

Validation of Methods for Measurement of Insulin Secretion in Humans In Vivo

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To detect and understand the changes in β -cell function in the pathogenesis of type 2 diabetes, an accurate and precise estimation of prehepatic insulin secretion rate (ISR) is essential. There are two common methods to assess ISR, the deconvolution method (by Eaton and Polonsky)—considered the “gold standard”—and the combined model (by Vølund et al.). The deconvolution method is a 2-day method, which generally requires separate assessment of C-peptide kinetics, whereas the combined model is a single-day method that uses insulin and C-peptide data from a single test of interest. The validity of these mathematical techniques for quantification of insulin secretion have been tested in dogs, but not in humans. In the present studies, we examined the validity of both methods to recover the known infusion rates of insulin and C-peptide mimicking ISR during an oral glucose tolerance test. ISR from both the combined model and the deconvolution method were accurate, i.e., recovery of true ISR was not significantly different from 100%. Furthermore, both maximal and total ISRs from the combined model were strongly correlated to those obtained by the deconvolution method ($r = 0.89$ and $r = 0.82$, respectively). These results indicate that both approaches provide accurate assessment of prehepatic ISRs in type 2 diabetic patients and control subjects. A simplified version of the deconvolution method based on standard kinetic parameters for C-peptide (Van Cauter et al.) was compared with the 2-day deconvolution method, and a close agreement was found for the results of an oral glucose tolerance test. We also studied whether C-peptide kinetics are influenced by somatostatin infusion. The decay curves after bolus injection of exogenous biosynthetic human C-peptide, the kinetic parameters, and the metabolic clearance rate were similar whether measured during constant peripheral somatostatin infusion or without somatostatin infusion. Assessment of C-peptide kinetics can be performed without infusion of somatostatin, because the endogenous insulin concentration remains constant. Assessment of C-peptide kinetics with and without infusion of somatostatin results in nearly identical secretion rates for insulin during an oral glucose tolerance test. *Diabetes* 49:580–588, 2000

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ANOVA, analysis of variance; ISEC, insulin secretion; ISR, insulin secretion rate; OGTT, oral glucose tolerance test; SMS, somatostatin.

Type 2 diabetes is characterized by a defect in insulin secretion (1–9). β -Cell defects are often poorly characterized, and controversy still persists concerning the importance of abnormalities in insulin secretion in the pathogenesis of type 2 diabetes (10–12). Reliable methods for estimation of insulin secretion are thus needed to complement insulin sensitivity in understanding the pathogenesis of type 2 diabetes and other pathogenic states, e.g., the pathophysiology of patients with pancreas transplants. Insulin secretion cannot be accurately estimated from peripheral insulin concentrations because of the large and variable hepatic extraction of the hormone from the portal vein secondary to the levels of insulin, disease state, and nutrient administration (13,14). Based on a review of the literature (13), it was concluded, however, that there was no convincing evidence that ingestion of glucose and other nutrients influenced the fractional hepatic extraction, provided insulin concentrations were in the physiological range. Because C-peptide is co-secreted in an equimolar amount, but not extracted by the liver, peripheral concentrations of C-peptide have been used as an index of insulin secretion in the basal state, as well as after β -cell stimuli (15–20). But because of the long half-life of C-peptide compared with insulin, it is difficult to evaluate fast changes in insulin secretion from peripheral C-peptide measurements.

To avoid these limitations, methods for evaluation of insulin secretion by mathematical models have been developed (15,16,21–24). The most common method has been to evaluate β -cell function by calculating insulin secretion rates (ISRs) by deconvolution of peripheral C-peptide concentrations. This technique was originally proposed by Eaton et al. (15) and was refined and validated by Polonsky et al. (16). With this 2-day method, C-peptide kinetics are determined in individual subjects after a bolus injection of biosynthetic C-peptide while endogenous insulin secretion is suppressed by an infusion of somatostatin. Thereafter, the kinetic parameters are used on the day of experiment to calculate ISR by deconvolution of peripheral C-peptide concentrations. Another approach based on kinetic modeling of concomitantly measured concentrations of insulin and C-peptide is termed the “combined model” (21,24). With this method, ISR, C-peptide, and insulin kinetics are calculated from measured profiles of the two peptides. In contrast to the deconvolution method, a separate day for assessment of C-peptide kinetics is not necessary. The validity of these mathematical techniques for quantification of insulin secretion has been tested in dogs, but not in humans. Furthermore, in relation to the Eaton-Polonsky approach, the

TABLE 1
Clinical characteristics of the two groups

	F/M	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m ²)	Fasting blood glucose (mmol/l)
Normal subjects	4/4	52.9 ± 4.0	90.9 ± 5.6	173.4 ± 4.4	30.15 ± 1.31	5.5 ± 0.1
Type 2 diabetic patients	4/4	47.9 ± 3.4	87.8 ± 3.9	169.6 ± 2.5	30.62 ± 1.52	10.0 ± 1.3

All subjects had normal kidney function, determined by serum creatinine.

effect of a somatostatin infusion on the kinetics of C-peptide is unknown.

The present studies were undertaken to examine the degree of accuracy with which the deconvolution and the combined model methods can calculate insulin secretion. This was accomplished by mimicking the insulin secretion during an oral glucose tolerance test in type 2 diabetic patients and matched nondiabetic subjects by a programmed insulin and C-peptide infusion. Thereafter, the insulin appearance rate was calculated on the basis of both the deconvolution technique using individual C-peptide kinetics and the combined model, and it was compared with the actual insulin and C-peptide infusion rate. Furthermore, we measured C-peptide kinetics in all subjects with and without somatostatin infusion to determine if somatostatin affects the clearance rate, the distribution volume, and kinetic parameters of C-peptide and thereby biases the ISRs when calculated by the deconvolution method.

RESEARCH DESIGN AND METHODS

Subjects. Eight type 2 diabetic patients and eight healthy nondiabetic control subjects were examined. The two groups were matched according to age, sex, and BMI as shown in Table 1. The diabetic patients were treated only with diet and oral antidiabetic agents and had a mean disease duration of 7 years (range 1–12). None had vascular, renal, eye, or neurological complications, and none had hepatic or endocrine diseases other than their diabetes. The control subjects had no family history of diabetes, and none were taking any medication known to influence glucose metabolism or were suffering from any renal, hepatic, or endocrine diseases. All studies were carried out in the Department of Endocrinology, Hvidovre University Hospital, Copenhagen, Denmark. Subjects signed written informed consent forms. The protocols were approved by the local committees of ethics in Copenhagen and were performed in accordance with the Helsinki Declaration II.

Methods. First, to examine if a somatostatin (SMS) infusion affects C-peptide decay kinetics, each subject was studied with a bolus injection of C-peptide in the presence and absence of SMS infusion (protocols 1 and 2). Second, the ISRs during an oral glucose tolerance test (OGTT; protocol 3) were calculated with both sets of C-peptide kinetic parameters (with and without SMS) to assess whether the use of SMS changed the ISRs. Third, the accuracy of the deconvolution method and the combined model was tested during a stepwise infusion of insulin and C-peptide simulating the secretion profiles obtained during the OGTT. Each subject was studied on four separate occasions within 5–8 weeks. Protocols 1, 2, and 3 were performed in randomized order, whereas protocol 4 required that protocols 1 and 3 were performed first. During all the protocols, the subjects attended the ward in the morning after fasting overnight, including no smoking since 10:00 P.M. the evening before. During each experiment, an intravenous sampling catheter (heparinized polyethylene catheter; Viggo, Vingmed, Denmark) was inserted into the dorsum of the hand, and when needed an infusion catheter was inserted into a cephalic forearm vein on the opposite arm. The hand with the sampling 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, Melsungen, Germany) were used for all infusions of peptides to avoid adhesion of the peptides to the plastic material. **Protocol 1:** Individual C-peptide kinetics with SMS. The subjects were placed at bed rest and kept supine during the study. After an overnight fast, the subjects received a primed bolus of 500 µg SMS (Durascan Medical Products, Odense, Denmark) intravenously followed by a continuous SMS infusion (500 µg/h) starting 90 min before the bolus of C-peptide and continuing throughout the experiment.

Blood samples were taken at –10, –5, and 0 min. At 0 min, a bolus of 50 nmol biosynthetic human C-peptide (Bachem Feinchemikalien, Bubendorf, Switzerland) was given over 30 s. 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 for measurement of C-peptide and insulin. Blood glucose was measured every 15 min. **Protocol 2:** Individual C-peptide kinetics without SMS. This protocol was performed identically to protocol 1, but without infusion of SMS. The two protocols for assessment of individual C-peptide kinetic were performed in random order in the individuals, with a mean of 10 days in between.

Protocol 3: The OGTT. A standard OGTT with 75 g glucose and frequent sampling was performed. Blood samples were taken at –10, –5, and 0 min. At 0 min, 75 g D-glucose dissolved in 300 ml of water was ingested over a maximum of 5 min. The blood sampling took place at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 105, 120, 150, 180, and 210 min for measurement of insulin, C-peptide, and glucose. The individual secretion rates were calculated from the individual C-peptide kinetics obtained in the presence and absence of a SMS infusion, using the deconvolution approach. The ISRs obtained using the C-peptide decay kinetics during SMS were subsequently employed in protocol 4. The insulin secretion profiles during the OGTT were calculated with the insulin secretion (ISEC) method (19,39). **Protocol 4:** Stepwise infusion of C-peptide and insulin. In this protocol, an insulin and C-peptide infusion simulating the individual secretory pattern from the OGTT was performed. Insulin and C-peptide were infused at stepwise variable rates, equal to the individually calculated secretion rates from the OGTT in the molar ratio 1:2, to resemble a 50% hepatic first-pass extraction. The endogenous secretion was suppressed by infusion of SMS. A bolus of 500 µg SMS (Durascan Medical Products, Odense, Denmark) was given intravenously, followed by a continuous SMS (500 µg/h) starting 90 min before the insulin and C-peptide infusion and continuing throughout the experiment. Basal samples were taken at –10, –5, and 0 min. At 0 min, the variable infusions of insulin and C-peptide were started and continued for 210 min, changing the infusion rate every 10 min. Blood samples for measurement of insulin, C-peptide, and plasma glucose were taken every 10 min before changing the infusion rate. The blood glucose concentration was clamped at the basal level by a variable infusion of 20% D-glucose.

Sample collection and analytical techniques. Blood samples for insulin and C-peptide were drawn into tubes containing 500 KIU/ml aprotinin and heparin-fluoride and placed on ice. All samples were centrifuged immediately at 4°C for 20 min and pipetted into two separate tubes, one for insulin measurement and one for C-peptide. 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 two-site enzyme-linked immunoassay (25), based on two murine monoclonal antibodies (Novo Nordisk). The intra-assay coefficient of variation averaged 4%, and the detection limit was 5 pmol/l. The plasma glucose concentration was measured immediately by the glucose oxidase method on a Yellow Springs Glucose Analyzer (Yellow Springs Instruments, Yellow Springs, OH). Human C-peptide was measured by radioimmunoassay (26,27). Biosynthetic human C-peptide was used as assay standard and tracer, respectively. The intra-assay coefficient of variation averaged 4%. The detection limit of the assay was 100 pmol/l.

Data analysis and statistical methods. For each subject, insulin secretion or appearance rates were calculated by use of the deconvolution method and the combined model. For comparison between the methods, fasting and maximal rates and the total amount of C-peptide appearance were calculated. To quantify the accuracy of the estimated rates based on the two methods, we calculated the difference between estimated and infused rates in percent of the infused rates. Furthermore, the agreement between the calculated rates of appearance and the corresponding infusion rates of C-peptide was assessed by the root mean square measure.

Apart from the special statistical nonlinear regression analysis methods, which are integrated into the computerized kinetic model calculations (16,21), standard statistical methods were used for analysis and presentation of the results. Comparisons of unpaired results from the control subjects and type 2 diabetic patients were made using the Mann-Whitney test or Student's *t* tests, depending on whether the distribution assumptions were considered acceptable. Likewise, comparisons within subjects, such as results obtained by two different methods,

were made by use of Wilcoxon's test or the t test for paired data. Analysis of variance (ANOVA) was used for testing differences between methods and between the normal and diabetic subjects using appropriate estimates of the random variation within and between subjects. The correlation was assessed by the product moment correlation or Spearman's rank correlation. Unless otherwise stated, results are reported as means \pm SE. P values <0.05 were considered significant, except when the Bonferroni adjustment for multiple testing was made.

Mathematical models. The two-compartment kinetic model for C-peptide first proposed by Faber et al. (28) was applied. The equations for the kinetic model are:

$$dC_1(t)/dt = -(K_1 + K_3)C_1(t) + K_2C_2(t) + S(t)$$

$$dC_2(t)/dt = K_1C_1(t) - K_2C_2(t)$$

Sampling stems from the central vascular compartment, which consists of the plasma space and tissues in rapid equilibrium with plasma. Given that C-peptide is biologically inert, removal is assumed to occur only from the central compartment. The kinetic constants K_1 and K_2 describe the rates at which C-peptide is transferred from the central compartment to the peripheral extravascular compartment and back again to the central compartment, respectively. K_3 describes the rate at which C-peptide disappears irreversibly from the central compartment. $C_1(t)$ and $C_2(t)$ represent the mass at time t of C-peptide in the vascular and extravascular compartments, respectively, and $S(t)$ represents the rate of C-peptide secretion by the β -cells or the rate of C-peptide infusion. If the kinetic parameters K_1 , K_2 , and K_3 are known, it is possible by deconvolution methods to calculate $S(t)$ from measurements of C-peptide in plasma (16).

The individual values of the kinetic parameters can be estimated from the decay curve of C-peptide after a bolus injection of C-peptide. The C-peptide decay curve is according to the two-compartment model resolved into a sum of two exponentials, and the parameters are calculated as described by Polonsky et al. (16).

In the current experiments, the kinetic parameters were estimated separately in each subject from the measured values of C-peptide in the central compartment after bolus injection. The estimates were made using iterative weighted nonlinear regression analysis, whereby a modified version of the sum of two exponentials was fitted to the data. The modification consisted of including a constant term to account for the constant basal level of endogenous C-peptide that is present in the experiment without SMS infusion. The apparent volumes of distribution and the metabolic clearance rate were calculated as:

$$V_1 = D/C_1(0)$$

$$V_2 = V_1K_1/K_2$$

$$Cl = K_3V_1$$

D is the intravenous bolus dose of C-peptide, and Cl is the clearance of C-peptide. Deconvolution method. The deconvolution method has been described in detail elsewhere (16,23) and is briefly reviewed here. Whereas the original method (15) used a cubic spline function to smooth the C-peptide concentration profile followed by mathematical deconvolution, we used a cubic spline function to describe the secretion rate (23), which can be estimated by fitting the convoluted rate using the individual kinetic parameters to the measured C-peptide by a multiple linear regression analysis. The inherent tendency of deconvolution methods to exhibit large deviations was avoided by choosing the knot points of the splines to be preceded and followed by three or four sampling times. The deconvolution method assumes that C-peptide kinetics are linear over the range of C-peptide concentrations during the experiments, that the hepatic extraction of C-peptide is minimal, and that C-peptide and insulin are released from the β -cells at equimolar rates.

Combined model. The combined model describes the kinetics of endogenous plasma insulin and C-peptide concentrations during a single experimental procedure as the result of a common equimolar prehepatic secretion rate (21). The model is based on one-compartment approximations of insulin and C-peptide kinetics. The combined model also assumes that the fraction of insulin extracted by the liver remains constant during the experiment. This corresponds to the assumption that the insulin elimination kinetics are constant throughout the experiment.

Insulin and C-peptide appear in the systemic circulation at rates $F \cdot R(t)$ and $R(t)$, respectively. Assuming no or negligible hepatic uptake of C-peptide and that a fraction $(1 - F)$ of newly secreted insulin is taken up by the liver, the rate of appearance of insulin in the systemic blood is:

$$V_1 dI(t)/dt = F \cdot R(t) - k_1 V_1 I(t)$$

and the rate of appearance of C-peptide is:

$$V_c dC(t)/dt = R(t) - k_c V_c C(t)$$

$I(t)$ and $C(t)$ are the concentrations of insulin and C-peptide at time t in arterial plasma. V_1 and V_c are the insulin and C-peptide distribution volumes, respectively, and k_1 and k_c are the elimination rate constants of the peptides. Let $r(t) = R(t)/V_c$ be the appearance rate per unit distribution volume of C-peptide and $f = F \cdot V_c/V_1$. Using these substitutions, the equations above simplify to:

$$dI(t)/dt = -k_1 I(t) + f \cdot r(t)$$

$$dC(t)/dt = -k_c C(t) + r(t)$$

The important concept of this method is that $r(t)$, the relative prehepatic appearance rate, appears in both equations. This makes it possible to estimate the individual parameters f , k_1 , and k_c , as well as $r(t)$, knowing the concentrations of insulin and C-peptide as a function of time. A computer program written in APL was designed to perform the calculations (21), where $r(t)$ is determined as a cubic spline function with knot points at 0, 20, 40, 60, 90, 120, and 150 min or a subset of these time points, depending on the goodness of fit of the model to the data.

RESULTS

C-peptide kinetics in normal and type 2 diabetic subjects with and without SMS. The C-peptide decay curves, plasma glucose, and plasma insulin following the bolus injection of C-peptide in the presence and absence of SMS are shown in Fig. 1, Fig. 2A, and Fig. 2B, respectively. Fasting plasma insulin and C-peptide levels were similar in the diabetic patients and normal subjects, whereas fasting plasma glucose was significantly higher in the diabetic patients. In the presence of SMS, fasting C-peptide concentrations were suppressed in both groups to one-third com-

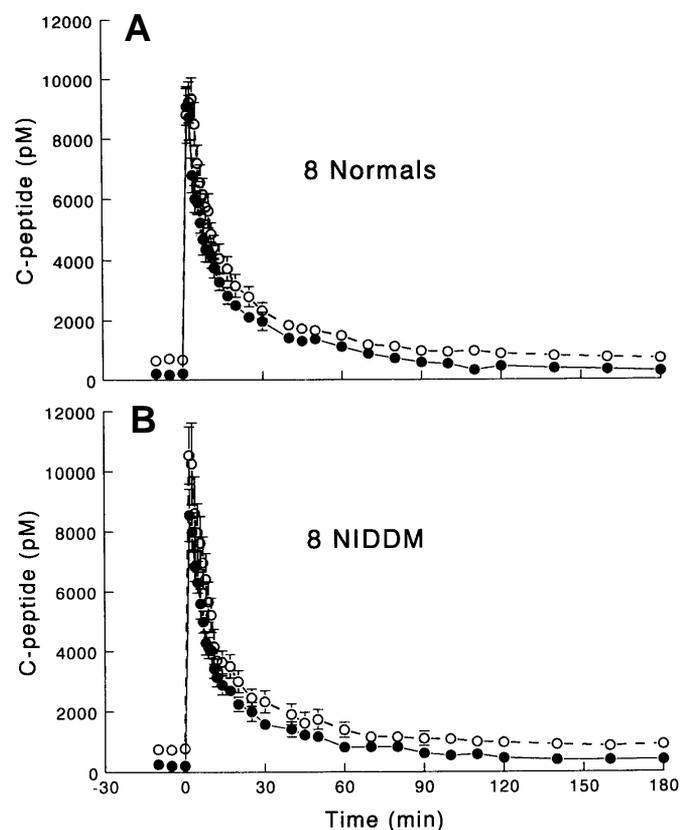


FIG. 1. C-peptide decay curves following an intravenous bolus injection of 50 nmol biosynthetic C-peptide at time 0 min, protocols 1 and 2. A illustrates the curves (mean and SE) for the nondiabetic control subjects in the absence (O) and in the presence (●) of SMS. The curves for the type 2 diabetic patients are displayed in B.

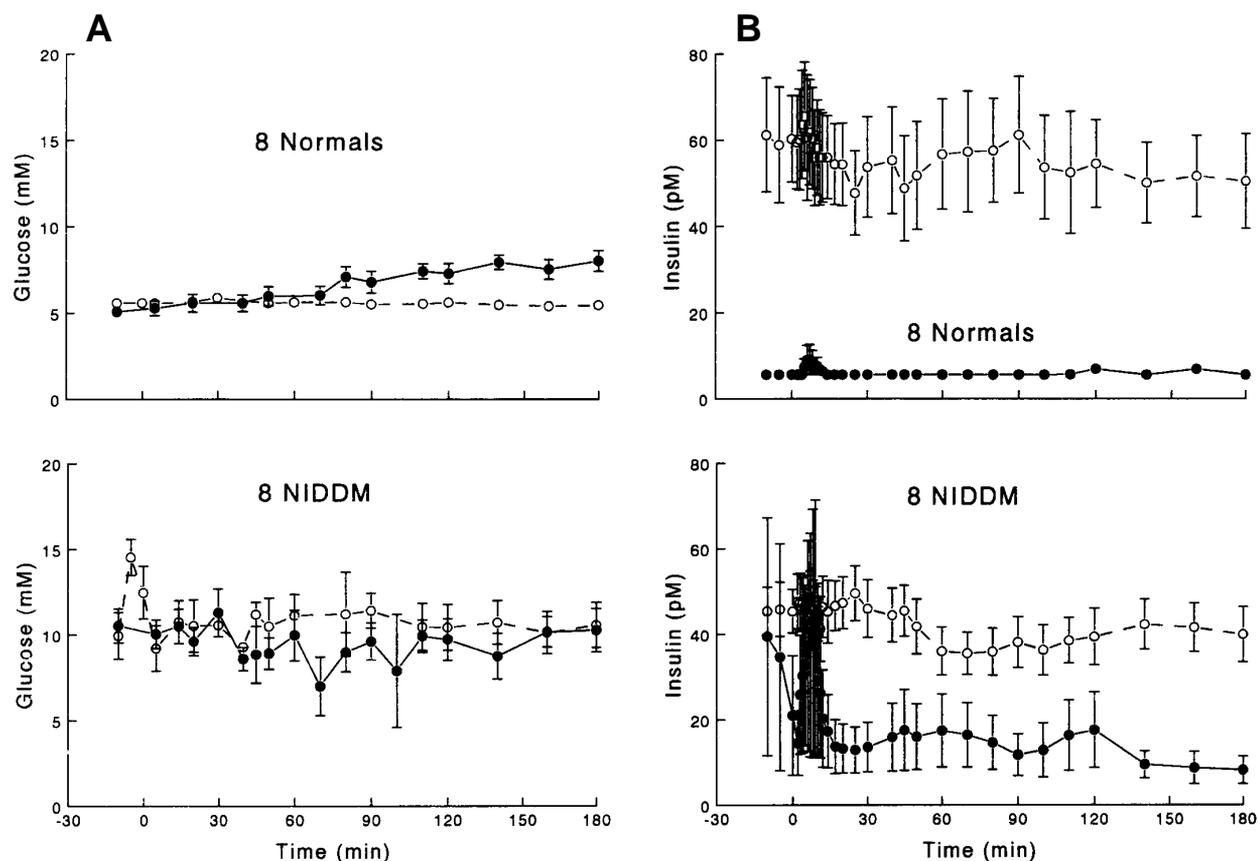


FIG. 2. A: Plasma glucose during C-peptide kinetic study in the absence (○) and presence (●) of SMS (protocol 1 and 2). Upper panel represents the nondiabetic control subjects, and the lower panel represents the type 2 diabetic patients (mean and SE). B: Plasma insulin during C-peptide kinetic study in the absence (○) and presence (●) of SMS (protocol 1 and 2). Upper panel represents the nondiabetic control subjects, and the lower panel represents the type 2 diabetic patients (mean and SE).

pared with the levels without SMS (Fig. 1). The C-peptide decay curves were similar on the days with and without SMS. After the bolus injection, plasma C-peptide increased to similar peak levels in diabetic and normal subjects (Fig. 1).

The SMS infusion suppressed the basal plasma insulin levels to concentrations at or below the detection limit of the assay (5 pmol/l) in the nondiabetic subjects, whereas the insulin levels in the type 2 diabetic patients decreased gradually by a factor of four during the study (Fig. 2B). Without SMS, the insulin concentrations remained constant through-

out the study period in both groups (Fig. 2B). Plasma glucose increased gradually in the nondiabetic subjects after initiation of SMS (from 5 to 8 mmol/l), whereas glucose levels were stable in the absence of SMS. No significant trend was observed in the type 2 diabetic patients, where the fasting glucose levels were similar on the 2 days (Fig. 2A).

The C-peptide mean kinetic parameters are given in Table 2. Individual parameters were determined with fractional standard deviations of about 10–20%. K_1 , K_2 , K_3 , and volumes of distribution were not statistically different between

TABLE 2

C-peptide kinetic parameters, distribution volumes, and metabolic clearance of C-peptide in the absence and presence of somatostatin (protocol 1 and 2)

Group	Normal subjects		Type 2 diabetic patients	
	Without SMS	With SMS	Without SMS	With SMS
n	8	8	7	8
K_1 (min^{-1})	0.039 ± 0.005	0.084 ± 0.017	0.071 ± 0.011	0.081 ± 0.013
K_2 (min^{-1})	0.051 ± 0.010	0.076 ± 0.012	0.054 ± 0.009	0.055 ± 0.008
K_3 (min^{-1})	0.062 ± 0.006	0.066 ± 0.006	0.074 ± 0.007	0.065 ± 0.007
V_1 (liters)	4.48 ± 0.26	4.51 ± 0.51	3.64 ± 0.44	4.68 ± 0.45
V_2 (liters)	4.75 ± 1.67	4.88 ± 0.78	4.71 ± 0.41	7.60 ± 1.53
V_{tot} (liters)	9.23 ± 1.68	9.39 ± 1.03	8.35 ± 0.51	12.28 ± 1.73
Clearance ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	3.14 ± 0.38	3.08 ± 0.13	2.96 ± 0.36	3.39 ± 0.41

Data are means \pm SE. K_1 was significantly higher with SMS ($P < 0.05$, ANOVA).

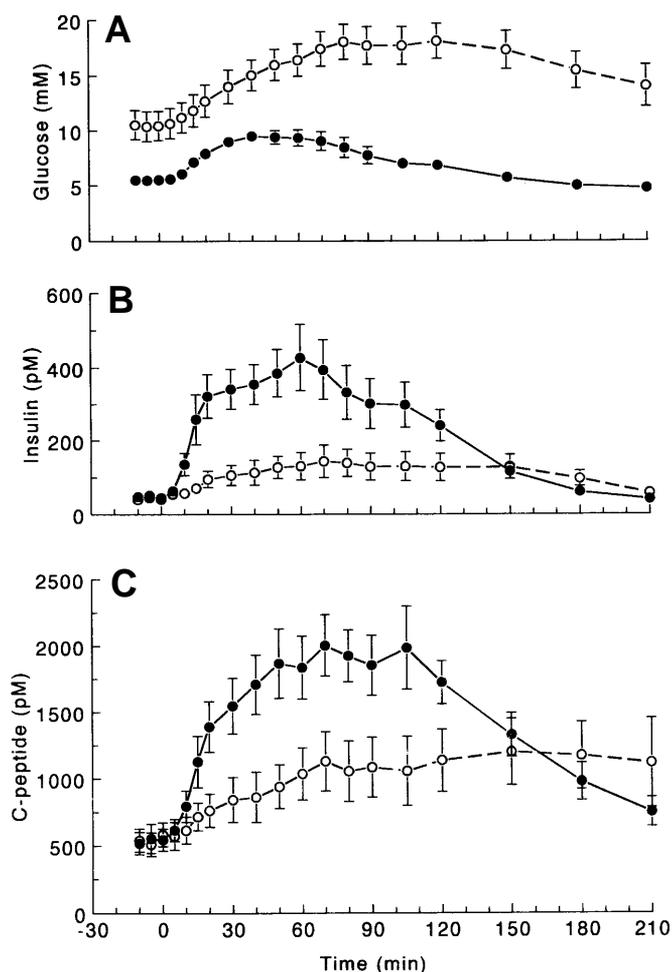


FIG. 3. Plasma glucose (A), plasma insulin (B), and C-peptide (C) profiles during the OGTT (protocol 3) in nondiabetic subjects (●) and type 2 diabetic patients (○) (mean and SE).

the normal subjects and the patients with type 2 diabetes. With SMS, K_1 was increased in both groups compared with the experimental day without SMS ($P < 0.05$ by ANOVA). K_2 and K_3 were not significantly different in the experiments with and without SMS. The peripheral volume of distribution V_2 tended to be increased in the patients with type 2 diabetes on the day with SMS, leading to a total volume of distribution ($V_1 + V_2$) being greater during SMS infusion, but the difference was not statistically significant. The volumes of distribution were similar in the normal subjects with and without SMS. The metabolic clearance rate of C-peptide was similar in the two groups and independent of the use of SMS, with a mean of about $3 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$.

OGTT in normal and type 2 diabetic subjects. The profiles of glucose, insulin, and C-peptide are shown in Fig. 3. As expected, the fasting and postprandial glucose concentrations were higher in patients with type 2 diabetes than in control subjects. The fasting plasma insulin and C-peptide concentrations were comparable between patients with type 2 diabetes and control subjects but were markedly lower in the patients after glucose ingestion.

The calculated rates of insulin secretion using the deconvolution method are shown in Fig. 4. The basal ISR did not differ between control subjects and patients with type 2 diabetes

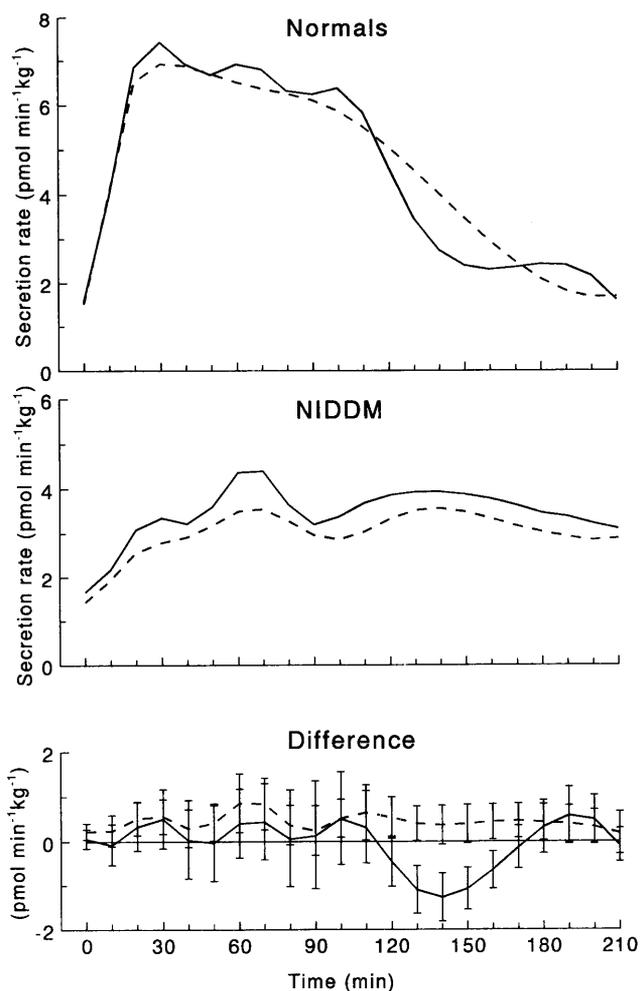


FIG. 4. Mean ISRs during OGTT in nondiabetic and diabetic subjects calculated by deconvolution based on C-peptide kinetics with (—) or without (---) SMS infusion, protocol 3. The lower curves show the differences between the rates with and without SMS for the nondiabetic (—) and diabetic subjects (---). The differences around 140 min were not statistically significant when adjusted for multiple comparisons.

(Table 3). The initial increase in ISR after the glucose load was blunted, and the maximal ISR was reduced ~40% in the diabetic subjects (Fig. 4 and Table 3). Using the C-peptide kinetics derived with or without the SMS infusion had no discernible impact on ISR (Fig. 4). The maximal ISRs in individuals calculated from the C-peptide kinetics in the presence and absence of SMS were highly correlated (control subjects: $r = 0.78$, $P < 0.05$; type 2 diabetic patients: $r = 0.81$, $P < 0.05$). ISR calculated with the ISEC method (data not shown) using population-based kinetic parameters for C-peptide did not differ significantly from ISR obtained with the deconvolution method based on individual C-peptide kinetics with SMS.

Insulin appearance during the validation study. The plasma glucose level was kept constant (5–6 mmol/l) in the control subjects, whereas a higher level (10–15 mmol/l) and some variation were allowed in the patients. The endogenous insulin secretion was suppressed by SMS in both groups before and during the insulin and C-peptide infusion. The mean infusion profiles of C-peptide in normal subjects

TABLE 3
ISRs during OGTT (protocol 3) obtained by deconvolution analysis

Insulin secretion summary results	Normal subjects		Type 2 diabetic patients	
	Without SMS (n = 8)	With SMS (n = 8)	Without SMS (n = 7)	With SMS (n = 8)
Relative to V_1				
Basal ($\text{pmol} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$)	32 ± 4	33 ± 5	43 ± 12	34 ± 5
Maximal rate ($\text{pmol} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$)	169 ± 21	180 ± 25	110 ± 23	102 ± 21
Total (nmol/l)	18.1 ± 2.0	17.7 ± 2.1	15.5 ± 3.4	12.8 ± 2.4
Relative to body weight				
Basal ($\text{pmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$)	1.5 ± 0.2	1.6 ± 0.3	1.4 ± 0.1	1.7 ± 0.2
Maximal rate ($\text{pmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$)	8.4 ± 1.2	9.2 ± 1.4	4.0 ± 0.7	4.9 ± 0.9
Total (nmol/kg)	0.92 ± 0.14	0.90 ± 0.11	0.55 ± 0.09	0.62 ± 0.10

Data are means \pm SE. Analysis was done using the C-peptide parameters in Table 2. Differences between groups and methods were not statistically significant by ANOVA.

and type 2 diabetic patients are shown in Fig. 5 together with the calculated appearance rates obtained from the combined model and the deconvolution method using the C-peptide kinetic parameters with SMS. The kinetic parameters estimated with the combined model are shown in Table 4. Individual parameters were determined with fractional standard deviations of ~ 10 – 20% for given values of the others.

The appearance rates were calculated over 10-min intervals for both the deconvolution method and the combined model to make the curves comparable to the infusion rates. In the normal subjects, the mean appearance rate for the deconvolution method differed significantly from the actual infusion rate of C-peptide at 20–30 min ($P < 0.05$ after adjustment for

multiple comparisons). In the type 2 diabetic patients, there was a similar difference (Fig. 5), which did not reach statistical significance. However, when the groups are combined, the deconvolution method gave significantly lower rates during the first three time intervals ($P < 0.05$ [adjusted]).

With the combined model, no statistically significant differences were observed between calculated appearance rates and actual infusion rates of C-peptide at any time intervals from 0–180 min in the groups of normal subjects and type 2 diabetic patients.

The ability of the two methods to recover the known infused profile is shown in Table 5. The recovery did not differ between the two groups or between the methods, and it

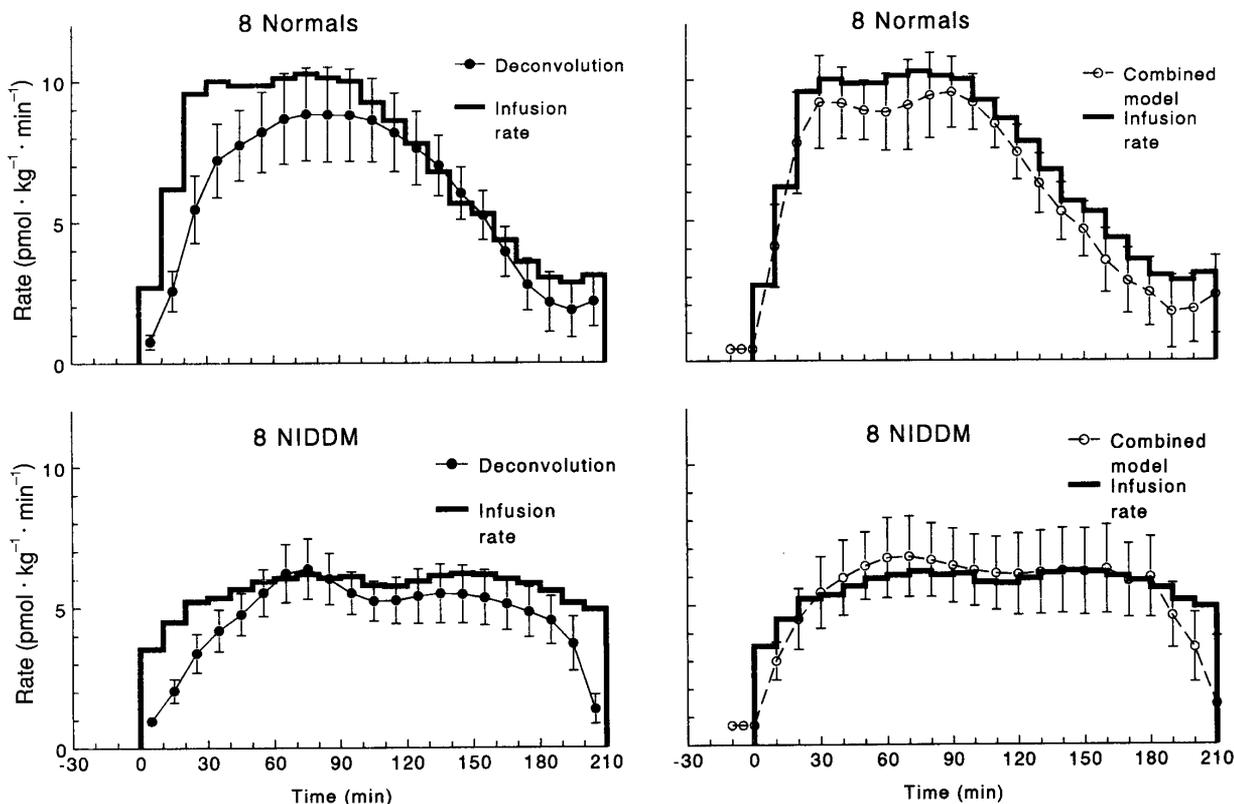


FIG. 5. Relationship between the estimated C-peptide appearance rates (mean and SE) based on the deconvolution method (●) and the combined model (○) and the known rates of C-peptide infusion (—) for nondiabetic subjects (upper panels) and type 2 diabetic patients (lower panels), protocol 4.

TABLE 4
Kinetic parameters from the combined model analysis of the insulin and C-peptide infusion profiles (protocol 4)

	Normal subjects	Type 2 diabetic patients
n	8	8
$f = F \cdot V_c / V_1$	0.45 ± 0.14	0.20 ± 0.05
k_1 (min ⁻¹)	0.094 ± 0.030	0.053 ± 0.011
k_c (min ⁻¹)	0.028 ± 0.003	0.031 ± 0.004

Data are means \pm SE. There were no statistically significant differences between groups.

was not significantly different from 100% by ANOVA. The correlation coefficients between the calculated appearances by the two methods were 0.82 (total amount, $P < 0.01$) and 0.89 (maximal rate, $P < 0.001$). The root mean square measures the systematic and random deviations between the individually calculated and known rates over the infusion period. The root mean squared deviations were, on the average, $\sim 2\text{--}3$ pmol \cdot kg⁻¹ \cdot min⁻¹, corresponding to $\sim 30\%$ of the mean maximal infusion rate (Table 5 and Fig. 5). They did not differ significantly between the two methods nor between the normal subjects and the type 2 diabetic patients.

DISCUSSION

It has been widely used and accepted that secretion rates of insulin can be derived from mathematical analyses of peripheral C-peptide concentrations, and the basic assumptions for the use of C-peptide as a measure of insulin secretion have previously been discussed in detail (17). However, the present study is the first direct in vivo demonstration of the accuracy of such methods in normal subjects and type 2 diabetic patients. The deconvolution method as well as the combined model was validated in humans by comparing the calculated ISRs with a known C-peptide and insulin infusion in a pattern simulating the changes in insulin secretion after oral glucose ingestion. Furthermore, we determined the effect of an SMS infusion on the decay kinetics of C-peptide in plasma after a bolus injection of C-peptide. Subsequently, we used the two sets of C-peptide kinetic parameters to calculate the ISRs by the deconvolution method during an OGTT in patients with type 2 diabetes and healthy control subjects. The results indi-

cate that both methods give reliable results of ISRs during the relatively slow changes in insulin secretion.

It has become increasingly accepted that a major defect contributing to type 2 diabetes is deficient insulin secretion (1–9,29–31). In fact, defects of insulin secretion can be detected in patients at risk of developing type 2 diabetes before the onset of an abnormal glucose tolerance (2–4,9). Over the years, it has become apparent that the pulsatile component in the overall insulin secretory pattern is important, because at least 70% of insulin is secreted in a pulsatile fashion (pulse frequency every 4–12 min). To obtain a reliable quantification of the pulsatile mode, sampling every 1 min is necessary. It is therefore a very laborious and expensive procedure, which is not feasible when large numbers of subjects or animals are studied. Strategies are being developed to enhance insulin secretion in patients with type 2 diabetes in new ways to treat this disease (32,33). To accurately determine the impact of these novel therapies on insulin secretion in patients with type 2 diabetes, effective and simple methods are required for measurement of insulin secretion. Because the subtle defect of insulin secretion is often only present during stimulation, it is also important to have methods available to measure the rate of insulin secretion after different perturbation tests.

One such approach was the development of the deconvolution technique of Eaton and Polonsky et al. (16). This approach overcomes the difficulties inherent in the non-steady state by using measured C-peptide decay kinetics to calculate secretion rates from measured C-peptide concentrations in individual patients. The assessment of the individual C-peptide kinetics was performed during an SMS infusion in order to suppress the endogenous C-peptide secretion. However, this is inconvenient because it requires admission of the study subject on a separate occasion, as well as infusion of SMS and a C-peptide bolus, which are expensive and not readily available for clinical purposes. We therefore sought to establish if this procedure could be simplified by performing the C-peptide decay kinetics in the absence of SMS. No effect of SMS was observed on the C-peptide decay curve (Fig. 1) and kinetic parameters, except that K_1 was increased with SMS (Table 2). However, the estimated ISRs during an OGTT were not different when using C-peptide kinetic parameters with or without SMS (Fig. 4 and Table 3). This implies that it is valid to perform C-peptide decay kinet-

TABLE 5
Recovery of the infused C-peptide by the two methods and root mean square of the deviations between individually calculated and known infusion rates (protocol 4)

	n	Recovery % of total amount of infused C-peptide	Recovery % of maximal rate of infused C-peptide	Root mean square (pmol \cdot kg ⁻¹ \cdot min ⁻¹)
Deconvolution method				
All subjects	16	82.0 ± 4.3	94.7 ± 5.8	2.57 ± 0.35
Normal subjects	8	81.0 ± 7.5	87.0 ± 6.1	3.17 ± 0.60
Type 2 diabetic patients	8	82.6 ± 4.8	102.4 ± 9.4	1.97 ± 0.25
Combined model				
All subjects	16	96.4 ± 6.7	110.7 ± 8.6	2.55 ± 0.25
Normal subjects	8	95.0 ± 8.9	99.5 ± 7.7	2.64 ± 0.20
Type 2 diabetic patients	8	97.6 ± 10.7	121.9 ± 14.9	2.45 ± 0.47

Differences between groups and methods were not statistically significant by ANOVA, nor did mean recovery for methods or groups differ significantly from 100%.

ics without suppression of the endogenous ISR in both non-diabetic and diabetic individuals. It is, however, necessary to estimate the kinetic parameters from a sum of two exponential model that includes a constant term to account for the basal level of endogenous C-peptide. This modification is further justified by the demonstration of a constant basal endogenous insulin level during the C-peptide decay (Fig. 2B).

Polonsky et al. (16) examined the C-peptide in 10 normal subjects and 7 type 1 diabetic patients. Metabolic clearance rates and kinetic parameters were obtained following an intravenous bolus of C-peptide. C-peptide concentrations were measured during constant and variable infusions of C-peptide, simulating the pattern of change in insulin secretion reported to occur after oral glucose ingestion. The two-compartment model, using the individual model parameters, was able to estimate infusion rates with considerable accuracy seen in almost superimposable curves, when comparing the actual infusion rates to the mean infusion rates.

To make the Eaton-Polonsky approach less laborious, Van Cauter et al. (19) derived population-based C-peptide kinetic parameters based on regression analysis of individual parameters obtained from 250 normal, obese, and type 2 diabetic subjects. These standard kinetics parameters were utilized in a computer program, ISEC (39), for calculations of ISRs, and the method was employed by Hovorka et al. (40) to investigate the accuracy of calculation of ISR. Five patients with type 2 diabetes and five matched control subjects were given a variable intravenous infusion of C-peptide, mimicking meal-stimulated ISR, with short-term oscillations. The C-peptide infusion was constructed by averaging and smoothing C-peptide secretion calculated from meal profiles in 19 nondiabetic men. Hovorka et al. concluded that the method can estimate meal-stimulated C-peptide secretion with biases of 14 and 22% in nondiabetic and diabetic subjects, respectively, but it is not suitable for pulse detection.

We compared the ISEC method to the deconvolution method based on individual C-peptide kinetics on the OGTT results and found a very close agreement. This provides additional indirect evidence for the validation of the ISEC method in subjects who have standard C-peptide kinetics. Like the combined model, the ISEC method requires data only from the actual experimental test.

The deconvolution method should, in theory, give more accurate secretion rates than the combined model, because it is based on the two-compartment model for C-peptide, which is required for an adequate description of the rapid decay of C-peptide after a bolus injection. It is possible, however, that a one-compartment approximation may be sufficiently accurate to describe the relatively slow changes in C-peptide concentration that occur after, e.g., meals and OGTTs.

In the present studies, we were able to validate the alternative technique for measurement of insulin secretion based on the combined model (21) of insulin and C-peptide kinetics. The combined model can estimate prehepatic ISRs as well as values of the parameters for insulin and C-peptide kinetics. It only needs data from a single experimental test that must exhibit sufficient dynamics in plasma insulin and C-peptide time courses (34,35). The combined model was validated in dogs, where it gave accurate estimates of portally infused C-peptide and insulin at rates resembling responses to OGTT, but the calculated rates tended to peak too early

(21), which might be due to the use of venous blood sampling or the one-compartment C-peptide model. This led to a modification of the model by Watanabe et al. (24) with two compartments for C-peptide that introduces more unknown parameters to be estimated and may require more precise data exhibiting pronounced dynamics.

In the present study, the combined model tended to give an earlier and higher initial rate of appearance than the deconvolution method and was closer to the known infusion rates. This could be explained by a suppression of endogenous C-peptide due to the fact that exogenous insulin was infused together with C-peptide. Because the deconvolution method is dependent only on the C-peptide data, it is possible that it underestimates the appearance rate. The combined model would also underestimate the early rise in rate of appearance, but this effect is reduced due to the use of the one-compartment model for C-peptide. Nevertheless, there was a close relation between the estimated appearance rates and the known infusion rates for both methods, as seen in Fig. 5, and it is concluded that both methods are valid for calculation of ISR in humans with a normal β -cell function as well as in type 2 diabetic patients with a heterogeneous β -cell defect and variable insulin response to oral glucose.

The present study extends our previous studies of pancreas and kidney transplanted type 1 diabetic patients as well as kidney-only transplanted nondiabetic subjects with impaired kidney function (23), where we compared the results obtained with the deconvolution method and the combined model during an oral glucose load (slow dynamics) and a glucagon test (fast insulin response). In parallel with the present results, we demonstrated a steeper rise in insulin secretion during OGTT when calculated from the combined model. The secretion rates for the normal subjects and the kidney transplanted decayed more rapidly when compared with the deconvolution method. During the glucagon test, the profile from the combined model seemed to overestimate ISR in the early poststimulatory phase of insulin secretion compared with the deconvolution method, this being most pronounced during the fast changes in insulin secretion. In that study, we could not determine which of the two methods gave the most correct and precise results. It is, however, likely that the deconvolution method gives the most reliable estimates of the fast changes in insulin secretion responses. When the additional efforts required to determine individual C-peptide kinetics are taken into account, the combined model or the simplified deconvolution method may be acceptable options. The latter requires, however, that standard C-peptide parameters can be applied, i.e., that the subjects are similar to those studied in Van Cauter et al. (19).

In summary, the present data provide direct evidence that 1) it is valid to examine the C-peptide decay kinetics after a C-peptide bolus in the absence of SMS in patients with type 2 diabetes or control subjects, and 2) that the deconvolution method and the combined model method are both valid for measurement of insulin secretion in the basal and postprandial state in patients with type 2 diabetes and control subjects during tests with relatively slow variation in β -cell secretion.

Using specific and precise assays for the measurement of plasma insulin and C-peptide, the combined model will be the method of choice because it does not require an additional experiment to determine individual C-peptide kinetics. It provides estimates of prehepatic ISR from a single protocol

of choice as long as there are sufficient dynamics in the plasma C-peptide and insulin concentration responses.

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