

Accurate Measurement of Endogenous Insulin Secretion Does Not Require Separate Assessment of C-Peptide Kinetics

Richard M. Watanabe and Richard N. Bergman

The implication of β -cell failure as an early defect in type 2 diabetes exacerbates the need for accurate but facile assessment of islet cell secretory rate, particularly in large group studies in which individual assessment of C-peptide kinetics is impractical. This study was designed to examine whether it is possible to obtain accurate secretory rates from the extended combined model, which provides insulin and C-peptide kinetics from plasma measurements of the two peptides. Equimolar intraportal infusions of insulin and C-peptide that are designed to simulate insulin secretion rates during both oral and intravenous glucose tolerance tests were used to generate plasma insulin and C-peptide data in conscious dogs that were examined under clamped glucose conditions. The plasma peptide kinetics were analyzed using the extended combined model to generate estimates of prehepatic insulin secretion that were then compared with the known intraportal infusion rates. The extended combined model was able to reproduce the known intraportal infusion profiles. The model-predicted rates were similar to those calculated with methods that require separate assessment of C-peptide kinetics. Simulation results supported lesser clearance of insulin during rapid changes of portal insulin (as measured by an intravenous glucose tolerance test) versus slow changes in portal insulin (as measured by an oral glucose tolerance test). The extended combined model accurately calculates prehepatic insulin appearance. It may be possible to apply this approach to large studies of β -cell function designed to identify changes in islet function in subjects at risk for diabetes. Such an approach could strengthen epidemiological and genetic studies of the pathogenesis of diabetes. *Diabetes* 49:373–382, 2000

From the Department of Biostatistics (R.M.W.), University of Michigan School of Public Health, Ann Arbor, Michigan; and the Department of Physiology and Biophysics (R.N.B.), University of Southern California School of Medicine, Los Angeles, California.

Address correspondence and reprint requests to Dr. Richard N. Bergman, Chairman, University of Southern California School of Medicine, Department of Physiology and Biophysics, 1333 San Pablo St., MMR-626, Los Angeles, CA 90033. E-mail: rbergman@syntax.hsc.usc.edu.

Received for publication 21 December 1998 and accepted in revised form 2 November 1999.

ID, internal diameter; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test; RIA, radioimmunoassay; SRIF, somatostatin.

Controversy has long characterized the questions of the time course and relative importance of factors that contribute to development of type 2 diabetes. Although insulin resistance has long been considered the preeminent factor that contributes to the development of type 2 diabetes, recent evidence has suggested that defects in β -cell insulin secretion are an important and, possibly, a primary defect (1–5). A cause of conflicting opinions has been the failure to normalize indices of insulin secretion to insulin resistance. Such normalization has demonstrated severe β -cell defects that have occurred earlier than previously understood; for example, insulin response is decreased 60–70% in the impaired glucose tolerance (IGT) state, even though fasting insulin levels are normal or increased (3).

Several important difficulties remain in assigning relative risk to insulin secretion per se in the pathogenesis of type 2 diabetes. The plasma insulin response is determined by a balance between insulin secretion and the rate of insulin clearance. Recent evidence has suggested that both are important components that mediate changes in plasma insulin response in the face of insulin resistance (6). Also, it is often difficult to perform the multiple protocols necessary to perform deconvolution of C-peptide to obtain assessment of insulin release per se, independent of changes in first-pass hepatic extraction of the peptide. Such knowledge can be difficult to obtain because human C-peptide for injection is not always readily available and, consequently, a separate experimental day for C-peptide kinetic assessment is required. While assumption of parameter values for C-peptide disappearance kinetics is one approach, such an assumption may not be accurate (7,8).

We introduced the combined model as an alternative approach that avoids potential pitfalls of the deconvolution method (9). The model uses single compartment descriptions for both insulin and C-peptide kinetics and relies upon simultaneous kinetic analysis of the dynamics of both peptides from a single experimental protocol to derive estimates of prehepatic insulin secretion rates (9). Under conditions devoid of rapid kinetics, the original model was able to reproduce known intraportal infusion patterns in vivo (9). However, computer simulation studies indicated the model was unable to account for rapid kinetics that might be encountered by protocols, such as the intravenous glucose tolerance test (IVGTT) (10). This led to the introduction of the extended combined model, which incorporates two compartmental

C-peptide kinetics (10). Computer simulation studies suggested that the extended combined model should be able to estimate correctly kinetic parameters for insulin and C-peptide and, more importantly, for prehepatic insulin secretion rates. The goal of the current study was to examine the accuracy of the extended combined model's ability to provide β -cell insulin secretion rates *in vivo*. Ready availability of such estimates would enable a reassessment of the role of insulin secretion *per se*, independent of hepatic insulin extraction, in the pathogenesis of states of reduced glucose tolerance, such as IGT and type 2 diabetes.

RESEARCH DESIGN AND METHODS

Surgery. Experiments were performed in six male dogs that were bred for research. All animals were instrumented with chronic indwelling catheters to allow infusion of peptides and sampling of blood. Dogs were housed under controlled kennel conditions (12-h light-dark cycle) in the University of Southern California (USC) School of Medicine Vivarium and fed standard chow (25% protein, 49% carbohydrate, and 9% fat) (Wayne Dog Chow; Alfred Mills, Chicago) once each day.

Surgical procedures were performed under strict aseptic conditions. Surgical and experimental protocols were approved by the USC Vivarium Institutional Animal Care and Use Committee. After an overnight fast, anesthesia was induced with sodium thiamylal (Biotal; Bio-Ceutic Laboratories, St. Joseph, MO) and maintained with 0.5–1.0% halothane and nitrous oxide. A tygon catheter (internal diameter [ID] 0.13 cm) was inserted in the left carotid artery through a lateral neck incision. This catheter was used for sampling of arterial blood. The catheter end was led subcutaneously to the back of the neck and exteriorized. The portal vein was accessed via a mid-line incision, and a silastic catheter (ID 0.04 cm) was inserted through a pinhole in the vessel wall and sutured in place ~3 cm from the porta hepatis. This catheter was used for infusion of insulin and C-peptide. Upon closure of the peritoneal cavity, the portal catheter end was led subcutaneously to the back of the neck and exteriorized. Catheters were filled with heparin saline (100 U/ml), were coiled and capped, and were placed in a small bag protected with a heavy denim collar.

Dogs were allowed to recover from surgery for a minimum of 5 days, during which time they were treated with antibiotics as needed and were used for experiments only if they had a normal body temperature and hematocrit and exhibited normal behavior and diet. Dogs were studied in a conscious relaxed state in a Pavlov sling. When all experimental protocols were completed, dogs were euthanized with sodium pentobarbital, and catheter locations were verified postmortem.

Experimental protocols. Each animal underwent three experimental protocols after an overnight fast: a bolus C-peptide injection and two insulin/C-peptide infusion protocols. Intraportal infusion profiles mimicking secretory responses that have been observed during an oral glucose tolerance test (OGTT) and an IVGTT were chosen. The OGTT profile was adapted from the studies of Shapiro et al. (11), and the IVGTT profile was adapted from studies from this laboratory (12). The C-peptide bolus injection study was performed to provide independent estimates of C-peptide kinetics parameters for comparison with combined model estimated values. The insulin/C-peptide infusion protocols were performed in random order, and the C-peptide bolus injection protocol was always performed last. This protocol order was necessary to maximize chances of completing protocols 1 and 2 with a patent portal catheter. Protocols were performed at least 1 week apart.

Each of the infusion experiments was initiated with the infusion of somatostatin (SRIF) ($0.8 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) to suppress endogenous pancreatic hormone secretion. Glucagon was then infused ($2.0 \text{ ng} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) to replace basal glucagon secretion (13), and a constant intraportal equimolar infusion of insulin and C-peptide (30 pmol/min) was initiated to replace basal insulin and C-peptide secretion. After an equilibration period of 120 min, the intraportal infusions of both insulin and C-peptide were altered according to a predetermined pattern, which simulated either the secretory profile for an OGTT or IVGTT. Blood was sampled during the basal and stimulated periods from the carotid artery for measurement of levels of insulin, C-peptide, and glucagon.

To prevent a decline in plasma glucose concentration during suprabasal insulin infusion, glucose was infused peripherally at predetermined rates for either intraportal insulin/C-peptide infusion protocol. The exogenous glucose infusion pattern was determined a priori by simulating insulin action during either protocol by use of the predicted plasma insulin profile as a known input to the minimal model. Average minimal model parameter values used for the simulation were derived from our previous application of the method and represents the "average" dog (14–16). A glucose infusion profile necessary to prevent the fall in glucose concentration was then derived from the minimal model predicted effect of the OGTT or IVGTT insulin profiles. The goal was not to clamp the glucose at a constant level; it was only to prevent large rapid fluctuations in glucose

that might engender SRIF breakthrough by the pancreas or generate a counter-regulatory response.

Protocol 1: simulated OGTT. The general protocol scheme is shown in Fig. 1A. Experiments simulating the OGTT were successfully completed in five of the six animals studied. On the morning of the experiment, 19-gauge intracatheters (Deseret Medical, Sandy, UT) were inserted into a cephalic vein for infusion of SRIF ($0.8 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) (Bachem, Torrance, CA) and glucagon ($2.0 \text{ ng} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, bovine/porcine mix) (Sigma, St. Louis, MO) and into a saphenous vein for infusion of glucose (variable rate, 50% dextrose) (McGaw, Irvine, CA). At -120 min , we initiated SRIF and glucagon infusions and the intraportal infusion of equimolar porcine insulin (Sigma) and human C-peptide (Bachem) at 30 pmol/min to re-establish basal insulin and C-peptide concentrations. At $t = 0$, the intraportal equimolar insulin/C-peptide infusion was changed according to the predetermined infusion pattern. Concomitantly, the peripheral variable glucose infusion was initiated to prevent changes in the glucose concentration.

Carotid arterial blood samples (5 ml) were taken every 10 min during the equilibration period and at the following times after initiation of the simulated OGTT for determination of insulin and C-peptide: 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120, 140, 160, 180, 210, 240, 270, and 300 min. Extra blood (3 ml) was drawn every 30 min during the equilibration period and at the following times for determination of glucagon: 10, 30, 60, 90, 180, and 300 min. Glucose levels were measured online with an autoanalyzer (YSI-2700; Yellow Springs Instruments, Yellow Springs, OH) to monitor the glucose concentration.

Protocol 2: IVGTT simulation. The IVGTT simulation protocol was analogous to that of protocol 1 (Fig. 1B), except that the intraportal equimolar insulin/C-peptide infusion profile was designed to simulate insulin secretion during an IVGTT. The peripheral glucose infusion was also altered to match the insulin action profile during the simulated IVGTT. Blood samples were collected at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 35, 40, 45, 50, 55, 60, 75, 90, 105, 120, 150, and 180 min for arterial insulin/C-peptide samples. Glucagon samples were taken at 5, 10, 25, 40, 60, 100, and 180 min.

Experiments were successfully completed in all six animals studied. However, in a single animal, C-peptide measurements were not obtained, and protocol 2 for this animal was excluded from further analyses.

Protocol 3: bolus C-peptide injection. After an overnight fast, basal blood samples were taken from the carotid arterial catheter at -20 , -10 , and -1 min . At $t = 0$, a bolus of human C-peptide (50 nmol/l) (Bachem) was injected over 30 s and was followed by a saline flush of equal volume. Additional blood samples for C-peptide were then taken at 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 35, 40, 45, 50, 55, 60, 75, 90, 100, 120, 150, and 180 min.

Sample handling. All blood samples were collected in chilled tubes that contained sodium fluoride, heparin, and trasylol ($1,000 \text{ U/ml}$ blood) (FBA Pharmaceuticals, New York). Samples were kept on ice until centrifugation at 4°C . Plasma was aliquotted and frozen for later assay. During protocols 1 and 2, small aliquots of blood (0.5 ml) were immediately centrifuged for online measurement of plasma glucose levels. All samples were stored at -70°C .

Assays. Glucose was measured in triplicate on an autoanalyzer (YSI-2700; Yellow Springs Instruments) using the glucose oxidase method. Insulin was measured in duplicate using a radioimmunoassay (RIA) with dextran-charcoal separation (17). C-peptide samples were first extracted using polyethylene glycol, and then the supernatant was measured by RIA (18). Glucagon was assayed by RIA according to Faloona and Unger (19). Materials for all RIAs, including standards, antibody, and labeled peptide, were purchased from Novo Nordisk (Bagsvaerd, Denmark).

All plasma measurements were corrected for the volume of trasylol that was added to the sampling tube and for hematocrit that was measured periodically throughout the experiments. Hematocrit was assumed to change linearly between measurements.

Data analysis

The extended combined model. The extended combined model (Fig. 2) is described by the following system of differential equations:

$$V_I \frac{dI(t)}{dt} = FR(t) - K_I I(t) V_I \quad (1)$$

$$V_{C_1} \frac{dC_1(t)}{dt} = R(t) - (K_{21} + K_{01}) C_1(t) V_{C_1} + K_{12} C_2(t) V_{C_2} \quad (2)$$

$$V_{C_2} \frac{dC_2(t)}{dt} = K_{21} C_1(t) V_{C_1} - K_{12} C_2(t) V_{C_2} \quad (3)$$

Where $R(t)$ is the equimolar release of insulin and C-peptide by the β -cells, F is the fraction of insulin surviving hepatic transit; K_I is the fractional disappearance rate for insulin; K_{21} , K_{12} , and K_{01} are the kinetic parameters for C-peptide; V_I is the distribution volume for insulin; and V_{C_1} and V_{C_2} are the distribution volumes for C-peptide. By making the following substitutions,

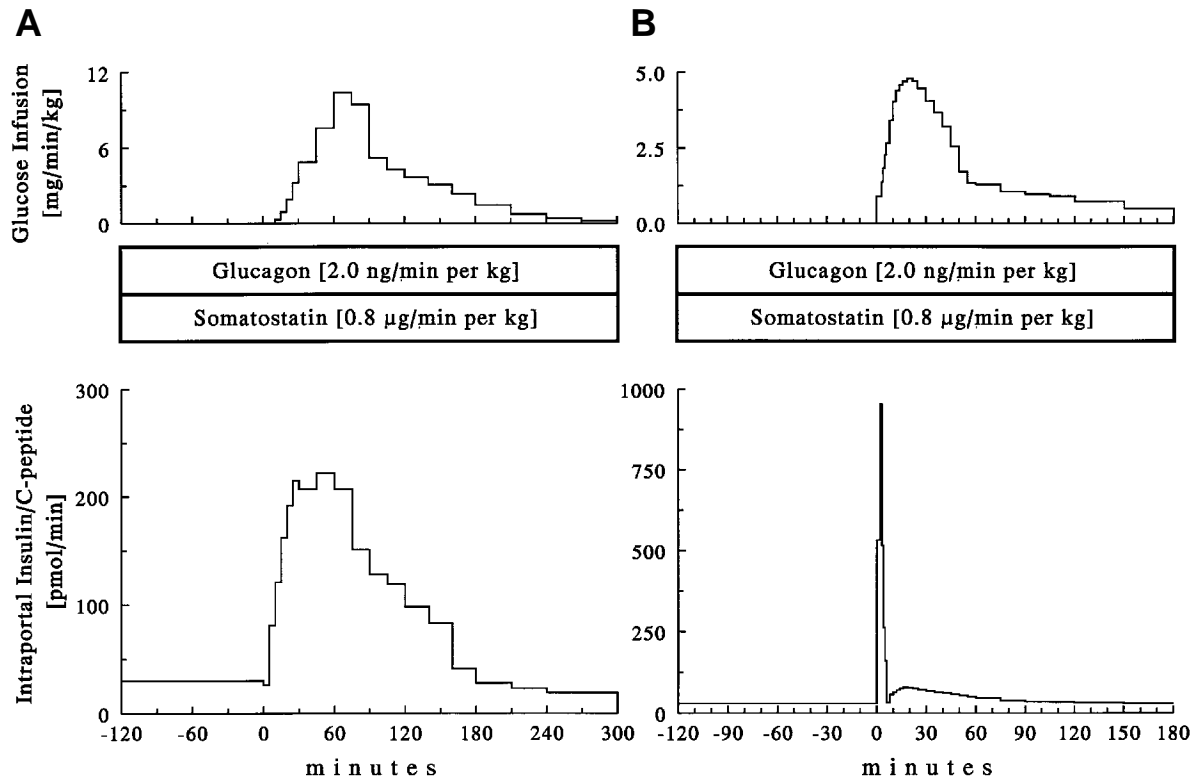


FIG. 1. Experimental protocols. A: The simulated OGTT protocol. Intraportal equimolar insulin and C-peptide infusion rates used to generate plasma peptide profiles that mimicked the OGTT. SRIF is infused to suppress endogenous insulin and C-peptide secretion and glucagon infused to restore basal glucagon levels. Glucose is infused peripherally in the pattern shown to prevent large fluctuations in plasma glucose. B: The simulated IVGTT protocol. Intraportal equimolar insulin and C-peptide infusion rates are designed to mimic an IVGTT.

$$r(t) = \frac{R(t)}{V_{C_1}} \quad (4)$$

$$f = F \frac{V_{C_1}}{V_I} \quad (5)$$

Eqs. 1–3 become

$$\frac{dI(t)}{dt} = fr(t) - K_I I(t) \quad (6)$$

$$\frac{dC_1(t)}{dt} = r(t) - (K_{21} + K_{01})C_1(t) + K_{12}C_2(t) \quad (7)$$

$$\frac{dC_2(t)}{dt} = K_{21}C_1(t) - K_{12}C_2(t) \quad (8)$$

where insulin secretion is now estimated per unit C-peptide volume in the first, presumably plasma, compartment (V_{C_1}).

Numerical methods

Identification of the extended combined model. The method used for identification of the extended combined model has been previously described (10) and is briefly reviewed in the APPENDIX. The principle behind the identification is to algebraically manipulate the model equations (Eqs. 6–8) in the Laplace domain to factor out the insulin secretion rate $r(t)$ from the system of equations. This yields a set of equations, from which the secretion rate $r(t)$ has been eliminated, that relates the concentrations of insulin and C-peptide in plasma. The equations are converted back into the time domain, and the parameters of these new equations can be estimated by fitting the C-peptide concentration, given insulin, or the converse.

Independent assessment of C-peptide kinetics. Protocol 3 was performed to provide independent estimates of kinetic parameters for plasma C-peptide and to allow estimation of insulin secretion rates by use of the Eaton deconvolution approach (24) as described by Polonsky and colleagues (7,20,21). C-peptide disappearance data from protocol 3 were fitted to the following exponential equation:

$$C_1(t) = Ae^{-\alpha t} + Be^{-\beta t} \quad (9)$$

The parameter estimates from this equation can be resolved into the individual rate constants for the two-compartment C-peptide model by using the following relationships:

$$K_{12} = \frac{-(A\beta + B\alpha)}{(A + B)} \quad (10)$$

$$K_{01} = \frac{\alpha\beta}{K_{12}} \quad (11)$$

$$K_{21} = \alpha + \beta - K_{12} - K_{01} \quad (12)$$

$$V_{C_1} = \frac{\text{dose}}{C_1(0)} \quad (13)$$

$C_1(0)$ is the predicted C-peptide concentration at $t = 0$ and assumes instantaneous distribution of the injected bolus of C-peptide.

Statistics. All parameter identifications were performed by use of MLAB (Civilized Software, Bethesda, MD). MLAB uses a Marquardt-Levenberg-weighted least-squares algorithm for parameter identifications. Weights for identifications were estimated through the use of MLAB's internal EWT weighting function. This function uses a five-point moving average to derive an estimate of the standard deviation in the data then computes the weight as the inverse variance. Model fits to the data were assessed by residual analysis. Studentized residuals were calculated according to Draper and Smith (22), and residual time courses were tested for systematic patterns by using the Runs Test.

Prehepatic insulin secretion rates estimated from both the extended combined model and the Eaton deconvolution approach were compared with the known intraportal infusion rates by use of regression analysis. Because the model-predicted insulin secretion rates are in units of mass/time per C-peptide distribution volume, and because the intraportal infusions are in units of mass/time, the slope of the relationship between them provides an estimate of the C-peptide distribution space (V_{C_1}). Because all three protocols were not completed in all animals, estimates of C-peptide kinetics parameters that were derived from fitting proto-

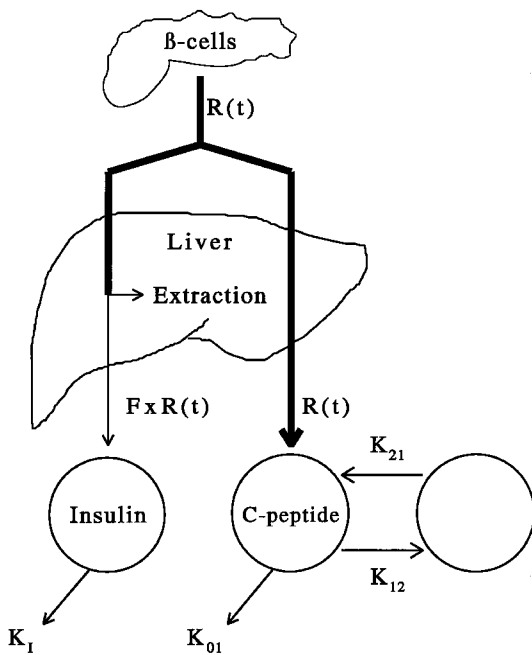


FIG. 2. Schematic diagram of the extended combined model. Insulin and C-peptide are assumed to be secreted in equimolar fashion [$R(t)$] by the β -cells. Insulin that survives hepatic transit appears in the systemic circulation as $F \times R(t)$ and distributes within a single compartment. C-peptide survives hepatic transit and is assumed to follow two-compartment distribution kinetics where compartment 1 represents plasma and compartment 2 represents the extravascular space. Details are provided in RESEARCH DESIGN AND METHODS.

col 3 data and the extended combined model were compared by one-way analysis of variance. All statistical analyses were performed using SAS for the personal computer (Version 6.04; SAS, Cary, ID). Data are reported as means \pm SE, unless specified otherwise.

RESULTS

Plasma time courses

Protocol 1: simulated OGTT. Average plasma glucose, glucagon, insulin, and C-peptide time courses are shown in Fig. 3A. Plasma glucose was maintained at a steady-state level of 160 ± 22 mg/dl during the equilibration period, reached an average peak concentration of 219 ± 30 mg/dl at 75 min, and was on average lower at the end of the experiment (last 60 min: 127 ± 0.4 mg/dl). At no point during the experiment was the glucose level statistically different from the initial equilibration period. This indicates that despite the use of identical glucose infusion rates for all experiments, the model-predicted glucose infusion profile adequately prevented the fall in plasma glucose concentration. Glucagon reached a steady-state level of 110 ± 34 pg/ml and showed a tendency to rise over the course of the experiment, but it did not significantly deviate from the equilibration level. The mean plasma glucagon level was 179 ± 36 pg/ml at the end of the experiment.

During the last 30 min of the equilibration period, the mean plasma insulin level was 26 ± 4 pmol/l, and the mean C-peptide level was 141 ± 22 pmol/l. At $t = 0$, the intraportal infusion was altered according to the predetermined profile, and mean levels of insulin and C-peptide gradually increased to peaks of 231 ± 12 and 558 ± 36 pmol/l at 60 min, respectively.

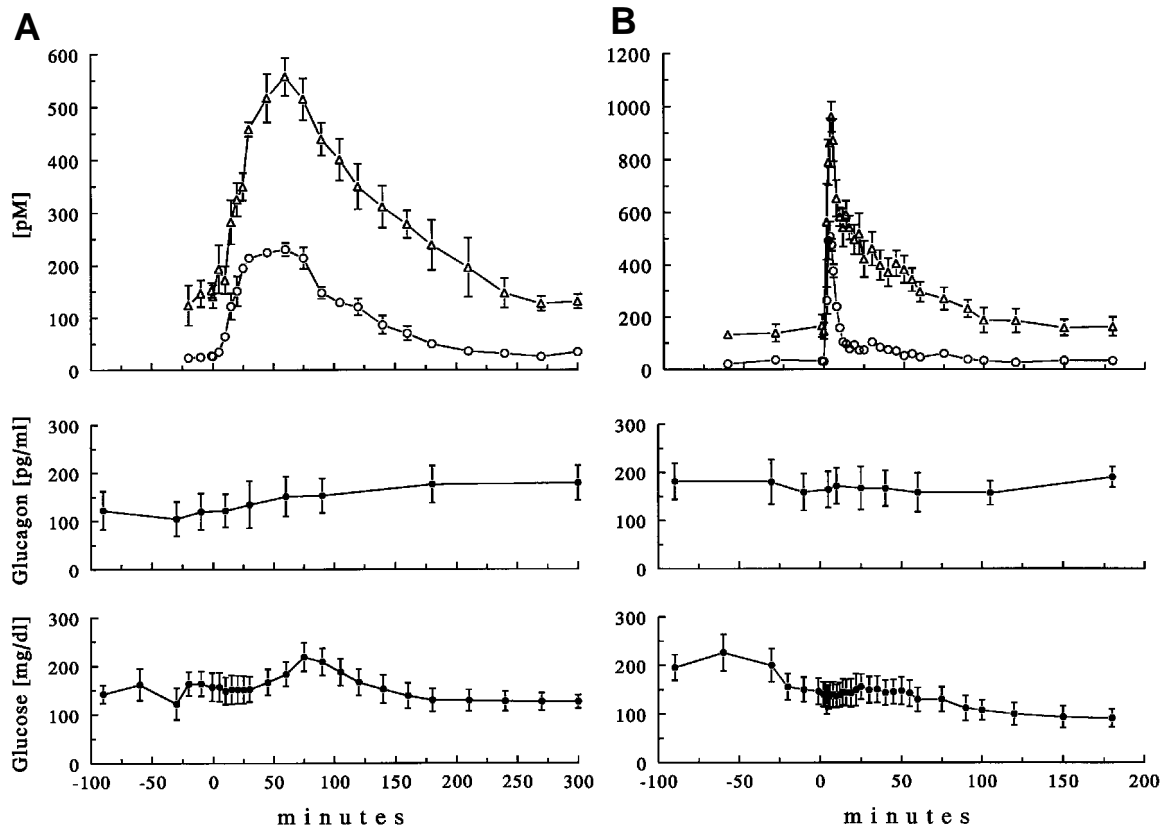


FIG. 3. Plasma profiles. Mean (\pm SE) plasma glucose, glucagon, insulin, and C-peptide time courses are shown for the simulated OGTT (A) and the simulated IVGTT (B) experiments. Top panels show insulin (O) and C-peptide (Δ) time courses.

Levels of both insulin and C-peptide then gradually decreased as the infusion rate was lowered and reached mean levels of 34 ± 4 and 131 ± 13 pmol/l by the end of the experiment.

Protocol 2: simulated IVGTT. Average plasma glucose, glucagon, insulin, and C-peptide time courses for the simulated IVGTT are shown in Fig. 3B. The average glucose concentration during the equilibration period was 179 ± 14 mg/dl, but it decreased to 151 ± 3 mg/dl during the 30-min period before the initiation of the simulated IVGTT. Plasma glucose levels remained relatively constant during the majority of the experimental period but gradually decreased to 95 ± 3 mg/dl during the last 60 min of the experiment. There were no rapid changes in the glucose concentration over the course of the experiment. The plasma glucose concentration at the end of the experiment was not statistically different from the average glucose concentration during the equilibration period or during the initial 10 min after the simulated IVGTT was initiated ($P > 0.10$ for both). The initial steady-state glucagon level was 179 ± 40 pg/ml and did not change significantly over the course of experiment.

During the last 30 min of the equilibration period, the mean plasma insulin level was 30 ± 6 pmol/l, and the mean C-peptide level was 147 ± 32 pmol/l. At $t = 0$, the intraportal infusion was altered, and mean levels of insulin and C-peptide abruptly increased to peaks of 508 ± 56 pmol/l by 4 min for insulin and 962 ± 57 pmol/l by 5 min for C-peptide. Plasma concentrations of both peptides gradually decreased and thereby exhibited dynamics typical of an IVGTT. Final insulin and C-peptide concentrations at the end of the experiment were 33 ± 2 and 164 ± 37 pmol/l, respectively.

Protocol 3: bolus C-peptide injection. Preinjection C-peptide levels were below detection limits of the assay. As previously mentioned, this experiment used human C-peptide, which is significantly different from the canine variant (23), and injection of human C-peptide resulted in an immediate increase in concentration to $7,690 \pm 1,890$ pmol/l by 1 min. The C-peptide concentration then fell in a biexponential fashion to 37 ± 10 pmol/ml by the end of the experiment at 180 min (data not shown).

Modeling results. The extended combined model was able to fit and provide physiologically plausible parameter estimates for four of the five OGTT simulations and for the remaining five IVGTT simulation experiments. For the one OGTT that failed, physiologically implausible negative parameter values were obtained. Thus, the remaining analyses were performed while excluding this single case. Exponential fits to the C-peptide disappearance curves from protocol 3 were successfully completed on all six experiments.

Combined model fits to the OGTT-simulated data had the identical shape, but they did not appear to follow the measured insulin and C-peptide time courses (Fig. 4A). Although none of the residuals were significantly different from zero (Fig. 4A, inset), Runs test revealed a systematic pattern in the residual time course ($P < 0.025$). Model predictions for insulin were consistently lower than the measured data, while C-peptide predictions were consistently higher. In contrast, model fits to IVGTT-simulated data were better than those obtained for the OGTT simulation (Fig. 4B). The residual time courses were randomly distributed about zero and exhibited no systematic patterns (Fig. 4B, inset). Exponential fits to the C-peptide disappearance curves from the bolus injection experi-

ments were excellent and displayed no detectable pattern in the residual time course (data not shown).

Average parameter estimates from all three protocols are shown in Table 1. Fractional insulin disappearance (K_i) was not significantly different between the two infusion protocols. Parameter f , the index of fractional hepatic insulin survival, can be converted to a value that reflects fractional hepatic insulin extraction (9). Fractional hepatic insulin extraction was significantly lower during the simulated IVGTT compared with the simulated OGTT ($P < 0.025$).

Data from the extensive work by Polonsky et al. (7) suggest that C-peptide parameters have a low intrasubject coefficient of variation. Based on these results, one would expect the C-peptide parameters to be similar among the three protocols. Comparison of C-peptide parameters among the three protocols revealed no statistically significant differences (Table 2). However, in some cases, parameters derived from the simulated OGTT were different from those derived from the simulated IVGTT and the bolus injection experiments.

Prehepatic insulin secretion rates. The comparison of the average insulin secretion rates estimated by the extended combined model to the known equimolar insulin/C-peptide intraportal infusion rates is shown in Fig. 5. When the estimated insulin secretion rates were compared with the known intraportal infusion rate, a high degree of correlation was obtained for both infusion profiles (OGTT, $r = 0.938 \pm 0.018$; IVGTT, $r = 0.943 \pm 0.005$) (Fig. 6). Because the extended combined model estimates prehepatic insulin secretion per unit C-peptide volume and the intraportal infusion is in units of mass per time, the slope of the relationship between the two provides an estimate of the C-peptide distribution volume. For the simulated OGTT data, an average C-peptide distribution space of 2.49 ± 0.37 liters was estimated, and a similar value was obtained for the simulated IVGTT (2.66 ± 0.84 liters). These volumes were not different from the distribution volume that was estimated from the bolus injection protocol (2.40 ± 0.36 liters, $P > 0.50$). When the model-estimated insulin secretion rates are corrected for the estimated C-peptide distribution space and superimposed upon the known intraportal infusion rates, the profiles are near identical, which indicates that the extended combined model is capable of accurately reproducing the known intraportal infusion rates.

Insulin secretion rates were also estimated by use of the deconvolution approach introduced by Eaton et al. (24) and refined by Van Cauter et al. (7) and Polonsky et al. (25). Secretion estimates derived from the Eaton approach did not match the intraportal infusion rate in three of the four animals (Fig. 6). In two cases, secretion estimates were significantly underestimated; in one case, they were overestimated. Given the dissimilarity in C-peptide kinetic parameters that were derived from the bolus injection protocol versus the extended combined model (Table 2), this outcome is somewhat expected. In contrast, the secretion estimates from the Eaton approach for the IVGTT simulation were well matched with the known intraportal infusion rates.

DISCUSSION

Insulin resistance alone does not result in type 2 diabetes (3,26). This realization has refocused interest on the β -cells as a companion or, possibly, a primary factor in the pathogenesis of this prevalent disease. With accurate measures of β -cell function and insulin secretion, diabetes risk can be

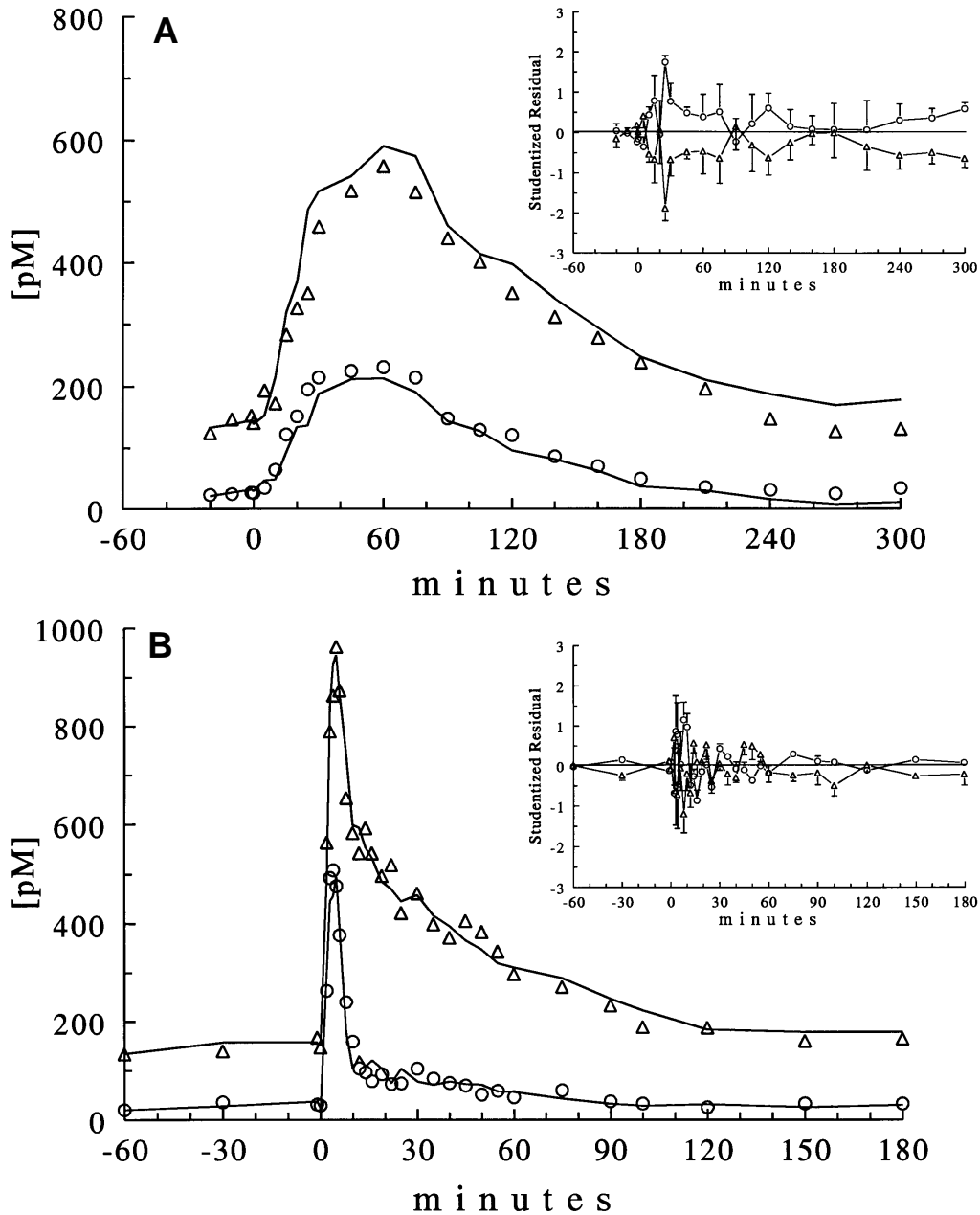


FIG. 4. Extended combined model fits to the data. Average model fits are superimposed on the average insulin (\circ) and C-peptide (Δ) data for the simulated OGTT (A) and the simulated IVGTT (B). Error bars have been excluded for clarity. Insets show studentized residual time courses for the model fits. Details are provided in RESEARCH DESIGN AND METHODS.

expressed in terms of the product of these parameters, the so-called disposition index (4,27). The disposition index has been shown to exhibit high heritability (28,29) in kindred with type 2 diabetes. Thus, there is a perceived need to assess islet function to identify those individuals most at risk for type 2 diabetes.

Measurement of islet function is confounded by variations in first-pass liver insulin extraction. Given the importance of the β -cell, it is of paramount importance to be able to measure insulin secretion in subjects at risk for type 2 diabetes. While the deconvolution approach introduced by Eaton et al. (24) and improved by Polonsky and colleagues (7,20) has proven immensely useful, one disadvantage is the necessity of a priori knowledge of C-peptide kinetics.

Cobelli and Pacini (30) introduced an alternative approach based on the minimal model of insulin kinetics (31). Toffolo et al. (32) have expanded the initial work and have evaluated variations on the original model to assess insulin secretion during the IVGTT. While the simulation work by Toffolo et al. (32) has provided interesting insights into the characteristics of insulin secretion from the IVGTT, both the original model and its variations were designed specifically for the intravenous test and thus cannot be applied globally to different clinical or experimental protocols.

The original combined model used single-compartment representations for the kinetics of both insulin and C-peptide (9) and obviated the need for assumption or independent measurement of C-peptide disappearance kinetic parameters.

TABLE 1
Average model parameters

	OGTT simulation	IVGTT simulation	Exponential fits
n	4	5	6
f	0.397 ± 0.097	0.774 ± 0.053*	—
Hepatic extraction (%)	64.5 ± 3.6	33.4 ± 0.1*	—
K ₁ (min ⁻¹)	0.167 ± 0.041	0.274 ± 0.051	—
K ₁₂ (min ⁻¹)	0.085 ± 0.039	0.095 ± 0.058	—
λ ₁ (min ⁻¹)	0.448 ± 0.153	0.218 ± 0.112	0.264 ± 0.040
λ ₂ (min ⁻¹)	0.029 ± 0.012	0.031 ± 0.010	0.032 ± 0.006

Data are means ± SE. *P < 0.05 vs. OGTT simulation.

Recently, the model structure was extended to include the two-compartment configuration for C-peptide, but it retained single compartment kinetics for insulin (10). Computer simulation studies suggested that this extended model should provide accurate estimates of parameters for insulin and C-peptide kinetics and prehepatic insulin secretion rates under both slow and rapid secretory kinetics. The current experimental study was performed to examine directly whether it is possible to calculate accurate estimates of insulin secretion rates by use of the extended combined model. If so, it might be possible to identify subtle changes in β-cell secretion rates in the absence of separate assessment of C-peptide disappearance kinetics.

Because accurate direct measurement of insulin release from the pancreas is extremely difficult due to sampling problems and incomplete mixing in the portal vein, we chose to create experimental conditions that allowed for the rate of delivery of insulin and C-peptide into the portal circulation to be known with precision. If the extended combined model could reproduce the known intraportal infusion rates, then it should be able to reproduce accurately the appearance of endogenously secreted insulin/C-peptide release into the portal circulation. Secretory profiles from two common protocols used in clinical research, the OGTT and IVGTT, were simulated. The overall results of this study clearly indicate that the extended combined model provides accurate and precise estimates of prehepatic insulin secretion. Furthermore, these results are not significantly different from those that would be obtained by use of the deconvolution approach introduced by Eaton and refined by Polonsky.

TABLE 2
Comparison of C-peptide kinetic parameters

	Bolus injection			OGTT simulation			IVGTT simulation		
	K ₂₁	K ₁₂	K ₀₁	K ₂₁	K ₁₂	K ₀₁	K ₂₁	K ₁₂	K ₀₁
Animal									
A	0.0226	0.0122	0.2367	—	—	—	0.0313	0.0135	0.0295
B	0.0638	0.0521	0.3621	0.4307	0.1566	0.1817	0.2782	0.3215	0.1147
C	0.0313	0.0328	0.1288	0.0884	0.0095	0.0624	0.0137	0.0247	0.0575
D	0.0847	0.0684	0.1403	0.0704	0.0257	0.1344	—	—	—
E	0.0243	0.0312	0.1499	0.3304	0.1483	0.2726	0.0305	0.0354	0.0730
F	0.0719	0.0761	0.1733	—	—	—	0.0328	0.0803	0.1071
Mean	0.0498	0.0455	0.1985	0.2300	0.0850	0.1628	0.0773	0.0951	0.0764
SE	0.0110	0.0100	0.0363	0.0894	0.0391	0.0441	0.0503	0.0577	0.0158

Data are expressed as min⁻¹.

Interestingly, C-peptide model parameters estimated from the simulated OGTT, while not statistically different, in some cases did not match those from the simulated IVGTT or the bolus injection experiments (Tables 1 and 2), whereas the latter two provided relatively consistent parameter estimates. Kinetic insulin parameters were also different when comparing combined model estimated values for the OGTT and IVGTT simulations. The relatively small sample size in this study precludes conclusions regarding absolute differences in parameter estimates among protocols; however, it is important to note that our results are in contrast to the work of Van Cauter et al. (7), who report low intra- and interindividual coefficient of variations in kinetic C-peptide parameters (7).

Despite these differences, it is also worth noting that if large differences in kinetic parameters were critical, prehepatic insulin secretion estimated from deconvolution using incorrect parameters should not have matched the known intraportal insulin/C-peptide infusion profiles used for the OGTT simulation. When prehepatic insulin secretion rates were estimated using the parameters from the bolus injection protocol rather than the values estimated by the Extended Combined Model, the secretion estimates for the simulated OGTT did not match the known intraportal infusion rate. The estimated prehepatic insulin secretion matched the intraportal infusion pattern only when parameters estimated from the Extended Combined Model were used for the deconvolution. The current study cannot formally address the issue of parameter sensitivity. However, based on the observations above and previous computer simulation study (10) one could argue that variability in kinetic parameters exists under differing experimental conditions and inaccurate parameter estimates or assumption of parameter values can lead to inaccurate assessment of insulin secretion rates.

Another interesting observation concerns the difference in the estimated hepatic insulin extraction rate between the simulated OGTT and IVGTT experiments. In the simulated OGTT, the estimated fractional hepatic extraction of insulin is similar to literature values suggesting that ~50% of insulin is extracted by the liver (33). However, in the simulated IVGTT, this value drops to 33% (Table 1). There are two explanations for this difference. The first is an error in the model that allows for an underestimation in the true hepatic extraction rate. This could be because the model assumes constant fractional hepatic insulin extraction. The extended combined model would likely provide incorrect estimates

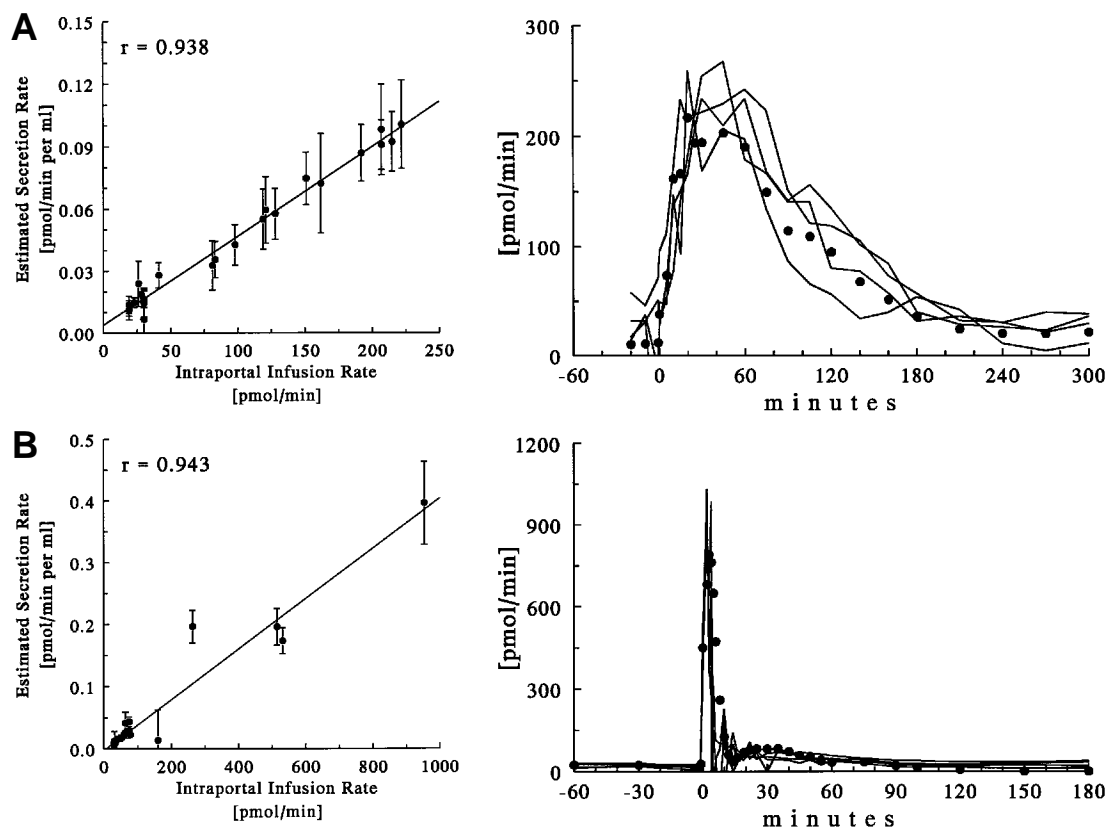


FIG. 5. Comparison of model-predicted insulin secretion to known intraportal infusion. Insulin secretion rates estimated by the extended combined model are compared with the known intraportal infusion rates for the simulated OGTT (A) and the simulated IVGTT (B). The left panels show correlations between the average estimated secretion rates and known infusion rates. The right panels show the estimated secretion time courses for each animal (solid lines) superimposed upon the known intraportal infusion rate (●).

of the prehepatic insulin secretion profile if large changes in hepatic insulin extraction occurred over the course of the experiment. However, we have found no evidence for this possibility: the extended combined model accurately reproduces the intraportal infusion rates for both the simulated OGTT and IVGTT. Furthermore, computer simulation studies (10) showed no evidence for such a structural deficiency.

Alternatively, the large mass of insulin appearing in the portal circulation during first-phase secretion could partially saturate the ability of liver insulin receptors to bind to and internalize insulin, which would result in a lower extraction rate. Previous work in this laboratory provides compelling evidence for this alternative. Steil et al. (34) performed paired intraportal and peripheral insulin infusion experiments with the goal of matching peripheral insulin concentrations between the two experiments. The plasma insulin profile they attempted to match was that from the IVGTT. They found that the only way to match the insulin concentrations observed in the intraportal infusion experiment was by assuming a 33% fractional hepatic extraction rate for the peripheral infusion experiment.

The model fits to the simulated OGTT displayed a distinct pattern in the residual time course for both insulin and C-peptide (Fig. 4). Although such patterns typically suggest a deficiency in model structure, the result from the simulated IVGTT protocol discounts this possibility because the model is able to provide accurate predictions of insulin and C-peptide. Furthermore, despite the residuals, the model-predicted

plasma concentrations had the appropriate shape, and the final calculation of prehepatic insulin secretion matched the intraportal infusion profile. Thus, there is no clear explanation for the imperfect model fits to the simulated OGTT data. One possibility is a slight mismatch in the insulin and C-peptide because of incomplete suppression of endogenous hormone secretion. Because the plasma profile of one peptide is used as input for the other, it is possible that a shift in either or both peptide measurements could result in a slightly poorer model fit. Evidence for this possibility exists in the comparison of the estimated secretion rate with the known intraportal infusion rate after correction for the estimated C-peptide distribution space. This relationship parallels the line of unity with a positive intercept of 0.0039 pmol/min. Although not statistically different from zero, this intercept hints at an absolute offset possibly due to incomplete suppression of endogenous insulin and C-peptide release by SRIF. Given that human and canine insulin are indistinguishable and their C-peptides are different, incomplete SRIF suppression of endogenous hormone release would result in differential confounding effects on the insulin and C-peptide time courses. Therefore, it should be noted that for the simulated IVGTT experiments, there was no evidence for incomplete SRIF suppression, and the model better fits the insulin and C-peptide data.

In summary, *in vivo* experimental conditions that allowed for the equimolar portal appearance of insulin and C-peptide to be known were created. Peripheral concentrations of both peptides were measured under conditions that simulated

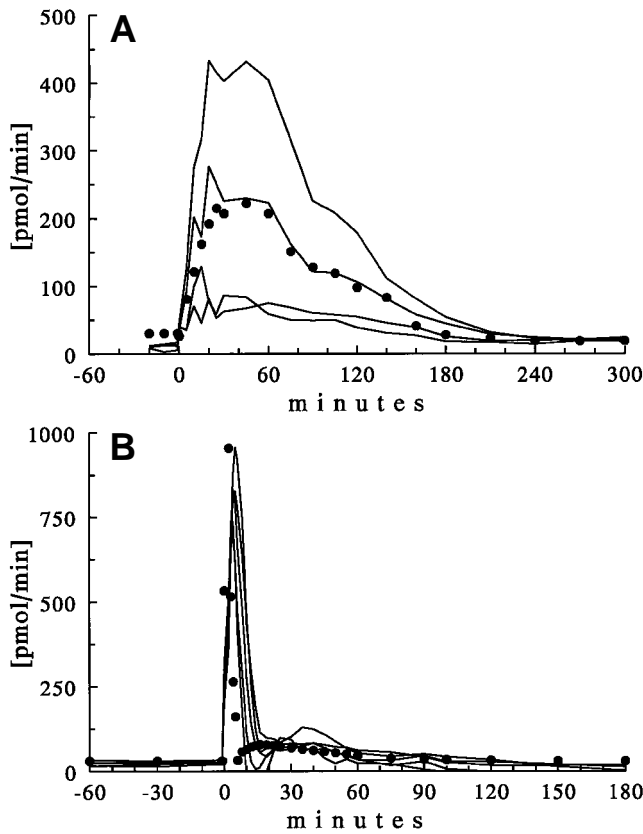


FIG. 6. Comparison of insulin secretion rates. Insulin secretion rates estimated using the Eaton deconvolution approach (solid lines) are compared with the known intraportal infusion rates (●) for each animal. A: Results for the simulated OGTT. B: Results for the simulated IVGTT.

insulin responses during an OGTT or IVGTT. The plasma data were analyzed using the extended combined model, and the model-estimated insulin secretion rates were compared with the known intraportal infusion rates. The model was able to reproduce accurately both secretory profiles. In addition, the model-estimated secretion rates were compared with rates that were estimated by using the Eaton deconvolution approach. Again, the combined model estimated rates were not different from those derived by using the more laborious Eaton deconvolution approach. These studies provide direct evidence that the extended combined model is capable of accurately estimating prehepatic insulin secretion rates without independent measurement of C-peptide kinetics. In addition, because the approach is successful for slow versus rapid secretion rates, the extended combined model can be applied to a wide variety of clinical and experimental protocols without a priori assumption regarding the insulin secretory pattern that an investigator expects to observe (30). This is particularly important for conditions in which the ability of the β -cell to respond to changes in metabolic status may be compromised. Finally, the current study, coupled with the results from the previous computer simulation study (10), suggests the combined model approach can be a useful tool to examine the role of β -cell function in the pathogenesis of type 2 diabetes.

APPENDIX

The final system of equations used to estimate the kinetic parameters for the extended combined model are as follows:

$$\frac{dY_1(t)}{dt} = fK_1I(t) - \lambda_1Y_1(t) - \lambda_1fI(t) \quad (A1)$$

$$\frac{dY_2(t)}{dt} = fI(t)(K_{12} - \lambda_2) + (K_{12} - \lambda_2)Y_1(t) - \lambda_2Y_2(t) \quad (A2)$$

$$C_1(t) = Y_1 + Y_2 + fI(t) \quad (A3)$$

$$\frac{dZ_1(t)}{dt} = f(\lambda_1 - K_1)[Z_2(t) + C_1(t)] - K_1Z_1(t) \quad (A4)$$

$$\frac{dZ_2(t)}{dt} = (\lambda_2 - K_{12})C_1(t) - K_{12}Z_2(t) \quad (A5)$$

$$I(t) = Z_1(t) + f[Z_2(t) + C_1(t)] \quad (A6)$$

Equations 9–11 describe the kinetics of C-peptide in plasma $[C_1(t)]$ where the plasma insulin profile $[I(t)]$ is used as a surrogate representation of the prehepatic insulin secretion rate. Parameters λ_1 and λ_2 characterize the kinetics of C-peptide in plasma, and the inverse of these two parameters represent the fast and slow time constants for C-peptide disappearance. Equations A1–A6 describe the alternative formulation of the model where the kinetics of plasma insulin are described by use of plasma C-peptide as the surrogate representation of the prehepatic insulin secretion rate.

Once individual parameter estimates are obtained, the prehepatic secretory rate is reconstructed by using deconvolution. The extended combined model parameters for C-peptide kinetics can be resolved into the parameters from the standard two-compartment configuration by the following relationships:

$$K_{12} = K_{12} \quad (A7)$$

$$K_{01} = \frac{\lambda_1\lambda_2}{K_{12}} \quad (A8)$$

$$K_{21} = \lambda_1 + \lambda_2 - K_{12} - K_{01} \quad (A9)$$

ACKNOWLEDGMENTS

These studies were supported by a National Institutes of Health (NIH) grant to R.N.B. (DK-29867). R.M.W. was an predoctoral trainee (AG-00093) when these studies were performed, was previously supported by NIH postdoctoral fellowships (T32-HG-0042 and F32-DK-09525), and is currently supported by a Career Development Award from the American Diabetes Association.

The authors would like to thank Donna Maria Moore for performing all the surgical procedures and assisting in the performance of experiments. We would also like to thank Dr. Marianthe Hamilton-Wessler for assistance in performing experiments and Elza Dermirchyan for performing all of the assays.

REFERENCES

1. Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Beebe C, Frank BH, Galloway JA, Van Cauter E: Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. *N Engl J Med* 318:1231-1239, 1988
2. Polonsky KS, Given BD, Van Cauter E: Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. *J Clin Invest* 81:442-448, 1988
3. Bergman RN: Toward physiological understanding of glucose tolerance: minimal model approach. *Diabetes* 38:1512-1527, 1989
4. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP, Porte D Jr: Quantification of the relationship between insulin sensitivity and β -cell function in human subjects: evidence for a hyperbolic function. *Diabetes* 42:1663-1672, 1993
5. Byrne MM, Sturis J, Clement K, Vionnet N, Pueyo ME, Stoffel M, Takeda J, Passa P, Cohen D, Bell GI, Velho G, Froguel P, Polonsky KS: Insulin secretory abnormalities in subjects with hyperglycemia due to glucokinase mutations. *J Clin Invest* 93:1120-1130, 1994
6. Mittelman SD, Van Citters GW, Bergman RN: Temporal pattern of β -cell compensation in fat-fed dog (Abstract). *Diabetes* 48 (Suppl. 1):A309, 1999
7. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368-377, 1992
8. O'Meara NM, Blackman JD, Sturis J, Polonsky KS: Alterations in the kinetics of C-peptide and insulin secretion in hyperthyroidism. *J Clin Endocrinol Metab* 76:79-84, 1993
9. Vølund A, Polonsky KS, Bergman RN: Calculated pattern of intraportal insulin appearance without independent assessment of C-peptide kinetics. *Diabetes* 36:1195-1202, 1987
10. Watanabe RM, Steil GM, Bergman RN: Critical evaluation of the combined model approach for estimation of prehepatic insulin secretion. *Am J Physiol* 274:E172-E183, 1998
11. Shapiro ET, Tillil H, Miller MA, Frank BH, Galloway JA, Rubenstein AH, Polonsky KS: Insulin secretion and clearance: comparison after oral and intravenous glucose. *Diabetes* 36:1365-1371, 1987
12. Watanabe RM, Vølund A, Roy S, Bergman RN: Prehepatic β -cell secretion during the intravenous glucose tolerance test in humans: application of a combined model of insulin and C-peptide kinetics. *J Clin Endocrinol Metab* 69:790-797, 1989
13. Bradley DC, Bergman RN: Restoration of stable metabolic conditions during islet suppression in dogs. *Am J Physiol* 262:E532-E538, 1992
14. Bergman RN, Ider YZ, Bowden CR, Cobelli C: Quantitative estimation of insulin sensitivity. *Am J Physiol* 236:E667-E677, 1979
15. Pacini G, Finegood DT, Bergman RN: A minimal-model-based glucose clamp yielding insulin sensitivity independent of glycemia. *Diabetes* 31:432-441, 1982
16. Finegood DT, Pacini G, Bergman RN: The insulin sensitivity index: correlation in dogs between values determined from the intravenous glucose tolerance test and the euglycemic glucose clamp. *Diabetes* 33:362-368, 1984
17. Herbert V, Law K, Gottlieb C, Bleicher S: Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375-1384, 1965
18. Heding LG: Radioimmunological determination of human C-peptide in serum. *Diabetologia* 11:541-548, 1975
19. Faloon GR, Unger RH: Glucagon. In *Methods of Hormone Radioimmunoassay*. Jaffe BM, Behrman HR, Eds. New York, Academic Press, 1974, p. 317-330
20. Polonsky KS, Licinio-Paixao J, Given BD, Pugh W, Rue P, Galloway J, Karrison T, Frank B: Use of biosynthetic human C-peptide in the measurement of insulin secretion rates in normal volunteers and type I diabetic patients. *J Clin Invest* 77:98-105, 1986
21. Polonsky KS, Given BD, Hirsch L, Shapiro ET, Tillil H, Beebe C, Galloway JA, Frank BH, Karrison T, Van Cauter E: Quantitative study of insulin secretion and clearance in normal and obese subjects. *J Clin Invest* 81:435-441, 1988
22. Draper NR, Smith H: *Applied Regression Analysis*. 2nd ed. New York, John Wiley & Sons, 1981
23. Peterson JD, Nehrlich S, Oyer PE, Steiner DF: Determination of the amino acid sequence of the monkey, sheep, and dog proinsulin C-peptides by a semi-micro Edman degradation procedure. *J Biol Chem* 247:4866-4871, 1972
24. Eaton RP, Allen RC, Schade DS, Erickson KM, Standefer J: Prehepatic insulin production in man: kinetic analysis using peripheral connecting peptide behavior. *J Clin Endocrinol Metab* 51:520-528, 1980
25. Polonsky KS, Pugh W, Jaspan JB, Cohen DM, Karrison T, Tager HS, Rubenstein AH: C-peptide and insulin secretion: relationship between peripheral concentrations of C-peptide and insulin and their secretion rates in the dog. *J Clin Invest* 74:1821-1829, 1984
26. Araki E, Lipes MA, Patti ME, Bruning JC, Haag B, Johnson RS, Kahn CR: Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* 372:186-190, 1994
27. Bergman RN, Phillips LS, Cobelli C: Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and β -cell glucose sensitivity from the response to intravenous glucose. *J Clin Invest* 68:1456-1467, 1981
28. Elbein SC, Hasstedt SJ, Wegner K, Kahn SE: Heritability of pancreatic β -cell function among nondiabetic members of Caucasian familial type 2 diabetic kindreds. *J Clin Endocrinol Metab* 84:1398-1403, 1999
29. Watanabe RM, Valle T, Hauser ER, Ghosh S, Eriksson J, Kohtamäki K, Ehnholm C, Tuomilehto J, Collins FS, Bergman RN, Boehnke M: Familiality of quantitative metabolic traits in Finnish families with non-insulin-dependent diabetes mellitus. *Hum Hered* 49:159-168, 1999
30. Cobelli C, Pacini G: Insulin secretion and hepatic extraction in humans by minimal modeling of C-peptide and insulin kinetics. *Diabetes* 37:223-231, 1988
31. Toffolo G, Bergman RN, Finegood DT, Bowden CR, Cobelli C: Quantitative estimation of β -cell sensitivity to glucose in the intact organism: a minimal model of insulin kinetics in the dog. *Diabetes* 29:979-990, 1980
32. Toffolo G, De Grandi F, Cobelli C: Estimation of β -cell sensitivity from intravenous glucose tolerance test C-peptide data: knowledge of the kinetics avoids errors in modeling the secretion. *Diabetes* 44:845-854, 1995
33. Field JB: Extraction of insulin by liver. *Annu Rev Med* 24:309-314, 1973
34. Steil GM, Rebrin K, Mittelman SD, Bergman RN: Role of portal insulin delivery in the disappearance of intravenous glucose and assessment of insulin sensitivity. *Diabetes* 47:714-720, 1998