Insulin and C-peptide secretion and kinetics in humans: direct and model-based measurements during OGTT

ANDREA TURA,1 BERNHARD LUDVIK,2 JOHN J. NOLAN,3 GIOVANNI PACINI,1 AND KARL THOMASETH1
1Institute of Systems Science and Biomedical Engineering, Italian National Research Council, 35127 Padua, Italy; 2Division of Endocrinology and Metabolism, Department of Medicine 3, University of Vienna Medical School, A-1090 Vienna, Austria; and 3Department of Endocrinology, St. James’s Hospital, Trinity College, Dublin 8, Ireland

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Tura, Andrea, Bernhard Ludvik, John J. Nolan, Giovanni Pacini, and Karl Thomaseth. Insulin and C-peptide secretion and kinetics in humans: direct and model-based measurements during OGTT. Am J Physiol Endocrinol Metab 281: E966–E974, 2001.—To directly evaluate prehepatic secretion of pancreatic hormones during a 3-h oral glucose tolerance test (OGTT), we measured insulin and C-peptide in six healthy control, six obese, and six type 2 diabetic subjects in the femoral artery and hepatic vein by means of the hepatic catheterization technique. Hypersecretion in obesity was confirmed (309 ± 66 nmol in obese vs. 117 ± 22 in control and 79 ± 13 in diabetic subjects, P ≤ 0.01), whereas early phase secretion was impaired in diabetics. We also measured hepatic insulin extraction (higher in diabetic than in control subjects, P = 0.03) and insulin clearance. The measured data were also used to validate a previously proposed mathematical model, developed to quantify prehepatic secretion, hepatic insulin extraction, and insulin clearance during OGTT, when C-peptide and insulin concentrations are systemically measured. We found good correspondence between experimental data and model estimates for prehepatic insulin secretion (P > 0.3, r2 = 0.93), whereas estimation of hepatic insulin extraction and insulin clearance needs further investigation for improvement.

Potential interactions of the liver on glucose metabolism. Thus one of the aims of this study was to directly evaluate endogenous secretion of C-peptide and insulin and their kinetics during an oral glucose tolerance test (OGTT). This is a physiological test involving the normal route of glucose intake. We applied the hepatic catheterization technique, which allows the direct assessment of the transsplanchnic balance of pancreatic hormones.

A second aim was the validation, against the experimental data obtained from the hepatic catheterization technique, of a mathematical model (23) that yields quantitative information on prehepatic β-cell secretion during the OGTT when C-peptide and insulin concentrations are systemically measured.

Glossary

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS(t)</td>
<td>C-peptide and insulin secretion rate (pmol/min) from measurements (Eqs. 2–5 and 7–8)</td>
</tr>
<tr>
<td>ClearanceC-Pep</td>
<td>Systemic C-peptide clearance (l/min) from measurements (Eq. 7)</td>
</tr>
<tr>
<td>ClearanceIns</td>
<td>Systemic insulin clearance (l/min) from measurements (Eq. 8)</td>
</tr>
<tr>
<td>CP(t)</td>
<td>C-peptide plasma concentration (pmol/l) predicted by the model (Eq. 9)</td>
</tr>
<tr>
<td>CPa(t)</td>
<td>C-peptide concentration (pmol/l) measured in the artery (Eqs. 2 and 7)</td>
</tr>
<tr>
<td>CPv(t)</td>
<td>C-peptide concentration (pmol/l) measured in the hepatic vein (Eq. 2)</td>
</tr>
<tr>
<td>CPS(t)</td>
<td>C-peptide and insulin secretion rate (pmol·l−1·min−1) from model estimations (Eqs. 9 and 10)</td>
</tr>
<tr>
<td>F</td>
<td>Posthepatic insulin fractional appearance (dimensionless) from model estimations (Eq. 10)</td>
</tr>
</tbody>
</table>

Address for reprint requests and other correspondence: K. Thomaseth, LADSEB-CNR, Corso Stati Uniti, 4, 35127 Padua, Italy (E-mail: Karl.Thomaseth@ladseb.pd.cnr.it).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
HBF(t)  Hepatic blood flow (l/min)  (Eqs. 2–6)
HCL(t)  Hepatic insulin clearance (l/min) from measurements (Eq. 6)
HE(t)  Hepatic insulin degradation (pmol/min) from measurements (Eqs. 3 and 4)
HIFC(t)  Hepatic insulin fractional extraction (dimensionless) from measurements (Eqs. 4–6 and 8)
I(t)  Insulin plasma concentration (pmol/l) predicted by the model (Eq. 10)
Ia(t)  Insulin concentration (pmol/l) measured in the artery (Eqs. 3–5 and 8)
Iv(t)  Insulin concentration (pmol/l) measured in the hepatic vein (Eqs. 3 and 5)
$k_{01}$  Systemic C-peptide fractional clearance (min$^{-1}$). It is a fixed parameter in model estimations (Eq. 9)
$n$  Systemic insulin fractional clearance (min$^{-1}$) from model estimations (Eq. 10)

**METHODS**

**Subjects**

Six male lean, nondiabetic [age 44.7 ± 4.4 yr, body mass index (BMI) 26.5 ± 0.9 kg/m$^2$], six male obese, nondiabetic (44.7 ± 2.4 yr, BMI 35.3 ± 1.2 kg/m$^2$), and six diabetic subjects (5 male, 1 female, 50.5 ± 5.3 yr, BMI 30.8 ± 1.1 kg/m$^2$, diabetes duration 7.7 ± 2.7 yr) participated in the study. All subjects were admitted 3 days before the respective study to the San Diego Veterans Affairs Medical Center’s Special Diagnostic and Treatment Unit and consumed a special diet known to affect glucose metabolism. The purpose, positive family history for diabetes or was taking any medication known to affect glucose metabolism. The protocol was reviewed and approved by the Human Subjects Committee of the University of California San Diego. All studies were performed at 8.00 AM after a 10- to 12-h overnight fast.

**Experimental Procedure**

Under local anesthesia with 2% lidocaine, the femoral artery was punctured with an 18-gauge needle, and a 5-French Teflon catheter was introduced and positioned fluoroscopically at the level of the inferior end of the saccular joint. The femoral vein was similarly punctured, and a 6.5-French polyethylene catheter was advanced under fluoroscopic control via the inferior vena cava into the right-sided hepatic vein in an area of adequate blood flow.

Hepatic blood flow was estimated by a primed continuous infusion of indocyanine green (19). The dye infusion was started via an antecubital vein 75 min before glucose ingestion and continued throughout the study. Blood was sampled simultaneously from the artery and the hepatic vein at 10-min intervals starting 45 min after the beginning of green dye infusion. At time 0, the subjects ingested 300 ml of a 75-g glucose solution over 5 min. Arterial and hepatic venous blood was sampled at 15-min intervals to determine the concentrations of glucose, C-peptide, insulin, and indocyanine green for 3 h after glucose ingestion. Hepatic plasma flow was calculated by dividing the green dye infusion rate by arterial-hepatic venous dye concentration difference. Hepatic blood flow was estimated by dividing hepatic plasma flow by 1 − hematocrit.

Glucose was measured with a YSI automated glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH) and C-peptide as described in Ref. 5. Insulin was assayed by double-antibody radioimmunoassay (4). Indocyanine green was analyzed by spectrophotometer after precipitation with sodium deoxycholate (8). The measurement errors, expressed as interassay coefficient of variation, were 1.5% for glucose, 5% for insulin, and 10% for C-peptide.

**Direct-Measurement Data Analysis**

Whole body kinetics were described with a circulatory model that includes the main processes involving the liver. In particular, this organ is represented as a compartment with inputs from the portal vein (pancreatic secretion) and the hepatic artery, and output in the hepatic vein. As a process of substrate disappearance from the liver, degradation in the hepatocytes is considered (Fig. 1). As the overall system can be assumed to be in a quasisteady state, given the slow dynamics of the OGTT, the mass flux of peptide across the liver was described as the steady-state equation

\[
\text{outflow} = \text{inflow} + \text{secretion} - \text{extraction} \tag{1}
\]

which can be applied to both C-peptide and insulin.

For C-peptide, it is known that only a negligible proportion is degraded in the liver (20); therefore, Eq. 1 becomes

\[
\text{CPv}(t) \times \text{HBF}(t) = \text{CPa}(t) \times \text{HBF}(t) + \text{BCS}(t) \tag{2}
\]

where \(\text{CPv}(t)\) and \(\text{CPa}(t)\) are C-peptide concentrations (pmol/l) in the hepatic vein and in the artery, respectively. \(\text{HBF}(t)\) is the measured hepatic blood flow (l/min), and \(\text{BCS}(t)\) is \(\beta\)-cell C-peptide secretion rate (pmol/min). The only unknown is \(\text{BCS}(t)\), which can thus be calculated. Given the equimolar release of C-peptide and insulin, \(\text{BCS}(t)\) also represents \(\beta\)-cell insulin secretion. The integral between 0 and 180 min of \(\text{BCS}(t)\) gives the total amount of insulin secretion (nmol). The ratio of total amount of insulin secretion to area under the curve (AUC) of glucose concentration provides an index of \(\beta\)-cell sensitivity to glucose stimulation.

**Equation 1 applied to insulin is**

\[
\text{Iv}(t) \times \text{HBF}(t) = \text{Ia}(t) \times \text{HBF}(t) + \text{BCS}(t) - \text{HE}(t) \tag{3}
\]

where \(\text{Iv}(t)\) and \(\text{Ia}(t)\) are insulin concentrations (pmol/l) in the hepatic vein and in the artery, respectively, and \(\text{HE}(t)\) is the
hepatic insulin degradation (pmol/min) and is expressed as a fraction of the amount of the hormone entering the liver, i.e.

$$HE(t) = HIFC(t)[1a(t)HBF(t) + BCS(t)]$$  (4)

where HIFC(t) is the hepatic insulin fractional extraction (dimensionless), calculated by substituting Eq. 4 in Eq. 3

$$HIFC(t) = 1 - [I(t)HBF(t)/[1a(t)HBF(t) + BCS(t)]]$$  (5)

Hepatic insulin clearance HCL(t) (l/min) can be computed as

$$HCL(t) = HIFC(t)HBF(t)$$  (6)

Systemic C-peptide clearance (l/min) was calculated as the ratio of the time integral of secretion rate to that of C-peptide concentration in the artery (which is equal to mixed venous blood concentration)

$$\text{clearance}_{C-Pep} = \frac{\int_0^{180} BCS(t)dt}{\int_0^{180} CP(t)dt}$$  (7)

Similarly, systemic insulin clearance (l/min), which does not include first-pass hepatic degradation, was calculated as the ratio of the time integral of secretion rate multiplied by hepatic insulin fractional delivery, $1 - HIFC(t)$, to that of arterial insulin concentration

$$\text{clearance}_{Ins} = \frac{\int_0^{180} \left[1 - HIFC(t)\right]BCS(t)dt}{\int_0^{180} 1a(t)dt}$$  (8)

**Model-Based Data Analysis**

The aforementioned circulatory model was used for analyzing experimental data. Here, we briefly recall the model of insulin secretion and kinetics from OGTT that we want to validating against the measurements directly obtained with the hepatic catheterization protocol. A detailed description of the model with all the assumptions and hypotheses has been reported in Ref. 23.

For C-peptide, the following mathematical description was adopted

$$dCP(t)/dt = -k_{03}CP(t) + CPS(t)$$  (9)

where CP(t) is the measured plasma C-peptide concentration (pmol/l), $k_{03}$ is the disappearance constant, which represents the systemic C-peptide fractional clearance (min$^{-1}$), and CPS(t) is the C-peptide secretion rate estimated by the model (pmol/l.min$^{-1}$); it also represents insulin secretion, since C-peptide is released equimolarly with insulin. The initial condition is provided by the basal C-peptide level, measured immediately before the glucose load. A considerable amount of insulin is extracted by the liver, and only a fraction F of CPS(t) constitutes the posthepatic appearance of the hormone in the peripheral circulation.

Insulin kinetics are described by

$$dI(t)/dt = -nI(t) + F \times CPS(t)$$  (10)

where I(t) is the measured plasma insulin concentration (pmol/l), n is the systemic insulin fractional clearance (min$^{-1}$), and $F \times CPS(t)$ is the posthepatic insulin delivery; (1$-F$) represents the hepatic insulin fractional extraction (dimensionless). The initial condition is given by the basal insulin value. In this study, CP(t) and I(t) were fitted to CPS(t) and 1a(t) from the hepatic catheterization experiment, respectively. Equations 9 and 10 represent a monocompartmental description for C-peptide and insulin kinetics, which is an approach already adopted in other kinetic models (1, 2, 26).

Individualized time courses of C-peptide and insulin secretion rate CPS(t) were estimated by use of the approach proposed previously (23), which is based on a parametric mathematical representation of CPS(t) in terms of continuous piecewise polynomials (splines). These are made up of a series of quadratic polynomials that are joined at specific knot points (3), i.e., 0, 0, 15, 30, 60, 90, 120, 150, 180, 180, and 180. This sequence is slightly different from that used in Ref. 23 to maintain close resemblance to the sampling schedule of the hepatic catheterization experiments, i.e., 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180 min. Double knots at $t = 0$ were introduced to enable the description of a rapid increase of insulin secretion at the beginning of the experiment; triple knots at $t = 180$ min were used to describe the sustained insulin release at the end of the observation interval (23).

Nonlinear weighted least squares were used for estimating the unknown parameters in the spline representation of the C-peptide and insulin secretion rate CPS(t) and the fraction F characterizing insulin appearance. The adopted weights were the inverse of the variance of the measurement errors. Total amount of insulin secretion (nmol) was estimated by integrating CPS(t) over 180 min. In the original model (23), insulin fractional clearance n was a fixed parameter assumed equal to that calculated from other studies and not estimated. Here, n was estimated as a model parameter to increase the possibility of using the method in subjects who can exhibit variations at the level of systemic insulin clearance. The C-peptide fractional clearance $k_{01}$ was still maintained constant (=0.062 min$^{-1}$), in accord with previous studies (13, 24).

**Comparison Between Model Estimates and Direct-Measurement Data**

For each set of data, the individualized reconstructed time course CPS(t) was compared with BCS(t) directly measured from the hepatic catheterization experiments. This comparison was also performed for the total amount of insulin secretion in the 180-min interval. It must be noticed that BCS(t) is expressed in picomoles per minute, whereas CPS(t) is expressed per unit volume. Thus, to compare the two variables with the same units, it was necessary to divide BCS(t) by the individual insulin distribution volume, calculated on the assumption of a distribution volume per unit of body weight of 78 ml/kg (6) (6.0 ± 0.2, 7.9 ± 0.3, and 6.9 ± 0.2 l for control, obese, and diabetic subjects, respectively). The measured clearances of insulin and C-peptide, calculated according to Eqs. 7 and 8, were compared with the corresponding model parameters n and $k_{01}$, respectively. For this purpose, measured clearances were divided by the distribution volume to obtain fractional clearances (min$^{-1}$). To compare measured and model estimated hepatic insulin fractional extraction, the time average of the measured extraction over the 180-min interval was computed.
Calculations and Statistical Analysis

All numerical calculations and parameter estimations were performed using MATLAB (The Mathworks), and numerical simulations were performed using the PANSYM software (22).

Results are presented as means $\pm$ SE unless otherwise designated. Nonparametric tests were used for statistical comparisons. In particular, comparisons between different groups were performed by the Mann-Whitney U-test, whereas those between model and experimental results were done by the Wilcoxon signed-rank test. The relationship between model and experimental results was also investigated by linear regression analysis. Regression was also used to investigate the relationship between measured hepatic blood flow and hepatic insulin fractional extraction and hepatic insulin clearance.

RESULTS

Direct Measurements from Hepatic Catheterization

OGTT. The time courses of the concentrations in the hepatic artery of the measured compounds after the administration of the oral glucose load are presented in Fig. 2. The patterns of C-peptide and insulin were qualitatively similar, with the typical hyperinsulinemia in obese and hypoinsulinemia in diabetic subjects. Glucose levels were not different between control and obese subjects, whereas diabetic subjects showed a marked hyperglycemia. Similar patterns, but higher values, were observed for the concentrations in the hepatic vein (not shown). The basal values and the AUCs for C-peptide, insulin, and glucose are reported in Table 1.

Table 1. Measured values and calculated parameters from the hepatic catheterization experiments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>C</th>
<th>O</th>
<th>D</th>
<th>C vs. O</th>
<th>C vs. D</th>
<th>O vs. D</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic blood flow</td>
<td>ml/min</td>
<td>1,596 ± 67</td>
<td>1,648 ± 129</td>
<td>1,447 ± 129</td>
<td>0.87</td>
<td>0.26</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Basal glucose</td>
<td>mmol/l</td>
<td>5.45 ± 0.20</td>
<td>5.61 ± 0.06</td>
<td>11.27 ± 1.54</td>
<td>0.42</td>
<td>0.004</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Basal insulin</td>
<td>pmol/l</td>
<td>21.0 ± 3.4</td>
<td>114.1 ± 32.5</td>
<td>47.0 ± 15.3</td>
<td>0.037</td>
<td>0.078</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Basal C-peptide</td>
<td>pmol/l</td>
<td>417 ± 42</td>
<td>976 ± 126</td>
<td>509 ± 78</td>
<td>0.004</td>
<td>0.069</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>OGTT AUC glucose</td>
<td>mol-l$^{-1}$.min</td>
<td>26.0 ± 1.6</td>
<td>28.2 ± 1.2</td>
<td>58.2 ± 4.4</td>
<td>0.20</td>
<td>0.004</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>OGTT AUC insulin</td>
<td>nmol-l$^{-1}$.min</td>
<td>53.3 ± 5.0</td>
<td>137.4 ± 25.8</td>
<td>18.2 ± 3.3</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>OGTT AUC C-peptide</td>
<td>nmol-l$^{-1}$.min</td>
<td>323 ± 37</td>
<td>534 ± 61</td>
<td>166 ± 23</td>
<td>0.006</td>
<td>0.004</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Total amount of insulin secretion</td>
<td>nmol</td>
<td>117 ± 22</td>
<td>309 ± 66</td>
<td>79 ± 13</td>
<td>0.010</td>
<td>0.15</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Hepatic insulin extraction</td>
<td>%</td>
<td>41.3 ± 4.2</td>
<td>45.7 ± 3.1</td>
<td>56.7 ± 5.3</td>
<td>0.34</td>
<td>0.037</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Hepatic insulin clearance</td>
<td>l/min</td>
<td>0.66 ± 0.09</td>
<td>0.72 ± 0.03</td>
<td>0.80 ± 0.07</td>
<td>0.42</td>
<td>0.26</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Systemic insulin clearance</td>
<td>l/min</td>
<td>1.19 ± 0.14</td>
<td>1.15 ± 0.12</td>
<td>1.61 ± 0.19</td>
<td>&gt;0.99</td>
<td>0.078</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Systemic C-peptide clearance</td>
<td>l/min</td>
<td>0.36 ± 0.04</td>
<td>0.57 ± 0.07</td>
<td>0.52 ± 0.11</td>
<td>0.025</td>
<td>0.52</td>
<td>0.20</td>
<td></td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE. C, control; O, obese; D, diabetic; OGTT, oral glucose tolerance test; AUC, area under the curve.
The control subjects in the 180-min interval also (Table 2). Moreover, when the total amount of insulin secretion was compared, no difference was found; however, when only the first 60 min were considered, the two secretory patterns were different (41 ± 11 nmol in control and 17 ± 4 in diabetic subjects, \( P = 0.037 \)), confirming the lack of early-phase release typical of diabetic patients. However, when the total amount of insulin secretion was normalized to the distribution volume of each subject, it was found to be lower in diabetic than in control subjects in the 180-min interval also (Table 2). The \( \beta \)-cell sensitivity index was \( 0.08 \pm 0.01 \text{ nmol/} (\text{nmol}^{-1} \text{min}^{-1}) \) in control, \( 0.19 \pm 0.04 \) in obese and \( 0.03 \pm 0.005 \) in diabetic subjects, different in each class with respect to the others (\( P < 0.03 \)).

Confidence intervals in Table 1 show the model fit for the mean C-peptide and insulin concentration data along with the pattern of residuals. In Table 2, the model-estimated parameters are shown normalized to the distribution volume.

### Table 2. Statistical comparison between model-estimated parameters and the corresponding parameters obtained from the hepatic catheterization experiments

<table>
<thead>
<tr>
<th>Unit</th>
<th>C</th>
<th>O</th>
<th>D</th>
<th>C vs. O</th>
<th>C vs. D</th>
<th>O vs. D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total amount of insulin secretion unit volume</td>
<td>nmol/l</td>
<td>19.8 ± 2.3</td>
<td>34.2 ± 4.1</td>
<td>9.5 ± 1.4</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>Model estimates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental data</td>
<td>19.8 ± 3.9</td>
<td>39.6 ± 9.5</td>
<td>11.3 ± 1.9</td>
<td>0.037</td>
<td>0.045</td>
<td>0.004</td>
</tr>
<tr>
<td>Comparison between model and experiment</td>
<td>( P = 0.92 )</td>
<td>( P = 0.60 )</td>
<td>( P = 0.35 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic insulin extraction</td>
<td>%</td>
<td>58.8 ± 7.0</td>
<td>56.1 ± 5.9</td>
<td>65.6 ± 8.7</td>
<td>0.52</td>
<td>0.42</td>
</tr>
<tr>
<td>Model estimates</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Experimental data</td>
<td>41.3 ± 4.2</td>
<td>45.7 ± 3.1</td>
<td>56.7 ± 5.3</td>
<td>0.34</td>
<td>0.037</td>
<td>0.15</td>
</tr>
<tr>
<td>Comparison between model and experiment</td>
<td>( P = 0.12 )</td>
<td>( P = 0.12 )</td>
<td>( P = 0.60 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic C-peptide fractional clearance</td>
<td>min (^{-1})</td>
<td>0.19 ± 0.05</td>
<td>0.13 ± 0.03</td>
<td>0.36 ± 0.12</td>
<td>0.26</td>
<td>0.52</td>
</tr>
<tr>
<td>Model estimates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental data</td>
<td>0.20 ± 0.03</td>
<td>0.14 ± 0.01</td>
<td>0.24 ± 0.03</td>
<td>0.078</td>
<td>0.42</td>
<td>0.037</td>
</tr>
<tr>
<td>Comparison between model and experiment</td>
<td>( P = 0.92 )</td>
<td>( P = 0.75 )</td>
<td>( P = 0.35 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic insulin fractional clearance</td>
<td>min (^{-1})</td>
<td>0.062</td>
<td>0.062</td>
<td>0.062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model estimates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental data</td>
<td>0.061 ± 0.008</td>
<td>0.072 ± 0.009</td>
<td>0.078 ± 0.019</td>
<td>0.34</td>
<td>0.63</td>
<td>0.87</td>
</tr>
<tr>
<td>Comparison between model and experiment</td>
<td>( P = 0.92 )</td>
<td>( P = 0.35 )</td>
<td>( P = 0.46 )</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The model-derived estimates of insulin and C-peptide secretion and kinetics were compared, no difference was found; however, when only the first 60 min were considered, the two secretory patterns were different (41 ± 11 nmol in control and 17 ± 4 in diabetic subjects, \( P = 0.037 \)), confirming the lack of early-phase release typical of diabetic patients. Moreover, when the total amount of insulin secretion was normalized to the distribution volume of each subject, it was found to be lower in diabetic than in control subjects in the 180-min interval also (Table 2). The \( \beta \)-cell sensitivity index was \( 0.08 \pm 0.01 \text{ nmol/} (\text{nmol}^{-1} \text{min}^{-1}) \) in control, \( 0.19 \pm 0.04 \) in obese and \( 0.03 \pm 0.005 \) in diabetic subjects, different in each class with respect to the others (\( P < 0.03 \)).

**Clearances and hepatic extraction.** Time average over the 180-min interval of the hepatic insulin fractional extraction (expressed as percentage of the insulin amount entering the liver) was found to be higher in diabetic than in control but not in obese subjects (Table 1), whereas hepatic insulin clearance was not different in the three groups (Table 2). A significant negative linear relationship (regression coefficient \( \beta = -4.46 \pm 1.05 \times 10^{-4}, P < 0.0001, r^2 = 0.37 \) was found between hepatic blood flow and hepatic insulin fractional extraction in obese subjects (Fig. 4). Conversely, no significant relationship between the two variables was found in control (\( P = 0.07 \)) and diabetic subjects (\( P = 0.2 \). Regression between hepatic blood flow and hepatic insulin clearance was not significant in any class (\( P = 0.9 \) in control, \( P = 0.16 \) in obese, and \( P = 0.10 \) in diabetic subjects). The time average over the 180-min interval of the hepatic blood flow is reported in Table 1.

**Systemic C-peptide and insulin clearances** are reported in Table 1. That of C-peptide was higher in obese than in control subjects, but when it was normalized to the distribution volume to obtain the C-peptide fractional clearance, no difference was observed (Table 2). Insulin clearance was not different in the three groups (Table 1). Fractional insulin clearance of obese subjects was lower than in diabetic but not lower than in control subjects (Table 2).

**Model-Derived Estimates**

**Model fit of C-peptide and insulin data.** Figure 5 shows the model fit for the mean C-peptide and insulin concentration data along with the pattern of residuals. In Table 2, the model-estimated parameters are shown for the three groups. Their precision, assessed by the coefficient of variation, i.e., the ratio of the diagonal element of the covariance matrix to the parameter value, was 47% for F, 35% for n, and 3% for CPS(t).

**Comparison between model-derived and measured variables.** Model-reconstructed CPS(t) was compared with the measured insulin secretion BCS(t) normalized to the distribution volume, and the two patterns were similar in every group (Fig. 6). A good correlation was found between measured and model-estimated mean values at any sampling time points (Fig. 7). Similarly, the normalized total amount of insulin secretion (Table 2) was not different from the corresponding measured quantity (Table 2), and the two were highly correlated (Fig. 8, A). Moreover, according to experimental results, model estimates confirmed the difference in the
normalized total amount of insulin secretion in each class with respect to the others (Table 2).

Although the correlation among individual values was not excellent (Fig. 8, B), estimated hepatic insulin fractional extraction was not different from that directly measured (Table 2). Despite the fact that individual values were found to be poorly correlated (Fig. 8, C), estimated insulin fractional clearance was not different from the corresponding measured quantity (Table 2). The value assumed for C-peptide fractional clearance was not different from the corresponding measured values (Table 2).

**DISCUSSION**

The present study addresses two main issues: the analysis of experimental data from hepatic catheterization and the validation of a mathematical model of insulin secretion and kinetics.

By use of hepatic catheterization, direct measurements were obtained for secretion and kinetics of insulin and C-peptide during an OGTT. This test was chosen because it is simple to perform, its use is widespread, and it is considered a “physiological” dynamic test. This study confirms insulin hypersecretion in obese compared with lean subjects and impairment of insulin secretion in type 2 diabetic patients. These patients also showed increased arterial glucose levels, as expected, mirrored by their decreased β-cell sensitivity to glucose.

In many studies, it is assumed that the level of insulinemia depends almost entirely on insulin secretion; however, the role of metabolic clearance should also be taken into account. Hepatic catheterization allowed the direct assessment of hepatic extraction and clearance as well as systemic insulin and C-peptide clearances. Because no difference in absolute or fractional insulin clearance was observed in obese and diabetic compared with control subjects, the hyperinsulinemia typical of obesity and the hypoinsulinemia of diabetes seem to be due only to enhanced or reduced secretion, respectively. A minor role, however, is played in diabetic subjects by hepatic extraction, which was found to be slightly higher in this group, contributing, therefore, to further lowering of systemic insulin. C-peptide clearance was higher in obese subjects, probably due to the larger distribution volume in these subjects. In fact, when C-peptide clearance was normalized to the distribution volume, no difference was observed between the three groups.

The second aim of this study was the validation of a mathematical model (23) developed to assess prehepatic C-peptide and insulin secretion and hepatic insulin extraction from systemic measurements during an OGTT. Mathematical modeling plays a key role in the estimation of these metabolic parameters that can hardly be measured directly in human subjects, and certainly not in the clinical routine. For that validation, we compared model estimates of insulin secretion
time courses and parameters with the corresponding variables directly measured with the hepatic catheterization experiment.

As shown in Fig. 6, the average time courses of prehepatic secretion that the model reconstructs were similar to those measured directly, despite measured patterns that were quite variable. From a quantitative point of view, the values of the total amount of released insulin were very similar. To compare the prehepatic secretion from the experiments with the prehepatic secretion estimated by the model, it was necessary to normalize the former to the insulin distribution volume to have both variables in the same units. The same procedure was used to compare clearances. The choice of the insulin distribution volume is therefore a critical aspect, but there is still a lack of knowledge about this physiological variable. Because we were unable to measure the distribution volume from our experiments, we had to choose a value reported in the literature (6), obtained from a noncompartmental analysis of pork insulin bolus during a hypoglycemic clamp. The same distribution volume per unit of body weight was used for control, obese, and diabetic subjects. In fact, to the best of our knowledge, only a few studies have been carried out on possible differences in the
insulin distribution volume between different classes of subjects. McGuire et al. (14) found a reduced distribution volume in obese with respect to nonobese subjects (~30%), and in diabetic with respect to nondiabetic subjects (10–20%). However, the absolute value of the estimate varied by as much as a factor of five, depending on the insulin kinetics model used. Opposite of the estimate varied by as much as a factor of five, depending on the insulin kinetics model used. Opposite of the estimate varied by as much as a factor of five, depending on the insulin kinetics model used.

Hepatic insulin extraction in each class of subjects as estimated by the model was in accord with the measured mean value, although individual correlation was not excellent. It is worth noting, however, that hepatic insulin extraction from experimental data is likely to be less accurate than the measurement of insulin secretion, due to error propagation in its calculation (see Eqs. 2–5). This is reflected in uncertainty in the comparison between model-based and experimental findings. Another possible reason for the low correlation may be the small number of subjects. However, because the experimental protocol is complex and invasive in humans, it has been possible to carry out this investigation only in a few subjects per class.

One of the assumptions of the model is a constant hepatic extraction for each subject. However, experimental data revealed a significant relationship between hepatic blood flow and hepatic extraction in obese subjects, in accord with conclusions from a previous study (24). Thus these results support the hypothesis that hepatic extraction is not constant in these subjects and that it depends on hepatic blood flow, which is not constant during an OGTT. Consequently, at least for obese subjects, the model estimation of a constant parameter has to be considered an approximation. On the other hand, it is not possible to further increase the number of parameters estimated by the model and maintain an acceptable accuracy. Moreover, both hepatic blood flow and hepatic extraction from experimental data do not actually exhibit marked changes with respect to their time average (standard error with respect to time average equal to 1.8, 2.4, and 2.8% in control, obese, and diabetic subjects, respectively, for hepatic blood flow and 7.5, 4.1, and 4.0% for hepatic extraction). Thus the assumption of a constant model parameter for representing hepatic extraction can be considered a reasonable choice, although it may partially contribute to the observed differences between model estimates and experimental results.

The model presented in this study also allows estimation of systemic insulin clearance; that in the original version (23) was fixed to values obtained from other studies (10–12) or from the literature (18, 21). The data set of the present study, in fact, allowed estimation of at least one more parameter, maintaining an acceptable accuracy in the estimates. We chose to estimate insulin clearance, because it has been reported to exhibit changes in different conditions and to be quite variable, even in normal subjects (6). On the contrary, C-peptide clearance was left at a fixed value, as in the original version, because several studies (16–18, 25) have reported a virtually unchanged C-peptide clearance in many different pathophysiological conditions. Those authors’ experience with the intravenous glucose test demonstrated that only in subjects with a clear impairment in renal function was C-peptide clearance significantly reduced (9).

Similarly to the hepatic extraction, the model estimated well in each class the mean values of the measured systemic insulin clearance, but individual values were poorly correlated. This could be due to the limits of the description of insulin kinetics of the model used, although in this case also problems of error propagation in the computation of this variable from experimental data (see Eq. 8), as well as the modest number of subjects, could have played a significant role. A larger number of subjects would help to clarify the reason for the observed poor correlation. C-peptide clearances from experimental results confirmed the adequacy of the choice of a fixed parameter, being similar in the three groups and not different from the value assumed in the model.

In conclusion, in the experimental part of this study, we provided direct confirmation of altered insulin secretion in obese and diabetic subjects. Information was obtained on clearance, which is less often investigated. Finally, it was shown that a previously introduced model of insulin secretion and kinetics during an OGTT in humans provides accurate estimates of prehepatic insulin secretion, as well as some information on hepatic insulin extraction and clearance, in a single subject. Thus, because the direct measure of these variables is not feasible in the clinical routine, the presented model-based approach is a useful tool for the assessment of insulin secretion and kinetics during an OGTT in different pathophysiological conditions, although changes in the model formulation are probably necessary to improve the estimation of insulin clearance and hepatic extraction.

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