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# A numerical deconvolution method to estimate C-peptide secretion in humans after an intravenous glucose tolerance test

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**Abstract**

Quantitative assessment of pancreatic insulin secretion rate in individuals may help advance our understanding and treatment of diabetes. We describe for the first time the application of a long-established numerical deconvolution procedure in which a prescribed input function is used to represent first-phase pancreatic secretion response to an intravenous glucose challenge (intravenous glucose tolerance test [IVGTT]) in individual subjects. We identify that C-peptide secretory response to an IVGTT can be described by a basal secretion rate ($S_b$) (picomoles per liter per minute) and a first-phase secretory response characterized by a Gaussian function. The Gaussian function contains 3 parameters: $P_1$ (picomoles per liter per minute), which represents the peak rate secretion; $P_2$ (per square minute), which is related to the inverse of peak width at half-peak height; and $P_3$ (minutes), which is the time of the peak secretion rate. When applied to data from 8 healthy Chinese subjects, the estimated parameter values (mean ± SD) were 19.2 ± 12.9 pmol $L^{-1}$ $min^{-1}$, 1548 ± 1143 pmol $L^{-1}$ $min^{-1}$, 1.09 ± 1.21 $min^{-2}$, and 2.94 ± 1.13 minutes for $S_b$, $P_1$, $P_2$, and $P_3$, respectively. The Gaussian input functions are shown to have similar shapes and to be highly concordant in magnitude with secretory responses estimated by means of the method of Eaton et al (1980) and by the ISEC computer program. In conclusion, we have presented a simple, integrated, validated, and easily implemented method suitable for quantifying pancreatic C-peptide and insulin secretion in individual human subjects. The superiority of our method in comparison with other methods is that it uses as an input function the Gaussian, which has been experimentally verified as describing in vivo the profile of pulsatile insulin secretion. The particular strength of our method is that the Gaussian parameters and simple indices derived from them provide a standardized and interpretable means for carrying out comparative investigations aimed at quantifying how pancreatic secretory responses to an IVGTT differ in various demographic groups or in response to therapeutic treatments.


## 1. Introduction

Quantitative assessment of pancreatic insulin secretion rate (ISR) in an individual in a basal state and/or during a glucose challenge is necessary if we are to be able to understand the mechanistic changes in $\beta$-cell function during the progression from health to diabetes or if we are to assess how the $\beta$ cells respond to therapeutic treatments. The 4 most common glucose challenge protocols are the intravenous glucose challenge (intravenous glucose tolerance test [IVGTT]), the insulin-modified glucose tolerance test, the oral glucose tolerance test, and the meal tolerance test [1]. Although ISR has been estimated after each of these different challenge protocols and slight variations on these protocols, the work presented here is specifically focused on ISR with respect to the standard IVGTT.

Direct measurement of ISR is generally not practical or desirable because it requires invasive protocols involving catheters placed in the hepatic artery and vein. Indirect procedures for estimation of ISR generally use a glucose challenge and 1 of 5 distinct mathematical methods to analyze the resulting plasma C-peptide and insulin data [2]. These indirect procedures are based on the fact that, for every mole of insulin secreted by the pancreas, the pancreas also secretes 1 mole of connecting peptide (C-peptide) [3,4] and that C-peptide is not appreciably extracted by the liver [5]. Thus, the ISR is equal to the rate of secretion of

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C-peptide (CPSR). The estimation of CPSR is not a trivial problem because both the secretion of C-peptide by the pancreas and the clearance of C-peptide from peripheral circulation occur simultaneously. The mathematical solutions to this problem use, in either an implicit or explicit form, a deconvolution approach with the CPSR treated as the unknown input function, the plasma response to a unit impulse considered as the transfer function (or disposition model), and the concentration of C-peptide in plasma as the output of the C-peptide system. Thus, C-peptide plasma concentration and CPSR are related by means of the convolution integral [6,7]:

$$C(t) = \int_0^t h(t-\tau)\mathrm{CPSR}(\tau)d\tau \quad (1)$$

Where $C(t)$ is the C-peptide plasma concentration (picomoles per liter) at $t$ minutes after administration of glucose, $h(t)$ is the C-peptide impulse response (per liter), and CPSR($t$) is the C-peptide secretion rate (picomoles per minute).

The approach of Eaton et al [8], which for a long time has been regarded as the criterion standard of deconvolution techniques, specifically assumes that C-peptide inertly distributes in 2 pools in the body; that, as stated above, insulin is cosecreted, mole per mole, with C-peptide; and that the C-peptide system is in equilibrium at the start of the study [8,9]. Using the approach of Eaton et al, the CPSR is given by:

$$CPSR(t) = -e^{-k_2 t}\left[k_1 C(t_1)e^{k_2 t_1} + k_1 k_2 \int_{t_1}^{t} e^{k_2 s} C(s)ds\right] + \frac{dC(t)}{dt} + (k_1 + k_3)C(t) \quad (2)$$

Where $k_1$, $k_2$, and $k_3$ are fractional rate constants (per minute) describing the movement of C-peptide from compartment 1 (the accessible compartment) to compartment 2 (the peripheral compartment) and from compartment 2 to compartment 1, and the elimination from compartment 1, respectively. $C(t_1)$ is the C-peptide concentration (picomoles per liter) at time zero, that is, the time of the glucose challenge. Eaton et al [8] used cubic splines of the C-peptide data because these provide a "smoothed" representation of $C(t)$ and, most importantly, spline functions can be differentiated and integrated, which is a prerequisite of the approach of Eaton et al. In addition, Eaton et al used mean C-peptide disposition parameters that they derived from 2-compartment analysis of the disappearance from plasma of C-peptide injected into 7 diabetic subjects [8,10]. Since Eaton et al first published their method in 1980, there have been in excess of 250 scientific articles that have referred to their work, including more than 70 articles since 2000, many of which describe the use of the method of Eaton et al for estimating insulin secretion [11].

A major disadvantage of the approach of Eaton et al [8] is that it requires an extra "substudy" to isolate the C-peptide disposition kinetics [12]. Polonsky et al [13-15] and Van Cauter et al [16] were able to show in a series of studies that the disposition kinetics of C-peptide for any given individual could be estimated from the metabolic state of the individual (normal, obese, diabetic), as well as from demographic data: the age and sex of the subject and the body surface area (BSA). Subsequently, Hovorka and colleagues [17] compiled the information from the experiments of Polonsky et al and Van Cauter et al into a computer program (ISEC) to facilitate the estimation of insulin secretion profiles.

The deconvolution approach used in ISEC involves constrained regularization and a stepwise approximation to insulin secretion. The ISEC program has been used in more than 30 published experiments and has facilitated the quantification of C-peptide secretion after the oral glucose tolerance test protocol [18], meal tolerance test [19], graded glucose infusions [20], standard IVGTT [21], tolbutamide-modified IVGTT [22], and 30-minute IVGTT [23]. The approach used in ISEC is somewhat similar to the "nonparametric" methods popularized by Sparacino and Cobelli [2] in that the method of Sparacino and Cobelli is also based on a regularization approach.

A third group of methods for determining C-peptide and/or insulin secretion involves direct modeling; and this relatively sophisticated approach has been refined over many years, and the details of a number of variations on the approach have been described in detail [24]. In the compartmental modeling approach of Volund et al [25], least squares data fitting is used to estimate not just the disposition parameters for both insulin and C-peptide, but also a function describing the pancreatic secretion of insulin and a parameter describing the fraction of pancreatic insulin secretion extracted by the liver. Thus, the 2 important advances of the approach of Volund et al are the estimation of the fraction of insulin extracted by the liver (HE) and obviating the necessity to carry out separate substudies to determine C-peptide kinetics [25]. Although the approach of Volund et al [25] involves fitting splines to the C-peptide data and differentiation of the spline, this approach has a more statistical/data fitting emphasis than the approach of Eaton et al [8]. The approach of Volund et al makes the important assumption that the liver extracts some insulin, but does not extract C-peptide. A further difference between the approach of Volund et al and that used by Eaton et al is that Volund et al assumed single-compartment models for both insulin and C-peptide disposition [25].

A fourth deconvolution method uses numerical stepwise integration and was first described by Turner and colleagues [26] in 1971, where they used their technique to investigate first-phase insulin delivery to the systemic circulation. The method described by Turner and colleagues involved an iterative analytical approach similar to "curve stripping." The

method of Turner et al was also subsequently described as a "waveform-independent method" [27]. Because Turner et al in 1971 applied their method to plasma insulin concentrations and did not take into account hepatic extraction of insulin, it did not describe ISR. Moreover, we have not found any articles in the scientific literature that have applied the method of Turner et al to C-peptide secretion. Nevertheless, if applied to C-peptide data, the method of Turner et al could be used to estimate ISR.

In some instances, researchers have modified the above-described deconvolution methods by including Bayesian approaches to assist with parameter estimation [28].

A fifth deconvolution approach involves the use of simulation procedures such as reversible jump Markov chain Monte Carlo sampling techniques [29].

Finally, in the sixth deconvolution technique, which is sometimes called *numerical deconvolution*, *parametric deconvolution*, or *Cutler's method of deconvolution*, the functional form of the input function is assumed so that the deconvolution problem becomes one of parameter estimation; and this can be performed by the method of least squares [6,7,30]. Thus, in numerical deconvolution, the main problem is the identification of a functional form that has the flexibility to plausibly describe the shape of the input function in question. A wide range of functional forms has been used in deconvolution procedures to estimate input functions for a large variety of biological systems [6,7,27,30-33]. In the example most relevant to hormone secretion, Gaussians were used as input functions to describe secretion of luteinizing hormone and growth hormone [27]. Cutler's method does not appear to have been applied to the ISR or CPSR problem with respect to the IVGTT. Furthermore, as far as we can ascertain, no authors have identified a functional form suitable for this specific purpose.

Based on a variety of estimation approaches, there is some evidence that the insulin secretory pulse after an IVGTT can be loosely described as Gaussian in shape, with a peak occurring between 2 and 4 minutes [23,34,35]. Furthermore, in the classic article of Eaton et al [8] on the ISR in response to a standard IVGTT, the pattern of ISR (see the original Fig. 5 in Eaton et al) appears to approximate a Gaussian function.

We point out that, although compartmental modeling techniques as well as the methods of Eaton et al [8], Turner et al [26], and even the ISEC computer program can be used to produce estimates of the first-phase secretory response to an IVGTT, the resulting secretion profiles must be subjected to analysis to obtain indices to describe the secretory pulse. In contrast, an advantage of Cutler's method of using a prescribed input function is that the parameters per se of the input function can serve directly as a way of quantifying the input pulse. Alternatively, simple arithmetic transformation of the parameters may provide useful indices of the input pulse. Thus, if the ISR($t$) in response to a standard IVGTT could in fact be shown to approximate a Gaussian function, this could facilitate ISR($t$) estimation by means of a prescribed input function and, most importantly, provide a standard means for quantifying and describing the principal features of ISR responses.

With respect to the standard IVGTT and resulting C-peptide secretion pulse, the principal aims of the work presented here are as follows:

1. To use the deconvolution approach of Eaton et al [8] and the ISEC computer program to investigate the functional form of the CPSR($t$) and to determine if the insulin secretion pulses can be approximated by a Gaussian function.
2. To use a numerical deconvolution technique using a Gaussian input function to estimate the Gaussian parameters describing CPSR in healthy Chinese subjects.
3. To demonstrate concordance and/or correlation of attributes of secretion pulses as estimated by the numerical deconvolution technique with the same pulse attributes as estimated by the standard approach of Eaton et al [8] and the ISEC computer program.
4. To present a robust, user-friendly, easily implementable method that can be used to quantify the principal features of the C-peptide response to an IVGTT in individual subjects.

## 2. Investigations and methods

### 2.1. Subjects and experimental protocol

Six healthy male subjects and 2 healthy female subjects participated in this study. All subjects were Chinese. Their individual demographic features are listed in Table 1 The purpose and nature of this study were explained to all subjects before their written consent was obtained. The study protocol was reviewed and approved by the Human Subjects Research Committee of the Tri-Service General Hospital, National Defense Medical Center, Taipei. All IVGTT studies were performed at 8:00 AM after an overnight fast. The

Table 1
Individual subject demographic features and C-peptide rate constants ($k_1$, $k_2$, and $k_3$) as calculated by the equations of Van Cauter and colleagues [16]

| Subject | Sex | Age (y) | BMI (kg m$^{-2}$) | BSA (m$^2$) | $k_1$ (min$^{-1}$) | $k_2$ (min$^{-1}$) | $k_3$ (min$^{-1}$) |
|---|---|---|---|---|---|---|---|
| 1 | F | 56 | 22.03 | 1.518 | 0.0561 | 0.0478 | 0.0548 |
| 2 | F | 31 | 18.12 | 1.425 | 0.0527 | 0.0493 | 0.0587 |
| 3 | M | 23 | 19.86 | 1.709 | 0.0515 | 0.0499 | 0.0601 |
| 4 | M | 26 | 22.03 | 1.739 | 0.0520 | 0.0496 | 0.0595 |
| 5 | M | 20 | 21.46 | 1.688 | 0.0512 | 0.0501 | 0.0606 |
| 6 | M | 21 | 25.54 | 1.937 | 0.0510 | 0.0500 | 0.0604 |
| 7 | M | 20 | 24.1 | 1.924 | 0.0510 | 0.0501 | 0.0606 |
| 8 | M | 20 | 20.44 | 1.811 | 0.0510 | 0.0501 | 0.0606 |
| Mean | | 27.1 | 21.7 | 1.719 | 0.0521 | 0.0496 | 0.0594 |
| SD | | 12.3 | 2.3 | 0.180 | 0.0017 | 0.0008 | 0.0020 |

BMI indicates body mass index.

IVGTT studies involved the standard frequently sampled protocol without administration of exogenous insulin. Briefly, catheters were placed in both femoral veins. Glucose (300 mg $kg^{-1}$) was injected through 1 catheter over a 1-minute interval. Blood samples were collected from the ipsilateral catheter at −5, 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 40, 50, 60, 70, 80, 90, 100, 150, 180, 210, and 240 minutes in relation to the glucose injection. Plasma C-peptide was determined using a Food and Drug Administration–approved radioimmunoassay (Dia-Sorin, Stillwater, MN). Sensitivity and intra- and interassay precision of the C-peptide were 30 pmol $L^{-1}$, 6.0% ± 2.5%, and 5.9% ± 0.9%, respectively.

### 2.2. Modeling C-peptide secretion using a Gaussian input function

Our numerical deconvolution technique uses the principals outlined by Cutler [6,7] and is somewhat similar to some previous modeling procedures [27,30]. Like many other researchers, we use a 2-compartment model to describe C-peptide disposition [10,14,16,24]. The main difference between our method and previous approaches is that we assume that, immediately after an IVGTT, C-peptide secretion from the pancreas is described by 2 processes: constant basal C-peptide secretion and a Gaussian function to represent first-phase C-peptide secretion. A schematic of a compartmental model of C-peptide kinetics for analyzing data from an IVGTT is shown in Fig. 1. The equations describing our model are as follows:

$$\frac{dC_1(t)}{dt} = S(t) + k_2 C_2(t) - (k_1 + k_3) C_1(t) \tag{3}$$

$$\frac{dC_2(t)}{dt} = k_1 C_1(t) - k_2 C_2(t) \tag{4}$$

Where

$$C_1(0) = C_b$$

$$C_2(0) = \frac{C_b k_1}{k_2}$$

$$S(t) = S_b + G_1(t)$$

$$G_1(t) = P_1 \exp\left\{-P_2 (t - P_3)^2\right\}$$

Where $C_1(t)$ and $C_2(t)$ are C-peptide concentrations (picomoles per liter) in compartments 1 and 2, respectively, at time $t$ minutes after the start of the IVGTT. $C_b$ is the end-test C-peptide concentration; and $k_1$, $k_2$, and $k_3$ (per minute) are transfer rate constants. $S(t)$ is a function describing the rate (picomoles per liter per minute) of secretion of C-peptide. $S(t)$ is composed of a basal rate of secretion of C-peptide ($S_b$) and a Gaussian function $G_1(t)$ representing a first-phase C-peptide secretion pulses. $P_1$ (picomoles per liter per minute) represents the height of the first-phase secretory pulse, $P_2$ (per square minute) is related to the inverse of the pulse width at half-peak height, and $P_3$ (minutes) represents the time of the peak of the first-phase C-peptide secretion rate. The use here of a Gaussian input function is similar to a recently published procedure in which Gaussians were used as input functions describing the rate of meal consumption during a day [31].

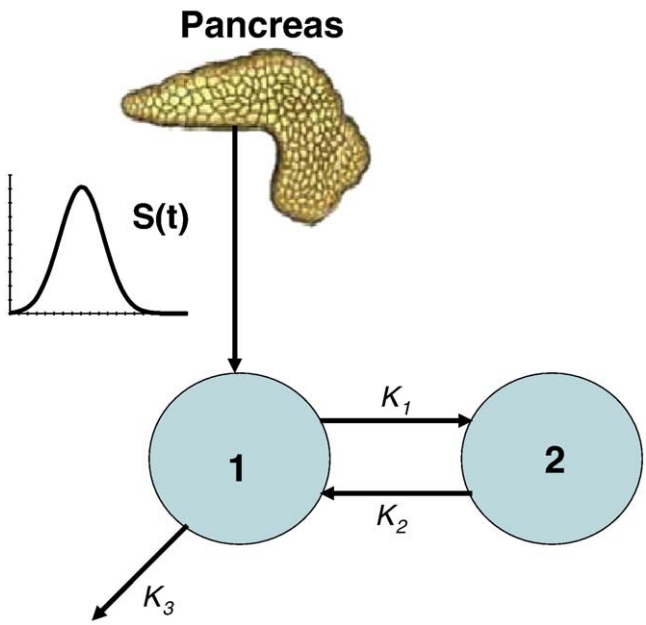


Fig. 1. A schematic of a compartmental model of insulin and C-peptide kinetics for analyzing data from an IVGTT. $S(t)$ is a function describing the rate of $\beta$-cell secretion into the portal vein; $S(t) = G_1(t) + S_b$, where $G_1(t)$ is a Gaussian function describing the first-phase insulin response to a glucose challenge and $S_b$ is the basal ISR. $C_1(t)$ and $C_2(t)$ are C-peptide concentrations (picomoles per liter) in the plasma and a distribution compartment, respectively. The first-order rate constants $k_1$ and $k_2$ (per minute) describe the distribution of C-peptide, whereas $k_3$ (per minute) describes the elimination of C-peptide.

The total cumulative secretion of C-peptide, AS($T$), up to time $T$ minutes can be estimated by evaluation of the integral:

$$\mathrm{AS}(T) = \int_0^T S(t)dt \tag{5}$$

In practice, AS($T$) evaluated between 0 and 10 minutes provides, in most cases, an estimate of total first-phase C-peptide secretion (picomoles per liter). Using the Gaussian parameters, a number of additional useful indices can be easily calculated. The width ($W_1$, minutes) at half-peak height of the first-secretory pulse is calculated as follows:

$$W_1 = 2\sqrt{\frac{\ln 2}{P_2}} \tag{6}$$

The pulsatility of the first pulse can be described by the ratio of the height to the width of the pulse ($HW_1$, picomoles per liter per square minute):

$$HW_1 = P_1 / W_1 \quad (7)$$

Modeling was performed with WinSAAM, which can be downloaded free from http://www.winsaam.com. The use of WinSAAM for modeling nonlinear compartmental models has been described previously [36,37]. The adjustable parameters $S_b$, $P_1$, $P_2$, and $P_3$ were determined by fitting the plasma C-peptide data to function $C_1(t)$. For each individual subject, the fixed parameters $k_1$, $k_2$, and $k_3$ were calculated from the demographic features sex, age, and BSA by the equations described by Van Cauter et al [16]. Parameter identifiability was established using frequently quoted standard procedures [38,39]. The fitting criterion used was that of nonlinear weighted least squares, with a fractional standard deviation weighting scheme [37].

### 2.3. Implementation of the deconvolution scheme of Eaton et al

The deconvolution scheme of Eaton et al [8] with a small number of necessary modifications/improvements was implemented using STATA statistical analysis software [40]. The modifications related to the method of smoothing and the method for choosing knot locations. Smoothing of the plasma C-peptide data from individual subjects to obtain a functional expression was accomplished by means of the bspline (a series of cubic polynomials that are joined at specific knot points) feature of STATA [41]. It should be noted that, in all deconvolution schemes that involve splines, the choice of number and location of knots appears somewhat arbitrary and problematic because, in the hands of an inexperienced investigator, poor choice of knot number and location can substantially distort the shape of derived ISR curves. In the article of Eaton et al [8], the number and location of knots for each individual subject's data set were determined with a computer program (KABIS). Unfortunately, this program appears to be no longer available. Therefore, we investigated the consequences of various numbers and locations for knots and found that, with respect to our IVGTT C-peptide data sets, knots at 0, 1, 2.5, 3.5, 6, 10, 20, 40, 140, and 240 minutes produced usable smoothed representations of the C-peptide profiles (Fig. 2A).

### 2.4. Analysis of data sets using ISEC

The same 8 data sets as described above were also analyzed using the ISEC computer program Version 3.4a [17]. When comparing results concerning secretion rates (picomoles per liter per minute) obtained from the compartmental model with Gaussian input against results from the ISEC computer program (picomoles per kilogram per minute), a conversion factor of 0.0655 L $kg^{-1}$ was used to take into account the C-peptide distribution volume [15].

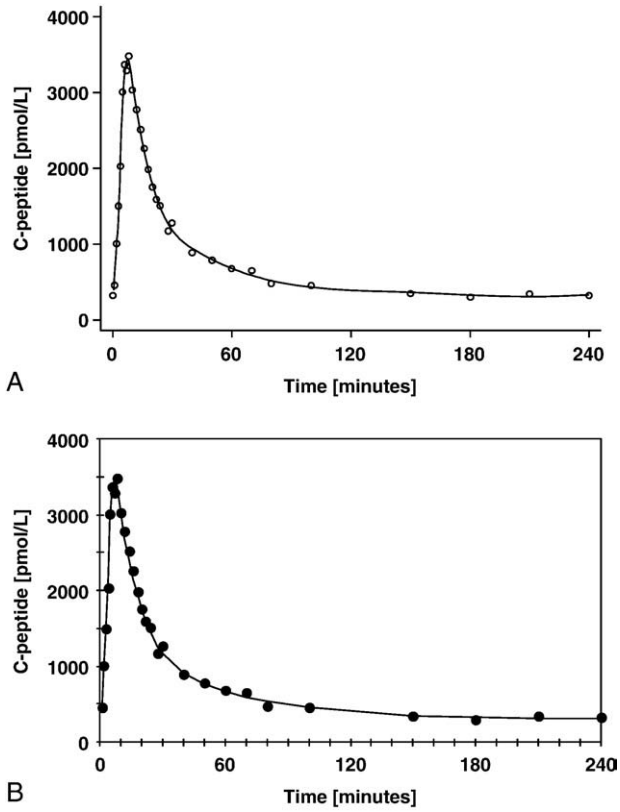


Fig. 2. A, Comparison of the C-peptide observed (circles) concentrations (picomoles per liter) and the concentrations as predicted by the spline technique used in the approach of Eaton et al to deconvolution of the C-peptide data from subject 7. B, The same C-peptide observed (dots) concentrations (picomoles per liter) for subject 7 and the concentrations as predicted by the numerical deconvolution method (solid line).

### 2.5. Statistical analyses

All statistical analyses were conducted using STATA software, and statistical significance was declared if $P$ was less than .05. Lin's [42] concordance correlation coefficient was used to quantify concordance between the method of Eaton et al [8] and the novel compartmental modeling method and also ISEC with respect to corresponding attributes of the first-phase secretory pulse.

## 3. Results

### 3.1. Deconvolution and modeling

Fig. 2A shows a typical example of the observed plasma C-peptide concentrations in a healthy Chinese subject after an IVGTT. Also shown in Fig. 2A are the predicted smoothed estimates of C-peptide concentrations obtained by the bspline technique as used in the deconvolution method of Eaton et al [8]. Shown in Fig. 2B are, for the same subject, the observed plasma C-peptide concentrations and the predicted C-peptide concentration as obtained using our numerical deconvolution method. In all 8 subjects, both

Table 2
Parameters[a] and indices[b] describing basal plasma C-peptide concentration ($C_b$), basal C-peptide secretion rate ($S_b$), and first phase of C-peptide secretion in 8 Chinese subjects

| Subject | $C_b$ (pmol $L^{-1}$) | $P_1$ (pmol $L^{-1}$ $min^{-1}$) | $P_2$ ($min^{-2}$) | $P_3$ (min) | $S_b$ (pmol $L^{-1}$ $min^{-1}$) | $W_1$ (min) | $HW_1$ (pmol $L^{-1}$ $min^{-2}$) | AS(10) (pmol $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 1 | 145.6 | 269 | 0.245 | 4.23 | 5.8 | 3.37 | 80 | 1638 |
| 2 | 228.4 | 153 | 0.012 | 4.42 | 13.0 | 15.24 | 10 | 1522 |
| 3 | 168.8 | 917 | 0.428 | 1.74 | 8.6 | 2.54 | 360 | 2438 |
| 4 | 370.7 | 2238 | 3.632 | 1.68 | 21.7 | 0.87 | 2562 | 3079 |
| 5 | 374.0 | 2193 | 1.148 | 2.54 | 22.2 | 1.55 | 1412 | 5083 |
| 6 | 820.9 | 3340 | 1.677 | 2.03 | 47.5 | 1.29 | 2597 | 7820 |
| 7 | 317.8 | 912 | 0.132 | 3.97 | 18.6 | 4.58 | 199 | 4992 |
| 8 | 307.8 | 2361 | 1.459 | 2.92 | 16.2 | 1.38 | 1713 | 4633 |
| Mean | 341.8 | 1548 | 1.092 | 2.94 | 19.2 | 3.85 | 1117 | 3901 |
| SD | 211.9 | 1143 | 1.206 | 1.13 | 12.9 | 4.77 | 1098 | 2141 |
| Median | 312.8 | 1555 | 0.788 | 2.73 | 17.4 | 2.0 | 886 | 3856 |
| Min | 145.6 | 153 | 0.012 | 1.68 | 5.8 | 0.9 | 10 | 1522 |
| Max | 145.6 | 3340 | 3.632 | 4.42 | 47.5 | 15.2 | 2597 | 7820 |

[a] $P_1$ is a Gaussian parameter describing the height of the first-phase secretory pulse, $P_2$ is related to the inverse of its width at half-peak height, and $P_3$ represents the time of the peak of the secretory pulse.
[b] $W_1$ represents the width at half-peak height of the first secretory pulse, $HW_1$ represents the ratio of its height to width, and AS(10) represents the accumulated secretion between zero and 10 minutes.

methods accurately described C-peptide concentrations, with $R^2$ values for all subjects in excess of 0.98 and the root mean square error for all subjects less than 75 pmol $L^{-1}$.

Table 2 presents, for all 8 subjects, the Gaussian parameters and indices describing the first phase of C-peptide secretion and basal C-peptide secretion rate ($S_b$) as determined by our numerical deconvolution method.

With regard to the numerical deconvolution method, all adjustable rate constants and parameters were resolved, having coefficients of variation less than 20%; and most parameters and rate constants had coefficients of variation less than 10%. For each subject, an examination of the correlation matrix revealed that the Gaussian parameters $P_1$, $P_2$, and $P_3$ had low correlations (generally <0.5) with each other.

In all 8 subjects, the method of Eaton et al [8], the Gaussian method, and the ISEC method all produced relatively similar estimates for C-peptide secretion profiles during the first 10 minutes after an IVGTT; and Fig. 3 instantiates this for subject 7. In all subjects, the time of the peak (Tmax) in C-peptide secretion (mean ± SD) occurred at 2.75 ± 2.0, 2.94 ± 1.13, and 3.24 ± 0.65 minutes by the method of Eaton et al, our numerical deconvolution method, and the ISEC method, respectively. Lin's [42] concordance correlation coefficient for Tmax by our method and the method of Eaton et al was 0.909 ± 0.061, the 95% confidence limits were 0.791 to 1.028, and the Pearson correlation coefficient was 0.941. In contrast, Lin's concordance correlation coefficient for Tmax by the ISEC method and the method of Eaton et al was 0.281 ± 0.254, the 95% confidence limits were −0.217 to 0.778, and the Pearson correlation coefficient was 0.407.

The estimated maximum secretion rates were 1383 ± 951, 1548 ± 1143, and 890.5 ± 463 pmol $L^{-1}$ $min^{-1}$ for the method of Eaton et al [8], our method, and the ISEC method, respectively. Lin's [42] concordance correlation coefficient for maximum C-peptide secretion rate for our method compared with the method of Eaton et al was 0.960 ± 0.023, the 95% confidence limits were 0.916 to 0.997, and the Pearson correlation coefficient was 0.99. Lin's concordance correlation coefficient for maximum secretion rate by the ISEC method compared with the method of Eaton et al was 0.590 ± 0.140, the 95% confidence limits were 0.315 to 0.865, and the Pearson correlation coefficient was 0.935.

Fig. 4 compares the cumulative C-peptide secretion from zero to 10 minutes, AS(10), as calculated by our method and the method of Eaton et al [8]. Lin's [42] concordance correlation coefficient was 0.982 ± 0.013, with 95% confidence limits of 0.956 to 0.998. The Pearson correlation coefficient was 0.992. Fig. 4 also compares the cumulative C-peptide secretion from zero to 10 minutes, AS(10), as

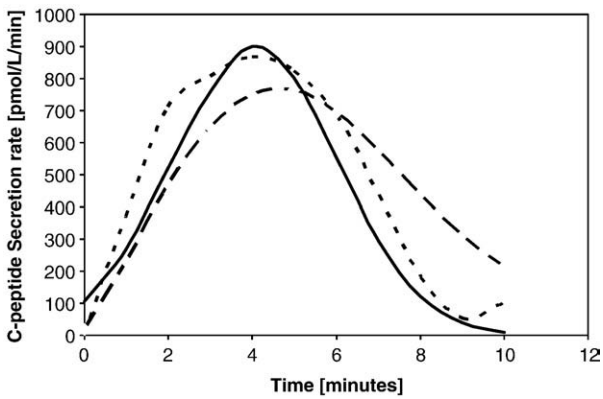


Fig. 3. Comparison of the first 10 minutes of C-peptide secretion rate (picomoles per liter per minute) profiles for subject 7, as estimated by the numerical deconvolution method (solid line), by the method of Eaton et al (short dash), and by ISEC (long dash).

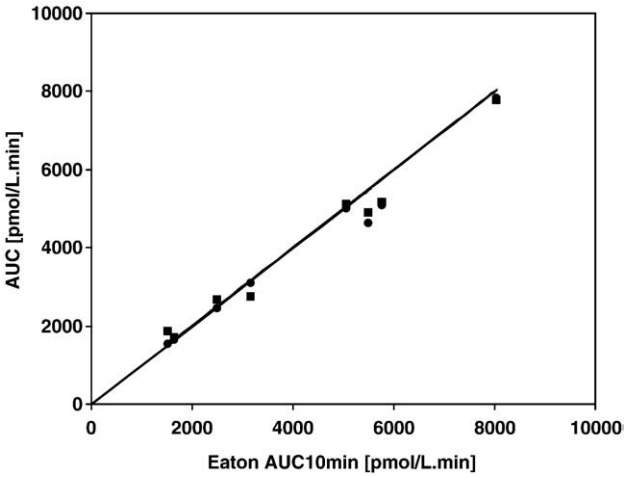


Fig. 4. Comparison of first-phase C-peptide accumulated secretion (AS[10] between 0 and 10 minutes, picomoles per liter) as calculated by the method of Eaton et al and our numerical deconvolution method (dots). Lin's concordance correlation coefficient was 0.982 ± 0.013, with 95% confidence limits of 0.956 to 0.998. The Pearson correlation coefficient was 0.992. Also shown are the ISEC computer program's predictions of the area under the curve (AUC) (squares). The comparison statistics between the prediction of AUC by the method of Eaton et al and the ISEC prediction are as follows: Lin's concordance correlation coefficient was 0.984 ± 0.011, with 95% confidence limits of 0.962 to 0.997. The Pearson correlation coefficient was 0.991. The solid line is the line of identity. For the sake of clarity, concordance regression lines are not shown because they are almost coincident with the line of identity. Note, AUC as estimated by the ISEC computer program has units of picomoles per kilogram. To enable comparison, the units of this index were converted to picomoles per liter with the assumption that the C-peptide distribution volume is 0.0655 L $kg^{-1}$.

calculated by our method and ISEC. Lin's concordance correlation coefficient was 0.994 ± 0.005, with 95% confidence limits of 0.985 to 0.996. The Pearson correlation coefficient was 0.995. From Fig. 4, it can also be seen that the accumulated secretions between zero and 10 minutes as calculated by the method of Eaton et al and ISEC are similar. For this comparison, Lin's concordance correlation coefficient was 0.984 ± 0.011, with 95% confidence limits of 0.962 to 0.997. The Pearson correlation coefficient was 0.991. These statistics indicate that the accumulated C-peptide secretion amounts between zero and 10 minutes, as calculated by all 3 methods, are highly concordant.

## 4. Discussion

As far as we are aware, this investigation appears to be the first to use a compartmental model with a prescribed input function to quantify CPSR apropos the IVGTT and appears to be the first to quantify C-peptide secretion in Chinese subjects.

The major finding of this work is that our numerical deconvolution method using a Gaussian input function to describe a prehepatic first-phase C-peptide secretion pulse in response to an IVGTT predicts input pulses that are similar to those elucidated by means of the deconvolution technique of Eaton et al [8] with respect to peak secretion rate, time of peak secretion rate, and accumulated secretion up to 10 minutes.

The graphs in Figs. 3 and 4 show that a Gaussian input function is similar in shape, magnitude, and time of peak secretion to the input functions derived using the approach of Eaton et al [8]. Moreover, our method and the ISEC computer program also produced estimated secretion profiles that are similar in terms of the area under the curve during the first 10 minutes (Fig. 4). Both our method and the method of Eaton et al differed substantially from ISEC in terms of the estimated magnitude of peak secretion rate and the time of the peak secretion rate. The reasons for these discrepancies are not clear but may be related to different levels of "smoothing" or "regularization" used in the different methods. With respect to the difficulties associated with choosing the appropriate extent of smoothing, the authors of the ISEC software state, "At a low level of smoothing, the appearance peaks and troughs are easily exaggerated, and at a high level, the true oscillatory pattern is not recovered" [43]. Another possible reason for discrepancies between our method and the ISEC predictions is that our method relies on the Gaussian function, which can be considered a "natural" function to prescribe pulsatile hormone secretion. In contrast, the secretion function predicted by ISEC has no definitive or a priori prescribed form; and random fluctuations in data may therefore substantially distort particular features of the predicted secretion profile.

These difficulties potentially influence all deconvolution procedures. Nevertheless, the accuracy and robustness of our method are attested to by our studies with a simulated mean C-peptide profile (with 10% added random error), where we were able to retrieve almost the exact same Gaussian input pulse (in terms of $P_1$, $P_2$, and $P_3$) that we had used to simulate the plasma C-peptide profile (results not shown). Our method is further supported by the fact that, in a study using the same simulated data, the deconvolution method of Turner et al [26] also retrieved a pulse almost the same as the known Gaussian input pulse (results not shown). Although estimation of the magnitude of peak secretion rate may be method dependent, if a standard method is used for comparisons, it may still be a useful metric to investigate differences between individuals and treatment or demographic groups.

One major criticism of the prescribed input has been that it involves the "heavy-handed" assumption of the nature of the input shape [2]. However, we consider that there is nothing heavy-handed about the prescribed Gaussian input function and that it is the most natural input function to be used to describe hormonal secretion. The major finding of this research is that the Gaussian is indeed an appropriate functional form to describe the insulin and C-peptide secretory pulses. We have examined other potential functional forms, but considered the Gaussian to be the most appropriate for a number of theoretical and practical reasons.

A $\gamma$ function has the requisite pulsatile pattern; but unlike the Gaussian, its parameters do not directly and immediately relate to attributes of a pulse. Moreover, in our experience, it is not a trivial process to obtain initial estimates for the parameters of a $\gamma$ function; and as previously discussed, from the practical point of data fitting, unstable parameter estimates may occur for pulses that occur at a substantial distance from the origin [31]. Other pulse forms that we investigated included a triangular wave, a square wave, a sum of 2 exponentials, and a polynomial input form. However, these impulse functions were rejected because they led to poor fits to the C-peptide plasma data.

The modeling method described here has a number of compelling attributes compared with other techniques. Chief among these is that the method uses a plausible functional description of the pulsatile pattern of prehepatic insulin secretion during first-phase pancreatic response to an IVGTT. Second, the parameters of a Gaussian pulse describe the major features of the secretory pulse (amplitude, inverse of width, and time of peak). Furthermore, the parameters can be used to calculate a number of useful indices (width of pulse at half height and pulsatility). Alternative approaches that use spline functions or regularization methods such as ISEC do not easily allow such direct descriptions and quantification of the C-peptide responses.

Gaussian input functions are consistent with biology in that they impose that insulin and C-peptide secretion occur at nonnegative rates and in pulsatile form. It is well known that normal steady-state insulin secretion takes place by a series of rapid pulses that occur at intervals of between 4 and 15 minutes and that these pulses have widths of approximately 1 to 4 minutes [44,45]. Although these rapid insulin secretory pulses have previously been described using a sinusoidal equation [46], we point out that the sine wave cycle probability density function under a Fisher transformation resembles a Gaussian probability density function [47]. Therefore, a Gaussian function is in agreement with the experimentally verified form of the in vivo profile of C-peptide and insulin secretion.

Although Veldhuis and Johnson [30] and Meier et al [44] have used Gaussian equations in a "multiparameter deconvolution approach" to quantify normal steady-state insulin secretion, the use of a Gaussian function to describe C-peptide secretion pulses after an IVGTT is a novel application of this approach.

In contrast to our method, deconvolution techniques using splines often encounter problems with predictions of transient negative secretion rates and are extremely sensitive to noisy data that may result in implausible predicted input patterns [48]. The use of splines in deconvolution also introduces the practical problem of deciding the numbers and locations for knots. In our experience, for some individual C-peptide data sets, the addition or removal of just one or two knots or changing the location of a single knot may result in quite diverse predicted secretion profiles. Thus, from the practical viewpoint of data fitting, deconvolution techniques using splines are themselves not without arbitrary assumptions that can have potentially significant effects on derived input functions.

An important feature of the method of Eaton et al [8], our method, and ISEC is that they all use fixed C-peptide disposition rate constants ($k_1$, $k_2$, and $k_3$) based on values determined in a white population by Van Cauter et al [16]. We acknowledge that the use of the equations of Van Cauter et al to make estimates of $k_1$, $k_2$, and $k_3$ for Chinese subjects may not be entirely appropriate. Despite a thorough search of the scientific literature, we were unable to find C-peptide disposition kinetics determined for Asian subjects in general or more specifically for Chinese subjects. However, we follow the precedent set by other researchers who, when estimating C-peptide secretion in Japanese subjects, have routinely used the estimates of Van Cauter et al for C-peptide disposition parameters [49].

Many studies have also reported on the basal rate of insulin secretion, but the plethora of units used means that it is difficult to summarize or compare estimates. Eaton et al [8] reported a basal prehepatic ISR of 15 ± 4 mU $min^{-1}$ 70 $kg^{-1}$ body weight in healthy white subjects. Assuming plasma volume of 0.0413 L $kg^{-1}$ body weight, this is equivalent to an ISR of 31.1 ± 8 pmol $L^{-1}$ $min^{-1}$. In a more recent study, also on white subjects, basal ISR was reported as 35 ± 5 pmol $L^{-1}$ $min^{-1}$ [50]. In this study of Chinese subjects, our estimate of a basal ISR of 19.2 ± 12.9 pmol $L^{-1}$ $min^{-1}$ is somewhat smaller than that reported for white subjects; but because of the variability associated with this estimate (Table 2) and the small number of subjects involved, it is not possible at this stage to know if this is indicative of a possible racial difference.

Table 2 documents the variability in the quantitative parameters and indices that describe the first-phase secretory pulses. Although a recent review has qualitatively discussed first-phase secretory pulses and their shape [51], Table 2 presents, as far as we know, the first comprehensive parameterization and quantitative description of first-phase secretory pulses. We have conducted a preliminary investigation (data not shown) to see if any of the parameters and indices listed in Table 2 might have potential for use as metabolic diagnostics. We found that the total first-phase insulin secretion, that is, AS(10), and the peak rate of insulin secretion, that is, $P_1$, were each strongly negatively related to insulin sensitivity ($S_I$). A thorough investigation of these relationships, using a larger data set, is warranted. Furthermore, of the 8 subjects investigated here, in only 1 subject was there any evidence from the C-peptide disposition data of a significant second-phase C-peptide response occurring between 20 and 40 minutes after the start of the IVGTT. Therefore, in future studies with a larger number of subjects, we postulate that it may be appropriate to use a second Gaussian function to quantify second-phase secretory response.

A common practice when documenting the area under the first-phase secretory response is to assume that the first-phase secretion pulse is finished by 5 minutes [49]. Fig. 3

shows that our method, the method of Eaton et al [8], and ISEC all predict that, for subject 7, the first-phase secretion pulse extends well past 5 minutes. For this reason and to be consistent with another commonly used index of first-phase insulin secretion during an IVGTT, the area under the plasma insulin curve from zero to 10 minutes ($AIR_G$), we would urge researchers to consider the merits of estimating first-phase secretion as occurring during the period between 0 and 10 minutes.

### 4.1. Perspectives and significance

Although there is an extensive scientific literature describing the use of prescribed input functions in deconvolution procedures related to a wide variety of biological problems [6,7,26,27,30-33], to our knowledge, this is the first time that this approach has been used to estimate pancreatic secretion of C-peptide in response to an IVGTT. A feature of our method is that it uses a Gaussian function as a quantitative and interpretable description of the first-phase response to a glucose challenge. The Gaussian input function is shown to have similar attributes, that is, peak secretion rate, time of peak secretion rate, and magnitudes of the accumulated secretion between zero and 10 minutes, as the corresponding attributes estimated from the secretion profiles predicted by means of the method of Eaton et al [8]. Our numerical deconvolution method is also concordant with ISEC in terms of the accumulated secretion between zero and 10 minutes. Although other deconvolution methods [8,17] produce predicted secretion profiles, these profiles must be subjected to further analysis to obtain an integrated measure of secretion and estimates of peak secretion rate, the time of peak secretion, and the width of the secretion pulse at half height. Furthermore, because the input pulses derived by other deconvolution procedures do not conform to a consistent shape, it is sometimes problematic to develop parameters or indices that can be used for comparative purposes. The major benefit of our method is that the parameters of the Gaussian input function and simple indices derived from these parameters serve directly as a means to describe and quantify C-peptide and, by inference, insulin response to an IVGTT. The significance of our relatively simple numerical deconvolution method is that it will facilitate the between-subject comparison of secretion profiles, the identification of differences in patterns of secretion in different demographic groups, and the investigation of therapeutic and other treatments on specific features of the secretion process. We also speculate that, because of the standardized form of a Gaussian secretion profile, our method may have future application in the area of Gaussian learning [52] and automatic “machine” diagnostic algorithms that are based on pattern matching of insulin secretion profiles.

In summary, our method uses the well-established procedure of deconvolution by a prescribed input function; and a Gaussian is used as the input function. Our method is concordant with other methods. However, the superiority of our method compared with other methods is in major part due to the fact that our method is the only one that uses a functional form that has been experimentally verified in vivo as describing the secretion profile and the rate of insulin secretion. It is acknowledged that validation studies are desirable to enable assessment of the method under a range of circumstances such as different demographic groups and treatment regimens. An example WinSAAM file containing our novel model with Gaussian input function is available from the principal author on request.

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