Electronic Supplementary Material to the article entitled “Altered pattern of the incretin effect as assessed by modelling in individuals with glucose tolerance ranging from normal to diabetic”

Integrated model of insulin secretion and incretin effect

This appendix describes the integrated model of insulin secretion and incretin effect in detail and reports some supplementary results.

Experimental procedures

The model requires plasma glucose and C-peptide data from the oral glucose tolerance test (OGTT) and the isoglycemic intravenous glucose infusion test (IIGI) (see main text). Plasma glucose was measured by the glucose oxidase technique (Beckman Glucose Analyzers; Beckman, Fullerton, CA). Plasma insulin and C-peptide were measured by radioimmunoassay (Linco Research Inc., St. Louis, Missouri). The study also included the measurement of incretin hormones. Total C-terminal amidated GLP-1 was assayed by RIA using the polyclonal antiserum n° 89390, raised in rabbits, which has an absolute requirement for the amidated C-terminus of GLP-1, and does not cross-react with C-terminally truncated metabolites nor with the glycine-extended forms. Intact (N-terminal) GIP was assayed by RIA using a polyclonal antiserum 98171, raised in rabbits, that is N-terminally directed and not recognizing N-terminally truncated peptides. Intra- and inter-assay coefficients of variation are < 6% and <15%, respectively [1]. Regarding measurement of glucose fluxes, 6,6-$[^2]$H$_2$]glucose and [1-$^2$H]-glucose enrichment were measured by gas chromatography/mass spectrometry [2].
The model is illustrated in ESM Figure 1. During the IIGI test, the relationship between glucose concentration and insulin secretion is the same as in the classical model [3, 4]:

\[
S_{IIGI}(t) = P_{GLU}(t) \cdot f(G_{IIGI}(t)) + r_{s_{IIGI}} \cdot \frac{d^+G_{IIGI}(t)}{dt}
\]  

(1)

where \(S_{IIGI}(t)\) is insulin secretion during the IIGI test, \(r_{s_{IIGI}}\) is rate sensitivity, \(d^+G_{IIGI}(t)/dt\) is the positive derivative of glucose concentration (i.e. \(dG_{IIGI}(t)/dt\) when \(dG_{IIGI}(t)/dt \geq 0\) and otherwise zero), \(f(G)\) is the dose-response function relating insulin secretion to glucose concentration described previously [3, 4] and \(P_{GLU}(t)\) is the potentiation factor that modulates the dose-response. The potentiation factor accounts for the fact that insulin secretion at a given glucose level may differ during the test [3, 4]; \(P_{GLU}(t)\) is assumed to average one during the IIGI test. In intravenous tests, this modulation has been classically attributed to exposure to relative hyperglycemia [5]; thus, \(P_{GLU}(t)\) is denoted as glucose-induced potentiation.

During the OGTT, the expression of insulin secretion is analogous, but another potentiation term \(P_{INCR}(t)\), denoted as incretin potentiation, amplifies the contribution of the dose-response. In contrast to \(P_{GLU}(t)\), \(P_{INCR}(t)\) is not constrained to average one. Furthermore, the rate sensitivity parameter differs from the IIGI test. Thus, insulin secretion during the OGTT \(S_{OGTT}(t)\) is given by:

\[
S_{OGTT}(t) = P_{INCR}(t) \cdot P_{GLU}(t) \cdot f(G_{OGTT}(t)) + r_{s_{OGTT}} \cdot \frac{d^+G_{OGTT}(t)}{dt}
\]  

(2)

Therefore, the incretin effect is quantified by the incretin potentiation \(P_{INCR}(t)\), which is a function of time, and by the OGTT-specific rate sensitivity \(r_{s_{OGTT}}\). The dose-response \(f(G)\) and the glucose-induced potentiation \(P_{GLU}(t)\) are the same as in the IIGI test (Eq. 1).
The model also includes equations for fitting glucose concentration in both tests ($G_{HGI}(t)$ and $G_{OGTT}(t)$), the purpose of which is to smooth and interpolate the measured values as in the previous model [3, 4]:

\[
\frac{dG_{HGI}(t)}{dt} = -k \cdot G_{HGI}(t) + R_{HGI}(t)
\]

(3)

\[
\frac{dG_{OGTT}(t)}{dt} = -k \cdot G_{OGTT}(t) + R_{OGTT}(t)
\]

(4)

where $k = 0.012 \text{ min}^{-1}$ is an assigned constant, and $R_{HGI}(t)$ and $R_{OGTT}(t)$ are functions of time.

Finally, the model includes the two-exponential model of C-peptide kinetics proposed by Van Cauter et al. [6], with which C-peptide concentration is calculated from insulin secretion obtained from Eqs. 1-2.

Parameter estimation involves the determination of the four parameters of the beta-cell dose-response function ($G$), the two rate sensitivity parameters ($r_{s_{HGI}}$ and $r_{s_{OGTT}}$), and the four functions of time representing the potentiating factors ($P_{GLU}(t)$ and $P_{INCR}(t)$) and the glucose smoothing terms, $R_{HGI}(t)$ and $R_{OGTT}(t)$. This has been achieved by representing $R(t)$ and $P(t)$ as piecewise linear functions over a 5-min time grid, and using a regularized least-squares approach for parameter estimation. The function that is minimized by the least-squares algorithm includes the classical squared residual terms and the regularization terms that ensure smoothness of $R(t)$ and $P(t)$:
\[
\begin{align*}
&\sum \left( w_{\text{OGTT}}(t) \cdot (G_{\text{OGTT}}(t) - \hat{G}_{\text{OGTT}}(t)) \right)^2 + \sum \left( w_{\text{IIGI}}(t) \cdot (G_{\text{IIGI}}(t) - \hat{G}_{\text{IIGI}}(t)) \right)^2 \\
&+ \sum \left( w_{\text{OGTT}}(t) \cdot (C_{\text{OGTT}}(t) - \hat{C}_{\text{OGTT}}(t)) \right)^2 \\
&+ \sum \left( w_{\text{IIGI}}(t) \cdot (C_{\text{IIGI}}(t) - \hat{C}_{\text{IIGI}}(t)) \right)^2 + \sum \left( w_{\text{OGTT}}(t) \cdot R''_{\text{OGTT}}(t) \right)^2 \\
&+ \sum \left( w_{\text{IIGI}}(t) \cdot R''_{\text{IIGI}}(t) \right)^2 + \sum \left( w_{\text{INCR}}(t) \cdot P''_{\text{INCR}}(t) \right)^2 \\
&+ \sum \left( w_{\text{GLU}}(t) \cdot P''_{\text{GLU}}(t) \right)^2 \\
&+ \sum \left( w_{\text{GLU}}(t) \cdot P(t) \right)^2 
\end{align*}
\] (5)

In Eq. 5, \( G \) and \( C \) represent glucose and C-peptide concentration, respectively, and the subscripts indicate the test; the hat distinguishes the model prediction from the measured values; the double quote is the second derivative with respect to time, and the \( w \)'s are data and regularization weights.

The first four terms are the standard weighted sums of squares for the experimental data (glucose and C-peptide during OGTT and IIGI), the following five terms are the regularization terms. The four regularization terms that include the second derivative of \( R(t) \) and \( P(t) \) ensure that the corresponding functions are smooth; the last term ensures that glucose-induced potentiation does not exhibit excessive excursions. Of note is that this term is not included for incretin potentiation, as it excursions can be arbitrary. Because \( R(t) \) and \( P(t) \) are represented in discrete form, the second derivatives are calculated by finite differences. The summations in the standard least-squares terms are over all the measured values; those of the regularization terms include all the discretized values of \( R(t) \) and \( P(t) \).

The data weights \( w_{\text{OGTT}}, w_{\text{IIGI}}, w_{\text{OGTT}}, \) and \( w_{\text{IIGI}} \) are set to the inverse of the expected standard deviation of the measurement error for glucose and C-peptide concentration. For glucose, this was estimated as a constant value equal to 1% of mean glucose levels. For C-peptide, the standard deviation was concentration-dependent, with an average of ~4%. The regularization weights were
chosen iteratively to achieve a glucose and C-peptide mean coefficient of variation close to that of
the expected measurement error, as with the classical model [3, 4]. Parameter estimation was
performed using Matlab. The data and model fit are shown in ESM Figure 2. As expected from the
flexibility originating from the potentiation factors, the model correctly predicted glucose and C-
peptide concentrations.

Additional calculations

The excursions of glucose-induced potentiation were quantified as the ratio between the 3-h and the
basal values. Basal insulin secretion and the integral of insulin secretion during the test (total insulin
secretion) were also calculated. Areas under time curves (AUC) of the measured variables were
calculated by the trapezium rule.

The oral glucose rate of appearance (expressed in μmol/min per kg of estimated fat-free mass, μmol
min⁻¹kgFFM⁻¹) was calculated using Steele’s equation, as previously reported [2]. The time course of
the oral glucose rate of appearance is shown in ESM Figure 3.

Comparison with the classical modelling analysis

The model parameters obtained using the new model were compared with those obtained using the
classical model [3, 4], with which the IIGI and the OGTT were analyzed separately [7]. All the
corresponding parameters, i.e., basal and total insulin secretion and rate sensitivity during the IIGI
and OGTT, glucose sensitivity, and glucose-induced potentiation ratio, were strongly correlated
(Pearson’s correlation coefficient ranging from 0.81 to 0.99, P<0.0001).

The classical incretin effect, calculated as the ratio between total insulin secretion from OGTT and
IIGI, was strongly correlated with the \( P_{INCt} \) AUC (Spearman’s correlation coefficient 0.94,
P<0.0001).
References


