Critical evaluation of the combined model approach for estimation of prehepatic insulin secretion

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Watanabe, Richard M., Garry M. Steil, and Richard N. Bergman. Critical evaluation of the combined model approach for estimation of prehepatic insulin secretion. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E172–E183, 1998.—The combined model approach uses kinetic analysis of both plasma insulin and C-peptide dynamics to estimate prehepatic insulin secretion rates and parameters of insulin and C-peptide kinetics. The original model used single-compartment kinetics to describe both insulin and C-peptide despite knowledge that C-peptide follows two-compartment kinetics. The performance of the model under rapidly changing secretory conditions has come into question. Thus a more complex combined model is introduced, incorporating two-compartmental C-peptide disappearance. The addition of two-compartment C-peptide kinetics required a novel numerical approach to allow estimation of model parameters. This simulation study was undertaken to 1) compare the performance of the original combined model and 2) examine the numerical method used to identify parameters for the extended combined model with two-compartment C-peptide kinetics under simulated conditions of rapidly changing insulin and C-peptide. Monte Carlo simulation revealed that the original combined model does not provide accurate estimates of prehepatic insulin secretion under rapid kinetics. However, the extended combined model provides accurate reconstruction of prehepatic insulin secretory profile without separate quantification of C-peptide kinetics.

Both insulin resistance and β-cell failure are hallmarks of non-insulin-dependent diabetes mellitus (NIDDM) (9, 17). Although it is clear that a relative insulin secretory defect is a critical element in the pathogenesis of NIDDM, the temporal development of metabolic defects in the natural history of the disease is not yet clarified. Thus the ability to derive accurate and precise measures of insulin secretion is paramount in detecting and understanding the pathogenesis of NIDDM. The traditional measure of insulin secretory function has been the plasma insulin concentration either at fasting or after perturbation by glucose or other insulin secretagogues. However, the peripheral insulin concentration does not accurately reflect insulin secretory activity at the level of the β-cell due to significant hepatic extraction of insulin by the liver during first pass (7, 14, 21). Furthermore, there is evidence for variation in hepatic extraction among physiological conditions (7, 14, 21) that confounds comparison of plasma insulin concentrations.

The discovery of equimolar release of insulin and C-peptide by the pancreatic β-cells (23, 25) led to development of improved methods for assessment of insulin secretion in vivo (8, 11–13, 22, 28). However, as previously noted (19, 27, 28, 30), insulin secretion estimates based on kinetic analysis of peripheral C-peptide concentration alone involve multiple experimental protocols or a priori assumption of C-peptide kinetic parameters. As a simple alternative, we introduced the “combined model” approach, which uses both plasma insulin and C-peptide measurements from a single experimental protocol to derive estimates of prehepatic insulin secretion (28, 30). Analysis of the kinetics of both peptides in plasma provides two sources of information regarding the insulin-C-peptide secretory profile. The combined model uses the information that can be gleaned from the kinetics of both plasma insulin and C-peptide to derive prehepatic insulin secretion rates. This allows for estimation of prehepatic insulin secretion rates without separate experimental assessment or a priori assumption of the kinetic parameters of the model.

The accuracy of secretion estimates derived from the combined model has been questioned due to the use of a single-compartment characterization for plasma C-peptide kinetics (18, 27). The two-compartment nature of C-peptide has been confirmed (11, 23, 24) following the original report by Faber et al. (13). Whereas the second-order nature of C-peptide was not disputed (28), the simplifying assumption of single-compartment C-peptide kinetics in the combined model was motivated by the desire to minimize model complexity (3, 4). It was hoped that this simplification would be an adequate representation of the system to allow characterization of prehepatic insulin secretion. Although this assumption apparently holds true under conditions of slow secretory dynamics (28), it is likely that the performance of single-compartment C-peptide kinetics will degrade as insulin secretion, and therefore changes in plasma C-peptide kinetics, becomes increasingly rapid and the effect of the “fast” C-peptide distribution compartment becomes more important.

To deal with rapid C-peptide kinetics, a more complex combined model is introduced, which incorporates the correct two-compartmental structure for C-peptide disappearance. The addition of two-compartment C-peptide kinetics requires a novel numerical approach to allow estimation of the model parameters. Most importantly, this extended combined model retains the ability to derive estimates of prehepatic insulin secretion without separate assessment or a priori assumption of C-peptide kinetics.

The current simulation study was undertaken with two goals. The first goal was to examine the perfor-
mance of the original combined model under conditions of rapidly changing insulin and C-peptide. The second goal was to examine the performance of the numerical identification scheme used for the extended combined model with two-compartment C-peptide kinetics under the identical computer-generated conditions. The a priori expectation is that the extended combined model would perform better than the original combined model simply due to the additional parameters introduced by the inclusion of the second compartment. The goal for the extended combined model was to examine whether the numerical approach used for the parameter identification has a negative impact on the model’s ability to reproduce the insulin secretory profile.

The simulation study shows that, under conditions of rapid kinetics, the original combined model is not able to provide accurate reconstruction of the insulin secretory profile. However, the extended combined model, incorporating two-compartment C-peptide kinetics and utilizing an alternative parameter identification scheme, does provide an accurate representation of prehepatic insulin secretion from insulin and C-peptide measurements, even under conditions of rapidly changing secretion. Thus, in principle, the extended combined model can be used to calculate insulin secretion correctly without separate quantification of C-peptide kinetics.

**METHODS**

The Models

The original combined model (model 1). Details of the original combined model (Fig. 1A) have been previously described (28, 30) and are briefly reviewed here. R(t) denotes the equimolar insulin-C-peptide release by the β-cells. For the insulin component of the model, a constant fraction of insulin is assumed to be lost due to first-pass hepatic extraction. Thus Fᵢ R(t) represents insulin that survives hepatic transit and distributes in the systemic circulation with single-compartment kinetics. In contrast, R(t) remains unchanged for C-peptide, since C-peptide is known to survive hepatic transit (22, 24). Model equations were derived as follows

\[
\frac{dI(t)}{dt} = FR(t) - K_{II}(t)V_I
\]

\[
\frac{dC(t)}{dt} = R(t) - K_{CC}(t)V_C
\]

Where \( V_I \) and \( V_C \) are the single-compartment distribution spaces for insulin and C-peptide, respectively. Allowing \( r(t) \) to be the secretion rate per unit C-peptide distribution volume \( [R(t)/V_C] \) and normalizing the fractional hepatic extraction rate (F) to the ratio of the C-peptide and insulin distribution spaces \( [F = F × (V_C/V_I)] \), Eqs. 1 and 2 simplify to

\[
\frac{dI(t)}{dt} = fr(t) - K_{II}(t)
\]

\[
\frac{dC(t)}{dt} = r(t) - K_{CC}(t)
\]

Because the model is expressed in terms of insulin secretion per unit volume C-peptide distribution, secretion rates in mass per time can be obtained by correcting for the C-peptide distribution space, which has been previously assessed to be 12.5% body weight for the single-compartment assumption (28).

The extended combined model (model 2). The extended combined model (Fig. 1B) is identical to model 1 in every aspect, except for incorporation of two-compartment C-peptide kinetics as described by Faber et al. (13). Model
equations can be written as follows

\[ V_1 \frac{dl(t)}{dt} = FR(t) - K_{11}l(t)V_1 \]  
\[ V_{C1} \frac{dC_1(t)}{dt} = R(t) - (K_{21} + K_{01})C_1(t) + K_{12}C_2(t)V_{C1} \]  
\[ V_{C2} \frac{dC_2(t)}{dt} = K_{21}C_1(t) - K_{12}C_2(t) \]

where \( C_1 \) represents the plasma, \( C_2 \) represents the extravascular compartments for C-peptide, and parameters \( K_{21} \) and \( K_{12} \) are the exchange rates between the first and second compartments. If similar substitutions are made for the secretion rate and fractional hepatic extraction as in model 1, new equations can be derived as

\[ \frac{dl(t)}{dt} = fr(t) - K_{11}l(t) \]  
\[ \frac{dC_1(t)}{dt} = r(t) - (K_{21} + K_{01})C_1(t) + \frac{V_{C2}}{V_{C1}} \]  
\[ \frac{dC_2(t)}{dt} = K_{21}C_1(t) - K_{12}C_2(t) \]

where insulin secretion is now estimated per unit C-peptide volume in the first, presumably plasma, compartment \( [r(t) = R(t)V_{C1}] \) and \( f = F \times (V_{C1}/V_{I}) \). Secretion rates estimated by this model can be corrected to mass/time by assuming a C-peptide distribution space of 6.02% body weight (27), the estimate for the plasma compartment for a two-compartment representation.

The Simulation Study Protocol

The study was designed to 1) determine how well the original combined model (model 1) could account for the rapid plasma insulin and C-peptide kinetics observed during a typical intravenous glucose tolerance test (IVGTT) and 2) assess the practical applicability and accuracy of the new numerical approach applied to the extended combined model (model 2). To test this, it was assumed that single-compartment insulin and two-compartment C-peptide kinetics were a correct representation of the in vivo system.

The simulation study protocol is outlined schematically in Fig. 2. Known kinetic parameters and IVGTT-like secretory profiles were submitted to model 2 to simulate plasma insulin and C-peptide data. Data representing typical “experimental” IVGTT data were generated by adding random error to each of the “perfect” simulated sets of data. Random Gaussian error with coefficients of variation of 3, 5, and 10% were added to the insulin and C-peptide time courses. Thirty sets of “experimental” data were generated at each error level (2 profiles \( \times 3 \) error levels \( \times 30 \) sets/error level, \( n = 180 \) overall). Each of the “experimental” data sets was analyzed using both models to assess the ability of each to reproduce the known kinetic parameters and insulin secretion rates.

From a theoretical perspective, the a priori expectation would be for model 2 to perform better than model 1 and provide accurate estimates of both the model parameters and prehepatic insulin secretion rates. This is simply due to the fact that model 2 is used to generate the simulated data to be analyzed. It must be emphasized that the goal of the simulation study was not to compare the performance of model 1 with model 2. In terms of model 2, it was equivocal whether the numerical approach used to estimate the kinetic parameters would perform well in the face of experimental data, i.e., data with error. Thus the evaluation of model 2 was for the assessment of the numerical method used for parameter identification rather than the actual performance of the model per se.

The secretory profiles used for the simulation study are shown in Fig. 3. Profile A was taken from work previously published by this group (30) and profile B was adapted from the studies by Shapiro et al. (26). Parameters for the simulations were taken from the literature (26, 27, 30) and are shown in Table 1. The plasma insulin and C-peptide time courses generated by simulating with these known parameters and secretory profiles are shown in Fig. 3.

Numerical Methods

Identification of model 1. Nonlinear weighted least-squares methods were used for the identification of parameters and insulin secretion rates for both models. Details of numerical analyses for model 1 have previously been described (30). The only difference in the current analysis was a modification in the weighting matrix. For identification of model 2 parame-
eters an algorithmic weighting function was used (see Data analysis). Thus, to maintain relative compatibility with the identification of model 2, model 1 identifications used the weighting matrix generated from model 2 analyses.

Identification of model 2. The traditional approach of simultaneously identifying model parameters and secretion rates, such as that used for model 1, was not applicable to model 2. This is because the additional two parameters required to identify parameters for two-compartment C-peptide kinetics, coupled with the large number of parameters associated with characterizing the prehepatic insulin secretion rate (n-1 samples), result in a system that is unidentifiable; i.e., unique solutions for the individual model parameters and the insulin secretion rates cannot be determined. Therefore, a unique numerical approach was used to circumvent these problems, which allowed for identification of the kinetic parameters independent of the secretion rate.

The method used for estimation of both prehepatic insulin secretion and kinetic parameters for insulin and C-peptide exploits the fact that insulin and C-peptide secretion rates are equal. Algebraic manipulation is used to factor out the prehepatic C-peptide disappearance rate. VC1, single compartment distribution for C-peptide in the 1st compartment.

Table 1. Parameters used for simulations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.4652</td>
</tr>
<tr>
<td>K1</td>
<td>0.2414 min⁻¹</td>
</tr>
<tr>
<td>V1</td>
<td>4.529 liters</td>
</tr>
<tr>
<td>C-Peptide</td>
<td></td>
</tr>
<tr>
<td>K21</td>
<td>0.053 min⁻¹</td>
</tr>
<tr>
<td>K12</td>
<td>0.051 min⁻¹</td>
</tr>
<tr>
<td>K01</td>
<td>0.062 min⁻¹</td>
</tr>
<tr>
<td>Vc1</td>
<td>4.214 liters</td>
</tr>
</tbody>
</table>

F, fraction of insulin that survives hepatic transit. K1, fractional insulin disappearance rate. V1, single compartment distribution for insulin. K21, K12, exchange rates between compartments. K01, fractional C-peptide disappearance rate. Vc1, single compartment distribution for C-peptide in the 1st compartment.

which are identical to the parameters of model 2, can then be estimated by fitting the C-peptide concentration, given insulin, or the opposite. The numerical approach applied to model 2 is described in detail in the appendix. The final equations used to estimate the kinetic parameters for model 2 are

\[
\frac{dY_1(t)}{dt} = \frac{1}{f} (K_1 - \lambda_1)[Y_2(t) + I(t)] - \lambda_1 Y_1(t) \quad (11)
\]

\[
\frac{dY_2(t)}{dt} = I(t)(K_{12} - \lambda_2) - \lambda_2 Y_2(t) \quad (12)
\]

\[
C(t) = Y_1(t) + \frac{1}{f} [I(t) + Y_2(t)] \quad (13)
\]

\[
\frac{dZ_1(t)}{dt} = f(\lambda_1 - K_1)[Z_2(t) + C_1(t)] - K_1 Z_1(t) \quad (14)
\]

\[
\frac{dZ_2(t)}{dt} = (\lambda_2 - K_12)C_1(t) - K_12 Z_2(t) \quad (15)
\]

\[
I(t) = Z_1(t) + f[Z_2(t) + C_1(t)] \quad (16)
\]

Equations 11-13 describe the kinetics of C-peptide in plasma \([C_1(t)]\), in which the plasma insulin profile \([I(t)]\) is used as a surrogate representation of the prehepatic insulin secretion rate. Parameters \(\lambda_1\) and \(\lambda_2\) characterize the kinetics of C-peptide in plasma, and the inverse of these two parameters represents the fast and slow time constants for C-peptide disappearance. Equations 14-16 describe the alternative formulation of the model in which the kinetics of plasma insulin are described using plasma C-peptide as the surrogate representation of the prehepatic insulin secretion rate.

This alteration of the model equations allows identification of model parameters independent of the insulin secretion rate. Once estimates for the model parameters are obtained, deconvolution is used to calculate the insulin secretion rates. The extended combined model differs from the Eaton method in that the estimations of model parameters are derived from plasma insulin and C-peptide measurements of the experi-
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Comparison of model fits between the differences in the number of estimated parameters. The degrees of freedom between the two sets of model fits due to tested for systematic patterns using the Runs Test. Use of the ing to Draper and Smith (10), and residual time courses were residual analysis. Studentized residuals were calculated accord-
simulated insulin and C-peptide data was assessed by re-
courses and comparison of the sums-of-squares using the

equations of interest alone; i.e., the advantages of the original combined model method are retained.

For model 2 parameter identifications, the simulated plasma insulin and C-peptide data were simultaneously fit to Eqs. 11-16 to estimate parameters f, K1, K12, λ1, and λ2 using weighted nonlinear least squares. Once individual parameter estimates were obtained, the prehepatic secretory rate was reconstructed using deconvolution. Deconvolution was performed independently on both insulin and C-peptide data; then the results were averaged to provide a final estimate of the prehepatic insulin secretion rate. The model-predicted insulin and C-peptide concentrations and their respective derivatives were used for the deconvolution calculation. Model 2 parameters for C-peptide kinetics can be resolved into the parameters from the standard two-compartment configuration by the following relationships

\[ K_{12} = K_{12} \]  
\[ K_{01} = \frac{\lambda_1 \lambda_2}{K_{12}} \]  
\[ K_{21} = \lambda_1 + \lambda_2 - K_{12} - K_{01} \]

Data analysis. Simulations for data generation and all identifications for model 2 were performed using MLAB (Civilized Software, Bethesda, MD) on a personal computer. MLAB uses a Marquardt-Levenburg weighted least-squares algorithm for parameter identifications. Weights for model 2 identifications were estimated using MLAB’s internal EWT weighting function. This function uses a five-point moving average to derive an estimate of the standard deviation in the data and then computes the weight as the inverse variance. All identifications for model 1 were performed on an Alliant FX/2 super minicomputer or a SUN workstation using in-house software written in FORTRAN-77 using IMSL subrou-
tines, as previously described (30). Weighting matrices from the model 2 identifications performed using MLAB were used for all model 1 identifications.

All statistics were performed using SAS implemented on a personal computer (SAS, Cary, NC). Data are reported as means ± SE unless otherwise stated.

Residuals. The ability of a given model to account for the simulated insulin and C-peptide data was assessed by resid-
ual analysis. Studentized residuals were calculated accord-
ing to Draper and Smith (10), and residual time courses were tested for systematic patterns using the Runs Test. Use of the Studentized residual partially accounts for the differences in degrees of freedom between the two sets of model fits due to the differences in the number of estimated parameters. Comparison of model fits between models 1 and 2 was accomplished by qualitative comparison of the residual time courses and comparison of the sums-of-squares using the Akaike Information Criterion (AIC) (1).

Parameter estimates. Model 1 estimates for both the fractional disappearance rate for insulin (K1) and index of fractional hepatic extraction (f) can be directly compared with the known values used for the simulation. For the simulation, parameter f represented the true fraction normalized to the ratio of the insulin and the two-compartmental C-peptide distribution spaces

\[ f_{\text{model}} = F \frac{V_C}{V_I} \]  

Therefore, parameter f estimated from model 1 should differ from the known value by the ratio of the differing C-peptide distribution spaces V C/V I. If the known parameter is corrected to this ratio, assuming V C to be 6.02% body weight and V I as 12.5% body weight, the model 1 estimated f should be 0.9659. Given the differences in model structure, C-peptide parameters estimated using model 1 cannot be directly compared with the known parameter values used in the original simulation. Parameter estimates from model 2 were directly compared with the known parameters used for the simulation by paired t-test.

Prehepatic secretion rates. All secretion rates were converted to units of mass per time assuming distribution volumes of 8,750 (12.5% body wt) and 4,214 ml (6.02% body wt) for models 1 and 2, respectively. The two different C-peptide distribution spaces are required due to the difference in model structures. The single-compartment representation used for model 1 requires an estimate of the total C-peptide distribution space, whereas only the volume of the plasma compartment is required for model 2.

Secretion profiles were contrasted in three ways. First, the average estimated secretory profiles were superimposed on the known profile and visually inspected for differences. Second, regression analysis was performed between the known profile and the individual estimated profiles at each error level. Although regression analysis is not completely appropriate for comparison of time course data, it should provide an indication of time shifts (reflected as hysteresis), relative degree of agreement (reflected as the slope), and relative offset (reflected as the intercept).

Results

Model 1

Examination of the residuals for model 1 fits to simulated insulin and C-peptide data revealed clear systematic patterns at all three error levels (Fig. 4). This distinct pattern in the residual time course is suggestive of a structural deficiency in the model. For profile A, model 1 exhibits a consistent underestimation of both the first-phase insulin and C-peptide peaks, which are then compensated for by subsequent over-
and underestimation in the remainder of the time courses. Analysis of the residual time courses revealed significant runs in all model fits (Runs test; P < 0.01). Model 1 fits to profile B appeared to be slightly better than those to profile A, in terms of relative magnitude, but consistent runs were also observed in the model fits (P < 0.01).

Table 2 shows the average model parameter estimates for both profiles. Like the model fits to the data, model 1-estimated parameters did not match those used for the simulation. For both profiles, the model estimates of the index of fractional hepatic insulin extraction (f) were underestimated by ~50% (cf. Table 2). This underestimation in fractional extraction was accompanied by an underestimation in the fractional disappearance rate for insulin (K1) of similar magni-
tude. For profile A, K1 was underestimated by an
average of ~40%, whereas for profile B the estimates were ~46% lower than the known value. Given that model 1 uses a single-compartment C-peptide representation, it is not possible to directly compare C-peptide parameters.

Model 1 was not able to accurately reproduce the known prehepatic insulin secretory profiles used to generate the simulation data (Fig. 5). For both profiles, the model predicts a biphasic secretory pattern but exaggerates both the first-phase response and the subsequent apparent refractory period at all three error levels. Although neither of the known profiles descends below the basal secretory rate during the first 30 min, in all cases model 1 incorrectly predicts a significant period of secretory inactivity with zero secretion. The exaggeration in the refractory period results in a loss of early second phase secretion in both profiles. However, the model appears to correctly estimate second phase insulin secretion from ~15 min until the end of the “experiment” at 180 min.

The distortion in the secretory estimates is more clearly depicted when the estimated rates are plotted against the known rate (Fig. 5A). The significant overestimation in first-phase secretion for both profiles results in average nonunity slopes of ~1.65 for profile A and ~1.72 for profile B with a significant degree of hysteresis. This distortion in the relationship between the known and estimated secretory profiles is dominated by the overestimation in first-phase secretion. When data for second-phase secretion are examined from 16 to 180 min, the slope of the relationship between the known and estimated secretory profiles is excellent at all three error levels: 0.947, 0.967, and 1.04 for profile A; 0.856, 0.875, and 0.943 for profile B.

Model 2

The simulated data without error were analyzed using model 2 to determine model consistency in an error-free environment. As shown in Table 3, the model was able to reproduce the known parameter estimates with acceptable accuracy. Identification of simulated data using model 2 with two-compartment C-peptide kinetics resulted in significantly improved fits com-

Table 2. Estimated model parameters using model 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Known</th>
<th>3% Error</th>
<th>5% Error</th>
<th>10% Error</th>
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<tbody>
<tr>
<td>Profile A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>0.9659</td>
<td>0.466±0.002†</td>
<td>0.467±0.004†</td>
<td>0.468±0.009†</td>
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<tr>
<td>K_(f) (min⁻¹)</td>
<td>0.2414</td>
<td>0.145±0.001†</td>
<td>0.147±0.001†</td>
<td>0.155±0.003†</td>
</tr>
<tr>
<td>K_(C) (min⁻¹)</td>
<td>0.0389</td>
<td>0.0394±0.0004</td>
<td>0.0417±0.0009</td>
<td></td>
</tr>
<tr>
<td>Profile B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>0.9659</td>
<td>0.426±0.002†</td>
<td>0.429±0.004†</td>
<td>0.441±0.011†</td>
</tr>
<tr>
<td>K_(f) (min⁻¹)</td>
<td>0.2414</td>
<td>0.109±0.001†</td>
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<td>0.126±0.005†</td>
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<td>K_(C) (min⁻¹)</td>
<td>0.0308</td>
<td>0.0316±0.0003</td>
<td>0.0344±0.0008</td>
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</tr>
</tbody>
</table>

Values for error are means ± SE. f = F × (V_C/V_I). K_C, fractional C-peptide disappearance rate. *Known value corrected for insulin and C-peptide distribution spaces. See METHODS for details. †P < 0.001 vs. known value.
pared with model 1 (Fig. 6). Model 2 showed an improved ability to account for the rapid first-phase spike. The improvement in model fits is reflected in both the AIC index and the residual plots. The AIC index was significantly lower with model 2 compared with model 1 at all three error levels (P < 0.001), which indicates that model 2 was able to better account for the rapid insulin and C-peptide kinetics. Furthermore, the residual plots revealed no distinct patterns by Runs test (P > 0.50), and overall the relative magnitude in the residuals was significantly reduced for both secretory profiles.

On the basis of the excellent model fits, one would expect good estimates of the model parameters. Comparison of the estimated model parameters with the known values is shown in Table 3. None were significantly different except for f, the index of hepatic insulin extraction, which was slightly underestimated in all cases. The accuracy with which the remaining parameters were estimated was excellent, with the average percent difference from known values not exceeding 12%.

As can be expected from the model fits and parameter estimates, calculated rates of prehepatic insulin secretion were virtually identical to the known infusion profiles (Fig. 7, top). Superimposing the average estimated secretion rates for each error level on the known rate shows near perfect agreement. This result is further emphasized when the known rates are plotted against the estimated rates (Fig. 7, bottom). For both profiles, there is no apparent hysteresis and the data fall randomly about the line of identity. When regression analyses are performed for each individual secretion profile, the average slopes of the relationships were not different from unity for both profiles: 0.997 ± 0.020, 1.024 ± 0.034, and 1.00 ± 0.031 for profile A and 0.996 ± 0.016, 1.028 ± 0.022, and 1.074 ± 0.039 for profile B.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Known</th>
<th>No Error*</th>
<th>3% Error</th>
<th>5% Error</th>
<th>10% Error</th>
</tr>
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<tbody>
<tr>
<td>Profile A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>0.4652</td>
<td>0.4534 (0.0133)</td>
<td>0.4436 ± 0.0218†</td>
<td>0.4305 ± 0.0394†</td>
<td>0.3918 ± 0.0732†</td>
</tr>
<tr>
<td>K1 (min⁻¹)</td>
<td>0.2414</td>
<td>0.247 (0.010)</td>
<td>0.233 ± 0.017</td>
<td>0.247 ± 0.029</td>
<td>0.214 ± 0.027</td>
</tr>
<tr>
<td>K12 (min⁻¹)</td>
<td>0.053</td>
<td>0.0602 (0.0015)</td>
<td>0.0514 ± 0.0022</td>
<td>0.0508 ± 0.0029</td>
<td>0.0479 ± 0.0037</td>
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<tr>
<td>λ1 (min⁻¹)</td>
<td>0.1440</td>
<td>0.173 (0.009)</td>
<td>0.152 ± 0.013</td>
<td>0.162 ± 0.021</td>
<td>0.141 ± 0.020</td>
</tr>
<tr>
<td>λ2 (min⁻¹)</td>
<td>0.02195</td>
<td>0.0238 (0.0002)</td>
<td>0.0221 ± 0.0006</td>
<td>0.0223 ± 0.0010</td>
<td>0.0224 ± 0.0014</td>
</tr>
<tr>
<td>Profile B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>0.4652</td>
<td>0.4707 (0.0108)</td>
<td>0.4383 ± 0.0293†</td>
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<td>K1 (min⁻¹)</td>
<td>0.2414</td>
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<td>0.210 ± 0.019</td>
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<td>K12 (min⁻¹)</td>
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<td>0.0577 (0.0005)</td>
<td>0.0502 ± 0.0027</td>
<td>0.0513 ± 0.0029</td>
<td>0.0473 ± 0.0035</td>
</tr>
<tr>
<td>λ1 (min⁻¹)</td>
<td>0.1440</td>
<td>0.162 (0.003)</td>
<td>0.143 ± 0.011</td>
<td>0.151 ± 0.013</td>
<td>0.142 ± 0.016</td>
</tr>
<tr>
<td>λ2 (min⁻¹)</td>
<td>0.02195</td>
<td>0.0246 (0.0001)</td>
<td>0.0229 ± 0.0007</td>
<td>0.0231 ± 0.0008</td>
<td>0.0228 ± 0.0014</td>
</tr>
</tbody>
</table>

*Represents analysis of the simulated data without addition of random error. Values within parentheses represent SE of the parameter estimate. †P < 0.05 vs. known value. λ1 and λ2, fast and slow time constants for C-peptide disappearance.
DISCUSSION

The combined model was introduced as an experimentally simple method for assessment of prehepatic insulin secretion rates (28, 30). The method has several advantages over alternative methodology. Noteworthy is the method's ability to provide estimates of prehepatic insulin secretory rates without independent assessment or assumption of parameters for C-peptide or insulin kinetics. This is in contrast to approaches that require an independent experiment to determine C-peptide kinetics (11) or assumption of the kinetic parameters for C-peptide (27). Also, unlike the approach introduced by Pacini and Cobelli (20), the combined model approach is not restricted to a single type of experimental protocol. The combined model approach can provide estimates of prehepatic insulin secretory rates from a single protocol of the investigator's choice so long as there are sufficient dynamics in

![Figure 6](image)

Fig. 6. Model 2 fits to simulated data. Average model 2 fits (solid line) are shown along with average simulated data (symbols) at each error level tested. Error bars are omitted for clarity. Model 2 predictions for profile A are shown on left and profile B on right. Symbols are identical to those in Fig. 4. Insets show average (means ± SE) Studentized residual time course.

![Figure 7](image)

Fig. 7. Model 2 estimated prehepatic insulin secretory profiles. Average model 2 estimated insulin secretion rates (dashed line) are superimposed on known rate used for simulation (solid line) for each error level tested. A: profile A; B: profile B. Insets show average estimated secretion rates plotted against the known rates. Solid line in each inset represents line of unity. Error bars have been omitted for clarity.
the plasma insulin and C-peptide time courses. The combined model achieves the ability to derive secretory rates without separate kinetic assessment through the simultaneous kinetic analysis of insulin and C-peptide dynamics from the experiment of interest alone. This integration of kinetic information provides added advantages. The combined model provides estimates of kinetic parameters for both insulin and C-peptide and provides an index of fractional hepatic insulin extraction. Thus one is able to obtain a near-complete kinetic profile of the insulin-C-peptide secretory system.

However, the structure of the combined model has recently been criticized for its simplified description of C-peptide kinetics (18, 27). The model was developed using the "minimal" model concept, which calls for the simplest model to account for the observed data (3, 5). Thus single-compartment representation was used under the assumption that such a description would provide an adequate, though admittedly imprecise, description of C-peptide kinetics. It should be noted that the possible deleterious effect of a simplification in C-peptide kinetics was not taken lightly, and validation experiments were performed, which apparently demonstrated the ability of the combined model to faithfully reproduce a known intraportal infusion pattern (28). However, under conditions of slow dynamics, it is possible for a single-compartment model to adequately account for two-compartment kinetics (K. Thomsen, personal communication). Thus the relatively slow infusion patterns used in the validation study and the resultant slow plasma dynamics may have created favorable conditions for using single-compartment C-peptide kinetics. It should be noted that single-compartment C-peptide kinetics have been successfully implemented for a variety of models and conditions (15, 16, 28–29).

The present study shows that, as plasma kinetics become more rapid, the second-order nature of C-peptide becomes readily apparent and the performance of the single-compartment model to account for such kinetics degrades significantly. The original combined model was unable to accurately account for insulin and C-peptide profiles simulating plasma concentrations during an IVGTT. Use of the single-compartment configuration for C-peptide kinetics resulted not only in poor model fits to the data, but also in grossly inaccurate parameter estimates for the insulin component of the model and presumably poor estimates for C-peptide parameters as well. The poor model fits and inaccurate model parameter estimates led to predictably poor reconstruction of the insulin secretory profile. Thus it is clear from this study and the previous validation study (28) that the original combined model with single-compartment insulin and C-peptide kinetics has limited applicability.

The apparent inability of the original combined model to account for rapid plasma insulin and C-peptide dynamics led to the consideration of an extended combined model with the more correct two-compartment C-peptide kinetics. The intent was to improve on the structure of the original combined model to allow application of the method to a wider range of kinetic conditions. This extended model was evaluated by analyzing the same simulation used to test the original combined model. Although this second component of the simulation study may appear to be circular logic, generating simulated data using a given model and then using that same model to identify parameters, there were several reasons such simulation testing was warranted.

First, to our knowledge the algebraic manipulation of model 2 equations that allowed for the successful identification of all the model parameters independent of the prehepatic insulin secretion rates has never been applied to a compartmental analysis problem of this nature. Although theoretically plausible, the robustness of the method in the face of error was unknown. It was possible that practical application of the method would be restricted due to an inability to extract kinetic information from data with error. This simulation study was designed with error levels assigned to simulate typical sampling and assay error that are likely to be encountered for IVGTT data. Thus this simulation study provides a good initial test of the robustness of the numerical approach used for the extended combined model (model 2).

Second, because the method relies on one peptide profile as a known input into the equations describing the kinetics of the other peptide, it was unclear what effect errors in the input profile would have on the parameter estimation. One potential possibility was the errors associated with the plasma measurements having a deleterious effect on the parameter identification process, leading to poor parameter estimates. There were several options to handle this problem.

One would have been to ignore the errors and hope that the weighted least squares would be immune to the effects of the input error. This was an undesirable choice, since the least-squares algorithm is not designed to handle errors of this type. Another possibility would have been to smooth the input profile before the identification. With the assumption of an unbiased smoothing algorithm being used, this option would clearly minimize errors in the input profile. However, this option has the negative effect of possible removal of physiological fluctuations in the secretion profile by the smoothing routine. Thus a certain amount of information loss is to be expected with this approach.

The approach finally chosen was to perform the simultaneous identification of both forms of the model: C-peptide-given insulin and insulin-given C-peptide. This approach has the advantage that the least-squares algorithm must provide best fits to both the insulin and C-peptide data, given the errors in both input profiles. Thus information from both the insulin and C-peptide model fit and both input profiles are contributing to the cost function in the overall identification process. The net result of this dual identification approach would be a dampening of the effect of measurement error associated with the profile used as the known input. This approach assumes that the errors in
insulin and C-peptide were random Gaussian and not biased in a correlated fashion.

The extended combined model provided reasonably accurate and precise estimates of all model parameters. However, there was a small, but systematic, underestimation of f, the index of fractional hepatic insulin extraction. There is no clear explanation for this inconsistency in the model. One possibility is that, because the model showed a modest inability to precisely fit the first-phase plasma responses (cf. Fig. 6), this translated into an inaccurate estimate of the f parameter. Although modifications to the numerics of the problem may relieve this problem, it should be noted that the relatively minor deviation in the model fits during the first phase and the underestimation in parameter f appear to have no negative effects on the model’s ability to estimate the other parameters or the final calculation of insulin secretion. The results clearly indicate an ability of the model to provide accurate and precise assessment of the parameters of insulin and C-peptide kinetics and the final outcome of interest, the prehepatic insulin secretion rates under the controlled conditions of the computer environment.

In summary, the current computer simulation study evaluated the ability of two different models to account for plasma insulin and C-peptide data simulating an IVGTT. The original combined model using single-compartment kinetics for both insulin and C-peptide was unable to account accurately for the simulated IVGTT plasma dynamics, resulting in poor parameter estimates and inaccurate reconstruction of the insulin secretory profiles. However, because previous work has shown that the original combined model was capable of accounting for alternative plasma dynamics (28), it would be biased to reject the original model outright. The original combined model can be applied under certain conditions but only after careful consideration of the type of data to be analyzed. Analysis of the identical set of simulated data using an extended combined model, which incorporates two-compartmental C-peptide kinetics, resulted in near-perfect reproduction of the known insulin secretory profiles. These results indicate that the novel numerical approach used for the extended combined model has no negative impact on the model’s ability to identify model parameters and estimate prehepatic insulin secretion. Thus we conclude that this new combined model with two-compartmental C-peptide kinetics provides accurate estimates of “prehepatic” insulin secretion without separate assessment of C-peptide kinetics or assumption of parameter values.

The ability of the extended combined model to easily provide a complete kinetic profile of the insulin secretory system from a single experimental protocol gives clinical investigators a powerful tool for the assessment of β-cell function in vivo. Furthermore, integrated application of the extended combined model and the Minimal Model of glucose kinetics (2, 5, 6) to intravenous glucose tolerance test data provides clinicians the ability to obtain a near complete metabolic profile for a given subject. This ability should allow for improved characterization of pathogenesis of NIDDM. However, the “acid test” of the efficacy of the extended combined model will require the demonstration of the ability to reconstruct known secretion rates in an in vivo model.

APPENDIX

The identification of kinetic parameters for model 2 is based on algebraic manipulation to factor out the insulin secretion rate R(t) from the model equations. The principle behind this manipulation is based on the fact that the plasma time courses of insulin and C-peptide are determined by the identical secretory profile. Thus it should be possible to use one peptide concentration profile as a surrogate representation of prehepatic insulin secretion to characterize the kinetics of the other peptide. In other words, it should be possible to use insulin as a known input representative of prehepatic secretion and fit the plasma C-peptide data to estimate the various kinetic parameters. The derivation of new model equations is described herein.

Consider the extended combined model with two-compartment C-peptide kinetics (cf., model 2, Fig. 1B) in incremental form, i.e., the increment in insulin secretion from basal. Initially focusing on the C-peptide component of the model independent of the insulin equation, differential equations describing the kinetics of C-peptide can be written as

\[
\frac{dC_1(t)}{dt} = -(K_{21} + K_{01})C_1(t) + K_{12}C_2(t) + \frac{1}{V_{C_1}}R(t) \quad (A1)
\]

\[
\frac{dC_2(t)}{dt} = -K_{21}C_1(t) - K_{12}C_2(t) \quad (A2)
\]

Equations A1 and A2 can be rewritten in matrix form by combining terms, yielding

\[
\begin{bmatrix}
\dot{C}_1(t) \\
\dot{C}_2(t)
\end{bmatrix} = \begin{bmatrix}
-A_{11} & A_{12} \\
-A_{21} & -A_{22}
\end{bmatrix} \begin{bmatrix}
C_1(t) \\
C_2(t)
\end{bmatrix} + \begin{bmatrix}
B \\
0
\end{bmatrix} R(t) \quad (A3)
\]

Application of the Laplace operator converts Eq. A3 from the time domain to the Laplace domain and yields the following transfer function

\[
\frac{C_1(s)}{R(s)} = \frac{(s + A_{22}B)}{(s + A_{11})(s + A_{22}) - A_{22}A_{12}} \quad (A4)
\]

A similar conversion can be made for the insulin component of the model. The single differential equation for insulin can be written as

\[
\frac{dl(t)}{dt} = -K_{11}l(t) + \frac{1}{V_{I}}R(t) \quad (A5)
\]

and the final transfer function in the Laplace domain is

\[
\frac{I(s)}{R(s)} = \frac{D}{(s + C_{11})} \quad (A6)
\]

Two items should be noted. First, the two-differential equation description for C-peptide kinetics has now been reduced to a single equation. Second, the insulin secretion rate in the Laplace domain [R(s)] now appears in both the C-peptide and insulin transfer functions (cf., Eqs. A4 and A6). It is now possible to solve for R(s) in one equation and substitute into the other. In this manner, the prehepatic secretion rate falls
out of the calculation after algebraic manipulation as shown in Eq. A7, when \( R(s) \) was solved for in the insulin transfer function and substituted into the C-peptide transfer function.

\[
C_I(s) = \frac{B}{D} \frac{(s + C_{11})(s + A_{22})l(s)}{s^2 + (A_{11} + A_{22})s + A_{11}A_{22} - A_{21}A_{12}} \tag{A7}
\]

This equation can be simplified to the single transfer function

\[
C_I(s) = \frac{1 + \lambda_1(s + \lambda_2)}{\frac{1}{f}(s + \lambda_1)(s + \lambda_2)} \tag{A8}
\]

where \( \lambda_1 \) and \( \lambda_2 \) are the quadratic solutions to the denominator in Eq. A7 and characterize the kinetics of C-peptide in plasma. The inverse of these two parameters represents the fast and slow time constants for C-peptide disappearance. More importantly, note that the insulin secretion rate \( [R(s)] \) has now disappeared from the equation, with plasma insulin now acting as a known input to the equation describing C-peptide kinetics. Equation A8 can now be converted back into the time domain by defining two new “state variables” \( Y_1 \) and \( Y_2 \)

\[
\frac{dY_1(t)}{dt} = f(K_I - \lambda_1)Y_2(t) + \lambda_1 Y_1(t) \tag{A9}
\]

\[
\frac{dY_2(t)}{dt} = -l(t)(K_{12} - \lambda_2) - \lambda_2 Y_2(t) \tag{A10}
\]

\[
C_I(t) = Y_1(t) + \frac{1}{f}[l(t) + Y_2(t)] \tag{A11}
\]

In this formulation of the model, the plasma insulin profile is used as a surrogate representation of the insulin secretion rate. With the use of the plasma insulin profile as a “known” input, the plasma C-peptide data can be fit to Eqs. A9-A11 to estimate the individual model parameters. It should be noted that the alternative formulation of using C-peptide as a known input to fit the plasma insulin data can also be derived as

\[
\frac{dZ_2(t)}{dt} = f(K_I - \lambda_1)Z_1(t) + C_I(t) - K_{12}Z_2(t) \tag{A12}
\]

\[
\frac{dZ_1(t)}{dt} = (\lambda_2 - K_{12})C_I(t) - K_{12}Z_2(t) \tag{A13}
\]

\[
l(t) = Z_2(t) + f[Z_2(t) + C_I(t)] \tag{A14}
\]

As noted earlier, this new modification to the parameter estimation and calculation of the prehepatic insulin secretion rates is based on the increment in prehepatic insulin secretion. The basal prehepatic insulin secretion rate \( r_b \) was determined as

\[
r_b = \lambda_b f K_I \text{ or } C_b K_{01} \tag{A15}
\]

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