

Use of Biosynthetic Human C-peptide in the Measurement of Insulin Secretion Rates in Normal Volunteers and Type I Diabetic Patients

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Abstract

We undertook this study to examine the accuracy of plasma C-peptide as a marker of insulin secretion. The peripheral kinetics of biosynthetic human C-peptide (BHCP) were studied in 10 normal volunteers and 7 insulin-dependent diabetic patients. Each subject received intravenous bolus injections of BHCP as well as constant and variable rate infusions. After intravenous bolus injections the metabolic clearance rate of BHCP (3.8 ± 0.1 ml/kg per min, mean \pm SEM) was not significantly different from the value obtained during its constant intravenous infusion (3.9 ± 0.1 ml/kg per min). The metabolic clearance rate of C-peptide measured during steady state intravenous infusions was constant over a wide concentration range.

During experiments in which BHCP was infused at a variable rate, the peripheral concentration of C-peptide did not change in proportion to the infusion rate. Thus, the infusion rate of BHCP could not be calculated accurately as the product of the C-peptide concentration and metabolic clearance rate. However, the non-steady infusion rate of BHCP could be accurately calculated from peripheral C-peptide concentrations using a two-compartment mathematical model when model parameters were derived from the C-peptide decay curve in each subject. Application of this model to predict constant infusions of C-peptide from peripheral C-peptide concentrations resulted in model generated estimates of the C-peptide infusion rate that were $101.5 \pm 3.4\%$ and $100.4 \pm 2.8\%$ of low and high dose rates, respectively. Estimates of the total quantity of C-peptide infused at a variable rate over 240 min based on the two-compartment model represented $104.6 \pm 2.4\%$ of the amount actually infused. Application of this approach to clinical studies will allow the secretion rate of insulin to be estimated with considerable accuracy.

The insulin secretion rate in normal subjects after an overnight fast was 89.1 pmol/min, which corresponds with a basal 24-h secretion of 18.6 U.

Introduction

The concentration of C-peptide in plasma and urine has been extensively used as a means of assessing beta cell secretory activity (1-5). The rationale for this approach depends on the fact that the liver is the major site of insulin metabolism, variably extracting $\sim 50\%$ of insulin delivered to it (6-11). C-peptide is

co-secreted from the beta cell on an equimolar basis with insulin (12) but does not undergo significant hepatic extraction (13-15). It has therefore been suggested that peripheral C-peptide levels more accurately reflect pancreatic insulin secretion rates than do peripheral insulin levels and that urinary C-peptide levels can be used to document changes in beta cell secretion.

Current knowledge of the kinetics of human C-peptide is incomplete and is based largely on a single study in which Faber and co-workers (16) administered intravenous bolus injections of synthetic human C-peptide to normal volunteers and Type I diabetic patients. The half-life of the peptide was 33.5 min and its metabolic clearance rate was 4.4 ml/kg per min in normal subjects. Because of lack of additional C-peptide, the metabolic clearance rate of C-peptide could only be studied by the constant infusion technique in a single subject.

A number of approaches have been proposed for the derivation of insulin secretion rates from peripheral C-peptide concentrations (1-5). However, due to lack of availability of sufficient quantities of human C-peptide for clinical studies, the accuracy with which insulin secretion can be derived from peripheral C-peptide concentrations using these indirect approaches has not been determined.

Application of recombinant DNA technology has led to the production of sufficient quantities of well characterized biosynthetic human C-peptide for human studies (17). Availability of this material has provided the opportunity to study how accurately the secretion rate of C-peptide and therefore insulin can be derived from peripheral C-peptide concentrations. The results of studies that have addressed these questions are detailed in this report.

Methods

Subjects

Experiments were performed in 10 normal volunteers (5 males, 5 females) and 7 insulin-deficient Type I diabetic patients (4 males, 3 females). Ages were similar in the normal (28.0 ± 1.7 yr) and diabetic subjects (28.0 ± 3.1 yr). All subjects were within 10% of ideal body weight. The diabetic patients were insulin-dependent with a disease duration of 9.4 ± 2.3 yr (range, 3-18). None had clinically significant vascular, renal, eye, or neurological complications. All studies were carried out in the Clinical Research Center of the University of Chicago, IL. Subjects signed written informed consent and the protocols were approved by the Institutional Review Board.

Biosynthetic human C-peptide

Biosynthetic human C-peptide (BHCP)¹ was produced by recombinant DNA technology as previously described (17). The material was shown to have the amino acid composition expected for human C-peptide and co-eluted with C-peptide isolated from human pancreas from both C8

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1. Abbreviations used in this paper: BHCP, biosynthetic human C-peptide; HPLC, high pressure liquid chromatography.

and C18 reverse-phase high pressure liquid chromatography (HPLC) systems. C-peptide immunoreactivity isolated from human plasma following its intravenous administration co-eluted on reverse-phase HPLC with the BHCP standard and with endogenous C-peptide extracted from normal human serum (data not shown).

Experimental protocols

We performed studies on volunteers after they had fasted overnight. Diabetic subjects received a continuous intravenous infusion of insulin adjusted to maintain the plasma glucose concentration in the euglycemic range. During each experiment, an intravenous sampling catheter was inserted into the dorsum of the hand and an infusion catheter was inserted into a vein on the opposite arm. The hand with the sampling catheter was maintained in a heating blanket to ensure arterialization of the venous sample. Each normal volunteer and diabetic subject was studied on four separate occasions over a period of 3–4 wk. The orders of the studies were randomized. During these four studies BHCP was administered by intravenous bolus injection as well as constant and variable rate intravenous infusions. Details of each study protocol are outlined below.

1. *Bolus intravenous injections of BHCP.* Each subject received an intravenous bolus injection of BHCP on two occasions. One bolus was administered in the presence of basal endogenous beta cell secretion, while on the other occasion somatostatin was infused to suppress endogenous beta cell secretion. This experimental design allowed us to determine (a) the reproducibility of the concentration response to a bolus injection of C-peptide; (b) the effect of somatostatin on the clearance kinetics of C-peptide and (c) the accuracy with which the kinetic parameters of administered BHCP could be determined in the presence of endogenous beta cell secretion. After a 60-min baseline during which endogenous C-peptide was measured at 10-min intervals, each subject received a bolus injection of 50 nmol BHCP.² Plasma C-peptide concentrations were measured at the following time points after the bolus: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 17, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 110, 120, 140, 160, and 180 min. Glucose and insulin concentrations were measured at 10-min intervals. The experiment utilizing somatostatin suppression followed a similar protocol except that after the 60-min baseline, subjects received a bolus (50 µg) and constant infusion of somatostatin (500 µg/h). The somatostatin was infused alone for 60 min during which C-peptide and insulin were measured at 10-min intervals to document suppression of endogenous beta cell secretion. 50 nmol of C-peptide was then injected intravenously and samples were drawn at the time points listed above. The somatostatin infusion was maintained throughout the experiment.

2. *Constant infusions of BHCP.* During a 60-min baseline period endogenous C-peptide concentrations were measured at 10-min intervals. A low dose BHCP infusion (3 pmol/kg per min) was then administered for 120 min. The infusion rate was subsequently increased in a single step to 30 pmol/kg per min for an additional 120 min. C-peptide, insulin, and glucose were measured at 5–10-min intervals during the BHCP infusions. In these and the experiments described below, the infusion rate of BHCP was derived using C-peptide concentrations in aliquots of the infusate drawn from the tip of the infusion catheter as it entered the subject.

3. *Variable infusions of BHCP.* Endogenous C-peptide, insulin, and glucose were measured at 10-min intervals during a 60-min baseline period. BHCP was then infused intravenously at a rate of 3 pmol/kg per min for 60 min. Subsequently, the infusion rate was progressively increased at 5-min intervals to reach a peak of 21 pmol/kg per min by 35 min. This rate was maintained for 15 min. The infusion rate was then progressively reduced at 10-min intervals over 70 min to the basal rate, which was administered for an additional 60 min. C-peptide was measured at 5–10-min intervals, and insulin and glucose were measured at 10–20-min intervals. This protocol was designed to simulate the pattern of change in insulin secretion reported to occur after oral glucose ingestion (2).

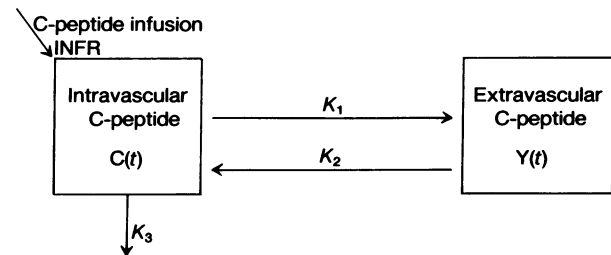
2. The molecular weight of human C-peptide is 3,021; therefore, to convert nanomoles to micrograms, multiply by 3.021.

Calculations

In all analyses the mean endogenous C-peptide concentration in the base line was subtracted from subsequent C-peptide levels measured after BHCP administration. The validity of this approach, which assumes that C-peptide does not affect its own secretion, was supported by the observation that peripheral insulin concentrations did not change as a result of the BHCP administration (see below). Preliminary experiments performed in seven normal volunteers showed that the current dose of somatostatin resulted in suppression of plasma C-peptide concentrations to below the detection limit of the assay (<0.02 pmol/ml) in all subjects. The baseline C-peptide concentration was therefore assumed to be zero in the experiments in which somatostatin was infused. In addition, preliminary experiments performed in four normal volunteers revealed that plasma C-peptide concentrations did not change significantly during a 4-h saline infusion administered after an overnight fast. C-peptide concentrations at the beginning and end of these experiments were 0.55 ± 0.08 pmol/ml and 0.51 ± 0.05 pmol/ml, respectively ($P = 0.2$).

The C-peptide decay curves were resolved into the sum of two exponentials by iterative, nonlinear, least squares regression analysis. The metabolic clearance rate of C-peptide was calculated from these decay curves as the ratio of the amount of C-peptide in the bolus to the integrated area under the plasma concentration curve. The metabolic clearance rate of BHCP was calculated during the constant infusion experiments as the ratio of the infusion rate to the steady state plasma concentration.

Since C-peptide and insulin are cosecreted in equal concentration from the beta cell but C-peptide is not extracted by the liver, the constant and variable infusions of BHCP described above can be considered to be analogous to the intraportal administration of C-peptide. To determine if the infusion rates of C-peptide during both the constant, and variable rate, infusion experiments could be accurately determined by analysis of peripheral C-peptide concentrations, we analyzed the concentrations of C-peptide achieved during these experiments, and we calculated the infusion rate of C-peptide according to a two-compartment mathematical model proposed by Eaton and co-workers (2). This model assumes that C-peptide distributes into a central compartment, from which sampling occurs, and into a peripheral extravascular compartment. The central compartment consists of the plasma space and tissues in rapid equilibrium with plasma. Fractional rate constants K_1 and K_2 describe the rate at which C-peptide passes from the central to the peripheral compartment (K_1) and back again to the plasma compartment (K_2). According to this model, C-peptide is irreversibly metabolized from the central compartment, K_3 being the rate constant that describes this process. The model is depicted in the diagram below.



Scheme I

$C(t)$ represents the total amount of C-peptide in the plasma compartment at time t , and $Y(t)$ is the amount of C-peptide in all extravascular sites.

The fractional rate constants and total initial distribution volume of C-peptide, which define the model parameters, can be derived by analysis of C-peptide decay curves using the equations of Metzler (18). In the current experiments K_1 , K_2 , and K_3 , and the C-peptide volume of distribution V_p were estimated separately in each subject using weighted, iterative, nonlinear least squares regression analysis of individual decay curves. The weight was defined as the reciprocal of the square of the C-peptide concentration. The appropriate equations utilized in this analysis are listed below.

$$C(t) = \frac{\text{Dose}}{V_p(\alpha - \beta)} [(\alpha - K_2)e^{-\alpha t} - (\beta - K_2)e^{-\beta t}],$$

where

$$\alpha = \frac{1}{2}[K_1 + K_2 + K_3 + \sqrt{(K_1 + K_2 + K_3)^2 - 4K_2K_3}]$$

and

$$\beta = \frac{1}{2}[K_1 + K_2 + K_3 - \sqrt{(K_1 + K_2 + K_3)^2 - 4K_2K_3}].$$

Since in addition to intravenous bolus injections of C-peptide, each subject also received constant and variable rate C-peptide infusions, it was possible to examine the accuracy with which this model could be applied to predicting the infusion rate of C-peptide from peripheral C-peptide concentrations. Individual model parameters derived in each normal subject or diabetic patient were used in the analysis. A least squares cubic spline was fit by computer to the discrete arterial C-peptide concentrations observed during the constant and variable C-peptide infusions to obtain a smooth representation of the concentration curves in each subject. Predicted C-peptide infusion rates, INFR (t), were generated according to the equation of Eaton et al. (2), which utilizes the smoothed C-peptide concentration curve and the estimated fractional rate constants and C-peptide distribution volume previously determined in the same subject by analysis of the C-peptide decay curves. At each sampling time during both the constant and variable BHCP infusions, the actual BHCP infusion rate was compared with the infusion rate derived by the above analyses of peripheral C-peptide concentrations. The absolute difference between the actual infusion rate and the rate predicted by the model was calculated at each sampling time. The mean difference in each experiment was expressed as a percentage of the mean actual infusion rate. This value was used as a measure of the overall error

associated with the use of the two-compartment model. Absolute differences between the actual and predicted infusion rates were used in the calculation so that positive and negative values would not cancel each other. As a further measure of the accuracy of the model, the area under the actual infusion rate curve and the curve predicted by the model were compared.

Sample collection and analytical techniques

Blood samples for insulin were allowed to clot at room temperature and the serum was stored at -20°C until assayed. C-peptide and glucose samples were drawn into tubes at 4°C containing 500 KIU/ml Trasylol and 1.2 mg/ml EDTA. Plasma was separated immediately and stored frozen until assayed.

Serum insulin was assayed by a double antibody technique (19). Plasma glucose was measured with a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). Human C-peptide immunoreactivity in plasma was measured as previously described. BHCP and ^{125}I -Tyr-BHCP were used as assay standard and tracer, respectively (20, 21).

Data analysis

The significance of differences between group means was evaluated by a two-tailed paired or unpaired t test or multivariate analysis where appropriate. Results are expressed as mean \pm SEM, and $P < 0.05$ was considered statistically significant. All calculations were performed using the Statistical Analysis System Version 82.3 (Statistical Analysis System Institute Inc., Cary, NC).

Results

Effect of BHCP administration on insulin and glucose concentrations in normal subjects. Fasting insulin and glucose concen-

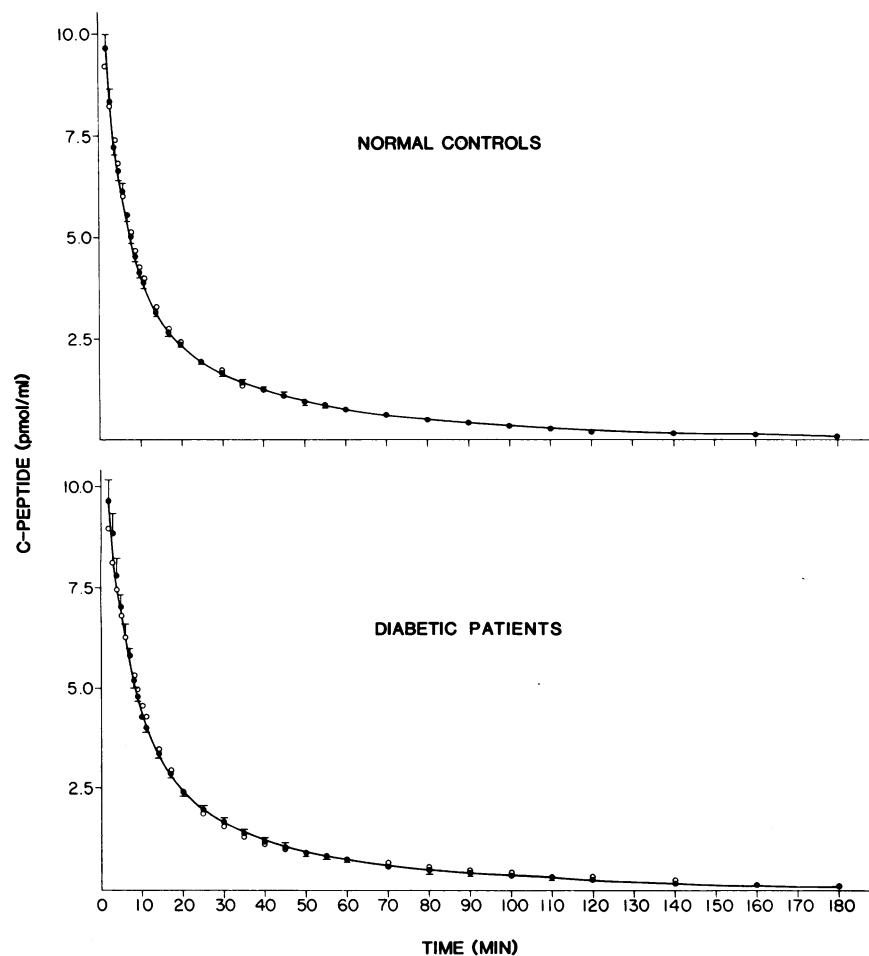


Figure 1. Mean plasma C-peptide concentrations (●) after intravenous bolus injections of biosynthetic human C-peptide in 10 normal subjects (top) and 7 Type 1 diabetic patients (bottom). Open circles depict mean fitted C-peptide concentrations derived from the two-compartment model applied to the data for each individual subject as described in Methods. Mean fitted values are shown only at time points where they were different from the average measured C-peptide concentrations. In this and subsequent groups the C-peptide level measured during the baseline endogenous sampling period in each experiment was subtracted from C-peptide levels measured after administration of BHCP.

trations before administration of the BHCP bolus were 7.2 ± 0.7 $\mu\text{U/ml}$ (49.7 ± 4.8 fmol/ml) and 90.1 ± 2.0 mg/dl (5.0 ± 0.10 mmol/liter), respectively. Mean levels after administration of the bolus were 6.1 ± 0.4 $\mu\text{U/ml}$ (42.1 ± 2.8 fmol/ml) and 90.0 ± 2.2 mg/dl (5.0 ± 0.12 mmol/liter), respectively. A similar lack of effect of BHCP on glucose and insulin concentrations was observed during the constant and variable rate infusion experiments. Somatostatin administration lowered the concentration of insulin to below the detection limit of the assay in all of these subjects.

Plasma concentrations of C-peptide. Mean plasma C-peptide concentrations after the intravenous bolus injections of BHCP are shown in Fig. 1. In the control subjects, the levels were not significantly different in the experiments with and without somatostatin after subtraction of the baseline C-peptide values in the latter experiments, and therefore, we report mean plasma levels and parameter estimates from the two protocols (Fig. 1). In the diabetic subjects, whose serum C-peptide concentrations were below the detection limit of the assay before administration of the bolus injections, the results of the decay curves with and without somatostatin were not statistically different. The concentration measured 2 min after the bolus was 9.7 ± 0.4 pmol/ml in the normals and 9.7 ± 0.6 pmol/ml in the diabetics. By 180 min, concentrations had fallen to 0.08 ± 0.01 and 0.14 ± 0.01 pmol/ml in the two groups, respectively.

During the constant infusions of C-peptide, steady state plasma levels were only achieved after 100 min at each infusion rate. The steady state plasma concentration during the low dose infusion was 0.837 ± 0.039 pmol/ml and during the high dose infusion the level increased to 9.123 ± 0.215 pmol/ml in the normal subjects. Corresponding values in the diabetics were

0.886 ± 0.021 and 9.476 ± 0.345 pmol/ml , respectively. The differences between the normals and diabetics were not significant and mean data are shown in Fig. 2 (top).

During the variable infusion protocol (Fig. 3, middle) the peripheral concentration of C-peptide at the end of the 60 min baseline infusion was 0.800 ± 0.08 pmol/ml . This concentration then increased progressively to reach a peak of 4.96 ± 0.17 pmol/ml by 70 min. The concentration fell progressively as the infusion rate was reduced but had not returned to baseline by the end of the experiment.

Metabolic clearance rate of C-peptide. The decay curves following the bolus injections of C-peptide were individually resolved into the sum of two linear exponentials. The respective half-lives of each exponential were 5.2 ± 0.4 and 36.5 ± 1.7 min in the normals and 6.9 ± 0.4 and 45.3 ± 1.4 min in the diabetics ($P < 0.02$ for each parameter). The metabolic clearance rate derived from analysis of the decay curves was 4.0 ± 0.2 ml/kg per min in the normals and 3.6 ± 0.2 ml/kg per min in the diabetics ($P < 0.001$).

In the normal subjects the metabolic clearance rate of C-peptide measured during its constant infusion was similar at the low (4.0 ± 0.2 ml/kg per min) and high (4.0 ± 0.2 ml/kg per min) infusion rates. Corresponding values in the diabetics were 3.6 ± 0.1 and 3.9 ± 0.2 ml/kg per min. As is apparent, calculation of the metabolic clearance rate after bolus injection and during steady state infusion gave similar values in both the normal and diabetic subjects.

Prediction of infusion rate of BHCP from peripheral C-peptide concentrations according to a two-compartment mathematical model. To determine if the C-peptide infusion rate could

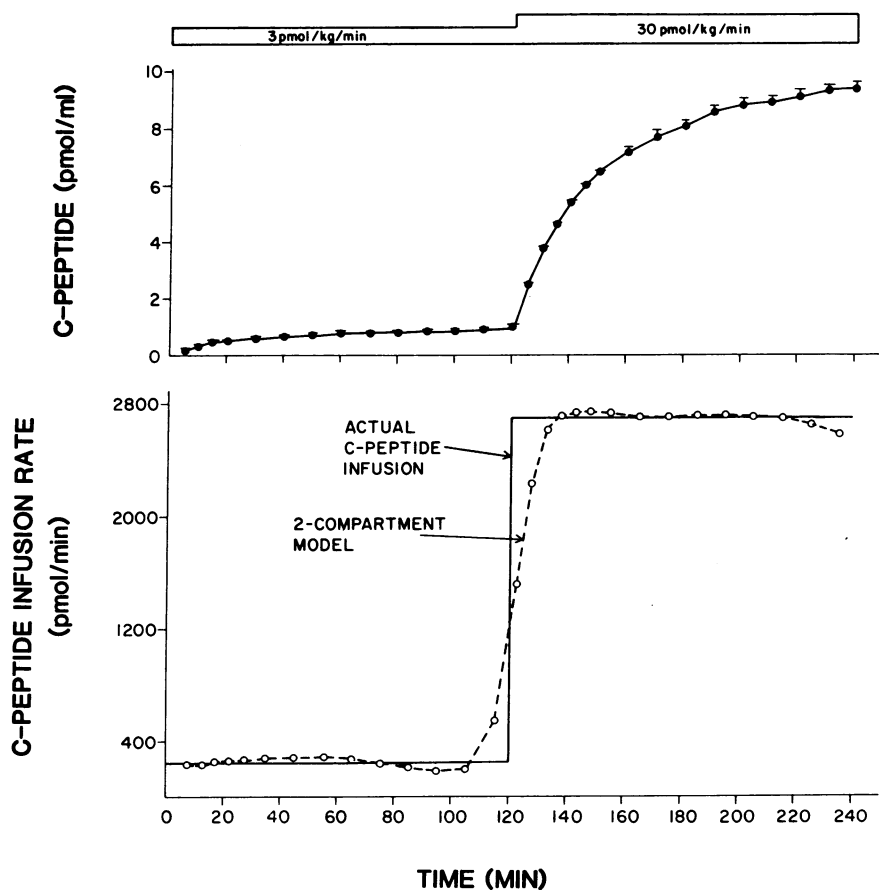


Figure 2. Constant infusion of biosynthetic human C-peptide in 10 normal volunteers and 7 Type I diabetic subjects at rates of 3 pmol/kg per min and 30 pmol/kg per min for 120 min each. Mean plasma concentrations of C-peptide from all 17 subjects are shown in the top panel. The actual infusion rate of C-peptide (—) is shown in the bottom panel in comparison to mean C-peptide infusion rates calculated according to a two-compartment mathematical model (—○—).

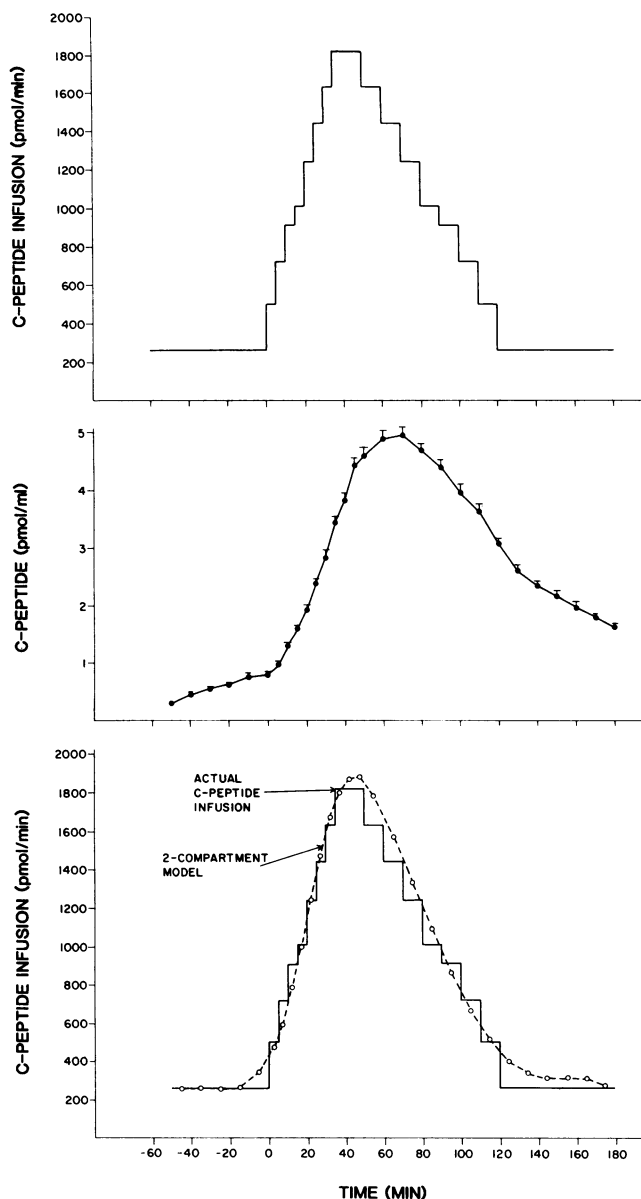


Figure 3. Variable infusions of biosynthetic human C-peptide in 10 normal volunteers and 7 Type I diabetic subjects. The infusion rate of C-peptide is shown in the *top* panel and mean plasma concentrations are in the *middle* panel. The *bottom* panel depicts the actual infusion rate of C-peptide (—) in comparison to mean infusion rates calculated according to a two-compartment mathematical model (---○---).

be estimated by the use of the two-compartment mathematical model described above, fractional rate constants and C-peptide distribution volume were derived for each subject from their individual decay curves as shown in Table I. Parameter values given for each subject are the average of the two values obtained from the two bolus injection protocols. The model parameters in the normal controls were: K_1 , $0.057 \pm 0.006 \text{ min}^{-1}$; K_2 , $0.054 \pm 0.006 \text{ min}^{-1}$; K_3 , $0.060 \pm 0.002 \text{ min}^{-1}$; and distribution volume, $65.5 \pm 2.5 \text{ ml/kg}$. Corresponding values in the diabetics ($0.037 \pm 0.004 \text{ min}^{-1}$, $0.031 \pm 0.002 \text{ min}^{-1}$, $0.052 \pm 0.003 \text{ min}^{-1}$, and $65.5 \pm 2.0 \text{ ml/kg}$) were significantly different from the normals by multivariate Hotelling-Hawley Trace statistic ($P < 0.0003$).

The accuracy with which this model could predict the constant infusion of C-peptide at the two rates utilized in these experiments is shown in Fig. 2 (middle and lower panels) and Table II. When the results for the whole group were considered, the infusion rates based on the model were $101.5 \pm 3.4\%$ of the actual low dose infusion rates, and $100.4 \pm 2.8\%$ of the actual high dose infusion rates.

Corresponding analyses applied to the variable infusions of C-peptide are shown in Fig. 3 (third and lower panels) and Table II. Estimates of the total amount of C-peptide infused during this experiment based on the two-compartment model were $104.6 \pm 2.4\%$ of the amount actually infused. The overall percentage error in individual subjects associated with the use of this model was $13.4 \pm 1.1\%$ (Table II).

Endogenous insulin secretion rates. At the start of the four experimental protocols described above, endogenous C-peptide concentrations were measured in each subject after an overnight fast during the 60-min baseline period. These C-peptide concentrations and individual model parameters derived during the subsequent bolus injection were used to calculate their basal insulin secretion rates. In the normal subjects the mean secretion rate was 89.1 pmol/min (range, 50.2–123.3), which corresponds to a 24-h basal insulin secretion rate of 128.3 nmol (range, 72.3–175.5). Assuming a molecular weight of 5,808 for insulin, this corresponds to a basal 24-h insulin secretion rate of 18.6 U (range, 10.5–25.8 U). The mean coefficient of variation of the insulin secretion rate measured in the same individual on four different days was 18.2% (range, 8–39.3%).

Discussion

C-peptide has been widely used as a test of beta cell function in human subjects in a variety of conditions including Type I diabetes (22), hypoglycemia (23), and surreptitious insulin administration (24). It has also been suggested that the C-peptide/insulin molar ratio reflects changes in hepatic insulin extraction in obesity, cirrhosis, diabetes, and following glucose administration (25–31). The secretion rate of insulin has been derived from hepatic venous (1, 32–34) and peripheral (2–5) C-peptide concentrations. Thus, Waldhäusl and co-workers (1) catheterized the hepatic vein in normal volunteers and measured splanchnic C-peptide production. In these studies the basal insulin secretion rate after an overnight fast was calculated to be 2.0 U/2 h . Meistas and co-workers (4) measured integrated 24-h C-peptide concentrations in blood samples obtained by a continuous withdrawal pump. They multiplied the integrated C-peptide concentration by the mean metabolic clearance rate previously reported by Faber et al. (16) and estimated the mean 24-h insulin secretion rate to be 54.8 U . Eaton et al. (2) proposed a two-compartment model that allows insulin secretion rates to be derived from peripheral C-peptide concentrations and estimated an insulin secretion rate of 15 mU/min in normal subjects after an overnight fast.

However, a systematic and critical study of the limitations of C-peptide as a peripheral marker of insulin secretion has not been possible due to lack of availability of sufficient quantities of human C-peptide for detailed and repeated clinical studies in control subjects and patients with various diseases (35). In this report, biosynthetic human C-peptide was used to assess the kinetics of the appearance of C-peptide in plasma, thus allowing determination of the accuracy with which plasma C-peptide levels reflect its rate of entry into the peripheral circulation. Since C-peptide is not extracted by the liver, its distribution and metabolic kinetics are similar during intraportal and peripheral in-

Table I. Metabolic and Kinetic Parameters of Biosynthetic Human C-peptide

Subject no.	MCR, constant infusion	MCR, bolus injection	K_1	K_2	K_3	Distribution volume
	<i>ml/kg per min</i>	<i>ml/kg per min</i>	<i>min⁻¹</i>	<i>min⁻¹</i>	<i>min⁻¹</i>	<i>ml/kg</i>
Normal controls						
1	4.4	3.8	0.036	0.035	0.060	60.3
2	4.2	4.2	0.044	0.034	0.054	71.5
3	3.6	4.0	0.080	0.069	0.062	61.8
4	4.1	4.0	0.030	0.042	0.060	72.8
5	3.1	3.9	0.082	0.069	0.059	59.6
6	4.5	4.3	0.042	0.034	0.053	79.9
7	3.7	3.9	0.072	0.083	0.059	63.4
8	4.3	4.3	0.060	0.051	0.063	65.6
9	3.8	3.8	0.052	0.048	0.053	68.7
10	<u>4.3</u>	<u>4.0</u>	<u>0.070</u>	<u>0.074</u>	<u>0.072</u>	<u>51.7</u>
Mean	4.0	4.0	0.057	0.054	0.060	65.5
±SEM	<u>0.1</u>	<u>0.1</u>	<u>0.006</u>	<u>0.006</u>	<u>0.002</u>	<u>2.5</u>
Diabetic patients						
1	3.6	3.4	0.046	0.031	0.069	46.8
2	3.7	3.6	0.032	0.026	0.050	65.7
3	4.0	3.7	0.022	0.025	0.055	60.9
4	3.5	3.4	0.051	0.042	0.046	69.1
5	3.9	3.7	0.041	0.030	0.047	73.9
6	4.0	3.2	0.036	0.034	0.048	67.8
7	<u>3.6</u>	<u>4.0</u>	<u>0.028</u>	<u>0.029</u>	<u>0.051</u>	<u>74.4</u>
Mean	3.8	3.6	0.037	0.031	0.052	65.5
±SEM	<u>0.1</u>	<u>0.1</u>	<u>0.004</u>	<u>0.002</u>	<u>0.003</u>	<u>3.6</u>
Mean	3.9	3.8	0.048	0.044	0.057	65.5
±SEM	0.1	0.1	0.004	0.004	0.002	2.0

The metabolic clearance rate (MCR) of biosynthetic human C-peptide was calculated during constant infusions and after intravenous bolus injections. Parameters for a two-compartment model were derived by iterative nonlinear least squares regression analysis of C-peptide decay curves.

fusions (13–15). The results of the present experiments, therefore, are equally applicable to both the physiological intraportal route and the peripheral route of C-peptide delivery.

The metabolic clearance rate of C-peptide was similar during low (3.9 ± 0.1 ml/kg per min) and high dose exogenous infusions (4.0 ± 0.1 ml/kg per min). The plasma levels during the latter infusions exceeded those encountered under most physiological circumstances. Furthermore, the metabolic clearance rate measured after intravenous bolus injection of C-peptide (3.8 ± 0.1 ml/kg per min) was not significantly different from the metabolic clearance rate measured during constant intravenous infusions (3.9 ± 0.1 ml/kg per min). These values are similar to those obtained by Faber et al. (16). Although the metabolic clearance of C-peptide is independent of its plasma concentration, the clearance rate varies amongst individuals (Table I). The range of C-peptide metabolic clearance rate in the present studies was 3.1 to 4.5 ml/kg per min and was lower in diabetic patients than in normal controls, although the difference was statistically significant only after the bolus injection. This suggests that derivation of kinetic parameters of C-peptide in individual subjects should increase the accuracy with which its secretion rate can be derived from peripheral plasma concentrations.

The slow equilibration time and clearance rate of C-peptide must be taken into account in the interpretation of peripheral C-peptide concentrations as shown during the variable rate C-

peptide infusions. During these experiments, the plasma C-peptide concentration increased more slowly than the infusion rate as it was increasing and fell more slowly than the infusion rate as it was being reduced. Furthermore, since the half-life of BHCP is greater than 30 min, its plasma concentration would not be expected to change rapidly with short-term changes in the infusion rate. Thus, under nonsteady state conditions, the peripheral concentration of C-peptide does not change in direct proportion to changes in its secretion rate and as a result the secretion rate of insulin cannot be accurately derived from plasma C-peptide concentrations by a simple single compartmental mathematical analysis.

Use of a two-compartment mathematical model (2) considerably increased the accuracy with which the C-peptide infusion rate could be estimated from peripheral C-peptide concentrations. According to this model, C-peptide distributes into two compartments: a central compartment consisting of plasma and tissues in rapid equilibration with plasma, and a peripheral extravascular compartment. Analysis of the C-peptide decay curves in this study has shown that on the average, $4.8 \pm 0.4\%$ of C-peptide in the central compartment moves to the peripheral compartment per minute and $4.4 \pm 0.4\%$ of C-peptide in the peripheral compartment is transferred back to plasma. $5.7 \pm 0.2\%$ of C-peptide is irreversibly lost from the central compartment per minute. As the infusion rate of C-peptide increases, move-

Table II. Prediction of C-peptide Infusion Rates Using a Two-compartment Model

Subject no.	Constant infusions		Variable rate infusions	
	Low dose A	High dose B	C	Total dose D
	Predicted/actual %	Predicted/actual %	% error	Predicted/actual %
Normal controls				
1	88.2	93.8	15.4	89.7
2	107.9	101.2	8.7	101.6
3	98.4	119.4	29.7	110.3
4	103.3	117.0	16.4	121.7
5	122.5	110.5	19.8	113.6
6	92.0	104.4	9.7	110.3
7	106.1	101.9	14.6	90.1
8	104.5	101.8	14.2	87.8
9	107.5	98.6	18.3	110.2
10	<u>94.2</u>	<u>78.5</u>	<u>17.6</u>	<u>109.0</u>
Mean	102.5	102.7	15.4	104.4
±SEM	<u>3.1</u>	<u>3.7</u>	<u>1.2</u>	<u>3.7</u>
Diabetic patients				
1	90.6	93.9	8.1	96.5
2	94.9	94.3	7.6	101.0
3	79.7	85.1	14.3	97.0
4	94.6	105.1	9.0	107.4
5	139.3	88.1	10.0	110.1
6	92.8	93.9	7.8	106.1
7	<u>108.2</u>	<u>119.0</u>	<u>15.9</u>	<u>116.0</u>
Mean	100.0	97.1	10.4	104.9
±SEM	<u>7.3</u>	<u>4.4</u>	<u>1.1</u>	<u>2.7</u>
Mean	101.5	100.4	13.4	104.6
±SEM	3.4	2.8	1.1	2.4

The constant C-peptide infusion rates derived by the two-compartment model are expressed as a percentage of the actual infusion rate (columns A and B). During the variable rate infusion experiments absolute differences between the actual and model generated infusion rates were calculated as a percentage of the actual infusion rate as shown in column C. The total amount of C-peptide infused during this experiment predicted by the two-compartment model expressed as a percentage of the actual amount is shown in column D.

ment of C-peptide from the central to peripheral compartment results in the finding that the plasma concentration of C-peptide increases more slowly than the infusion rate. As the secretion rate of C-peptide falls, C-peptide in the peripheral compartment moves into the plasma, thus slowing its disappearance from this compartment and contributing to its prolonged half-life. The compartmental distribution of C-peptide is also responsible for the observation shown in Fig. 3 that the same C-peptide infusion rate results in a higher peripheral C-peptide concentration when the overall direction of secretion is falling than when it is increasing. Note that Sherwin et al. (36) have proposed a three-compartment model for the kinetic analysis of insulin.

Application of the two-compartment model to analysis of peripheral concentrations of C-peptide measured during its intravenous infusion allowed the infusion rates to be accurately estimated. Thus, during constant infusions of BHCP, estimates of the infusion rate of C-peptide were 101.5 ± 3.4 and $100.4 \pm 2.8\%$ of the actual infusion rates at the low and high doses, respectively. Estimates of the total amount of C-peptide infused during the variable infusion experiments based on the model were $104.6 \pm 2.4\%$ of the amount actually infused. The mean absolute error associated with the use of the model at discrete time points during each experiment represented $13.4 \pm 1.1\%$ of the actual infusion rate of C-peptide (Table II). Using this model, we es-

timated a basal insulin secretion rate of 18.6 U/24 h, which is in close agreement with values of 21.64 and 24 U/24 h previously reported by Eaton et al. (2) and Waldhäusl et al. (1), respectively.

In summary, the present studies indicate that because C-peptide distributes into more than a single body compartment, its plasma concentration does not change in proportion to its secretion rate under nonsteady state conditions. This factor, combined with the fact that C-peptide has a half-life in excess of 30 min, limits the accuracy with which peripheral concentrations of C-peptide reflect changes in insulin secretion while it is increasing and decreasing. Nevertheless, the above studies indicate that insulin secretion rates can be accurately quantitated from peripheral C-peptide levels in different subjects, under nonsteady state conditions, if the appropriate kinetic parameters derived from analysis of C-peptide decay curves are applied to the compartmental analysis of endogenous C-peptide concentrations.

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