whole data set, the relationship was a direct one ( $\rho = 0.53, P < 0.0017$ ) (Fig. 5). The dual dependence of 2-h plasma glucose concentration on tissue sensitivity to insulin and  $\beta$ -cell glucose sensitivity for the entire study group divided into quartiles of each factor is displayed in Fig. 6. Individuals with the worst glucose tolerance were characterized by the lowest tissue sensitivity to insulin and the greatest reduction in  $\beta$ -cell glucose sensitivity. The impact on glucose tolerance of these two factors is less than additive ( $P = 0.01$  for the negative interaction term), in that in the most insulin-resistant group, there was little additional effect of severe  $\beta$ -cell deficiency and, conversely, in the group with the worst  $\beta$ -cell

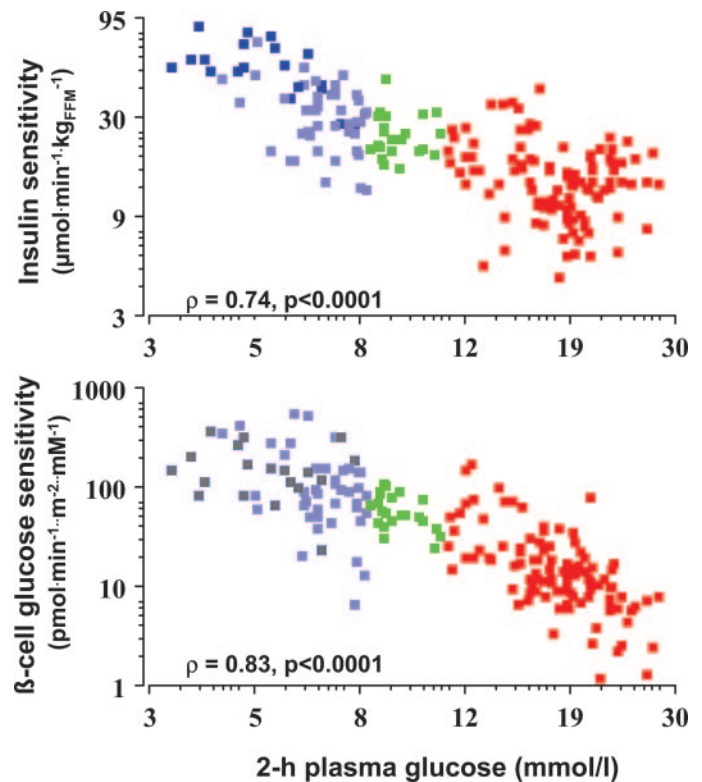


FIG. 4. Log-log plot of individual values of whole-body insulin sensitivity (top) and  $\beta$ -cell glucose sensitivity (bottom) against 2-h plasma glucose concentration. Lean NGT subjects are in blue, obese NGT in light blue, IGT in green, and T2DM in red.

glucose sensitivity, there was little additional effect of insulin resistance.

In multivariate analysis (Table 3), both fasting and 2-h plasma glucose levels were simultaneously and independently associated with  $\beta$ -cell glucose sensitivity and insulin sensitivity after accounting for gender, age, and BMI. Furthermore, rate sensitivity and potentiation made an independent contribution to glucose levels, especially at 2 h post glucose. Also of note is that fasting ISR was positively related to glucose levels.

**Discussion**

Over the last decade, the biochemical and molecular defects responsible for the insulin resistance in muscle and fat cells have been studied intensively (38). Much less is known about the time sequence and specific abnormalities responsible for the impairment in  $\beta$ -cell function. In the present cross-sectional study, our aims were to: 1) determine which of these  $\beta$ -cell functions was altered in states of progressively worse glucose tolerance; 2) define the earliest stage of glucose intolerance at which abnormalities in parameters of  $\beta$ -cell function could be detected; and 3) examine the relative contributions of impaired  $\beta$ -cell function and reduced insulin sensitivity to glucose tolerance.

The most striking finding was the divergent pattern of association of different parameters of  $\beta$ -cell function with 2-h plasma glucose levels. Thus, total insulin secretion showed the familiar approximately inverted U-shaped profile, also

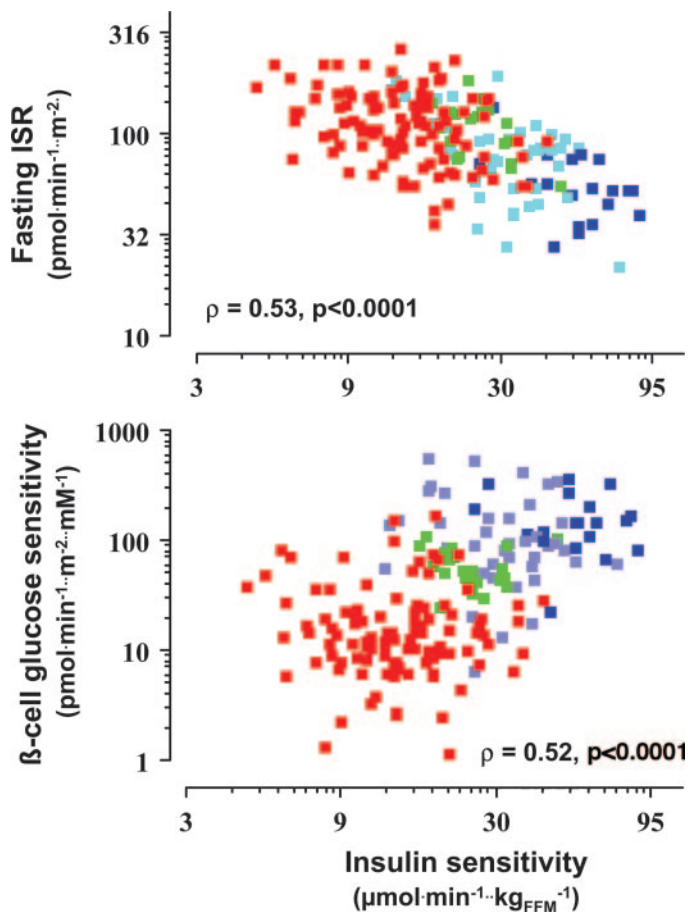


FIG. 5. Log-log plot of individual values of whole-body insulin sensitivity against fasting insulin secretion rate (*top*) and  $\beta$ -cell glucose sensitivity (*bottom*). Color code as in Fig. 4.

known as Starling's curve of the pancreas (Fig. 1). In contrast,  $\beta$ -cell glucose sensitivity decreased monophasically as a non-linear function of increasing 2-h glucose levels (Figs. 2–4). Within the NGT range (with fasting glucose ranging from 4.7 to 6.0 mmol/liter and 2-h glucose ranging from 4.1 to 7.7 mmol/liter),  $\beta$ -cell glucose sensitivity declined by 50–70%, whereas insulin sensitivity decreased by only 20% (Fig. 7). Importantly, the association between  $\beta$ -cell glucose sensitivity and 2-h glucose levels was unaffected by age, gender, BMI, or waist circumference, again in contrast with insulin sensitivity, which was closely dependent on BMI as expected [the largest drop occurring between lean and obese subjects within the NGT range (Fig. 2)]. Moreover,  $\beta$ -cell glucose sensitivity was only weakly related to insulin sensitivity, although it tended to be decreased in parallel with insulin sensitivity across glucose tolerance groups (Fig. 5). Of note also is that the product of insulin sensitivity and insulinogenic index (approximate disposition index) was only detectably impaired in T2DM. To the extent that this index reflects compensation of acute-phase insulin release for the prevailing insulin resistance, failure of such compensation was evident only in overt diabetes. Thus, basal insulin release increases as glucose levels rise (*cf.* Table 3) and insulin sensitivity declines (*cf.* Fig. 5). In contrast,  $\beta$ -cell glucose sensi-

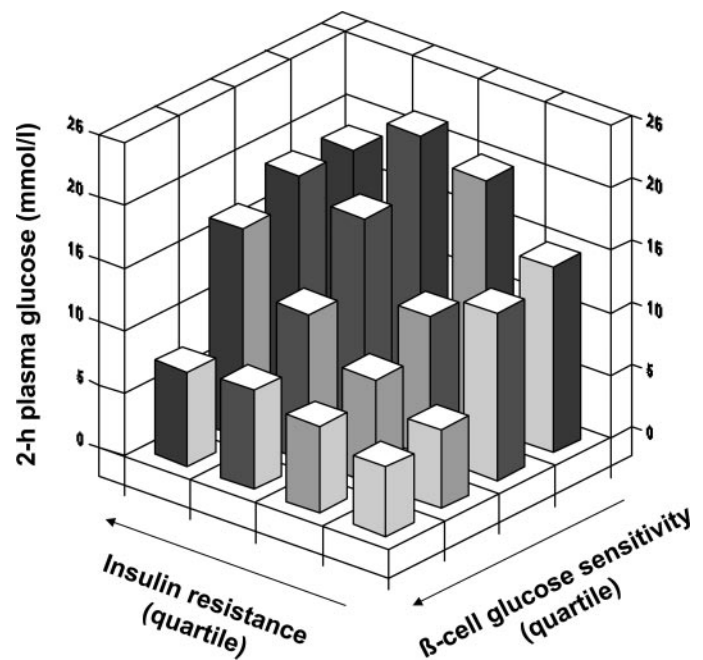


FIG. 6. Dual dependence of 2-h plasma glucose concentrations on tissue insulin sensitivity and  $\beta$ -cell glucose sensitivity. For this analysis, the entire group of 188 subjects was divided into quartiles of insulin sensitivity and  $\beta$ -cell glucose sensitivity. Quartile breaks are less than 13, less than 20, less than 31, and 31–86 or more  $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}_{\text{FFM}}^{-1}$  for insulin sensitivity and less than 12, less than 30, less than 86, and 86–561 or more  $\text{pmol}\cdot\text{min}^{-1}\cdot\text{m}^{-2}\cdot\text{mM}^{-1}$  for  $\beta$ -cell glucose sensitivity.

tivity, *i.e.* insulin secretion changes *viz.* plasma glucose changes, tracks only with plasma glucose levels.

What the cause-effect relationship is underlying the association between reduced glucose sensitivity of the  $\beta$ -cell and diminished glucose tolerance within the NGT group cannot be decided by these cross-sectional results. In the whole data set, we found an inverse association between  $\beta$ -cell glucose sensitivity and the mean FFA concentration during the OGTT ( $\rho = -0.39$ ,  $P < 0.0001$ , data not shown), which is reminiscent of the concept of lipotoxicity as initially predicated by Unger (39) and McGarry (40) in rodents and Golay *et al.* (41) in humans. Circulating FFAs, however, typically track with insulin resistance, and the relationship with  $\beta$ -cell glucose sensitivity may be spurious. Another possibility is

TABLE 3. Determinants of plasma glucose levels

	Fasting glucose		2-h glucose	
	Partial correlation	<i>p</i> value	Partial correlation	<i>p</i> value
Gender (F vs. M)	-0.001	NS	0.15	0.03
Age	0.08	NS	0.31	<0.00001
Ethnicity	-0.10	NS	0.13	NS
BMI	-0.08	NS	-0.14	NS
ISR	0.17	0.01	0.40	<0.00001
Insulin sensitivity	-0.25	0.0001	-0.37	<0.00001
$\beta$ -Cell glucose sensitivity	-0.73	<0.00001	-0.81	<0.00001
Rate sensitivity	-0.18	<0.04	-0.22	0.01
Potential	-0.10	NS	-0.33	<0.00001
Total explained variance	77%		89%	

Data represent partial correlation coefficients and their *p* values in multivariate models with fasting plasma glucose or 2-h plasma glucose as the dependent variable. ISR, Fasting insulin secretion rate; F, female; M, male; NS, not significant.

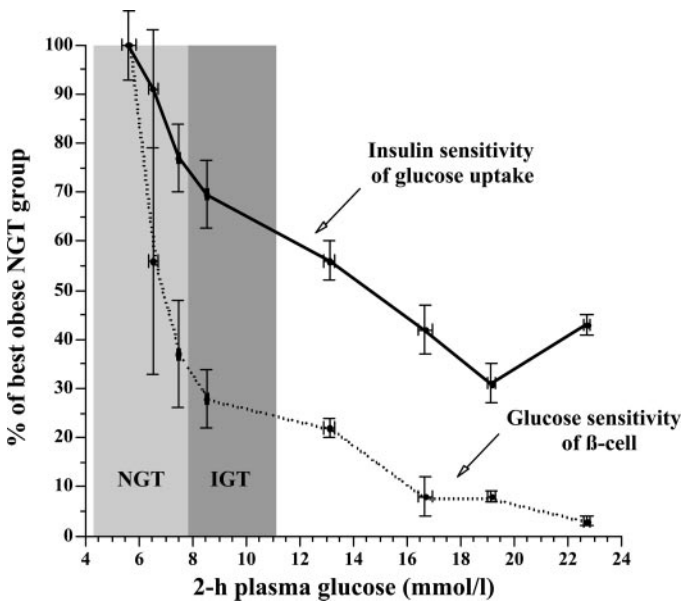


FIG. 7. Plot of  $\beta$ -cell glucose sensitivity and insulin sensitivity against 2-h plasma glucose concentration in obese NGT tertiles, IGT, and T2DM quartiles. Data here are expressed as percent of the best obese NGT group because all these groups have comparable BMI.

that it is the increase in postprandial plasma glucose concentration *per se* that is responsible for the loss of  $\beta$ -cell sensitivity, *i.e.* glucotoxicity (42). In partially pancreatectomized (60%) rats, a dietary-induced rise in the mean day-long plasma glucose concentration of only 1 mmol/liter resulted in a marked impairment in glucose-stimulated insulin secretion when the islets were removed from the animals and perfused *in vitro* (43). Our NGT subjects in the top tertile of 2-h glucose levels were presumably exposed to substantial around-the-clock hyperglycemia in comparison with subjects in the bottom tertile. Among other possibilities, the progressive deterioration in glucose sensitivity in NGT subjects may be explained by as-yet-unidentified metabolic abnormalities or represent a preprogrammed genetic  $\beta$ -cell defect (2).

In comparison with BMI-matched NGT, IGT was marked by further deterioration of both insulin sensitivity (44) and  $\beta$ -cell glucose sensitivity (Table 2); in addition, defects in potentiation became evident. Decreased plasma GLP-1 levels have been described in T2DM patients after meal ingestion (21) as well as, more recently, in IGT (45). Thus, incretin dysfunction may be one component of the observed impairment of potentiation in IGT and T2DM. It should, however, be pointed out that different phenomena (glucose potentiation, incretin potentiation, neural modulation) contribute to the potentiation factor as represented in the current glucose model. For example, the effect of GLP-1 to potentiate glucose-induced insulin secretion is in part lost to the fact that GLP-1 release is largely synchronous with glycemia itself and is therefore absorbed into glucose sensitivity rather than being read as potentiation.

Lastly, overt T2DM is associated with a further reduction in tissue sensitivity to insulin as well as  $\beta$ -cell glucose sensitivity (Figs. 2, 3, and 7). By multivariate analysis (Table 3), both insulin resistance and  $\beta$ -cell glucose insensitivity inde-

pendently contribute to raise 2-h plasma glucose levels;  $\beta$ -cell rate sensitivity and potentiation add significant contributions, thereby bringing total explained variance of 2-h plasma glucose levels to close to 90%. Of note is that the same model also explains the vast majority of the variance in fasting glucose levels. From the multiple regression equations (using mean population data), it is of interest to calculate that a halving of  $\beta$ -cell glucose sensitivity (from 120 to 60  $\text{pmol}\cdot\text{min}^{-1}\cdot\text{m}^{-2}\cdot\text{mm}^{-1}$ ) or a halving of insulin sensitivity (from 50 to 25  $\mu\text{mol}/\text{min}^{-1}\cdot\text{kg}_{\text{FFM}}^{-1}$ ) predicts a similar rise in 2-h plasma glucose levels of (+1.53 *vs.* +1.35 mmol/liter) as well as fasting plasma glucose levels (+0.85 *vs.* +0.56 mmol/liter).

The existence of complex interactions between the two main defects is depicted graphically in Fig. 6. In very insulin-resistant individuals, a relatively small change in  $\beta$ -cell glucose sensitivity (as occurs between quartile 1 and 2) is associated with severe hyperglycemia. Conversely, in subjects with poor  $\beta$ -cell glucose sensitivity (bottom quartile), an improvement in insulin sensitivity is associated with little change in 2-h plasma glucose concentrations (46).

In summary, the present study has quantitated insulin sensitivity and the major parameters of  $\beta$ -cell function (glucose sensitivity, rate sensitivity, potentiation) in a large number of individuals spanning the range of glucose tolerance from normal to overtly diabetic. Our results demonstrate that impaired  $\beta$ -cell glucose sensitivity is a characteristic feature of even minimally IGT. Prospective data are needed to establish the natural history of  $\beta$ -cell dysfunction (47), its interrelationships with insulin resistance, and its reversibility.

### Acknowledgments

The authors thank our nurses (Magda Ortiz, Diane Frantz, Socorro Mejorado, Janet Shapiro, John Kincaid, James King, Norma Diaz, Patricia Wolf) for their assistance in performing the insulin clamp and OGTT studies.

Received June 15, 2004. Accepted October 1, 2004.

Address all correspondence and requests for reprints to: Ele Ferrannini, M.D., Department of Internal Medicine, Via Roma, 67, I-56100 Pisa, Italy. E-mail: ferranni@ifc.cnr.it.

This work was supported by National Institutes of Health Grant DK24092, NIH General Clinical Research Center Grant MOI-RR-01346, a Veterans Affairs Merit Award, funds from the Veterans Affairs Research Foundation, an European Federation for the Study of Diabetes-Novo Nordisk Type 2 Programme Focused Research Grant, and funds from the Italian Ministry of University and Scientific Research (Ministero dell'Università e della Ricerca Scientifica e Tecnologica Protocollo 2001065883-001).

### References

1. Reaven GM, Hollenbeck CB, Chen YDI 1989 Relationship between glucose tolerance, insulin secretion, and insulin action in non-obese individuals with varying degrees of glucose tolerance. *Diabetologia* 32:52–55
2. DeFronzo RA 1997 Pathogenesis of type 2 diabetes mellitus: metabolic and molecular implications for identifying diabetes genes. *Diabetes* 5:117–269
3. Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G 1997 Insulin resistance and hypersecretion in obesity. *J Clin Invest* 100:1166–1173
4. Perley MJ, Kipnis DM 1967 Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. *J Clin Invest* 46:1954–1962
5. Ahren B, Taborsky GJ 2003  $\beta$ -Cell function and insulin secretion. In: Porte D, Sherwin RS, Baron A, eds. *Ellenberg and Rifkin's diabetes mellitus*. 6th ed. New York: McGraw Hill; 43–65
6. Porte Jr D 1991 Banting Lecture 1990.  $\beta$ -Cells in type II diabetes mellitus. *Diabetes* 40:166–180
7. Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Pettiti M, Natali A,

- Mari A, DeFronzo RA 2003 Predominant role of reduced  $\beta$ -cell sensitivity to glucose over insulin resistance in impaired glucose tolerance. *Diabetologia* 46:1211–1219
8. Brunzell JD, Robertson RP, Lerner RL, Hazzard WR, Ensink JW, Bierman EL, Porte Jr D 1976 Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance tests. *J Clin Endocrinol Metab* 2:222–229
  9. Tripathy D, Carlsson M, Almgren P, Osoma B, Raskinen M-R, Tuomi T, Groop LC 2000 Insulin secretion and insulin sensitivity in relation to glucose tolerance. Lessons from the Botnia Study. *Diabetes* 49:975–980
  10. Carnevale Schianca GP, Rossi A, Sainaghi PP, Maduli E, Bartoli E 2003 The significance of impaired fasting glucose versus impaired glucose tolerance: importance of insulin secretion and resistance. *Diabetes Care* 26:1333–1337
  11. Polonsky KS, Sturis J, Bell GI 1996 Non-insulin-dependent diabetes mellitus—a genetically programmed failure of the  $\beta$  cell to compensate for insulin resistance. *N Engl J Med* 334:777–783
  12. Hansen BC, Bodkin NH 1986 Heterogeneity of insulin responses: phases leading to type 2 (noninsulin-dependent) diabetes mellitus in the rhesus monkey. *Diabetologia* 29:713–719
  13. Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn RC 1992 Role of glucose and insulin resistance in development of type 2 diabetes mellitus: result of a 25-year follow-up study. *Lancet* 340:925–929
  14. Lillioja S, Mott DM, Howard BV, Bennett PH, Yki-Jarvinen H, Freymond D, Nyomba BL, Zurlo F, Swinburn B, Bogardus C 1988 Impaired glucose tolerance as a disorder of insulin action. Longitudinal and cross-sectional studies in Pima Indians. *New Engl J Med* 318:1217–1225
  15. Weyer C, Bogardus C, Mott DM, Pratley RE 1999 The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* 104:787–794
  16. Weyer C, Tataranni PA, Bogardus C, Pratley RE 2001 Insulin resistance and insulin secretory dysfunction are independent predictors of worsening of glucose tolerance during each stage of type 2 diabetes development. *Diabetes Care* 24:89–94
  17. Weyer C, Hanson RL, Tataranni PA, Bogardus C, Pratley RE 2000 A high fasting plasma insulin concentration predicts type 2 diabetes independent of insulin resistance. *Diabetes* 49:2094–2101
  18. Drucker DJ 2001 Minireview: the glucagon-like peptides. *Endocrinology* 142: 521–527
  19. Fehmanc HC, Goke R, Goke B 1995 Cell and molecular biology of the incretin hormones, glucagon-like peptide-1 and glucose-dependent insulin releasing polypeptide. *Endocr Rev* 16:390–410
  20. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W 1993 Preserved incretin activity of glucagon-like peptide 1 [7–36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type 2 diabetes mellitus. *J Clin Invest* 91:301–307
  21. Vilsboll T, Krarup T, Deacon CF, Madsbad S, Holst JJ 2001 Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 50:609–613
  22. Ahren B, Larsson H, Holst JJ 1997 Effects of glucagon-like peptide-1 on islet function and insulin sensitivity in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 82:473–478
  23. Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W 1993 Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7–36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 36:741–744
  24. D'Alessio DA, Vogel R, Prigeon R, Laschansky E, Koerker D, Eng J, Ensink JW 1996 Elimination of the action of glucagon-like peptide 1 causes an impairment of glucose tolerance after nutrient ingestion by healthy baboons. *J Clin Invest* 97:133–138
  25. Nauck MA, Bartels E, Orskov C, Ebert R, Creutzfeldt W 1993 Additive insulinotropic effects of exogenous synthetic human gastric inhibitory polypeptide and glucagon-like peptide-1-(7–36) amide infused at near-physiological insulinotropic hormone and glucose concentrations. *J Clin Endocrinol Metab* 76:912–917
  26. 1997 Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197
  27. Henquin JC, Ishiyama N, Nenquin M, Ravier MA, Jonas JC 2002 Signals and pools underlying biphasic insulin secretion. *Diabetes* 51(Suppl 1):S60–S67
  28. Bergman RN, Finegood DT, Kahn SE 2000 The evolution of  $\beta$ -cell dysfunction and insulin resistance in type 2 diabetes. *Eur J Clin Invest* 32:35–45
  29. DeFronzo RA, Tobin JD, Andres R 1979 The glucose clamp technique. A method for quantifying insulin secretion and resistance. *Am J Physiol* 6:E214–E223
  30. Mari A, Schmitz O, Gastaldelli A, Oestergaard T, Nyholm B, Ferrannini E 2002 Meal and oral glucose tests for assessment of  $\beta$ -cell action: modeling analysis in normal subjects. *Am J Physiol Endocrinol Metab* 283:E1159–E1166
  31. Mari A, Tura A, Gastaldelli A, Ferrannini E 2002 Assessing insulin secretion by modeling in multiple-meal tests: role of potentiation. *Diabetes* 51(Suppl 1):S221–S226
  32. Ferrannini E, Gastaldelli A, Matsuda M, Miyazaki Y, Pettiti M, Glass L, DeFronzo RA 2003 Influence of ethnicity and familial diabetes on glucose tolerance and insulin action: a physiological analysis. *J Clin Endocrinol Metab* 88:3251–3257
  33. Bonora E, Del Prato S, Bonadonna RC, Gulli G, Solini A, Shank ML, Ghiatas AA, Lancaster JL, Kilcoyne RF, Alyassin AM, DeFronzo RA 1992 Total body fat content and fat topography are associated differently with *in vivo* glucose metabolism in nonobese and obese nondiabetic women. *Diabetes* 41:1151–1159
  34. Steele RW, Wall JS, DeBodo RC, Altszuler N 1956 Measurements of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol* 187:15–24
  35. Groop L, Bonadonna RC, Del Prato S, Ratheiser K, Zych K, DeFronzo RA 1989 Effect of insulin on oxidative and nonoxidative pathways of glucose and FFA metabolism in NIDDM. Evidence for multiple sites of insulin resistance. *J Clin Invest* 84:205–213
  36. Van Cauter E, Mestrez F, Sturis J, Polonsky KS 1992 Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368–377
  37. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP, Porte Jr D 1993 Quantification of the relationship between insulin sensitivity and  $\beta$ -cell function in human subjects. Evidence for a hyperbolic function. *Diabetes* 42:1663–1672
  38. Saltiel AR, Kahn CR 2001 Insulin signaling and the regulation of glucose and lipid metabolism. *Nature* 414:799–806
  39. Unger RH 1995 Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* 44:863–870
  40. McGarry JD 2002 Banting Lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* 51:7–18
  41. Golay A, Felber JP, Jequier E, DeFronzo RA, Ferrannini E 1988 Metabolic basis of obesity and non-insulin dependent diabetes mellitus. *Diabetes Metab Rev* 4:727–747
  42. Rossetti L, Giaccari A, DeFronzo RA 1990 Glucose toxicity. *Diabetes Care* 13:610–630
  43. Leahy JL, Bonner-Weir S, Weir GC 1988 Minimal chronic hyperglycemia is a critical determinant of impaired insulin secretion after an incomplete pancreatectomy. *J Clin Invest* 81:1407–1414
  44. Festa A, D'Agostino Jr R, Hanley AJ, Karter AJ, Saad MF, Haffner SM 2004 Differences in insulin resistance in nondiabetic subjects with isolated impaired glucose tolerance or isolated impaired fasting glucose. *Diabetes* 53:1549–1555
  45. Rask E, Olsson T, Soderberg S, Holst JJ, Tura A, Pacini G, Ahren B 2004 Insulin secretion and incretin hormones after oral glucose in non-obese subjects with impaired glucose tolerance. *Metabolism* 53:624–631
  46. Bergman RN, Ader M, Huecking K, Van Citters G 2002 Accurate assessment of  $\beta$ -cell function: the hyperbolic correction. *Diabetes* 51(Suppl 1):S212–S220
  47. Chen KW, Boyko EJ, Bergstrom RW, Leonetti DL, Newell-Morris L, Wahl PW, Fujimoto WY 1995 Earlier appearance of impaired insulin secretion than of visceral adiposity in the pathogenesis of NIDDM: 5-year follow-up of initially nondiabetic Japanese-American men. *Diabetes Care* 18:747–753

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.