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Methods

Estimation of prehepatic insulin secretion: comparison between standardized C-peptide and insulin kinetic models

Andrea Tura[a,*], *Giovanni Pacini*[a], *Alexandra Kautzky-Willer*[b], *Amalia Gastaldelli*[c], *Ralph A. DeFronzo*[d], *Ele Ferrannini*[c,e], *Andrea Mari*[a]

[a] *Metabolic Unit, Institute of Biomedical Engineering, National Research Council, Padova, Italy*
[b] *Unit of Gender Medicine, Division of Endocrinology and Metabolism, Clinic of Internal Medicine III, Medical University of Vienna, Austria*
[c] *Institute of Clinical Physiology, National Research Council, Pisa, Italy*
[d] *Department of Medicine, Diabetes Division, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA*
[e] *Department of Internal Medicine, University of Pisa School of Medicine, Pisa, Italy*

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ABSTRACT

Our aim was to compare traditional C-peptide–based method and insulin-based method with standardized kinetic parameters in the estimation of prehepatic insulin secretion rate (ISR). One-hundred thirty-four subjects with varying degrees of glucose tolerance received an insulin-modified intravenous glucose tolerance test and a standard oral glucose tolerance test with measurement of plasma insulin and C-peptide. From the intravenous glucose tolerance test, we determined insulin kinetics parameters and selected standardized kinetic parameters based on mean values in a selected subgroup. We computed ISR from insulin concentration during the oral glucose tolerance test using these parameters and compared ISR with the standard C-peptide deconvolution approach. We then performed the same comparison in an independent data set (231 subjects). In the first data set, total ISRs from insulin and C-peptide were highly correlated (R^2 = 0.75, P < .0001), although on average different (103 ± 6 vs 108 ± 3 nmol, P < .001). Good correlation was also found in the second data set (R^2 = 0.54, P < .0001). The insulin method somewhat overestimated total ISR (85 ± 5 vs 67 ± 3 nmol, P = .002), in part because of differences in insulin assay. Similar results were obtained for fasting ISR. Despite the modest bias, the insulin and C-peptide methods were consistent in predicting differences between groups (eg, obese vs nonobese) and relationships with other physiological variables (eg, body mass index, insulin resistance). The insulin method estimated first-phase ISR peak similarly to the C-peptide method and better than the simple use of insulin concentration. The insulin-based ISR method compares favorably with the C-peptide approach. The method will be particularly useful in data sets lacking C-peptide to assess β-cell function through models requiring prehepatic secretion.

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* *Corresponding author*. ISIB-CNR Corso Stati Uniti, 4, 35127 Padova, Italy. Tel.: +39 049 829 5786; fax: +39 049 829 5763.
E-mail address: tura@isib.cnr.it (A. Tura).

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1. Introduction

The assessment of insulin secretion is of primary importance in the study of glucose homeostasis and its disturbances. Insulin secreted by the pancreatic β-cells enters the liver through the portal vein, where it is cleared from circulation. Insulin escaping hepatic removal reaches the peripheral circulation via the hepatic veins and sustains insulin concentration measured in plasma. Thus, peripheral insulin delivery (also denoted as *peripheral* or *posthepatic* insulin secretion) is a fraction of the actual pancreatic secretion (also denoted as *prehepatic* or *endogenous* insulin secretion). C-peptide, which is co-secreted with insulin in equimolar amounts, is not removed by the liver to a significant extent; and thus, peripheral C-peptide delivery equals prehepatic insulin secretion. In addition, the linearity of C-peptide kinetics has justified the use of deconvolution of plasma C-peptide concentration as the reference method for the calculation of prehepatic insulin secretion [1].

However, in several large cohorts, plasma C-peptide concentration has not been measured; and this has prevented the study of β-cell function with approaches requiring the knowledge of the actual insulin secretion [2]. Thus, it would be useful to have a method to calculate insulin secretion from the plasma insulin. Past attempts to calculate insulin secretion by deconvolution of plasma insulin concentration have provided important information [3,4]. However, these methods calculated the delivery of insulin to the peripheral circulation and not the prehepatic insulin secretion rate (ISR). We sought to present an insulin-based deconvolution method, using the same logic as the traditional C-peptide approach [5], and to compare this method to the C-peptide approach. To accomplish our aims, we derived a standardized insulin kinetics model from an insulin-modified intravenous glucose tolerance test (IVGTT) and determined insulin secretion using both the C-peptide approach and the insulin kinetic model. We then compared the results of the 2 methods in a separate large data set in which an oral glucose tolerance test (OGTT) was performed.

2. Methods

2.1. Subjects: Vienna and San Antonio data sets

The Vienna data set included 134 women, 98 of whom had a history of gestational diabetes and 36 had normal pregnancy. All women were recruited from the outpatient department of the University Clinic of Vienna and gave written informed consent for participation in the study, which was approved by the local Ethics Committee. Subjects were studied 4 to 6 months after delivery (they were not breast-feeding at the time). Among the women with former gestational diabetes, 75 had normal glucose tolerance (NGT), 18 had impaired glucose regulation (IGR) (impaired fasting glucose and/or impaired glucose tolerance), and 5 had type 2 diabetes mellitus (T2DM) according to the American Diabetes Association 1997 criteria; 64 women were lean (body mass index [BMI] <25 kg/m^2), 33 were overweight (BMI ≥25 and <30 kg/m^2), and 37 were obese (BMI ≥30 kg/m^2). Some of these subjects have been included in a previous report [6].

The San Antonio data set included 231 subjects (109 men, 122 women). All subjects were studied on the Clinical Research Center of the University of Texas Health Science Center, San Antonio, TX. The study protocol was approved by the Institutional Review Board of the University of Texas Health Science Center, San Antonio; and informed written consent was obtained from all subjects before their participation. Among the studied subjects, 44 had NGT, 22 had IGR, and 165 had T2DM. Based upon their BMI, 21 subjects were lean, 90 were overweight, and 120 were obese. All subjects had normal liver and kidney function. These subjects have been included in a previous study [7].

2.2. Metabolic tests

After a 10- to 12-hour overnight fast, all subjects in the Vienna data set underwent an insulin-modified IVGTT. Glucose (300 mg/kg) was injected at time 0 to 0.5 minute, and insulin (0.03 IU/kg) was infused intravenously at time 20 minutes over 1 minute. Venous blood samples for determination of plasma concentration of glucose, insulin, and C-peptide were collected at baseline and at 3, 4, 5, 6, 8, 10, 14, 19, 22, 27, 30, 35, 40, 50, 70, 100, 140, and 180 minutes after glucose injection. On a separate day, the same subjects underwent a standard 75-g OGTT. Venous blood samples were collected at baseline and at 10, 20, 30, 60, 90, 120, 150, and 180 minutes. Insulin (Serono Diagnostics, Freiburg, Germany) and C-peptide (CIS Bio International, Gif-sur-Yvette, France) were determined in duplicate by commercially available radioimmunoassay kits.

Similarly, after an overnight fast, all the subjects in the San Antonio data set underwent a 75-g OGTT; and blood samples were collected at –30, –15, 0, 30, 60, 90, and 120 minutes for the measurement of plasma glucose, insulin, and C-peptide. Some of the subjects (175 of 231) also underwent a euglycemic insulin clamp for determination of the rate of glucose disappearance (*M* value) [7]. Plasma insulin and C-peptide concentrations were measured by radioimmunoassay using specific kits (Linco Research, St Louis, MO).

2.3. Calculation of insulin kinetic parameters from the IVGTT in the Vienna data set

During the insulin-modified IVGTT, insulin enters the peripheral circulation exogenously from the infusion at 20 minutes as well as from the release of endogenously secreted insulin from 0 to 180 minutes, after passage through the liver. The posthepatic insulin delivery, $ISR_{PE}(t)$, is a fraction of prehepatic insulin secretion, ISR(*t*); that is, $ISR_{PE}(t) = F(t)ISR(t)$, with *F*(*t*) between 0 and 1. We described insulin kinetics with a linear model relating the plasma insulin concentration to peripheral insulin appearance, that is, the sum of exogenous insulin infusion, IINF(*t*), and posthepatic insulin delivery, $ISR_{PE}(t)$. Thus, insulin concentration, IC(*t*), is the convolution of the impulse response of the linear insulin kinetic model, *h*(*t*), and IINF(*t*) + *F*(*t*)ISR(*t*):

$$IC(t) = h(t) \otimes [IINF(t) + F(t)ISR(t)], \quad (1)$$

where ⊗ is the convolution operator. This approach involves an approximation, as it assumes that the time-varying hepatic insulin fractional extraction, 1 – *F*(*t*), affects the peripheral

insulin delivery (through $F[t]ISR[t]$), but not insulin clearance and $h(t)$ (see "Discussion" for further comments).The insulin kinetic impulse response was represented using the 2-exponential function:

$$h(t) = 1 / Cl_{INS_{PE}} \cdot \left(W \cdot \alpha \cdot e^{-\alpha t} + (1 - W) \cdot \beta \cdot e^{-\beta t}\right) \quad (2)$$

where $Cl_{INS_{PE}}$ is the peripheral (posthepatic) insulin clearance and W is the relative contribution of the first exponential term to the clearance (as the term in parentheses has unit integral and W is the fraction due to the first exponential).

Because the exogenous insulin infusion $IINF(t)$ is known (1-minute infusion of known dose at 20 minutes of the IVGTT) and the prehepatic insulin secretion $ISR(t)$ can be calculated from C-peptide deconvolution using the method by Van Cauter et al [5], it is possible to estimate the parameters of $h(t)$ and $F(t)$ from the plasma insulin concentration by using least squares, with the addition of a regularization term to obtain a smooth $F(t)$ (represented as a piecewise-linear function of time).

The factor $F(t)$ obtained from the IVGTT analysis is specific for this test. To obtain a model by which insulin secretion can be computed from plasma insulin concentration by deconvolution in the general case, as in the approach of Van Cauter et al, we used the mean value of $F(t)$, $\overline{F}$. Thus, the insulin kinetic impulse response used for deconvolution of the plasma insulin curve is the function:

$$r(t) = 1 / Cl_{INS} \cdot \left(W \cdot \alpha \cdot e^{-\alpha t} + (1 - W) \cdot \beta \cdot e^{-\beta t}\right), \quad (3)$$

where $Cl_{INS} = \frac{Cl_{INS_{PE}}}{\overline{F}}$ represents prehepatic insulin clearance.

To derive a standardized insulin kinetic model along the same approach used by Van Cauter et al, we tested the relationships between the individual insulin kinetic parameters (Cl_{INS}, W, α, β, and $\overline{F}$) and the anthropometric data. Because no significant relationship was found ("Results"), we adopted the mean group values as standardized parameters. To avoid a possible disturbing influence from the subjects in whom the agreement between insulin secretion calculated from C-peptide and insulin was not satisfactory, we calculated mean parameters in a selected group, as explained in the following section.

In the standardized parameters, insulin clearance was given in liters per minute per square meter of body surface area (BSA). Therefore, the insulin deconvolution method calculates insulin secretion in picomoles per minute per square meter, whereas the insulin concentration is in picomoles per liter. However, when comparing the insulin and C-peptide deconvolution methods, insulin secretion was expressed in picomoles per minute, as in the approach of Van Cauter et al, by multiplying by BSA.

2.4. *Calculation of insulin secretion from OGTT plasma insulin values in the Vienna data set and parameter determination for the standardized insulin kinetic model*

Insulin secretion was calculated from the OGTT plasma C-peptide curves of the Vienna data set with the approach of Van Cauter et al [5]. Briefly, with this method, insulin secretion is determined by deconvolution from plasma C-peptide concentration using individualized C-peptide kinetic parameters (distribution volume, short and long half-life, and short half-life associated fraction). Parameters are determined for each subject based on anthropometric data and diabetes status.

Insulin secretion from the approach of Van Cauter et al was compared with insulin secretion obtained from plasma insulin levels using the individual parameters of insulin kinetics (Cl_{INS}, W, α, β, and $\overline{F}$). From the insulin secretion profiles, basal insulin secretion and total insulin secretion (ie, the integral during the OGTT) were calculated.

Based on the comparison between insulin secretion obtained from C-peptide and insulin, a subgroup of subjects

Table 1 – Subjects' characteristics in the Vienna and San Antonio data sets (mean ± SE)

	Vienna data set			San Antonio data set		
	NGT	IGR	T2DM	NGT	IGR	T2DM
n	111	18	5	44	22	165
Age (y)	32.5 ± 0.5	35.2 ± 1.1	34.2 ± 1.7	37.0 ± 1.9	42.0 ± 2.8	52.4 ± 0.8
BMI (kg/m^2)	26.0 ± 0.5	30.2 ± 1.2	31.4 ± 3.1	29.6 ± 0.9	31.7 ± 0.9	31.3 ± 0.4
Sex (M/F)	0/111	0/18	0/5	18/26	9/13	82/83
OGTT						
Basal glucose (mmol/L)	4.76 ± 0.04	5.48 ± 0.15	6.83 ± 0.47	5.24 ± 0.05	5.56 ± 0.11	10.20 ± 0.22
Basal insulin (pmol/L)	57.0 ± 3.4	62.2 ± 5.2	97.4 ± 43.5	60.4 ± 5.9	80.0 ± 6.5	116 ± 7
Basal C-peptide (pmol/L)	596 ± 24	748 ± 87	759 ± 187	677 ± 52	849 ± 54	932 ± 36
Mean glucose (mmol/L)	5.97 ± 0.07	7.93 ± 0.23	10.25 ± 0.49	6.75 ± 0.14	8.39 ± 0.15	15.51 ± 0.25
Mean insulin (pmol/L)	248 ± 14	259 ± 24	320 ± 118	410 ± 38	554 ± 65	259 ± 20
Mean C-peptide (pmol/L)	1833 ± 57	1975 ± 109	1845 ± 334	2104 ± 134	2413 ± 177	1494 ± 67
Basal C-peptide to insulin ratio	11.8 ± 0.4	11.2 ± 0.6	11.7 ± 3.4	12.6 ± 0.7	11.5 ± 0.8	9.7 ± 0.5
Mean C-peptide to insulin ratio	8.6 ± 0.3	8.1 ± 0.5	7.2 ± 1.1	5.8 ± 0.3	5.2 ± 0.5	7.4 ± 0.3
IVGTT						
Basal glucose (mmol/L)	4.72 ± 0.04	5.29 ± 0.13	6.84 ± 0.28	–	–	–
Basal insulin (pmol/L)	53.5 ± 3.3	57.8 ± 4.1	86.4 ± 29.7	–	–	–
Basal C-peptide (pmol/L)	582 ± 24	694 ± 71	673 ± 137	–	–	–
Mean glucose (mmol/L)	9.04 ± 0.11	10.83 ± 0.51	12.07 ± 0.73	–	–	–
Mean insulin (pmol/L)	503 ± 23	523 ± 43	474 ± 84	–	–	–
Mean C-peptide (pmol/L)	1208 ± 49	1278 ± 118	904 ± 189	–	–	–

in which the agreement was best was selected to obtain more robust mean parameters for the standardized insulin kinetic model. The following quality criteria were used: (*a*) basal and total insulin secretion from insulin not exceeding twice the basal secretion from C-peptide, and vice versa; (*b*) ratio of basal C-peptide to basal insulin (from OGTT and IVGTT) not exceeding twice the ratio of insulin to C-peptide clearances, and vice versa; and (*c*) ratio of the OGTT areas under the curve of C-peptide and insulin not exceeding twice the ratio of insulin to C-peptide clearances, and vice versa.

We selected the mean parameters in this group as standardized parameters for the insulin kinetic model of Eq. (3). We then recalculated insulin secretion in all the subjects of this study with this model and using the same deconvolution method used for C-peptide analysis.

2.5. *Calculation of insulin secretion from OGTT plasma insulin in the San Antonio data set*

In the San Antonio data set, we calculated insulin secretion from plasma insulin concentration with the standardized insulin kinetic model and from plasma C-peptide concentration using the approach of Van Cauter et al [5]. Appropriate comparison was performed.

Table 2 – Insulin kinetic parameters computed in the whole Vienna data set and in the group of subjects meeting the quality criteria (mean ± SE)

	Whole Vienna data set (N = 134)	Vienna data set selection (n = 74)
$Cl_{INS_{PE}}$ (L/min)	0.68 ± 0.02	0.68 ± 0.03
Cl_{INS} (L/min)	1.82 ± 0.06	1.85 ± 0.06
$Cl_{INS_{BSA}}$ (L min^{-1} m^{-2})	1.00 ± 0.03	**1.01** ± 0.04
W (dimensionless)	0.52 ± 0.03	**0.54** ± 0.04
α (1/min)	0.63 ± 0.05	**0.58** ± 0.06
β (1/min)	0.08 ± 0.01	**0.08** ± 0.01
Cl_{CP} (L/min)	0.24 ± 0.01	0.24 ± 0.01
$\overline{F}$ (dimensionless)	0.38 ± 0.01	0.37 ± 0.01

The standardized insulin kinetic parameters for Eq. (3) are in bold.

2.6. *Statistical analysis*

Data are given as mean ± SE unless otherwise specified. Linear regression analysis was performed by standard techniques. Differences were tested using paired or unpaired *t* test, as appropriate, on logarithmically transformed values.

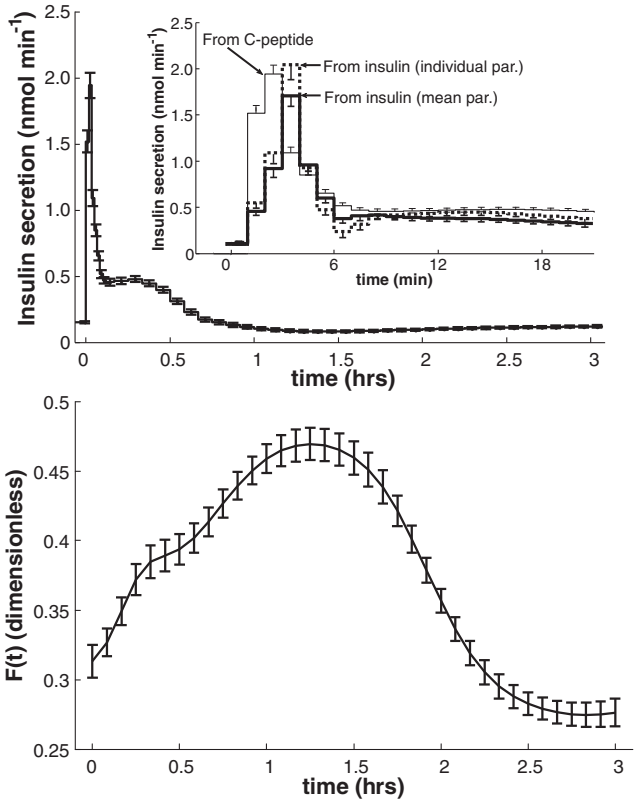

Fig. 1 – Insulin secretion from the plasma C-peptide concentration during the IVGTT in the Vienna data set (mean ± SE); the inset shows insulin secretion during the first 20 minutes from plasma C-peptide (solid, thin line) and from plasma insulin, with individual kinetic parameters (dashed line) and mean kinetic parameters (solid, thick line) (top). Pattern of *F*(*t*) parameter during the IVGTT in the Vienna data set (mean ± SE) (bottom).

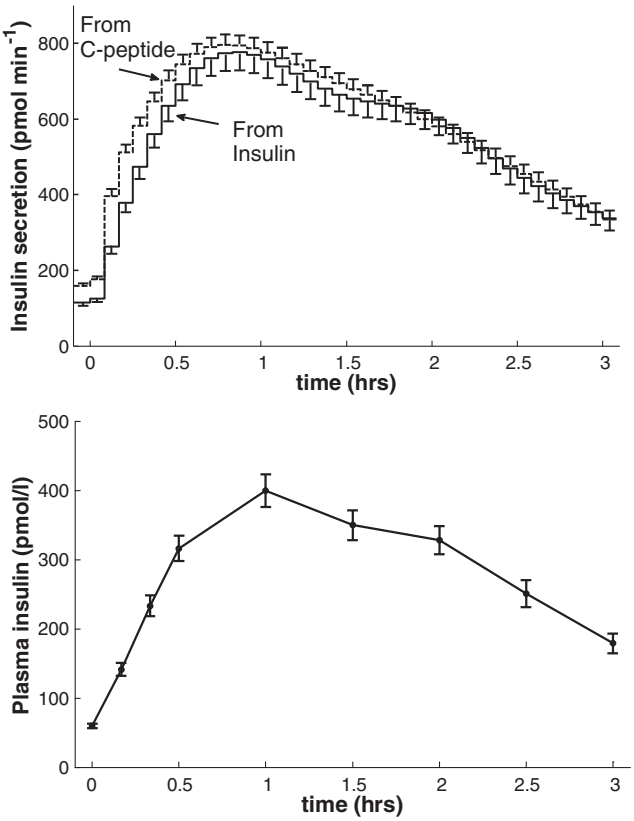

Fig. 2 – Insulin secretion from plasma C-peptide (dashed line) and plasma insulin (solid line) in the Vienna data set (top). Plasma insulin concentration is also reported (bottom). Data are mean ± SE.

Statistically significant differences were assumed for $P < .05$. Bland-Altman analyses were also performed.

3. Results

3.1. *Insulin secretion and kinetic parameters from the IVGTT in the Vienna data set*

The subjects' characteristics in the Vienna data set are reported in Table 1. Insulin secretion from C-peptide during the IVGTT in the whole data set is shown in Fig. 1 (top). The mean values for the insulin kinetic parameters are shown in Table 2 (left column). The pattern of factor $F(t)$ is reported in Fig. 1 (bottom). By either univariate or multivariate regression analysis, we did not find significant relationships between the insulin kinetic parameters and anthropometric data (age, BMI, BSA, lean body mass) or metabolic parameters (glucose levels, insulin sensitivity computed with the homeostasis model assessment of insulin resistance [8], and insulin sensitivity index from minimal model analysis [9]). After calculation of insulin secretion during the OGTT (see below), 74 subjects met the quality criteria specified in "Methods." The mean insulin kinetic parameters in these selected subjects are reported in Table 2 (right column); these mean values are the standardized insulin kinetic parameters used throughout this analysis. As can be appreciated from Table 2, the subject selection was virtually without effect on the mean values.

The inset of Fig. 1 shows the comparison of the insulin secretion profiles obtained from plasma C-peptide and from plasma insulin during the first 20 minutes of the IVGTT, that is, before insulin injection, in the whole Vienna data set. Insulin secretion rates calculated from C-peptide and those derived from insulin using the individual kinetic parameters were very similar, although the insulin peak was delayed by approximately 1 minute on average. When using mean kinetic parameters, the results deteriorated only slightly (the peak value was somewhat lower). The acute insulin secretory response (AIR), computed as the mean increase above baseline over the initial 0- to 8-minute interval, was 784 ± 37 pmol/min using C-peptide, 635 ± 45 pmol/min using insulin with individual kinetic parameters, and 627 ± 45 pmol/min with the standardized insulin parameters. By regression analysis, AIR from C-peptide was significantly related to the AIR from insulin with both individual ($R^2 = 0.46$, $P < .0001$) and standardized parameters ($R^2 = 0.61$, $P < .0001$). In the group of subjects with NGT (Table 1), the minimal model insulin sensitivity index was weakly but consistently related in a reciprocal fashion to logarithmically transformed AIR values calculated with all methods (C-peptide: $R^2 = 0.06$, $P = .008$; insulin with individual parameters: $R^2 = 0.05$, $P = .015$; insulin with standardized parameters: $R^2 = 0.11$, $P = .0003$). Peak insulin secretion (Fig. 1), expressed as the ratio to the basal value, was similar with all methods (14- to 16-fold), but considerably higher than the peak insulin concentration (~6-fold).

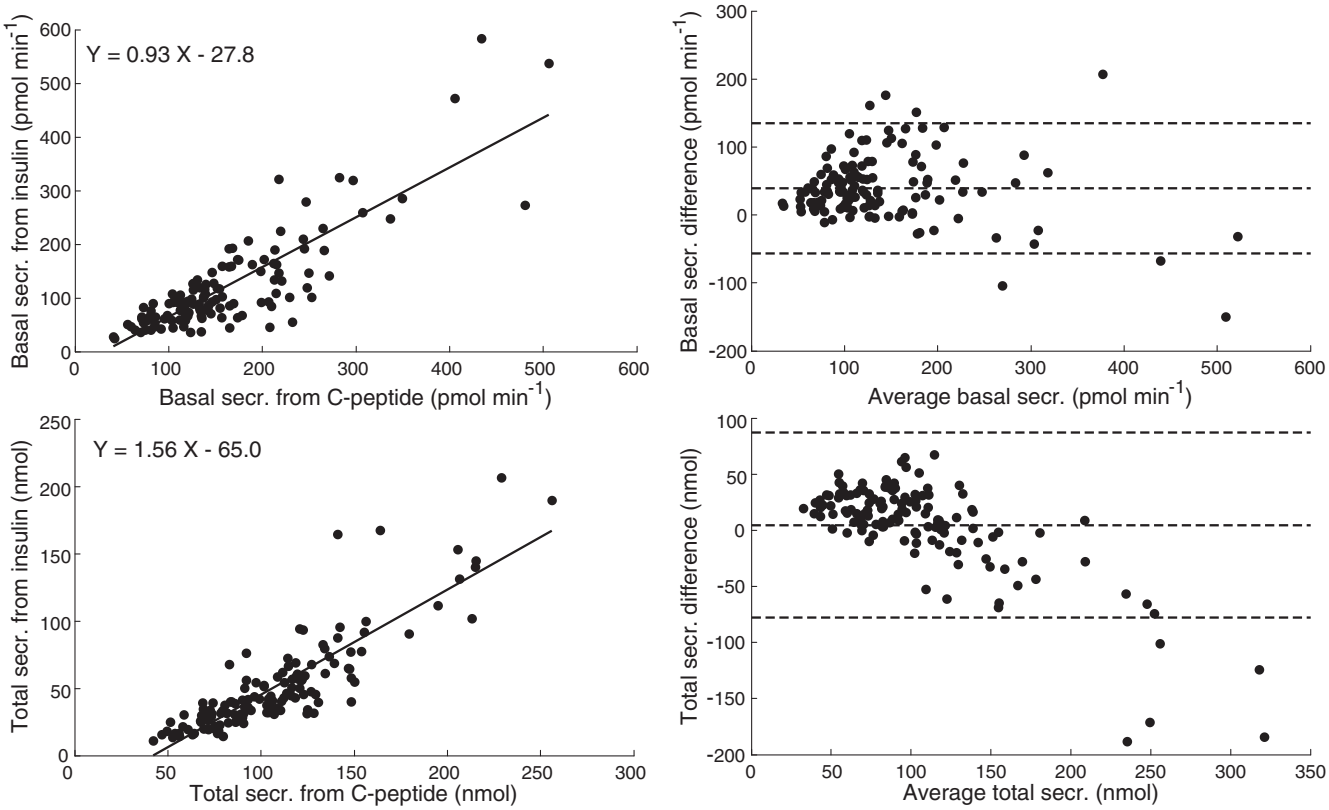

Fig. 3 – Basal (top panels) and total (bottom panels) insulin secretion from plasma C-peptide and insulin concentrations in the subjects from the Vienna data set. The left panels show the correlations (regression equations are reported); the right panels show the corresponding Bland-Altman plots.

3.2. *Insulin secretion from the OGTT in the Vienna data set*

As shown in Fig. 2 (top), insulin secretion calculated from the plasma insulin concentration using standardized parameters agreed closely with that derived from C-peptide analysis. Plasma insulin concentration was also reported (Fig. 2, bottom).

Mean basal and total insulin secretions from C-peptide were 158 ± 7 pmol/min and 108 ± 3 nmol, respectively. The corresponding values obtained from insulin were 113 ± 8 pmol/min and 100 ± 6 nmol with the individual insulin kinetic parameters and 119 ± 8 pmol/min and 103 ± 6 nmol with the standardized parameters. Although modest, the differences between the C-peptide method and the standardized insulin method were statistically significant ($P < .001$). However, regression analysis showed a strong relationship between secretion rates from C-peptide and from insulin ($R^2 = 0.71$ for basal secretion, $R^2 = 0.75$ for total secretion, both $P < .0001$; Fig. 3). This correlation was similar to that found between C-peptide and insulin levels at fasting and during the OGTT ($R^2 = 0.63$ for the fasting values, $R^2 = 0.71$ for the OGTT area under the curves, $P < .0001$). Bland-Altman analysis of these data showed that only a limited number of samples were outside the limits of agreement (average difference ± 1.96 standard deviation of the difference), that is, only 5 of 134 samples for total insulin secretion and 7 samples for basal secretion (Fig. 3, right).

3.3. *Insulin secretion from the OGTT in the San Antonio data set*

The subjects' characteristics in the San Antonio data set are reported in Table 1. Furthermore, Table 1 compares the glucose, insulin, and C-peptide levels in the Vienna and San Antonio subjects divided according to their glucose tolerance. The groups of corresponding glucose tolerance were relatively homogeneous, except for the diabetic patients. There was a significant difference in the plasma C-peptide to insulin ratios (8.59 ± 0.24 vs 6.82 ± 0.22, $P < .0001$, for ratios of area under curves), which most likely is explained by the different C-peptide and insulin assays used in Vienna and San Antonio. This clearly affects the relationship between the C-peptide and the insulin method for calculation of insulin secretory rate in this data set. As a consequence, higher ISRs were obtained with the insulin method as compared with the C-peptide method (Fig. 4, top). Plasma insulin concentration was also reported (Fig. 4, bottom).

Fig. 5 shows the regression analysis and the Bland-Altman plot for basal and total insulin secretion. The overall correlation coefficients were $R^2 = 0.45$ and $R^2 = 0.54$ for basal and total secretion, respectively ($P < .0001$). The slope of the regression line was not different from unity (95% confidence interval [CI], 0.98-1.31) for basal secretion, whereas it was greater than 1 for total insulin secretion (95% CI, 1.23-1.56).

The difference between basal insulin secretion calculated from the plasma C-peptide and insulin concentrations, although small, was statistically significant (225 ± 8 vs 228 ± 13 pmol/min, respectively, $P = .02$); a significant difference also was found for total insulin secretion (67 ± 3 vs 85 ± 5 nmol, $P = .002$). However, according to the Bland-Altman analysis, only

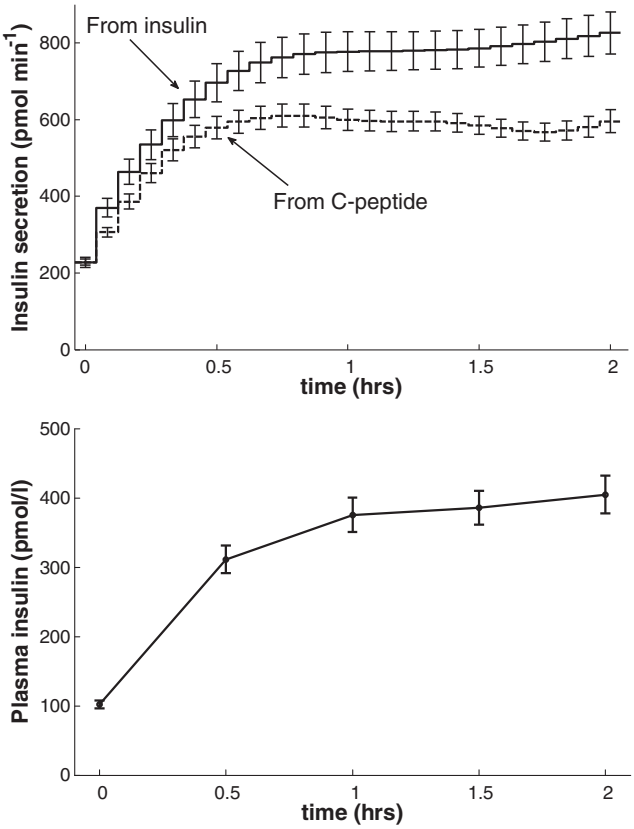

Fig. 4 – Insulin secretion from plasma C-peptide (dashed line) and plasma insulin (solid line) in the San Antonio data set (top). Plasma insulin concentration is also reported (bottom). Data are mean ± SE.

7 of 231 samples for basal insulin secretion and 9 samples for total insulin secretion fell outside the limits of agreement.

In the NGT subjects, we also investigated whether insulin secretion calculated from the plasma insulin and C-peptide concentrations showed similar relationships with the BMI and insulin sensitivity (*M* value). After logarithmic transformation of the variables, basal insulin secretion from either C-peptide or insulin was directly related to BMI ($R^2 = 0.34$ for C-peptide, $R^2 = 0.53$ for insulin, $P < .0001$); similar results were found for total insulin secretion ($R^2 = 0.22$, $R^2 = 0.52$, $P < .0012$). Inverse relationships were found between basal and total insulin secretion and the *M* value both with the C-peptide and with the insulin method ($R^2 = 0.34$, $R^2 = 0.31$ basal secretion; $R^2 = 0.28$, $R^2 = 0.36$ total secretion; $P < .0009$).

3.4. *Insulin secretion in subgroups of the San Antonio data set*

As summarized in Table 3, results in subgroups were in general agreement with those in the whole data set. Notably, despite absolute differences in ISRs calculated from the plasma C-peptide and insulin methods, the differences between groups were consistently detected with both approaches. Thus, with both methods, basal insulin secretion was higher in diabetic than nondiabetic subjects ($P < .03$ for both), whereas the opposite was true for stimulated total

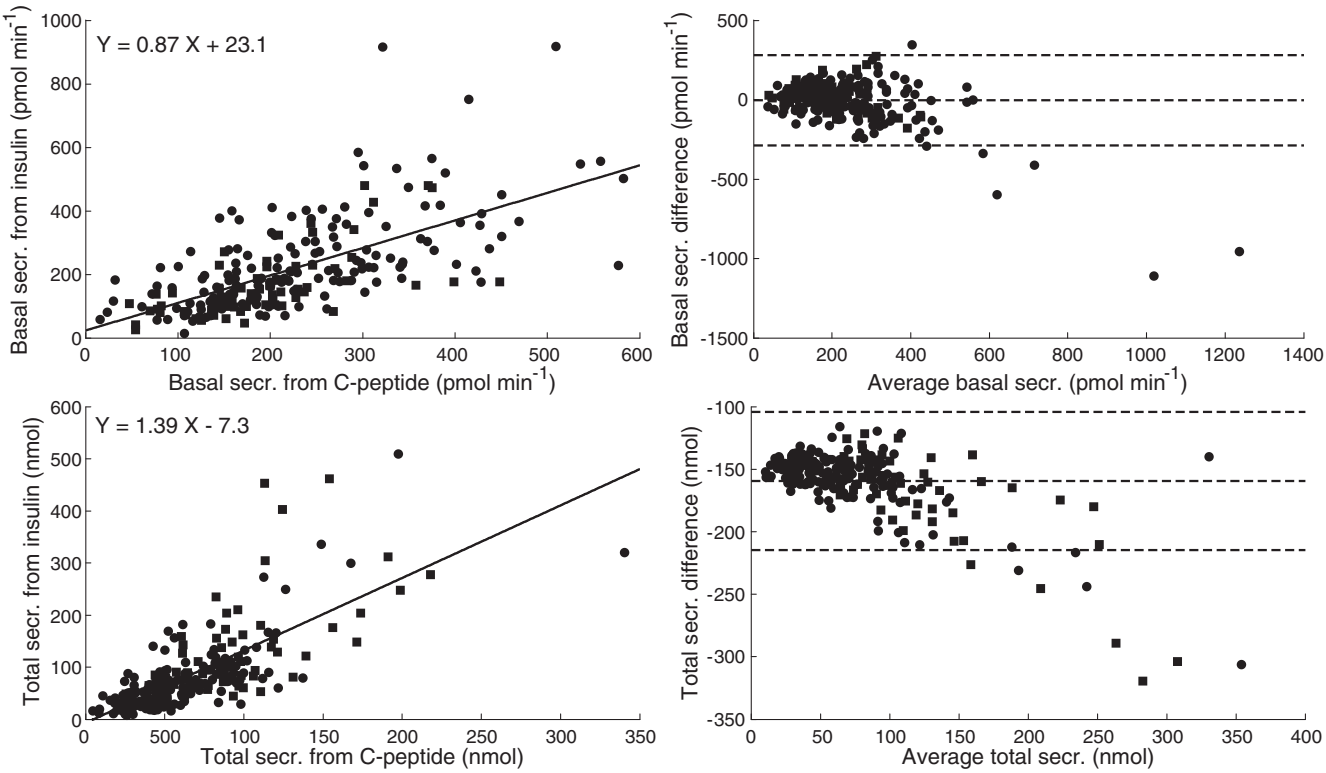

Fig. 5 – Basal (top panels) and total (bottom panels) insulin secretion from plasma C-peptide and insulin concentrations in the subjects from the San Antonio data set. The left panels show the correlations (regression equations are reported); the right panels show the corresponding Bland-Altman plots. Subjects are divided into diabetic (circle) and nondiabetic (square) groups.

Table 3 – Comparison of basal and total insulin secretion from plasma C-peptide and insulin concentrations in subjects of the San Antonio data set divided according to different criteria

	Secretion from C-peptide	Secretion from insulin	Regression analysis slope (95% CI), R^2	Bland-Altman analysis N1/N2 (samples over total out of agreement limits)
Basal secretion (pmol/min)				
Diabetic subjects	238 ± 9	252 ± 17	(0.98-1.39), R^2 = 0.44	6/165
Nondiabetic subjects	192 ± 11	166 ± 13	(0.56-1.03), R^2 = 0.41	4/66
Obese subjects [a]	265 ± 11	297 ± 22	(0.98-1.55), R^2 = 0.41	5/120
Nonobese subjects	184 ± 9	157 ± 10	(0.56-0.87), R^2 = 0.42	7/111
Men	218 ± 10	214 ± 15	(0.83-1.23), R^2 = 0.50	5/109
Women	231 ± 11.	240 ± 21	(0.96-1.47), R^2 = 0.43	3/122
Young subjects [b]	215 ± 12	200 ± 15	(0.49-1.02), R^2 = 0.37	2/60
Elderly subjects	204 ± 12	212 ± 17	(0.71-1.21), R^2 = 0.47	3/67
Insulin-resistant subjects [c]	276 ± 16	265 ± 23	(0.48-1.20), R^2 = 0.35	3/44
Insulin-sensitive subjects	158 ± 9	130 ± 12	(0.48-1.10), R^2 = 0.40	2/43
Total secretion (nmol)				
Diabetic subjects	56 ± 3	68 ± 5	(1.13-1.47), R^2 = 0.58	10/165
Nondiabetic subjects	93 ± 5	130 ± 12	(0.96-1.90), R^2 = 0.37	4/66
Obese subjects [a]	74 ± 5	106 ± 9	(1.15-1.63), R^2 = 0.54	5/120
Nonobese subjects	59 ± 3	64 ± 5	(1.02-1.46), R^2 = 0.52	1/111
Men	63 ± 4	80 ± 8	(1.20-1.71), R^2 = 0.55	5/109
Women	69 ± 4	90 ± 7	(1.10-1.55), R^2 = 0.54	4/122
Young subjects [b]	79 ± 6	109 ± 12	(0.90-1.78), R^2 = 0.39	3/60
Elderly subjects	53 ± 4	68 ± 7	(1.23-1.81), R^2 = 0.62	4/67
Insulin-resistant subjects [c]	96 ± 10	121 ± 14	(0.95-1.51), R^2 = 0.65	1/44
Insulin-sensitive subjects	49 ± 4	49 ± 7	(0.73-1.54), R^2 = 0.44	1/43

Regression analysis was always significant ($P < .0001$).

[a] BMI ≥30 kg/m^2.

[b] Age <41 years, >57 (lower and upper quartiles).

[c] M <13.0 mg min^{-1} kg^{-1}, M >26.7 (lower and upper quartiles).

secretion ($P < .0001$). The higher insulin secretion in obesity (without distinction between diabetic and nondiabetic subjects) also was consistently detected ($P < .0001$ and $P < .02$ for basal and total secretion, respectively). The same was true with respect to insulin resistance: with both methods, basal and total insulin secretions were greater in insulin-resistant subjects compared with insulin-sensitive subjects ($P < .0001$ for both). With both methods, young and elderly subjects had similar basal insulin secretion; but the young had higher total secretion ($P < .0005$). Some discrepancy was found in the comparison between men and women, where total insulin secretion was higher in men with the insulin method ($P = .02$) but not with the C-peptide method ($P = .2$). This was explained by similar area under the C-peptide curve in men and women ($P = .2$), but a slightly higher area under the insulin curve in men ($P = .045$).

The insulin method is expected to overestimate insulin secretion under conditions of reduced insulin clearance such as obesity and insulin resistance. To assess the degree of overestimation, in the obese subjects (Table 3), we calculated basal and total insulin secretion normalized to the corresponding mean value in the lean group. Similarly, in the insulin-resistant group, we normalized insulin secretion to the mean value of the insulin-sensitive group (Table 3). With the C-peptide method, basal and total insulin secretions in the obese subjects were 1.44 and 1.24 times greater than those in the lean subjects, respectively. The corresponding figures for the insulin method were 1.89 and 1.67. Basal and total insulin secretions in the insulin-resistant subjects were 1.75 and 1.96 times greater than those in the insulin-sensitive subjects with the C-peptide method, whereas they averaged 2.05 and 2.44 with the insulin method. Therefore, the insulin method overestimated secretion by approximately 33% in obese subjects and approximately 21% in insulin-resistant subjects.

4. Discussion

In this study, we present an insulin-based deconvolution method to calculate prehepatic insulin secretion and compare the results with those calculated using the traditional C-peptide approach. Our purpose was not to replace the C-peptide method, which remains the criterion standard. However, in studies in which C-peptide is not available, assessment of insulin secretion from plasma insulin is useful, despite its potential limitations. It must be acknowledged that the integral of plasma insulin concentration and that of the secretory profile are essentially proportional because of the use of constant kinetic parameters, and hence the former could be assumed as a surrogate measure of the latter. However, in data sets that lack C-peptide determination, our method allows the application of mathematical models of β-cell function [10,11], which have been developed in particular for the OGTT and proven to be useful in several large studies [12-14]. In these models, it is necessary to use *prehepatic* insulin secretion to obtain β-cell function parameters that can be correctly interpreted and compared with those of previous studies. Besides this application, the method is valuable for reconstructing the fast secretion phenomena, such as first-phase insulin secretion during the IVGTT. In fact, the peak of plasma insulin does not reflect that of secretion, which it is better estimated using our method, though with approximately 1-minute delay. In addition to the estimation of first-phase insulin secretion, this property could be useful in the study of insulin secretion pulsatility [15].

The approach we have used to derive the standardized insulin kinetics model clearly embeds approximations. In fact, insulin kinetics are assumed to be linear and time invariant, and described by Eqs. (1) to (3), with $F(t)$ constant. Thus, Eq. (1) assumes that the time-varying hepatic insulin fractional extraction, $1 - F(t)$, affects the peripheral insulin delivery but not insulin clearance. This would be a significant limitation if the goal were the accurate calculation of hepatic insulin extraction. In our study, however, we aimed at providing a reasonable estimate of prehepatic insulin secretion using insulin concentration. The approximation of a constant F is justified by the impossibility to predict $F(t)$ in general; the accuracy of this approximation is more dependent on the insulin clearance value used in Eq. (3) (see below) than on the approximation used in Eq. (1) and should be evaluated from the comparison with the C-peptide reference method. Anyhow, our estimate of the hepatic insulin fractional extraction, $1 - \overline{F}$ (~60%, Table 2), was consistent with previous studies [16,17].

Following the logic of the method of Van Cauter et al [5], we examined possible relationships between the insulin model parameters and simple anthropometric or metabolic data for possible individual adjustment of the parameters. Unfortunately, we were not able to find suitable allometric equations, as in the approach of Van Cauter et al. It should be noted that, even in the study of Van Cauter et al [5], the correlation of the kinetic parameters with the anthropometric parameters was statistically significant but quite weak (R^2 in linear regression analysis ranging from 0.08 to 0.25 in the best case).

The use of constant insulin kinetic parameters makes our method in principle analogous to the historical posthepatic insulin secretion approaches [3,4]. However, there are some important differences. First, the historical methods did not propose a standardized insulin kinetic model, that is, a method to calculate posthepatic insulin secretion in the general case. In our study, this standardized insulin kinetic model turned out to generate a model with constant parameters; but this is a result of a specific and novel analysis on a considerably large number of subjects. Second, although by simple scaling, we have proposed a method for calculating *prehepatic* insulin secretion and evaluated its performance against the reference C-peptide technique. This approach has not been considered in the historical studies. Last, to our knowledge, this is the first study examining in detail the potentialities and limitations of the insulin deconvolution method in a large number of subjects.

For the estimation of insulin kinetic parameters, we used a group of women with a history of gestational diabetes (Vienna data set), where the main limitations are the single sex and the relatively young age. However, for our purposes, it was essential to analyze a sufficiently large data set generated from 2 independent tests including both plasma insulin and C-peptide measurements: an insulin-modified IVGTT to derive insulin kinetic parameters and a 3-hour OGTT for testing the method. Other advantages of the Vienna data set were the following: (*a*) all tests were performed in the same

center, avoiding possible differences in insulin and C-peptide assays (still a vexing problem [18]); and (*b*) the data set included women with glucose levels and BMI varying over a wide range, but without concomitant complications that could act as confounding factors in the assessment of their metabolic status.

Our method for calculating insulin secretion from plasma insulin levels was then tested in an independent data set. As shown in Fig. 4, the insulin method tended to overestimate insulin secretion in the San Antonio data. One reason for this overestimation is likely due to the use of different assays in Vienna and San Antonio, resulting in a significantly lower C-peptide to insulin ratio in San Antonio (Table 1). If the calculated ISR from plasma insulin in San Antonio was corrected for the differences in mean C-peptide to insulin ratio between the 2 data sets (Table 1), total insulin secretion values from C-peptide and insulin would become very similar (67 ± 3 vs 68 ± 4 nmol, respectively). In this condition, the slope of the regression between insulin- and C-peptide–based secretion would not be different from 1 (1.11; 95% CI, 0.97-1.24). However, another potential explanation for the overestimation is intrinsic to the use of insulin, which overestimates insulin secretion in states of reduced insulin clearance [19,20]. This known fact was confirmed in our study because the insulin method overestimated total secretion in obese and insulin-resistant subjects by approximately 33% and approximately 21%, respectively. In these subjects, overestimation may indeed be due to reduced insulin clearance (and hepatic extraction), not accounted for in our model, which may be in part due to saturation phenomena in the presence of high insulin secretion; this is suggested by the Bland-Altman plot (Figs. 3 and 5), in which the larger deviation between the methods is observed at the highest secretion levels.

It should also be noted that the entity of the overestimation reported above, and evident from inspection of Table 3, refers to differences in total secretion. If we considered point-by-point absolute difference in the ISR, the differences in the results between C-peptide and insulin approaches may be even higher. This may happen if the shape of plasma C-peptide and insulin curves differ remarkably, although it is known that this is not a common condition [21]. On our data, the average point-by-point difference was around 35%.

Despite the potential for overestimating the absolute ISR with the insulin method, it is important to emphasize that the method reproduces known facts about insulin secretion. Thus, when grouping subjects by glucose tolerance, obesity, sex, insulin sensitivity, or age, the insulin method consistently reproduced the findings of the criterion standard, C-peptide method, both for basal and total insulin secretion. The disparity concerning the sex differences could be attributed to differences in the corresponding C-peptide to insulin ratio. Another important result was that the classic relationships between basal and total insulin secretion vs BMI and insulin sensitivity were similarly well described using the insulin method and the C-peptide method. This analysis reveals an underappreciated concordance between the 2 methods.

In conclusion, we have developed an insulin-based deconvolution method for the calculation of endogenous (prehepatic) insulin secretion and compared it to the traditional C-peptide–based method. The method can be useful for the application of β-cell function modeling methodologies in studies where plasma C-peptide concentrations are not available. However, it should be used with caution in conditions where insulin clearance is known to be remarkably different from the standardized value that we have used in our approach.

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Conflict of Interest

The authors have no conflict of interest relevant to this article to declare.

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