Insulin Release in Impaired Glucose Tolerance

Oral Minimal Model Predicts Normal Sensitivity to Glucose but Defective Response Times

Elena Breda, Gianna Toffolo, Kenneth S. Polonsky, and Claudio Cobelli

The availability of quantitative indexes describing β-cell function in normal life conditions is important for the characterization of impaired mechanisms of insulin secretion in pathophysiological states. Recently, an oral C-peptide minimal model has been proposed and applied to subjects with normal glucose tolerance (NGT) during graded up-and-down glucose infusion protocols (40-min periods at 4, 8, 16, 8, 4, and 0 mg·kg⁻¹·min⁻¹) and oral glucose tolerance tests. These tests are characterized by slow glucose and C-peptide dynamics, which reproduce prandial conditions. In view of the importance of β-cell dysfunction in the pathogenesis of type 2 diabetes, our aim was to test and use the oral minimal model in subjects with impaired glucose tolerance (IGT) to identify deranged mechanisms of β-cell function. Plasma C-peptide and glucose data from graded up-and-down glucose infusions were analyzed in nine NGT and four IGT subjects using the classic deconvolution approach and the oral minimal model, and indexes of β-cell function were derived. An index of insulin sensitivity was also obtained for each subject from minimal model analysis of glucose and insulin levels achieved during the test. Both deconvolution and minimal model analyses revealed that individuals with IGT have a relative defect in the ability to secrete enough insulin to adequately compensate for insulin resistance. Additionally, minimal model analysis suggests that insulin secretory defect in IGT arises from delays in the timing of the β-cell response to glucose. Diabetes 51 (Suppl. 1): S227–S233, 2002

Various methods are currently used to estimate β-cell function during the intravenous glucose tolerance test, including the calculation of the acute insulin response to glucose (AIRglucose) (1), the so-called combined model (2,3), and the C-peptide minimal model (4,5). Recently, the need to quantify β-cell function under normal life conditions has encouraged many investigators to use more physiological protocols, including graded up-and-down glucose infusions, meals, oral glucose tolerance tests (OGTTs), and free living conditions. These tests are all characterized by slow glucose and C-peptide dynamics. Under these circumstances, the insulin secretory profile and indexes of β-cell function have been derived from glucose and/or C-peptide data by using either deconvolution, in conjunction with the widely used quasi–steady-state approaches (6–8), or structural models (9–13). In particular, a C-peptide minimal model, hereafter called “oral,” has recently been proposed and applied to subjects with normal glucose tolerance (NGT) during graded up-and-down glucose infusion protocols (40-min periods at 4, 8, 16, 8, 4, and 0 mg·kg⁻¹·min⁻¹) (12) and OGTTs (13).

In view of the importance of β-cell dysfunction in the pathogenesis of type 2 diabetes, we used both deconvolution (DEC) and the oral minimal model (OMM) to study subjects with impaired glucose tolerance (IGT). In particular, our aims were to verify the necessity of OMM assumptions—static and dynamic controls of glucose on insulin secretion and delay between glucose stimulus and β-cell response—to adequately describe C-peptide data in IGT, and to assess DEC and OMM indexes in IGT compared with NGT.

RESEARCH DESIGN AND METHODS
Selection and definition of study subjects. Studies were performed in nine healthy subjects with NGT (seven women and two men; age, 33 ± 3 years [mean ± SE]; BMI, 26 ± 2 kg/m²) and four subjects with IGT (two women and two men; age, 37 ± 3 years; BMI, 38 ± 3 kg/m²). This database includes the database used in Toffolo et al. (12).

Glucose tolerance was determined by using the American Diabetes Association Expert Committee criteria (14). All subjects had a normal screening blood count and chemistries and none were taking medications known to affect insulin secretion or action. All protocols were approved by the Institutional Review Board of the University of Chicago. Written informed consent was obtained from each subject.

Experimental protocol. All studies were performed in the Clinical Research Center at the University of Chicago, starting at 8:00 A.M., after an overnight fast. Intravenous cannulas were placed in a forearm vein for blood withdrawal, and the forearm was warmed to arterialize the venous sample. A second catheter was placed in the contralateral forearm for administration of glucose.

Subjects received graded glucose infusions at progressively increasing (step-up) and then decreasing (step-down) rates (4, 8, 16, 8, 4, and 0 mg·kg⁻¹·min⁻¹). Each glucose infusion rate was administered for a total of 40 min. Glucose, C-peptide, and insulin levels were measured at 15-min intervals during a 40-min baseline period before the glucose infusion and throughout the 240-min glucose infusion.

Assay. Plasma glucose was measured immediately using a glucose analyzer (YSI Model 2300 STAT; Yellow Springs Instruments, Yellow Springs, OH). The coefficient of variation (CV) of this method is <2%. Serum insulin was assayed by a double-antibody technique (15) with a lower limit of sensitivity of 20
pmmol/l and an average intra-assay CV of 6%. The cross-reactivity of proinsulin in the radioimmunoassay for insulin is ~40%. Plasma C-peptide was measured as previously described (16). The lower limit of sensitivity of the assay is 0.02 nmol/l, and the intra-assay CV averaged 6%.

β-Cell sensitivity to glucose

Deconvolution. The insulin secretion rate (ISR) was derived by stochastic deconvolution of C-peptide levels (17). Mean ISR for each glucose infusion rate was then plotted against the corresponding mean glucose concentration to define the dose-response relationship (6–8). Indexes of β-cell function were derived by calculating the areas under the ISR curve (ISCUR) over the entire protocol as well as during the step-up and the step-down parts only, and by normalizing them to the glucose CUR calculated for the same intervals. These three indexes of β-cell responsivity to glucose (ISRDEC, ISCU DEC 1, and ISRDEC 1) are thus independent of the glucose levels achieved.

Oral minimal model. Insulin secretion was also quantified from C-peptide and glucose levels by using the OMM (12,13). C-peptide kinetics are described by the well-known two-compartment model originally proposed by Eaton et al. (18):

\[ CP_1(t) = -[k_{12} + k_{13}] CP_1(t) + k_1 CP_2(t) + SR(t) \]
\[ CP_2(t) = k_{21} CP_1(t) - k_2 CP_2(t) + SR_0 \]
where the overdot indicates time derivative; \( CP_1 \) and \( CP_2 \) (nmol/l) are C-peptide concentrations above basal in the accessible and peripheral compartments, respectively; \( k_{12} \) (min⁻¹) is C-peptide kinetic parameters, and secretion rate (SR) (pmol · l⁻¹ · min⁻¹) is pancreatic secretion rate above basal, entering the accessible compartment, and normalized by the volume of distribution of compartment 1.

Pancrotic SR has been described as the sum of two components controlled by glucose concentration (static glucose control, \( SR_b \)) and its rate of increase (dynamic glucose control, \( SR_d \)):

\[ SR(t) = SR_b(t) + SR_d(t) \]

\( SR_b \) is assumed to be equal to the provision of new insulin to the β-cells, \( Y \) (pmmol · l⁻¹ · min⁻¹):

\[ SR_b(t) = Y(t) \]

which is controlled by glucose according to the following equation:

\[ Y(t) = -a[Y(t) - \beta(G(t) - G_b)] \]
\[ Y(0) = 0 \]

Thus, \( SR_b \) is not linearly related to glucose concentration, but tends with a time constant \( 1/\alpha \) toward a steady-state value linearly related through parameter \( \beta \) to glucose concentration \( G \) above a threshold level \( G_b \) (nmol/l). \( SR_b \) represents the secretion of insulin stored in the β-cells in a promptly releasable form (labile insulin), and is proportional to the rate of increase of glucose:

\[ SR_d(t) = \left\{ \begin{array}{ll} k(G) \cdot G(t) & G(t) > 0 \\ 0 & G(t) \leq 0 \end{array} \right. \]

where:

\[ k(G) = \left\{ \begin{array}{ll} k_{01} \left( 1 - \frac{G(t) - G_b}{G_{max} - G_b} \right) & G_b \leq G(t) < G_{max} \\ 0 & \text{otherwise} \end{array} \right. \]

According to Eqs. 6 and 7, the dynamic control is maximum when glucose increases just above its basal value \( G_b \), then it decreases linearly with glucose concentration and vanishes when glucose concentration exceeds the threshold level \( G_{max} \), able to promote the secretion of all stored insulin. If \( G_b \) assumes an elevated value, \( k(G) \) approximates the constant \( k_{01} \).

The profile of ISR (pmmol/min) can be calculated as

\[ ISR(t) = [SR_b(t) + SR_d(t)] \cdot V_1 \]

where \( SR_b \) (pmmol · l⁻¹ · min⁻¹) is insulin secretion in the basal state, \( CP_{lab} \) (nmol/l) is basal C-peptide concentration, and \( V_1 \) (l) is the distribution volume of the accessible compartment.

OMM also provides indexes of β-cell function. The static sensitivity index \( \Phi_s \) (10⁻⁹ min⁻¹) equals parameter \( \beta \) and measures the effect of glucose on β-cell secretion at steady state:

\[ \Phi_s = \beta \]

The dynamic sensitivity index \( \Phi_d \) (10⁻⁹) is a measure of the stimulatory effect of the rate at which glucose increases upon the secretion of stored insulin. It is defined as the amount of insulin (per unit of C-peptide distribution volume) released in response to the maximum glucose concentration (\( G_{max} \)) achieved during the experiment, normalized by the glucose increase \( G_{max} - G_b \):

\[ \Phi_d = \frac{G_{max}}{G_b} \int_0^T \frac{k(G)dG}{G_{max} - G_b} \]

where:

\[ \left\{ \begin{array}{ll} k(G) & G_b < G(t) \\ 0 & \text{otherwise} \end{array} \right. \]

If \( G_b \) assumes an elevated value, \( \Phi_d \) approximates the constant \( k_{01} \).

Finally, the basal sensitivity index \( \Phi_b \) (10⁻⁹ min⁻¹) measures basal SR over basal glucose concentration:

\[ \Phi_b = \frac{SR_b}{\frac{G_{max} - G_b}{G_b}} \]

The OMM also allows one to quantify the β-cell response times (min) to both a decreasing (\( T_{down} \)) and an increasing (\( T_{up} \)) glucose stimulus. When glucose decreases,

\[ T_{down} = \frac{1}{\alpha} \int_0^T \frac{\Phi_s}{\Phi_{dec}} dt \]

since in this case secretion equals provision (described by Eq. 5) with \( 1/\alpha \) as time constant. When glucose increases, the additional amount \( \Delta G \) of insulin secreted due to the dynamic control of glucose accelerates the β-cell response time. As detailed in Toffolo et al. (12), this is equivalent to a reduction in the β-cell response time:

\[ T_{up} = \frac{1}{\alpha} \int_0^T \frac{\Phi_s}{\Phi_{dec}} dt \]

From the model parameters, a global index of β-cell sensitivity to glucose, \( \Phi \) (10⁻⁹ min⁻¹), similar to \( \Phi_{dec} \), can also be measured:

\[ \Phi = \frac{1}{\alpha} \int_0^T \frac{\Phi_s}{\Phi_{dec}} dt \]

where \( T \) (min) is the time at which the system returns to steady-state conditions after the perturbation. Equation 14 is slightly different from the global index already defined in Breda et al. (13), because it considers total pancreatic secretion, like \( \Phi_{dec} \), and not secretion above basal.

Insulin sensitivity. The glucose minimal model (19) was applied to glucose and insulin concentrations, and an estimate of insulin sensitivity (\( S_b \) (10⁻⁹ min⁻¹ per pmmol/l)) was derived for each subject.

Numerical identification. Deconvolution was performed by using the program WINSTODEC (STOchastic DEConvolution) (20), OMM and glucose minimal model parameters were estimated, together with a measure of their precision, by nonlinear least squares (21,22) using SAAM II software (23). Population parameters for C-peptide kinetics (24) were used in both DEC and OMM approaches. Measurement errors have been assumed to be independent and Gaussian, with zero mean. Errors in C-peptide measurements were assumed with a constant but unknown variance; errors in glucose measurement with a CV of 2% AUCs were calculated by using the trapezoidal rule.

Statistical analysis. The significance of differences was determined by either the Mann-Whitney U test or the Wilcoxon signed-rank test, as appropriate. For all analyses, a two-tailed P value of < 0.05 was considered to indicate statistical significance. All results are expressed as mean ± SE. Statistical analysis was performed using StatView 5.0 (SAS Institute, Cary, NC).

RESULTS

Plasma concentrations. Mean plasma glucose, C-peptide, and insulin concentrations achieved by NGT and IGT subjects during the graded up-and-down glucose infusion are shown in Fig. 1. IGT subjects achieved significantly higher glucose, C-peptide, and insulin levels than subjects with NGT. However, while glucose levels returned to basal
before the end of the experiment in both NGT and IGT, C-peptide and insulin levels remained very high in IGT.

**Deconvolution.** Mean ISR profiles obtained by deconvolution for both NGT and IGT subjects are shown in Fig. 2 and the dose-response relationship between glucose and ISR throughout the infusion protocol is shown in Fig. 3. This relationship shows a hysteresis during decreasing glucose steps, i.e., β-cell response to glucose appears to be higher than during increasing glucose steps, and this is more pronounced in IGT subjects.

Both ISR AUC ($10^{-3}$ pmol) and glucose AUC ($10^{-3}$ mmol·l$^{-1}$·min) were significantly higher in IGT compared with NGT (ISR AUC: $212 \pm 34$ vs. $114 \pm 11$, $P = 0.013$; glucose AUC: $3.3 \pm 0.2$ vs. $2.2 \pm 0.1$, $P = 0.0055$). As a result, $\Phi_{DEC}$ ($10^9$ l·min$^{-1}$) was not different between IGT and NGT subjects (NGT, 52 ± 5; IGT, 64 ± 10) (Fig. 4). $\Phi_{DEC\ UP}$ ($10^9$ l·min$^{-1}$) was not different between the groups either (NGT, 51 ± 5; IGT, 48 ± 9), whereas $\Phi_{DEC\ DOWN}$ ($10^9$ l·min$^{-1}$) was significantly higher in IGT than in NGT (NGT, 55 ± 5; IGT, 75 ± 10). These results suggest that β-cell response to glucose in IGT is similar to that in NGT during increasing steps but is significantly higher during decreasing steps. Alternatively, when comparing

FIG. 1. Average (mean ± SE) concentration of plasma glucose, C-peptide, and insulin obtained during the graded up-and-down glucose infusion (normal glucose tolerance [NGT] $n = 9$; impaired glucose tolerance [IGT] $n = 4$).

FIG. 2. Mean ISR during the graded up-and-down glucose infusion reconstructed by deconvolution (DEC, dark line) and by the oral minimal model (OMM, gray line).

FIG. 3. Relationship between average deconvolution-derived ISR and average glucose concentration during the graded up-and-down glucose infusion experiment. The relationship predicted by the oral minimal model is shown by the dashed line.
the step-up versus the step-down phase, $\Phi_{\text{DEC DOWN}}$ was significantly higher than $\Phi_{\text{DEC UP}}$ in IGT but not in NGT subjects ($P < 0.05$), suggesting that β-cell response to glucose in IGT is significantly higher during decreasing than during increasing glucose steps, consistent with the results observed from the dose-response curve.

**Oral minimal model.** The OMM well describes experimental data in both NGT and IGT subjects (Fig. 5). OMM predictions of ISR are shown in Fig. 2 and are not different from ISR obtained by deconvolution.

Index of basal β-cell sensitivity to glucose was higher in IGT compared with NGT ($\Phi_b [10^9 \text{ min}^{-1}]$ NGT 5.0 ± 0.4; IGT 8.5 ± 1.8), but the difference just failed to reach statistical significance ($P = 0.08$). Indexes of static, dynamic, and global β-cell sensitivity to glucose did not differ between groups ($\Phi_s [10^9 \text{ min}^{-1}]$ NGT 18.4 ± 1.7; IGT 20.0 ± 1.5).

**FIG. 4. Indexes (mean ± SE) of β-cell function and insulin sensitivity ($\Phi_{\text{DEC}}, \Phi_{\text{DEC UP}}, \Phi_{\text{DEC DOWN}}, \Phi, S_I, T_{\text{up}}, \text{ and } T_{\text{down}}$) calculated during the graded up-and-down glucose infusion experiment. *Comparison to NGT: $P < 0.05$.**
Insulin sensitivity. The index of insulin sensitivity with NGT (1.3\times10^{-6} with IGT, as revealed by the protocol adopted in this study. In fact, ISR AUC reflects not only pancreatic sensitivity to glucose, but also the β-cell response time to a glucose stimulus; it thus underestimates the steady-state value during the increasing steps and overestimates it during the decreasing steps.

**β-Cell indexes through modeling.** Various indexes of β-cell function have been recently proposed in the literature. They are based on modeling analyses of glucose and C-peptide data during protocols characterized by slowly increasing and then decreasing glucose and C-peptide concentrations, such as a meal (9), a 120-min OGTT (10), free living conditions (11), graded up-and-down glucose infusion protocols (12), and a 300-min OGTT (13). In particular, the model proposed in Hovorka et al. (9) simply assumes a linear immediate control of glucose on insulin secretion, while the model proposed in Cretti et al. (10) assumes a control of glucose on insulin secretion similar to the static glucose control of OMM, and thus characterized by a delay between glucose stimulus and β-cell response. The model proposed in Mari et al. (11) assumes a (nonlinear) control of glucose and a control of glucose rate of change on insulin secretion, but no delay between glucose stimulus and β-cell response. A third secretion term is also incorporated into the model, which is not constrained to a specific functional form but is allowed to take on arbitrary smooth zero-mean time course. This term is introduced to obtain a good fit to the data, and thus a good ISR profile, but makes it difficult to assess the assumptions of the model structure against the data. Finally, the model proposed in Toffolo et al. (12) and Breda et al. (13), here called OMM, assumes controls of both glucose and glucose rate of change on insulin secretion, as well as a delay between glucose stimulus and β-cell response.

Given this controversy in the assumptions built into the available models, a purpose of this study was to test whether the OMM assumptions on the mechanisms of insulin secretion (i.e., controls of glucose and of glucose rate of change as well as delay between glucose stimulus and β-cell response) are really necessary to adequately describe C-peptide data in NGT and IGT subjects during graded up-and-down glucose infusions.

**Oral C-peptide minimal model**

*Delay between glucose stimulus and β-cell response.* When using a model similar to the OMM, but not accounting for the delay between glucose-stimulus and β-cell response, we obtained model predictions shifted to the left with respect to real C-peptide data (Fig. 5) with residuals that were no longer independent, showing that such a
delay is real and necessary to appropriately describe C-peptide data in subjects with NGT or IGT.

**Dynamic glucose control.** The importance of a control of both glucose and glucose rate of change on insulin secretion during physiological glucose perturbations has already been shown in previous studies (12,13) and is confirmed by the results of the present study. The model fit obtained by coupling the model of C-peptide kinetics with a secretion rate controlled only by glucose and not by glucose rate of change, i.e., SR(t) = SRp produces a systematic underestimation, especially in the rising portion of C-peptide data, as shown in Fig. 5.

**β-Cell indexes.** OMM overcomes the problems associated with quasi-steady-state approaches, since model equations describe the non–steady-state relationships between glucose concentration and ISR. It assumes a linear steady-state relationship between glucose concentration and ISR and estimates the slope β of this relationship from non–steady-state data, such as those measured during a graded up-and-down glucose infusion experiment. The sensitivity β is thus the same during both the step-up and the step-down phases (Fig. 3). The difference between phases observed by the deconvolution approach is indeed a consequence of the β-cell response times Tdown and Tup. The former coincides with the time constant of insulin provision, whereas the second is an equivalent parameter, which also takes into account the ability of the dynamic glucose control to accelerate the rate with which β-cells respond to an increasing glucose stimulus (12). IGT subjects have similar static and dynamic β-cell sensitivities to glucose compared with NGT, but they have statistically higher response times Td and Tup. This means that the substantially higher insulin secreted in the down portion of the protocol by IGT does not reflect a higher β-cell sensitivity to glucose (as the hysteresis in the dose–response curve and the higher Td and Tup could suggest), but simply longer response times than NGT. This quantitative difference in the response times is evident visually on inspection of the glucose and C-peptide curves during the protocol (Fig. 1).

**Insulin sensitivity.** When insulin sensitivity was calculated by the glucose minimal model, it was evident that IGT subjects, besides being characterized by a higher delay between glucose stimulus and β-cell response, have significantly lower insulin sensitivity indexes compared with NGT subjects. These results suggest that IGT subjects have a relative defect in the ability to secrete insulin to adequately compensate for their insulin resistance.

From **graded up-and-down glucose infusions to OGTT.** We also tested and used the OMM on 10 NGT and 6 IGT subjects during a frequently sampled 300-min OGTT (unpublished observations). Also in this situation, all OMM assumptions (static and dynamic control of glucose on insulin secretion, as well as a delay between glucose stimulus and β-cell response) were necessary to adequately describe C-peptide data. The basal index of β-cell function $\Phi_b$ ($10^9 \text{min}^{-1}$) was higher (even if not significantly) in IGT than in NGT ($8.4 \pm 1.8 \text{ vs.} 5.7 \pm 0.5$), whereas indexes of β-cell function during the glucose stimulus were not different between the two groups (e.g., $\Phi_d [10^9] = 604 \pm 97$ in NGT and 515 ± 106 in IGT; $\Phi_s [10^9 \text{min}^{-1}] = 31 \pm 3$ in NGT and 28 ± 4 in IGT). Response time parameters were higher (even if not significantly) in IGT (e.g., $T_{\text{down}} [\text{min}] = 6.7 \pm 2.1$ in NGT and 16.4 ± 6.5 in IGT). Eight (two IGT and six NGT) of these 16 subjects also underwent a graded up-and-down glucose infusion protocol. Pancreatic indexes $\Phi_d$ ($10^9$) and $\Phi_s$ ($10^9 \text{min}^{-1}$) during the oral glucose perturbation were significantly higher than during the intravenous glucose infusion (OGTT: $\Phi_d = 587 \pm 125$, $\Phi_s = 33 \pm 4$; up-and-down: $\Phi_d = 114 \pm 54$, $\Phi_s = 17 \pm 3$). This can probably be ascribed to the presence of the insulin-stimulating gastrointestinal hormones, which are secreted in response to oral but not intravenous glucose administration (incretin effect) (25).

**CONCLUSIONS**

In conclusion, we have shown that the recently proposed OMM adequately describes C-peptide data during physiological glucose perturbations in subjects with NGT or IGT. It overcomes the problems associated with quasi–steady-state data analysis of non–steady-state situations and provides a quantitative assessment of pancreatic function in an individual. Its application has provided novel insights into the mechanisms of insulin secretion in IGT. Also, the simultaneous assessment of insulin sensitivity in a single individual should make the C-peptide and glucose minimal model approach a powerful tool to measure changes in insulin secretion and action under physiological conditions. Further work needs to be performed to better define the domain of validity of this approach throughout the whole range of glucose tolerance, including patients with overt diabetes.

**ACKNOWLEDGMENTS**

The authors wish to thank Dr. Melissa K. Cavaghan (Indiana University School of Medicine) and Dr. David A. Ehrmann (Department of Medicine, University of Chicago) for having kindly provided unpublished data.

**REFERENCES**

5. Toffolo G, Cefalu W, Cobelli C: Beta cell function during insulin modified IVGTT successfully assessed by the C-peptide minimal model. *Metabolism* **48**:1102–1109, 1999