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Quantification of beta-cell function during IVGTT in Type II and non-diabetic subjects: assessment of insulin secretion by mathematical methods

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Abstract

Aims/hypothesis. We compared four methods to assess their accuracy in measuring insulin secretion during an intravenous glucose tolerance test in patients with Type II (non-insulin-dependent) diabetes mellitus and with varying beta-cell function and matched control subjects.
Methods. Eight control subjects and eight Type II diabetic patients underwent an intravenous glucose tolerance test with tolbutamide and an intravenous bolus injection of C-peptide to assess C-peptide kinetics. Insulin secretion rates were determined by the Eaton deconvolution (reference method), the Insulin SECretion method (ISEC) based on population kinetic parameters as well as one-compartment and two-compartment versions of the combined model of insulin and C-peptide kinetics. To allow a comparison of the accuracy of the four methods, fasting rates and amounts of insulin secreted during the first phase (0–10 min) and the second phase (10–180 min) were calculated.
Results. All secretion responses from the ISEC method were strongly correlated to those obtained by the Eaton deconvolution method ($r = 0.83$–0.92). The one-compartment combined model, however, showed a high correlation to the reference method only for the first-phase insulin response ($r = 0.78$). The two-compartment combined model failed to provide reliable estimates of insulin secretion in three of the control subjects and in two patients with Type II diabetes. The four methods were accurate with respect to mean basal and first-phase secretion response. The one-compartment and two-compartment combined models were less accurate in measuring the second-phase response.
Conclusion/interpretation. The ISEC method can be applied to normal, obese or Type II diabetic patients. In patients with deviating kinetics of C-peptide the Eaton deconvolution method is the method of choice while the one-compartment combined model is suitable for measuring only the first-phase insulin secretion. [Diabetologia (2001) 44: 1339–1348]

Keywords Beta-cell function, Type II diabetes, intravenous glucose tolerance test, insulin secretion, mathematical models.

Type II (non-insulin-dependent) diabetes mellitus is characterised by a progressive functional beta-cell defect [1–5] and a precise measurement of insulin secretion is therefore important. However, an accurate evaluation of insulin secretion involves methodological difficulties [6]. Direct measurement of plasma insulin and C-peptide concentration responses or both to beta-cell stimuli is still the most commonly used method. The kinetics of insulin and C-peptide are influenced by various factors and consequently peripheral insulin or C-peptide concentrations do not always reflect the actual endogenous insulin secretion [6–14]. To overcome these problems, several mathematical methods have been developed to calculate beta-cell secretion from peripheral insulin or C-pep-

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Abbreviations: ISEC, Insulin SECretion method.

Table 1. Summary of clinical characteristics of the study subjects (means with SEM)

	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Fasting BG (mmol/l)
Control subjects: 4 females, 4 males	52.9 ± 4.0	90.9 ± 5.6	173.4 ± 4.4	30.15 ± 1.31	5.5 ± 0.1
Type II diabetic patients: 4 females, 4 males	47.9 ± 3.4	87.8 ± 3.9	169.6 ± 2.5	30.62 ± 1.52	10.0 ± 1.3

tide concentrations [15–27]. These include the Eaton deconvolution method [15, 16], the Insulin SECretion method (ISEC) [17–19] and the combined model with one-compartment [23, 24] or two-compartments for C-peptide kinetics [25, 26] (see Methods). The deconvolution technique has been used in many physiological and pathological conditions [5, 7, 10, 15, 16, 28–30] and has been simplified by using population-based kinetic parameters of C-peptide instead of individually determined parameters [17]. Investigators [17] determined population-based C-peptide kinetic parameters based on regression analysis of data from 250 normal, obese and Type II diabetic subjects and these were applied to the ISEC method [18, 19].

The combined model [23, 24] uses only insulin and C-peptide concentrations obtained during the day of experiment and is not restricted to a certain protocol or subjects from a specific population. This model has later been modified to encompass two-compartment C-peptide kinetics [25, 26]. We have recently assessed the accuracy of the original deconvolution method and the combined model during slow changes in insulin appearance rates, as seen during an oral glucose tolerance test [31]. However, limited information is available on the accuracy of these methods in estimating insulin secretion during fast dynamics, e.g. intravenous glucose tolerance test, in subjects with normal and impaired glucose tolerance. We aimed to assess these methods under the conditions of rapid dynamics of plasma insulin and C-peptide in relevant groups of human subjects with normal glucose tolerance and Type II diabetes mellitus.

We compared the accuracy of the four methods, using the deconvolution method [15, 16] as the reference method.

Subjects and methods

Subjects. Eight patients with Type II diabetes and eight control subjects, all Caucasian, were examined (subject characteristics are given in Table 1). The two groups were matched according to age, gender and BMI. The diabetic patients were only treated with diet and oral anti-diabetic agents. Their mean disease duration was 7 years (range 1–12 years) and glycated haemoglobin (HbA_{1C}) within the range of 5–12 %. All oral antidiabetic agents were withdrawn at least three days before the study. None had vascular, renal, eye or neurological complications and none had hepatic or endocrine diseases apart from diabetes mellitus. The control subjects had no family history of diabetes mellitus and none were taking any medication known to influence glucose metabolism. All studies were carried out in the Department of Endocrinology, Hvidovre University Hospital, Copenhagen, Denmark. Subjects gave their written informed consent and the protocols were approved by the local Committees of Ethics in Copenhagen and done in accordance with the Helsinki Declaration II.

Methods. Two experiments were done in random order on different days (at least 7 days apart). The subjects attended the ward in the morning after overnight fasting including no smoking. An intravenous sampling catheter (heparinised polyethylene catheter, Viggo, Vingmed, Denmark) was inserted into the dorsum of the hand and an infusion catheter was maintained in a heating blanket to ensure "arterialization" of the venous samples, and the cannula was kept open with 0.9 % saline. Specially coated syringes and tubing lines (Braun, Germany) were used for all infusions of peptides to avoid adhesion of the peptides to the plastic material.

Protocol 1: Assessment of C-peptide kinetics. Each subject received a priming bolus of 500 μg somatostatin (Durascan Medical Products, Odense, Denmark) intravenously, followed by a somatostatin infusion (500 μg/h) starting 90 min before injection of C-peptide and continuing throughout the experiment. Blood samples were taken at –10, –5 and 0 minutes. At 0 min, a bolus of 50 nmol biosynthetic human C-peptide (Bachem Feinkemikalien, Bubendorf, Switzerland) was given intravenously over 30 s and blood samples were taken at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 17, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 140, 160 and 180 min to measure plasma C-peptide

Protocol 2: Intravenous glucose tolerance test (IVGTT). A standard frequently sampled IVGTT with tolbutamide was done. Baseline blood samples were taken at –10, –5 and 0 min. At 0 min, 0.3 g/kg 50 % D-glucose (SAD, Copenhagen, Denmark) was given intravenously over 1 min and blood samples were taken at 3, 4, 5, 6, 7, 8, 10, 12, 14, 17 and 19 min. At 20 min a bolus of 300 mg tolbutamide (Hoechst, Germany) dissolved in saline was given intravenously over 30 s, and samples were taken at 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 140, 160 and 180 min to measure plasma insulin, C-peptide and glucose.

Sample collection and analytical techniques. Blood samples for insulin and C-peptide were drawn in chilled tubes containing 500 KIU/ml aprotinin and heparine fluoride and placed on ice. All samples were centrifuged immediately at 4 °C, 3000 rpm for 20 min and pipetted into three separate tubes; one for insulin measurement, one for C-peptide and one for additional measurements. The C-peptide samples were stored at –80 °C and the insulin samples were stored at –20 °C until assayed. Plasma insulin was assayed in duplicate by a highly sensitive two-site enzyme-linked immunosorbent assay [32]. The assay is based on two monoclonal antibodies (Novo Nordisk A/S) specific for intact human insulin. The intra-assay coefficient of variation was 4 % and the detection limit 5 pmol/l (assay range 5–2000 pmol/l). Human C-peptide was measured in duplicate with a radioimmunoassay [33, 34]. The intra-assay coef-

ficient of variation was 4 % and the detection limit of the assay 100 pmol/l. Plasma glucose concentration was measured immediately using the glucose oxidase method on a Yellow Spring Glucose Analyser (Yellow Spring Instruments Co., Yellow Spring, Ohio, USA).

Mathematical models. The two-compartment model for C-peptide was applied [35]. The following biexponential model was fitted to the individual C-peptide concentrations measured according to protocol 1:

$$C(t) = \alpha_0 + \alpha_1 \exp(-\beta_1 t) + \alpha_2 \exp(-\beta_2 t) \quad (1)$$

C(t) represents the concentration at time t of C-peptide in the plasma after injection of the dose D at time 0. The parameter α_0 represents the possibly incomplete suppressed concentration of endogenous C-peptide. The parameters α_1, β_1, α_2 and β_2 were used to calculate the secretion rate during the IVGTT by means of deconvolution of the measured C-peptide concentrations. The kinetic parameters of the two-compartment model for C-peptide k_{01} (elimination from the plasma compartment), k_{21}, k_{12} (transfer rates between compartments) and the apparent volume of the plasma compartment $V_1 = D/(\alpha_1 + \alpha_2)$ were calculated [16]. We also calculated the apparent volume of the second (extravascular) compartment $V_2 = V_1 k_{21}/k_{12}$ and the clearance rate $Cl = k_{01} V_1$.

Deconvolution methods. The original deconvolution method [15] used a cubic spline function to smooth the measured C-peptide concentration profile followed by calculation of the secretion rate from the equations for the two-compartment model:

$$dC_1(t)/dt = -(k_{01} + k_{12})C_1(t) + k_{21}C_2(t) + S(t) \quad (2)$$

$$dC_2(t)/dt = k_{21}C_1(t) - k_{12}C_2(t) \quad (3)$$

$C_1(t)$ and $C_2(t)$ represent the amounts of C-peptide in the two compartments and S(t) is the secretion rate at time t. We used a cubic spline function to describe the secretion rate, which can be estimated by fitting the convoluted spline function based on the estimated individual kinetic parameters to the measured C-peptide by a multiple linear regression analysis [23, 31]. The inherent tendency of deconvolution methods to show large fluctuations between sampling time intervals was avoided by choosing the knot points of the spline to be preceded and followed by three to four sampling times. The deconvolution method calculates the C-peptide secretion rate, which is equal to the insulin secretion rate if hepatic extraction of C-peptide is zero and insulin and C-peptide are secreted at equimolar rates.

To avoid measuring each individual's C-peptide kinetics, investigators calculated population-based parameters adjusted for clinical status (normal, obese or Type II diabetes), age, gender, height and body weight by regression analysis of a large number of individual kinetic parameters [17]. We used a method adapted to computer use, denoted ISEC [18, 19]. This ISEC method uses the population-based C-peptide kinetics parameters and a regularisation method of deconvolution constrained to non-negative values. For both deconvolution methods and both combined models a basal (initial) steady state was assumed and confirmed by the constant concentrations of insulin and C-peptide before IVGTT.

Combined models. The combined models describe the kinetics of endogenous plasma insulin and C-peptide concentrations during a single experimental test as the result of a common equimolar pre-hepatic secretion rate [23–26]. The combined models also assume that the fraction of insulin extracted by the liver remains constant during the experiment corresponding to the assumption that insulin elimination kinetics do not change. Insulin and C-peptide appear in the systemic circulation at rates $F \cdot R(t)$ and R(t), respectively, and the fraction (1-F) of newly, as well as previously, secreted insulin is taken up by the liver during each passage. Using the one-compartment models for insulin and C-peptide kinetics the equations for the combined model are [23]:

$$dI(t)/dt = -k_I I(t) + f \cdot r(t) \quad (4)$$

$$dC(t)/dt = -k_C \cdot C(t) + r(t) \quad (5)$$

I(t) and C(t) are the concentrations of insulin and C-peptide at the time t in arterial plasma. The V_I and V_C are the insulin and C-peptide distribution volumes, respectively, and k_I and k_C are the corresponding elimination rate constants of the peptides. The $r(t) = R(t)/V_C$ is the secretion rate per unit distribution volume of C-peptide and $f = F \cdot V_C/V_I$.

Of note, the pre-hepatic secretion rate r(t) appears in both the above equations, allowing the estimation of the individual parameters (f, k_I and k_C) as well as r(t) from the concentrations of insulin and C-peptide as a function of time. A computer programme written in APL was designed to perform the calculations [23]. The programme uses a cubic spline function to represent r(t) with knot points chosen at suitable time points as described above. To convert secretion rates expressed in pmol/min per unit distribution volume of C-peptide to pmol/min per kg body weight, a value of 0.125 l/kg was used for V_C [23].

This model has been extended to encompass two-compartment C-peptide kinetics [25, 26]. The insulin equation remained unchanged, but the C-peptide equation was replaced by equations (2) and (3) where $r(t) = S(t)/V_1$ and $f = F \cdot V_1/V_I$. Although only the insulin and C-peptide concentrations in the vascular compartment (I(t) and C(t)) can be measured, the five parameters f, k_I, k_{01}, k_{21}, k_{12} as well as r(t) can be identified by an ingenious method described [25]. r(t) describes now the rate of C-peptide appearance in the vascular compartment and to obtain the secretion rate per kg of body weight a conversion factor of 0.0602 l/kg was used [25]. The properties of the four methods are summarised in Table 2.

Data analysis and statistical methods. For each subject, insulin secretion rates were calculated, firstly, by the deconvolution methods based on individual C-peptide kinetics and, secondly, the ISEC method based on the population parameters as well as the two versions of the combined model. The goodness of fit was evaluated by calculating the deviations between measured and fitted concentrations. The accuracy was evaluated by calculating the 95 %-confidence limits for the differences between the secretion rates obtained with the reference method and the other methods. The precision of the individual kinetic parameters for the reference method and the combined models was estimated by non-linear regression analysis. The method based on population kinetic parameters did not, however, provide error estimates for the individual parameters. For comparison between the methods, fasting rates and the amounts of insulin secreted during the first (0–10 min) and second phase (10–180 min) were calculated. A comparison of results from the control subjects and diabetic patients was made using the Mann-Whitney test or *t* tests depending on whether the distribution assumptions were considered acceptable. Results obtained by two different methods in the same subjects were compared by Wilcoxon's test or the *t* test for paired

Table 2. Schematic description of the methods for assessing insulin secretion

Method	Reference method [15, 16]	Deconvolution method with population-based parameters for C-peptide [17, 18, 19]	Combined model, one-compartment of insulin and C-peptide [23]	Combined model, one-compartment of insulin and two-compartment for C-peptide [25, 26]
Principle	Determination of individual C-peptide kinetic parameters by analysis of C-peptide concentrations after bolus injection of human C-peptide and calculation of secretion by deconvolution of C-peptide concentrations measured at intervals during actual test period	Calculation of secretion by deconvolution of C-peptide based on kinetic parameters adjusted for subject type (normal, obese and Type II diabetes only)	Calculation of secretion rate from measurements of insulin and C-peptide by combined parameter estimation and deconvolution, exploiting the different kinetics of the two peptides	Same as for combined one-compartment model for insulin and C-peptide
Assumptions	Equimolar secretion rates of insulin and C-peptide, negligible first pass uptake of C-peptide prior to blood sampling, two-compartment model for C-peptide kinetics with elimination from the plasma compartment and exchange with a second compartment	Same as for reference method	Same as for reference method except for one-compartment models for C-peptide and insulin kinetics	Same as for reference method plus one-compartment model for insulin kinetics
Validation	Dogs, normal and diabetic subjects [7, 9, 16, 31]	Normal subjects [19]	Dogs and normal and diabetic subjects [23, 31]	Dogs [26]
Applicability	All kinds of secretion studies, all types of subjects	All kinds of secretion studies in normal, obese and Type II diabetes only	All kinds of studies and subjects exhibiting variation in secretion	All kinds of studies and subjects exhibiting variation in secretion
Resource requirements	Extra test with intravenous bolus injection in each subject to determine kinetic parameters, measurement of C-peptide but not insulin	Measurement of C-peptide only	Measurement of insulin and C-peptide	Measurement of insulin and C-peptide

data. The correlations were assessed by the product-moment correlation or the Spearman rank correlation. Unless otherwise stated, results are reported as means ± standard error of the means (SEM). A p values of less than 0.05 was considered to be statistically significant.

Results

Individual C-peptide kinetics. The mean C-peptide decay curves for the diabetic patients and the control subjects were almost identical (Fig. 1). The mean C-peptide kinetic parameters were obtained by fitting equation (1) to the individual data followed by conversion of the estimates of α_1, α_2, β_1, β_2 to the two-compartment rate constants k_{21}, k_{12}, k_{01} (Table 3). The mean squared relative deviation between measured and fitted C-peptide concentrations was about 10 % (range 5 to 18 %), and the precision of the individual estimates of the rate constants was about 10 to 20 %. Table 3 also shows the population-based kinetic parameters calculated according to methods described previously [17] and incorporated in the ISEC method [18, 19]. The mean population-based parameters and the mean individual counterparts based on analysis of the C-peptide decay curve after intravenous bolus injection do not deviate significantly. The SEM of the rate constants from the intravenous bolus test reported in Table 3 correspond to standard deviations($SD = SEM \cdot \sqrt{8}$) of 30 to 50 %, indicating a substantial variation between subjects compared to the precision of the individual estimates. In contrast, there was a much smaller variation between the individual population-based parameters, and the rate constants for the Type II diabetics were significantly higher than those of the controls athough the differences were relatively small. The rate constants from the intravenous bolus test and the derived parameters (V_1, V_2, Cl) were not significantly different between the diabetic patients and the control subjects.

Intravenous glucose tolerance test. As expected, the fasting and stimulated glucose concentrations were higher in the patients than in the control subjects,

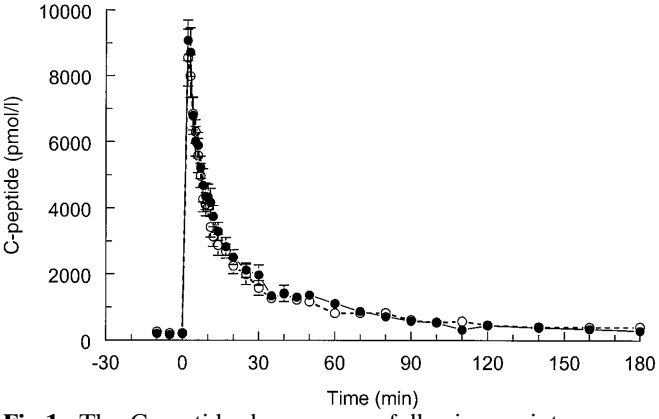

Fig. 1. The C-peptide decay curves following an intravenous bolus of 50 nmol biosynthetic C-peptide. The mean curve for the non-diabetic control subjects (●—●) and the mean curve for the Type II diabetic patients (○– –○)

whereas the fasting plasma insulin and C-peptide concentrations were comparable between the two groups (Fig. 2). Patients with diabetes lacked first-phase insulin and C-peptide responses to glucose and showed only a small, blunted second-phase response to glucose plus tolbutamide. In contrast, the control subjects responded well to the glucose injection with a distinct first-phase insulin response followed by a large second-phase response. As expected, C-peptide plasma concentrations were higher than those of insulin and also showed biphasic dynamics.

Insulin secretion rates. The mean insulin secretion rates obtained with the deconvolution methods are shown in Figure 3. The reference method using individually estimated kinetic parameters and the ISEC method using population parameters gave similar results for both groups. The mean squared relative deviation between measured and fitted C-peptide concentrations was typically 5 to 10%. Because the 95%-confidence limits for the mean difference include zero, there were no significant differences between the secretion rates obtained with the two methods. Control subjects showed a large first-phase secretion response lasting about 5 min. Between 5 and 20 min the secretion rate began to fluctuate. The second-phase secretion response peaked after 20 min when tolbutamide was given and decreased subsequently approaching the fasting rate after about 60 min. For the Type II diabetic patients, both methods were able to identify a small first-phase response where the concentration profiles had failed to do so. The second-phase secretion with a peak value similar

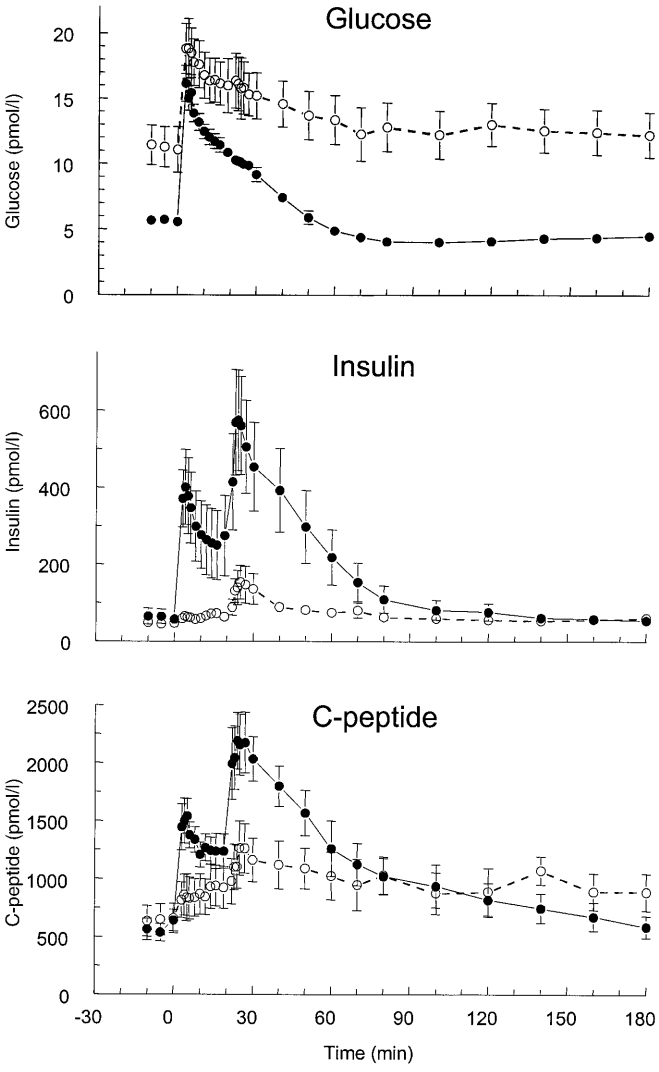

Fig. 2. Plasma glucose, insulin and C-peptide concentrations during the IVGTT. The means for the control subjects (●—●). The means of the Type II diabetic patients (○– –○)

Table 3. C-peptide kinetic parameters estimated after intravenous bolus injection of C-peptide and the population-based values used by the ISEC program (means with SEM)

	Control subjects (n = 8)		Type II diabetic patients (n = 8)	
Parameter	i.v. bolus exp.	ISEC	i.v. bolus exp.	ISEC
k_{21} (min^{-1})	0.084 ± 0.017	0.0556 ± 0.0005	0.081 ± 0.013	0.0632 ± 0.0005
k_{12} (min^{-1})	0.076 ± 0.012	0.0480 ± 0.0002	0.055 ± 0.008	0.0488 ± 0.0002
k_{01} (min^{-1})	0.066 ± 0.006	0.0553 ± 0.0006	0.065 ± 0.007	0.0607 ± 0.0005
V_1 (l)	4.51 ± 0.51	4.51 ± 0.17	4.68 ± 0.45	4.35 ± 0.07
V_2 (l)	4.88 ± 0.78	5.21 ± 0.15	7.60 ± 1.53	5.64 ± 0.16
Cl ($ml \cdot kg^{-1} \cdot min^{-1}$)	3.08 ± 0.13	2.78 ± 0.09	3.39 ± 0.41	3.04 ± 0.11

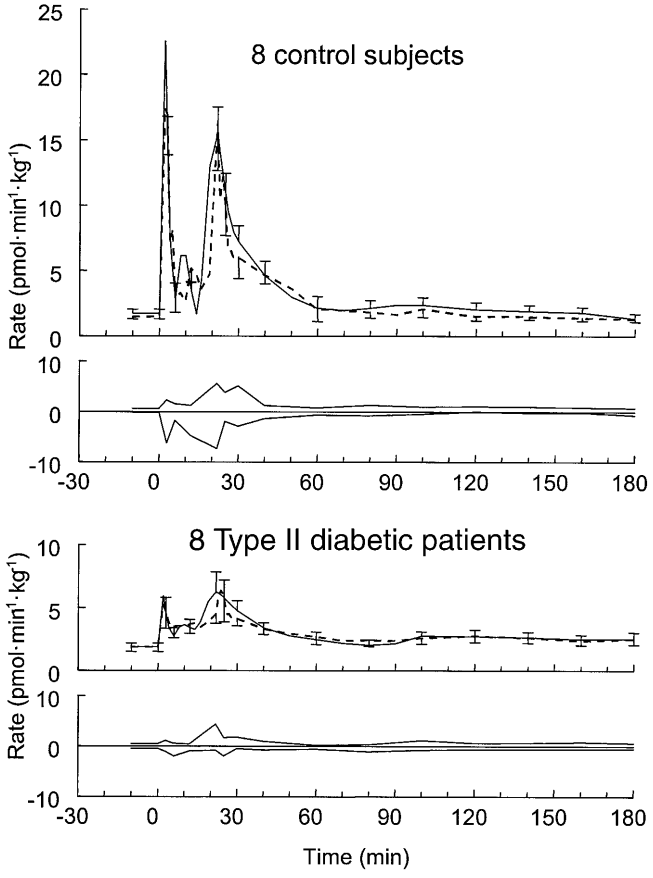

Fig. 3. Mean estimates and 95 %-confidence limits for the difference between secretion rates obtained with the deconvolution reference method based on individual C-peptide parameters (—) and the deconvolution method based on population parameters (- - -) for the control subjects in the two upper panels and for the Type II diabetic patients in the two lower panels

to the first phase peak and a prolonged duration as in the control subjects was also identified. The second-phase peak tended to be narrower with the ISEC than with the reference method.

The parameters of the combined models share only the insulin elimination rate constant k_I (Table 4). k_I is about twice as large with the two-compartment than with the one-compartment model. There was, however, a large variation between subjects and the accuracy of the individual parameter estimates was low and varied greatly from subject to subject with coefficients of variation around 50 %. The mean squared relative deviation between measured and fitted insulin and C-peptide concentrations was about 10 % for the combined models. The C-peptide kinetic parameters of the two-compartment combined model can be compared with corresponding values in Table 3. The k_{01} and k_{21} in Table 4 are about twice as large as in Table 3, while k_{12} is similar. The corresponding parameters from the Type II patients and the control subjects were not significantly different.

For the mean insulin secretion rates obtained with the combined one-compartment model and the deconvolution reference method, we found the profiles to be similar (Fig. 4). However, the peak rate of the control subjects seems to be somewhat overestimated using the combined model and the second-phase response tends to decrease more rapidly using the combined model than with the reference method. A similar pattern of differences is seen with the Type II patients. The 95 %-confidence limits confirm that there are no statistically significant differences between the rates obtained with the two methods. The two-compartment combined model failed to provide reliable estimates of insulin secretion in three of the control subjects and in two of the diabetic subjects. The estimation was considered as a failure when one or more of the parameter estimates was negative. The mean secretion profiles of the five control subjects and the six Type II diabetics compared to the mean secretion profiles obtained with the reference method is shown in Figure 5. There is good agreement with respect to the basal rate and the first-phase secretion. The two-compartment combined model tends to overestimate the second-phase response considerably, but the difference did not reach statistical significance according to the 95 %-confidence limits.

The mean basal rate and the secretion responses for the first phase (0–10 min) and second phase (10–180 min) as well as the total secretion (0–180 min) are shown in Table 5. By paired tests the only significant difference between the reference method and each of the three other methods was

Table 4. Kinetic parameters estimated for the one- and two-compartment combined models of insulin and C-peptide kinetics during IVGTT (means with SEM)

	Control subjects		Type II diabetic patients	
Parameter	One-compartment $n = 8$	Two-compartment $n = 5$	One-compartment $n = 8$	Two-compartment $n = 6$
$f = F \cdot V_I/V_C$	0.41 ± 0.05		0.29 ± 0.07	
k_I (min^{-1})	0.088 ± 0.028	0.198 ± 0.050	0.195 ± 0.115	0.355 ± 0.158
k_C (min^{-1})	0.024 ± 0.007		0.037 ± 0.012	
$F \cdot V_I/V_1$		4.05 ± 1.08		5.75 ± 0.87
k_{21} (min^{-1})		0.120 ± 0.077		0.177 ± 0.064
k_{12} (min^{-1})		0.030 ± 0.008		0.067 ± 0.018
k_{01} (min^{-1})		0.099 ± 0.053		0.141 ± 0.059

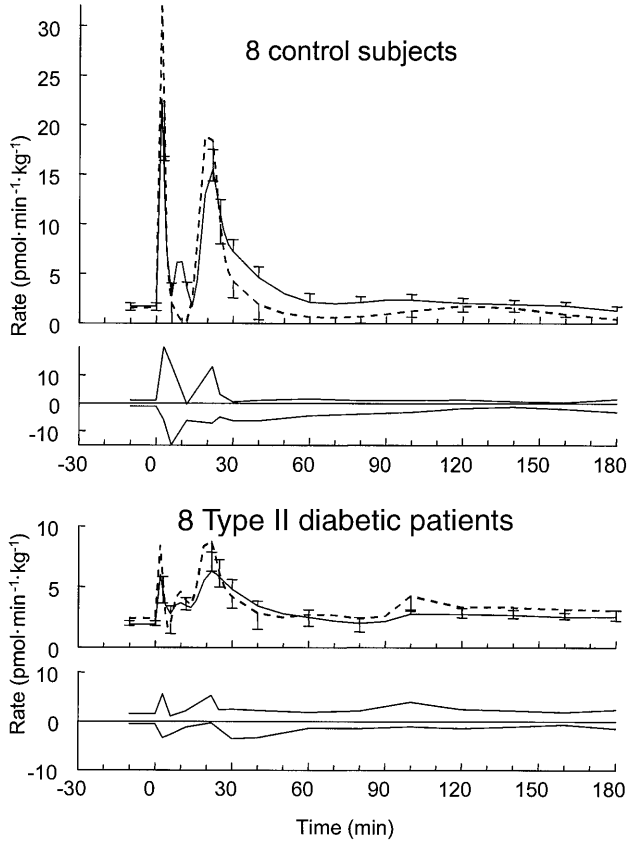

Fig. 4. Mean estimates and 95 %-confidence limits for the difference between secretion rates obtained with the deconvolution reference method based on individual C-peptide parameters (—) and the one-compartment combined model for the control subjects (---) in the two upper panels and for the Type II diabetic patients in the two lower panels

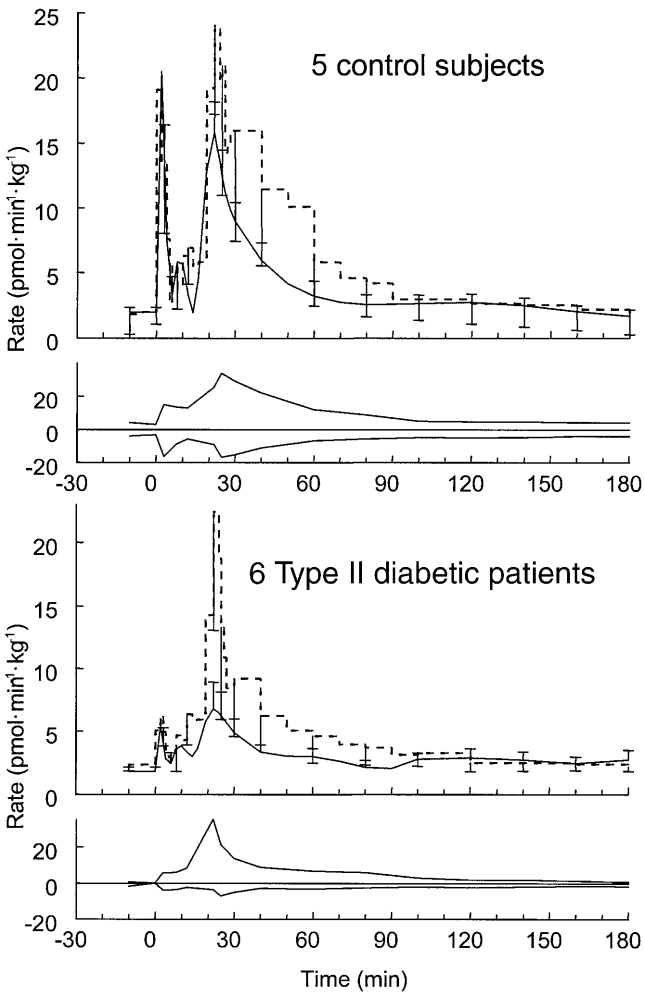

Fig. 5. Mean estimates and 95 %-confidence limits for the difference between secretion rates obtained with the deconvolution reference method based on individual C-peptide parameters (—) and the two-compartment combined model for the control subjects (---) in the two upper panels and for the Type II diabetic patients in the two lower panels. Because the two-compartment combined model was not able to calculate secretion rates for all subjects, only results from five of the control subjects and six of the Type II diabetic subjects are shown

seen with the first-phase response obtained by the ISEC method in the control group. The difference was, however, relatively small (about 15 %). Table 5 shows also the results of the statistical comparisons for each method and secretion response between the control subjects and the Type II patients. Both deconvolution methods and the one-compartment combined model show significant differences in the first-phase response between the two groups. The magnitude of the standard errors indicates that the variation between subjects is similar for the deconvolution methods and higher with the combined models, in particular for the two-compartment model and the second-phase and total secretion responses.

Table 6 shows the correlation coefficients between the secretion responses with the reference method and the three other methods. All secretion responses were highly correlated for the two deconvolution methods while only the first-phase response with the combined models showed a significant correlation to the reference method.

Discussion

In our study, the two deconvolution methods [15, 16, 18, 19] gave similar secretion results (Fig. 3, Tables 5 and 6) as expected from the similarity of the individually estimated and population-based kinetic parameters for C-peptide. The relatively small deviations (5–10 %) between measured and fitted C-peptide concentrations indicate that the two-compartment model was adequate. In the Type II diabetic patients, the methods were able to identify a small first-phase insulin response even when the concentration profiles of C-peptide did not. The second phase was also identified, although the second-phase peak tended to be narrower with the ISEC than the reference method. This could be explained by differences be-

Table 5. Insulin secretion responses during IVGTT with tolbutamide obtained with the four methods

		Basal secretion rate (pmol · min^{-1} · kg^{-1})		1st phase secretion 0–10 min (pmol/kg)		2nd phase secretion 10–180 min (pmol/kg)		Total secretion 0–180 min (pmol/kg)	
Method	Subjects	*n*	Means ± SEM	*n*	Means ± SEM	*n*	Means ± SEM	*n*	Means ± SEM
Deconvolution	Control subjects	8	1.7 ± 0.3	8	86 ± 9	8	531 ± 95	8	618 ± 95
individual parameters	Type II diabetic patients	8	1.9 ± 0.3	8	37 ± 7[c]	7	483 ± 68	7	519 ± 72
ISEC	Control subjects	8	1.5 ± 0.2	8	73 ± 8[a]	8	459 ± 62	8	532 ± 57
population parameters	Type II diabetic patients	8	1.9 ± 0.4	8	38 ± 9[b]	7	483 ± 82	7	520 ± 90
Combined	Control subjects	8	1.6 ± 0.8	8	91 ± 15	8	372 ± 105	8	463 ± 113
model, one-compartment	Type II diabetic patients	8	2.4 ± 0.6	8	41 ± 9[b]	7	600 ± 152	7	642 ± 158
Combined	Control subjects	5	1.9 ± 1.0	5	94 ± 22	5	1021 ± 536	5	1115 ± 555
model, two-compartment	Type II diabetic patients	6	2.4 ± 1.0	6	44 ± 14	5	562 ± 202	5	593 ± 204

[a] $p < 0.05$ Significant difference relative to reference deconvolution method (individual parameters)
[b] $p < 0.05$, [c] $p < 0.001$ Significant difference relative to control subjects

Table 6. Correlation coefficients between secretion responses obtained with the deconvolution reference method and the other methods

Method	Basal rate	1st phase 0–10 min	2nd phase 10–180 min	Total 0–180 min
ISEC population parameters	0.83[a] (16)	0.92[a] (16)	0.81[a] (15)	0.80[a] (15)
Combined one-compartment	0.40 (16)	0.78[a] (16)	0.12 (15)	0.08 (15)
Combined two-compartment	0.11 (11)	0.80[a] (11)	–0.05 (10)	–0.05 (10)

[a] $p < 0.001$ (product moment correlation)
Number in bracket is number of subjects

tween the two deconvolution methods, i.e. the smoothing factor in the ISEC programme and the spline representation of the secretion rate with the reference method based on individual C-peptide kinetics. The variation between the subjects is, however, always much larger with the experimentally determined parameters than for the population-based ones. This is presumably because the population-based method underestimates the variation between subjects as well as the intra-subject variation in the parameter estimates.

The relative deviations between the measured insulin and C-peptide concentration and those fitted according to the one-compartment combined model were also relatively small (about 10%) indicating that the model gives an acceptable approximation [23, 24]. Our data show that during rapid changes in secretion, the rates of pre-hepatic insulin secretion obtained with the one-compartment combined model were not significantly different from those obtained with the reference method (Fig. 4). The peak values seem to be somewhat overestimated and the second-phase response with the combined model tends to decrease more rapidly in the control subjects. The one-compartment combined model yields similar estimates for the first-phase secretion, while basal and second-phase secretion were more variable though not significantly different from the results obtained with the reference method (Tables 5 and 6). Only the first-phase secretion showed a significant correlation between the two methods. The combined model, contrary to expectations, seems to be more accurate in estimating the rapid first-phase response than the slower second phase and the basal insulin secretion. In previous validation studies, the combined model was able to estimate relatively slowly varying known rates of human insulin and C-peptide administered to dogs [23] as well as to control subjects and Type II diabetic patients [31]. Recently, investigators [14] compared the one-compartment combined model and the deconvolution technique in pancreas- and kidney-transplanted Type I diabetic patients as well as in normal control subjects and kidney-transplanted patients during an OGTT and a glucagon test. The combined model seemed to overestimate the rates in the early poststimulatory phase of insulin secretion and to underestimate in the later phase when compared to the deconvolution reference method.

The combined model with two-compartment kinetics for C-peptide which introduces more unknown

parameters to be estimated could require more precise data showing pronounced dynamics [25, 26] but it should in theory give a more accurate estimation of the insulin secretion than the one-compartment model [23, 24]. In this study the modified two-compartment combined model [25, 26] was only able to estimate insulin secretion profiles in five out of eight control subjects and in six out of eight patients with Type II diabetes. The two-compartment model gave similar estimates for basal and first-phase secretion as the other methods (Table 5). The second-phase secretion tended, however, to be overestimated probably because of the addition of a second compartment to the C-peptide kinetic model rendering parameter identification more difficult or impossible depending on the precision of the measurements and the biological variation. It is possible that some modification, e.g. reweighting of the data, might improve the performance of the two-compartment model.

A similar problem concerning estimates of the kinetic parameters of the two-compartment model for C-peptide has been observed in a study [21] which evaluated the accuracy of the minimal model of glucose-dependent C-peptide secretion for the IVGTT in non-diabetic, healthy subjects. As rather large differences were observed between the estimated parameters and the population-based values the authors suggested that the population-based values should be used. A comparison of the estimates of k_{21}, k_{12}, and k_{01} in Table 4 with the corresponding population-based values or the i.v. bolus test parameters in Table 3 also shows relatively large deviations, indicating the difficulties involved in identification of the parameters of the two-compartment combined model.

Estimates of k_I are about twice as large with the two-compartment model relative to the one-compartment model and in the Type II patients compared to the control subjects (Table 4). Of note, differences between the estimated secretion rates are much smaller than the differences between the estimates of the underlying kinetic parameters. This phenomenon has also been observed in a simulation study of the ability of the one-compartment combined model to estimate secretion rates when realistic random errors were added to the concentrations of insulin and C-peptide [24]. In other words, the secretion rates measured by the combined model are more accurate than the estimates of the underlying kinetic parameters. Overall, the combined models perform better in estimating the rapid first-phase secretion than the second phase. This could, however, be an advantage because it is the first-phase response that is of vital interest.

The rather small and insignificant correlation coefficients (Table 6) between the reference method and the combined model methods for the basal secretion, the second-phase and the total secretion responses, can be explained by the relatively large variation in these responses from the combined models (Table 5).

In summary, our data show that the deconvolution methods based on individual or population-based C-peptide kinetic parameters and the combined model all give similar estimates of the basal and first-phase insulin secretion during an IVGTT. The two-compartment combined model could not estimate secretion rates in 5 of the 16 subjects and tended to overestimate the second-phase secretion. Taking the deconvolution method with independent assessment of C-peptide kinetics as the reference method, it can be concluded that the population-parameter-based deconvolution method as well the one-compartment combined model are also accurate in assessing rapid changes in insulin secretion, i.e. the first-phase response of IVGTT in control subjects and Type II diabetic patients.

We recommend applying the ISEC method to subjects that can be classified as normal, obese or Type II diabetic and who can be expected to show normal C-peptide kinetics. In subjects where deviating kinetics of C-peptide cannot be excluded (e.g. pancreas- and kidney-transplanted patients), the one-compartment combined model or the deconvolution method with individual assessment of C-peptide kinetics is to be recommended.

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