β-Cell Function During Insulin-Modified Intravenous Glucose Tolerance Test Successfully Assessed by the C-Peptide Minimal Model

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The insulin-modified intravenous glucose tolerance test (IM-IVGTT) is increasingly used to measure insulin sensitivity. However, the assessment of β-cell secretion is usually made using rough indices. The aim here is to evaluate the ability of the minimal model of C-peptide secretion and kinetics recently proposed for the standard IVGTT (S-IVGTT) to also assess β-cell function during the IM-IVGTT. C-peptide and glucose data from the IM-IVGTT in 15 normal humans were analyzed. The results show that the same rich β-cell picture from the S-IVGTT can be obtained during an IM-IVGTT. In particular, in each individual, the time course of β-cell secretion can be reconstructed and the functional indices of glucose control on first-phase (Φ_1), second-phase (Φ_2), and basal (Φ_b) insulin secretion can be estimated ($\Phi_1 = 191 \pm 29$, $\Phi_2 = 10.9 \pm 1.4 \cdot 10^{-9} \cdot min^{-1}$, and $\Phi_b = 5.7 \pm 1.0 \cdot 10^{-9} \cdot min^{-1}$, mean ± SE). Finally, the comparison between IM-IVGTT and S-IVGTT Φ_1, Φ_2, and Φ_b values suggest they are not affected by insulin administration.

THE INSULIN-MODIFIED intravenous glucose tolerance test (IM-IVGTT)[1,2] is increasingly used[3] to obtain a quantitative picture of glucose disposal through the minimal model indices of insulin sensitivity and glucose effectiveness.[4] In contrast, the current IM-IVGTT assessment of β-cell function is rather approximate, being usually based on the acute insulin response to glucose (AIR) index, ie, the area under the initial portion of insulin data before insulin administration, which reflects not only first-phase secretion but also hepatic insulin extraction and the whole-body insulin clearance rate. This approximate IM-IVGTT description appears somewhat at odds with the description obtained by the standard IVGTT (S-IVGTT) in which C-peptide is measured, and several methods can be applied to obtain a rich parametric portrait of β-cell function.[5-8] In particular, the C-peptide minimal model originally proposed by our group[8] and applied in several studies[9-11] allows a reconstruction of the prehepatic insulin secretion profile and quantification of the indices of glucose control on first-phase, second-phase, and basal insulin secretion.

The aim herein is to investigate if a detailed portrait of β-cell function can also be obtained from an IM-IVGTT if glucose and C-peptide concentrations are interpreted with the S-IVGTT minimal model.[8]

SUBJECTS AND METHODS

Subjects

Studies were performed in 15 healthy, community-dwelling subjects (age, 51 ± 4 years; weight, 84 ± 5 kg; body mass index, 30.3 ± 1.6 kg/m^2). Participants underwent a standardized interview to obtain demographic data and information documenting eligibility. All subjects were fully ambulatory and normally active and used no medications known to affect glucose metabolism, blood pressure, or lipids. Participants were considered healthy as determined by a medical history, physical examination, electrocardiogram, complete blood cell count, routine blood and urine chemistry analysis, and thyroid function tests. All subjects provided written informed consent to participate in the study, which was approved by the Clinical Research Practices Committee of the Bowman Gray School of Medicine.

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Protocol

The IM-IVGTT study was initiated in the morning after an overnight fast. Two 18-gauge intravenous catheters were placed in each forearm and kept patent by controlled-flow saline infusion. Each line was equipped with a three-way stopcock. One line was used for intravenous administration of glucose and insulin, and the other was used to obtain blood samples. Blood samples of 1 mL for insulin and glucose were drawn from the line at 15 minutes, 5 minutes, and immediately before glucose (300 mg/kg) was injected intravenously, and the line was flushed with saline solution. Insulin (0.03 U/kg) was infused 20 to 25 minutes after glucose injection. Blood samples of 1 mL were drawn at 2, 3, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24, 28, 32, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 140, 160, and 180 minutes after glucose injection. The samples were centrifuged immediately, and the plasma was placed on ice. Glucose determinations were performed immediately after centrifugation using the glucose oxidase method on a Glucose Analyzer 2 (Beckman, Brea, CA; intraassay coefficient of variation [CV], 2%). C-peptide was assayed from frozen plasma by radioimmunoassay (IncStar, Stillwater, MN; intraassay CV, 6%).

C-Peptide Kinetic Model

IM-IVGTT C-peptide and glucose data were analyzed with the C-peptide minimal model.[8] We will summarize its salient features and describe the parameters it provides. The model is shown in Fig 1 and is described by the following:

$$\begin{aligned} CP_1(t) &= -[k_{01} + k_{21}]CP_1(t) + k_{12}CP_2(t) + mX(t) & CP_1(0) &= 0 \\ CP_2(t) &= k_{21}CP_1(t) - k_{12}CP_2(t) & CP_2(0) &= 0 \\ X(t) &= -mX(t) + Y(t) & X(0) &= X_0 \\ Y(t) &= -\alpha[Y(t) - \beta[G(t) - h]] & Y(0) &= 0 . \end{aligned} \qquad \text{Eq 1}$$

CP_1 and CP_2 (picomolars) are the C-peptide concentration (above basal) in the accessible and peripheral compartments, respectively: X (picomolars) and Y (picomoles per liter per minute) are, respectively, the C-peptide amount and provision in the β cells, both normalized to the distribution volume of compartment 1; k_{ij} (per minute) values are

C-peptide kinetic parameters: m (per minute), α (per minute), β (per minute), and h (millimolars) are secretory parameters. The secretion rate (above basal) is mX and enters compartment 1. The stored amount of C-peptide, X_0 (picomolars), is responsible for first-phase secretion, while second-phase secretion derives from provision Y, which is controlled by the glucose concentration, G (millimolars), through parameters β and h. By adding to mX the basal secretion rate given by $k_{01}CP_{1b}$ (suffix b denotes the end-test basal value), one obtains the β-cell secretion, SR (picomoles per liter per minute), as

$$SR(t) = k_{01}CP_{1b} + mX(t) . \quad \text{Eq 2}$$

In addition to SR in each subject, the model also allows the estimation of three indices of β-cell function. The first-phase sensitivity to glucose, Φ_1 (dimensionless), is given by the ratio between the incremental amount of C-peptide secreted during the first phase and the maximum increment of the plasma glucose concentration, ΔG (millimolars):

$$\Phi_1 = \frac{X_0}{\Delta G} . \quad \text{Eq 3}$$

The second-phase sensitivity to glucose, Φ_2 (per minute), is given by the parameter describing the stimulatory effect of the glucose concentration on provision

$$\Phi_2 = \beta . \quad \text{Eq 4}$$

Finally, the basal sensitivity to glucose, Φ_b (per minute), is given by

$$\Phi_b = k_{01}CP_{1b}/G_b . \quad \text{Eq 5}$$

where G_b is the end-test glucose concentration.

The numerical identification of the model requires a knowledge of C-peptide kinetics. Like in our study,[8] the kinetic parameters k_{21}, k_{12}, and k_{01} were fixed to standard values following the method proposed in Van Cauter et al.[12] The values (mean ± SE) were as follows: k_{01} = 0.0578 ± 0.0009 min^{-1}; k_{21} = 0.0585 ± 0.0008 min^{-1}; and k_{12} = 0.0483 ± 0.0002 min^{-1}. The unknown model parameters m, α, β, h, and X_0 were then estimated, together with a measure of their precision, by nonlinear least-squares.[13] Measurement errors are assumed to be independent, gaussian, and zero-mean with an experimentally determined CV equal to 6%. When Y decays very fast, (1/α small), parameter α is usually estimated with poor precision; in this case, the simplified model with four unknown parameters, m, β, h, and X_0, as described in Appendix B of our report[8] was used.

Statistical Analysis

Values are reported as the mean ± SE. The statistical significance of differences was calculated using a two-tailed Student's *t* test. The relationship between different variables was examined by linear regression. A *P* value less than .05 was considered statistically significant.

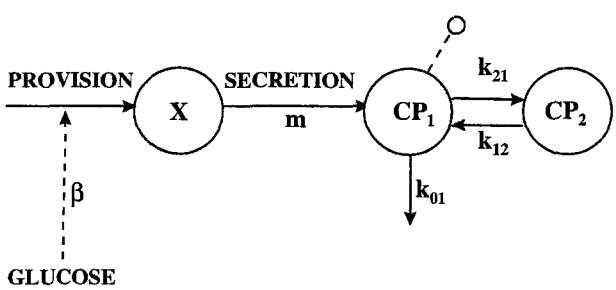

Fig 1. C-peptide minimal model. CP_1 and CP_2, C-peptide concentrations in the accessible and peripheral compartments, respectively; X, C-peptide level in β cells; k_{ij}, kinetic parameters; m and β, secretory parameters. (---) C-peptide measurement.

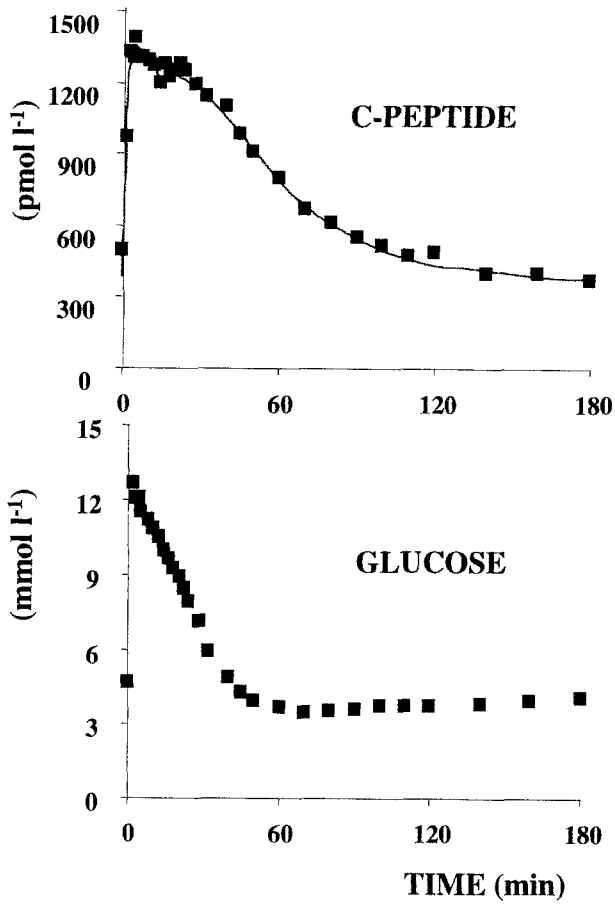

Fig 2. Mean (N = 15) plasma glucose and C-peptide concentrations during IM-IVGTT. For C-peptide, the mean value for the individual minimal model fit is also shown.

RESULTS

Mean plasma glucose and C-peptide concentrations during the IM-IVGTT are shown in Fig 2. The ability of the minimal model to describe C-peptide data is also shown in Fig 2, where

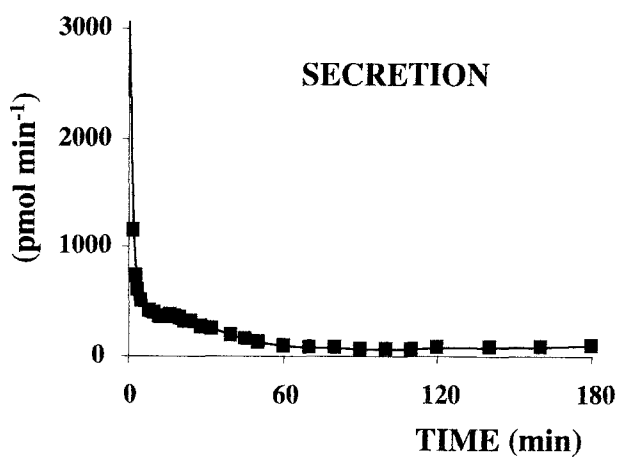

Fig 3. Mean (N = 15) β-cell secretion during IM-IVGTT as predicted by the minimal model, shown as mass per unit time. SR of Eq 2 was multiplied by the distribution volume of compartment 1 (4.19 ± 0.08 L^{12}).

Table 1. β-Cell Sensitivity Indices and Precision (CV)

Subject No.	Φ_1 (10^9)	CV (%)	Φ_2 (10^9 min^{-1})	CV (%)	Φ_b (10^9 min^{-1})	CV (%)*
1	141.8	5	4.0	8	1.2	8
2	253.6	8	6.0	15	5.3	8
3	198.9	25	10.3	14	3.2	8
4	154.7	8	4.8	12	2.3	8
5	296.2	6	18.6	7	7.4	8
6	163.0	7	12.6	6	9.2	8
7	173.5	4	7.3	6	2.0	8
8	93.1	5	8.7	6	2.7	8
9	81.4	5	12.3	26	4.5	8
10	64.7	58	10.4	14	15.7	8
11	188.1	5	18.0	5	6.7	8
12	178.0	7	9.4	7	5.9	8
13	135.5	4	13.8	4	6.4	8
14	217.1	8	21.8	5	4.4	8
15	533.2	6	5.7	33	9.1	8
Mean ± SE	191.5 ± 29.2	11	10.9 ± 1.4	10	5.7 ± 1.0	8

*Precision of Φ_b is 8% in all subjects since it depends only on the precision of basal C-peptide and glucose measurements.

the mean for the individual model fit is shown against C-peptide data.

The mean profile for β-cell secretion (Eq 2) is shown in Fig 3.

The sensitivity indices Φ_1, Φ_2, and Φ_b estimated by applying the minimal model to individual data are shown in Table 1 together with their precision. In subjects no. 1, 3, 4, and 14, the simplified version of the model was used.

DISCUSSION

The AIR index, usually used to assess β-cell function during an IM-IVGTT, provides a useful but approximate quantification of this process: it solely reflects first-phase insulin secretion in the first 10 minutes after glucose injection, and is confounded by insulin extraction by the liver and the insulin whole-body clearance rate. In contrast, for the S-IVGTT, a detailed picture of β-cell function can be obtained if the C-peptide concentration is also measured and interpreted with a model.[8] In particular, one can reconstruct the time course of β-cell secretion and estimate the functional indices (Φ_1, Φ_2, and Φ_b) of glucose control on first-phase, second-phase, and basal secretion in each individual.

The aim here was to determine if the S-IVGTT C-peptide minimal model can also be used to produce a similar β-cell picture during an IM-IVGTT. We analyzed an IM-IVGTT C-peptide and glucose data set obtained in 15 normal humans. The results show that the minimal model is able to reliably describe C-peptide secretion and kinetics during an IM-IVGTT, thus providing the same rich β-cell picture as the S-IVGTT.

The parameters obtained with the IM-IVGTT (Table 1) can be compared with those obtained in normal subjects with a S-IVGTT at the same dose.[9-11] The parameter values (N = 27; age, 54 ± 3 years; weight, 76 ± 2 kg; body mass index, 24.6 ± 0.4 kg/m^2; $\Phi_1 = 156 \pm 18$, $\Phi_2 = 10.5 \pm 0.6$, and $\Phi_b = 5.3 \pm 0.4$) are not significantly different from the IM-IVGTT values (Fig 4). The two groups are comparable with regard to age but not body mass index, since some IM-IVGTT subjects exhibit a high (>30 kg/m^2) index. However, this difference does not appear to influence the results. Parameter values in the subset of IM-IVGTT subjects with a body mass index similar to that of the S-IVGTT group (N = 8; age, 55 ± 5 years; weight, 73 ± 5 kg; body mass index, 26.0 ± 1.1 kg/m^2; $\Phi_1 = 184 \pm 23$, $\Phi_2 = 9.0 \pm 1.7$, and $\Phi_b = 4.1 \pm 1.0$) show no significant difference versus the S-IVGTT values. These results indicate that the sensitivity to glucose of first-phase, second-phase, and basal secretion is not affected by insulin administration.

Conversely, a difference arises if the IM-IVGTT results are compared with S-IVGTT results at a higher dose.[8] Parameter values in normal subjects with a S-IVGTT at a glucose dose of 500 mg/kg (N = 7; age, 24 ± 2 years; weight, 70 ± 6 kg; body mass index, 22.6 ± 1.0 kg/m^2) are $\Phi_1 = 92 \pm 15$, $\Phi_2 = 11.3 \pm 1.1$, and $\Phi_b = 4.1 \pm 0.5$. There is a difference in age with respect to subjects studied at a lower dose, but this is unlikely to influence the comparison since no correlation of sensitivity indices with age is observed in the wide range of S-IVGTT and IM-IVGTT subjects (22 to 81 years). Indices Φ_2 and Φ_b are similar in the IM-IVGTT (glucose dose 300 mg/kg) and S-IVGTT (500 mg/kg), as well as the S-IVGTT (300 mg/kg), indicating that the sensitivity to glucose of second-phase and basal secretion is not affected by either insulin administration or the glucose dose. A significant difference ($P < .01$) is found for Φ_1 when the 500-mg/kg dose results are compared with the 300-mg/kg dose (both S-IVGTT and IM-IVGTT), indicating

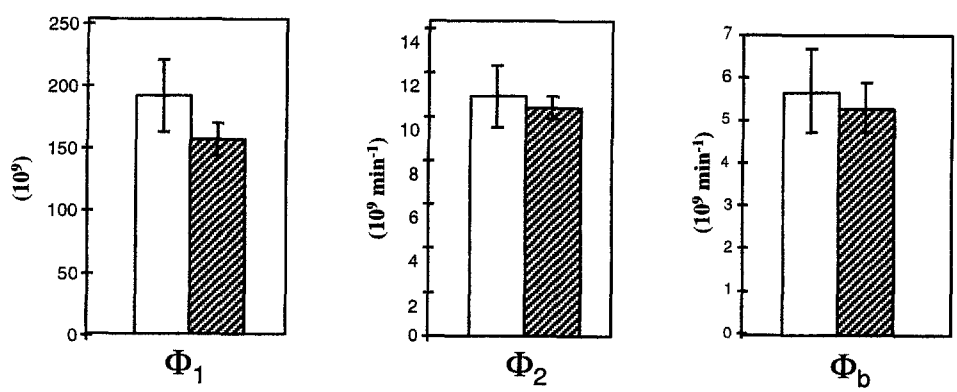

Fig 4. Sensitivity indices Φ_1, Φ_2, and Φ_b (mean ± SE) in IM-IVGTT (N = 15, □) and S-IVGTT at 300-mg/kg dose (N = 27, ▨).

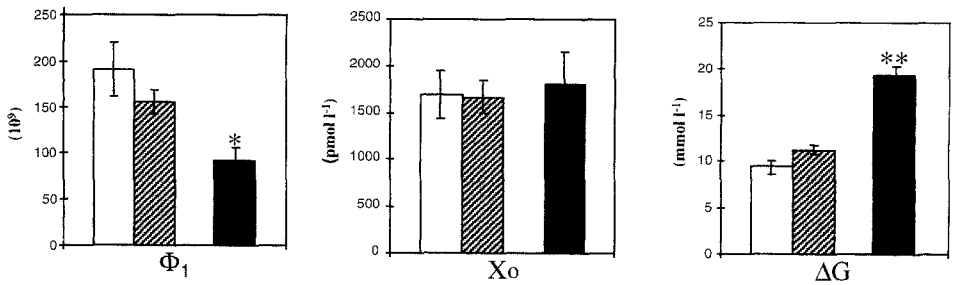

Fig 5. Sensitivity index Φ_1 and parameters X_0 and ΔG (mean ± SE) in IM-IVGTT (N = 15, □), and in S-IVGTT at 300 (N = 27, ▨) and 500 mg/kg (N = 7, ■). *$P < .01$, and **$P < .001$ v IM-IVGTT.

that the sensitivity to glucose of first-phase secretion depends on the glucose dose. This can be explained (Fig 5) by returning to the definition of Φ_1 (Eq 3). Φ_1 is the ratio between the total amount, X_0, of C-peptide secretion during the first phase and the glucose excursion, ΔG. While ΔG is significantly higher with the highest dose (IM-IVGTT 300 mg/kg, ΔG = 9.5 ± 0.8; S-IVGTT 300 mg/kg, ΔG = 11.2 ± 0.5; and S-IVGTT 500 mg/kg, ΔG = 19.3 ± 1.1, $P < .001$), the X_0 values, respectively, 1,698 ± 254, 1,670 ± 175, and 1,806 ± 356, do not increase significantly with the glucose dose. In other words, the glucose control on the first-phase secretion shows a saturation, since the initial elevation in plasma glucose observed with the lowest dose (ΔG = 10 to 12 mmol/L) is sufficient to elicit near-maximal first-phase secretion. At high ΔG levels, there is no correlation between ΔG and X_0, as confirmed by regression analysis between the individual values for X_0 and ΔG ($r = .14$, $P = .34$). Hence, X_0 could be a more accurate index of first-phase secretion, instead of its normalized value, $\Phi_1 = X_0/\Delta G$, especially when data from experiments at different doses are considered.

Finally, it is of interest to compare the IM-IVGTT minimal model parameters with the AIR index evaluated as the area under the insulin data at 0 to 10 minutes. The AIR is 1,865 ± 228 pmol/L/min, and no significant relationship is found with Φ_1 ($r = .36$, $P = .19$) or Φ_2 ($r = .36$, $P = .18$), while the relationship with X_0 just fails to reach statistical significance ($r = .503$, $P = .056$). A relationship between the AIR and X_0 is expected, since they both reflect the total amount of C-peptide secretion during first-phase secretion. However, in all likelihood, the correlation between the AIR and X_0 is not high due to their different definition. More precisely, X_0 (picomolars) is the model-based measure of the amount of C-peptide secretion during the first phase per unit of C-peptide volume, separated from the other two secretion components, second-phase and basal. On the contrary, the AIR (picomolars · minute) is based on an empirical definition of the first phase as occurring in the time interval of 0 to 10 minutes after the bolus injection, which makes it a composite parameter depending not only on the total amount of C-peptide secretion during the 0- to 10-minute interval but also on the hepatic extraction and plasma clearance rate of insulin.

In conclusion, we have shown that β-cell function can be assessed from IM-IVGTT data by using the C-peptide minimal model developed for the S-IVGTT. The model allows reconstruction of β-cell secretion and estimation of the pancreatic sensitivity indices of first-phase, second-phase, and basal secretion. Since the IM-IVGTT is frequently used to measure insulin sensitivity, by using the minimal model of glucose kinetics, the present results enrich the parametric picture of the glucose-insulin regulatory system obtainable with this test in an individual.

Further studies are needed to compare the β-cell portrait provided by the IM-IVGTT C-peptide model versus other tests such as graded glucose infusion and oscillatory glucose infusion.[14-16] An interesting feature of our approach is the ability to assess both β-cell function and insulin sensitivity in order to obtain an easy and straightforward normalization of β-cell function indices to insulin sensitivity. This normalization appears mandatory in assessing β-cell function in pathophysiological and drug therapy studies.[16]

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