Dose-response characteristics of insulin action on glucose metabolism: a non-steady-state approach

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1Metabolism Unit of the Consiglio Nazionale delle Ricerche Institute of Clinical Physiology and Department of Internal Medicine, University of Pisa, 56126 Pisa; and 2Consiglio Nazionale delle Ricerche Institute of Systems Science and Biomedical Engineering, 35127 Padua, Italy

Natali, Andrea, Amalia Gastaldelli, Stefania Camasta, Anna Maria Sironi, Elena Toschi, Antonio Masoni, Ele Ferrannini, and Andrea Mari. Dose-response characteristics of insulin action on glucose metabolism: a non-steady-state approach. Am J Physiol Endocrinol Metab 278: E794–E801, 2000.—The traditional methods for the assessment of insulin sensitivity yield only a single index, not the whole dose-response curve information. This curve is typically characterized by a maximally insulin-stimulated glucose clearance ($C_{L\text{max}}$) and an insulin concentration at half-maximal response ($E_{C50}$). We developed an approach for estimating the whole dose-response curve with a single in vivo test, based on the use of tracer glucose and exogenous insulin administration (two steps of 20 and 200 mU·min$^{-1}·m^{-2}$, 100 min each). The effect of insulin on plasma glucose clearance was calculated from non-steady-state data by use of a circulatory model of glucose kinetics and a model of insulin action in which glucose clearance is represented as a Michaelis-Menten function of insulin concentration with a delay ($t_{1/2}$). In seven nondiabetic subjects, the model predicted adequately the tracer concentration: the model residuals were unbiased, and their coefficient of variation was similar to the expected measurement error (~3%), indicating that the model did not introduce significant systematic errors. Lean (n = 4) and obese (n = 3) subjects had similar half-times for insulin action ($t_{1/2} = 25 ± 9$ vs. $25 ± 8$ min) and maximal responses ($C_{L\text{max}} = 705 ± 46$ vs. 668 ± 259 mU·m$^{-1}·m^{-2}$, respectively), whereas $E_{C50}$ was $240 ± 84$ µU/ml in the lean vs. $364 ± 229$ µU/ml in the obese ($P < 0.04$). $E_{C50}$ and the insulin sensitivity index (ISI, initial slope of the dose-response curve), but not $C_{L\text{max}}$ were related to body adiposity and fat distribution with r of 0.6–0.8 ($P < 0.05$). Thus, despite the small number of study subjects, we were able to reproduce information consistent with the literature. In addition, among the lean individuals, $t_{1/2}$ was positively related to the ISI ($r = 0.72, P < 0.02$). We conclude that the test here presented, based on a more elaborate representation of glucose kinetics and insulin action, allows a reliable quantitation of the insulin dose-response curve for whole body glucose utilization in a single session of relatively short duration. Insulin sensitivity

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steady-state data from a single euglycemic clamp experiment in which insulin concentrations and glucose disposal rates cover a large portion of the insulin sensitivity curve. Estimating glucose fluxes in the nonsteady state requires the use of a glucose tracer and a model of glucose kinetics. Although stable isotopes have conveniently replaced radioactive tracers, the traditional approaches to glucose modeling (one or two compartments) have intrinsic limitations (20). In the present study, we describe a new method by which modeling errors are minimized by the use of the specific activity clamp format coupled with a circulatory model (10, 11), a noncompartmental approach that relies on a physiological representation of the glucose system (20). A simple model was then incorporated to account for the delay in insulin action with respect to plasma concentrations, and the relationship between glucose clearance and insulin action was assumed to follow saturation (Michaelis-Menten) kinetics.

**METHODS**

Study subjects. Seven adult male subjects, either healthy lean volunteers (1-4) or obese nondiabetic patients attending our metabolism clinic (5-7), were recruited (Table 1). None had a family history of diabetes or was taking drugs known to affect glucose metabolism. Subject 5 had an unconfirmed diagnosis of essential hypertension; subject 4 was engaged in noncompetitive long-distance running. The potential risks of the study were carefully explained to each subject, who gave his informed written consent before the study. The study protocol was approved by the Institutional Ethics Committee.

Experimental protocol. Subjects were admitted as outpatients to the Metabolism Unit between 8:00 and 9:00 AM after an overnight (11-12 h) fast. The study was done with the subject resting supine in a comfortable bed in a quiet air-conditioned room. A 20-gauge catheter was inserted into an antecubital vein (for the infusion of test substances); another catheter was threaded retrogradely into a wrist vein of the same arm and was used for blood sampling. The hand was kept in a heated box for the entire duration of the study to achieve and maintain arterialization of venous blood. The study protocol was started and was continued for the entire basal period. During insulin infusion, plasma glucose concentration was measured every 10 min and maintained at basal values by means of a variable 20% glucose infusion according to the isoglycemic clamp technique (4). To minimize the changes in plasma [6,6-2H2]glucose enrichment, 2 g of tracer were added to 500 ml of the 20% glucose solution while the constant [6,6-2H2]glucose infusion was turned off in a stepwise fashion (by 25% every 10 min). Blood sampling for the assay of plasma [6,6-2H2]glucose enrichment was more frequent (every 2–5 min) during the first 50 min of each of the three study periods and was spaced at 10- to 15-min intervals thereafter. Three blood samples for plasma insulin determination were also taken at the end of the basal period, whereas during insulin infusion sampling for insulin was the same as for plasma [6,6-2H2]glucose.

Analytical procedures. Plasma glucose was assayed by the glucose oxidase method (Glucose Analyzer, Beckman Instruments, Fullerton, CA). Specific insulin was assayed in plasma by RIA (human insulin-specific RIA kit, Linco Research, St. Charles, MO). Plasma [6,6-2H2]glucose enrichment was measured in arterialized blood samples after deproteinization with barium hydroxide (0.3 N) and zinc sulfate (0.3 N). The supernatant was run through columns of ion-exchange resin, evaporated, and derivatized. The sample tracer-to-tracee ratio (TTR) was determined as previously described (19). Briefly, isotopic enrichment was determined on the pentaceta
derivative (1:1 acetic anhydride-pyridine) by gas chromatography-mass spectrometry (GC-MS) (Hewlett-Packard GC 5890-MS 5972, Palo Alto, CA) by use of electronic impact ionization and selectively monitoring ions of rounded molecular weight (rmw) 200, 201, and 202. Following the method used by Rosenblatt et al. (18), the apparent enrichment of rmw 202 (M + 2) was corrected for the contribution of singly labeled molecules [rmw 201, (M + 1)] by subtracting the product (TTR (M + 1)) x natural abundance of (M + 2) from the TTR (M + 2). This correction was very small and rather constant, ranging from 0.01 to 0.02% for plasma sample enrichments between 2 and 5%. All samples from the same study were processed and assayed in the same run.

Model of glucose kinetics. In the circulatory model (10, 11, 15), the body is schematized as the combination of the heart-lung block and the periphery block, which lumps together all the remaining tissues (Fig. 1). Each block is regarded as a single inlet-single outlet organ and can be described mathematically by an impulse response (9). The organ impulse response is defined as the tracer influx observed at the outlet after a bolus injection of a unit dose into the inlet (with assumption of no tracer recirculation). After bolus injection into a peripheral vein, the tracer disappearance curve will be the result of the combination of the impulse responses of the two interconnected blocks. When blood flow, i.e., cardiac output (F), and the impulse response of the heart/lung are known, the impulse response of the periphery can be calculated (12). Cardiac output was calculated as follows: F (milliliters per square meter of body surface area) = 3,200–30 × (years of age – 40). This quantity was assumed to remain constant during the test and was corrected for the ratio of whole blood to plasma glucose concentration (≈ 0.84) to obtain the actual glucose mass flux. Second, the impulse response of the heart-lung block was assumed to be known and not affected by insulin. It was represented by a two-exponential function starting from zero and returning to zero after rising to an early peak. The parameters of the heart-lung impulse response were set to match experimentally derived curves, as detailed in Ref. 15. In particular, the heart-lung glucose distribution volume was assumed to be

**Table 1. Subjects’ characteristics**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>BSA (m²)</th>
<th>W/H</th>
<th>MBP (mmHg)</th>
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<td>26.2</td>
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<td>0.90</td>
<td>98</td>
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<tr>
<td><strong>Mean</strong></td>
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<td><strong>174</strong></td>
<td><strong>76</strong></td>
<td><strong>25.1</strong></td>
<td><strong>1.93</strong></td>
<td><strong>0.87</strong></td>
<td><strong>101</strong></td>
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<td>113</td>
<td>34.9</td>
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<td>1.00</td>
<td>97</td>
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<tr>
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<td>176</td>
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<td><strong>34.1</strong></td>
<td><strong>2.32</strong></td>
<td><strong>1.01</strong></td>
<td><strong>109</strong></td>
</tr>
</tbody>
</table>

BSA, body surface area; W/H, waist-to-hip ratio; MBP, mean blood pressure.
400 ml/m², and glucose fractional extraction was assumed to be nil. Third, the impulse response of the periphery block was represented by a four-exponential function, starting from zero and gradually returning to zero after reaching a peak, with the fastest rising exponential term fixed. This impulse response can be conveniently represented as a convolution of a three-exponential function and a single-exponential function, γe⁻γt, representing the fastest rising exponential term in which γ = 10 min⁻¹ is fixed (15). Thus

\[ r_{\text{per}}(t) = \gamma e^{-\gamma t} \otimes [w_1 \lambda_1 e^{-\lambda_1 t} + w_2 \lambda_2 e^{-\lambda_2 t} + (1 - w_1 - w_2) \lambda_3 e^{-\lambda_3 t}](1 - E) \]  

(1)

where the symbol \( \otimes \) is the convolution operator. The three-exponential function in square brackets has the property that its integral from zero to infinity is one, and the parameter \( w_1 \) (dimensionless) represents the relative contribution of the exponential term of exponent \( \lambda_1 \) (min⁻¹) to the total integral. From this property, it follows that the integral from zero to infinity of \( r_{\text{per}}(t) \) is 1 - E, i.e., E (dimensionless or %) is the glucose fractional extraction of the periphery block (15), which is constant in the basal state and varies with insulin concentration during the clamp period.

Model of insulin action. To account for saturation of glucose fluxes (as observed in most in vivo and in vitro experimental studies), the model assumes that insulin action on fractional glucose extraction follows Michaelis-Menten kinetics (Fig. 1). In addition, to use also the non-steady-state data, the delay that is normally observed between changes in plasma insulin concentration and changes in E was modeled by use of a single linear differential equation (monoexponential delay). For this purpose, a new variable, Z(t), representing the delayed insulin time course, was introduced. Z(t) (in micromolars per milliliter) is related to the measured plasma insulin concentration increment from baseline by the equation

\[ \frac{dZ}{dt} = -\alpha Z(t) + \alpha[I(t) - I_b] \]  

(2)

where \( I(t) \) (in micromolars per milliliter) is the plasma insulin concentration at time \( t \), \( I_b \) (micromolars per milliliter) is plasma insulin concentration at baseline, and \( \alpha \) (min⁻¹) quantifies the delay; the half-time of insulin action is thus calculated as \( \ln(2)/\alpha \). In this model, therefore, it is Z(t) that determines fractional glucose extraction \( E(t) \) according to a Michaelis-Menten relationship

\[ E(t) = \frac{E_{\text{max}}Z(t)}{EC_{50} + Z(t)} \]  

(3)

where \( E_b \) (dimensionless or %) is the basal glucose fractional extraction, and \( E_{\text{max}} \) (dimensionless or %) and \( EC_{50} \) (in micromolars per milliliter) are the Michaelis-Menten parameters.

During the low-dose insulin infusion, the onset of insulin action was found to be somewhat irregular. To account for this, a further disturbance term \( E_d(t) \) (dimensionless or %) was introduced into Eq. 3 to add flexibility to \( E(t) \) in the initial phase of the clamp period, i.e.

\[ E(t) = \frac{E_{\text{max}}Z(t)}{EC_{50} + Z(t)} + E_d(t) \]  

(4)

where the disturbance term \( E_d(t) \) is nonzero only during the first 60 min of the low-insulin period and is represented as a generic piecewise constant function on 10-min intervals.

The dose-response curve relating glucose clearance (Cl) in milliliters per minute per square meter to insulin concentration at steady state is calculated from the Michaelis-Menten parameters. Therefore, because glucose clearance is the product of cardiac output (F) and fractional glucose extraction (E) at the insulin concentration I (in \( \mu \text{M} \)), glucose clearance is given by

\[ Cl = F \left[ \frac{E_b + E_{\text{max}}(I - I_b)}{EC_{50} + (I - I_b)} \right] \]  

(5)

From Eq. 5, the maximally insulin-stimulated glucose clearance is calculated as \( Cl_{\text{max}} = F(E_b + E_{\text{max}}) \). The glucose clearance at plasma insulin concentration of 100 \( \mu \text{M} \) (Cl₀) was calculated by fitting \( I = 100 \). For insulin concentrations well below \( EC_{50} \), the relationship between insulin concentration and glucose clearance is nearly linear. The slope of the line, which is the ratio of \( F \times E_{\text{max}} \) to \( EC_{50} \), is a traditional insulin sensitivity index, denoted here as ISI.

Tracer fit and parameter estimation. The six parameters of the periphery impulse response (Eq. 1) were estimated by fitting the circulatory model to the [6,6-2H₂]glucose concentra-
tion in the basal state. To analyze the data of the clamp period, the circulatory model of glucose kinetics was combined with the model of insulin action (Eqs. 2 and 4). We assumed that in this non-steady-state condition the major effect of insulin is on E, whereas the other parameters of the periphery impulse response are not modified by insulin, as supported by experimental studies using the clamp (10, 15). Thus the parameters $\lambda_3$, $\lambda_2$, $\lambda_3$, $W_3$, $W_2$ (Eq. 1), and $E_0$ (Eq. 4), were taken from the analysis of the basal tracer curves, whereas $EC_{50}$ and $E_{max}$ (Eq. 4) and $\alpha$ (Eq. 2) were estimated from [6,6-$^2$H$_2$]glucose concentration during the clamp. In this analysis, plasma insulin concentration, which is required as a continuous function of time, was smoothed and interpolated with the use of a simplified model of insulin kinetics (14).

Parameters were estimated by least squares with Matlab. We used equal weights for all tracer points, because with relatively stable [6,6-$^2$H$_2$]glucose concentrations the error variance was not expected to differ substantially among time points.

The experimental data were also analyzed by use of an approach that does not require assumptions on the mechanism of insulin action. The circulatory model of glucose kinetics and the parameters of the basal period were used to reconstruct the time course of the fractional glucose extraction E(t) from the [6,6-$^2$H$_2$]glucose concentration. This time-varying E(t) was approximated as a piecewise constant function of time on 5-min intervals. E(t) was thus represented by a total of 40 elements in the 200 min of non-steady state, which were estimated by least squares fit of the [6,6-$^2$H$_2$]glucose concentration according to a scheme analogous to deconvolution (15). The time-varying glucose clearance was then calculated as $F \times E(t)$.

After E(t) is estimated, the circulatory model makes it possible to calculate the plasma glucose concentration that is due to the exogenous glucose infusion. By subtracting these values from the measured plasma glucose concentrations, the component of glucose concentration due to endogenous glucose production (EGP) is obtained. EGP was calculated from the endogenous glucose concentration and the model by a deconvolution method (15). Briefly, EGP was estimated by minimizing the sum of the squared model residuals (observed minus model-predicted endogenous tracee concentration) plus the sum of the squared elements of the second derivative of EGP multiplied by a weighting factor. The latter term is necessary to eliminate the spurious oscillations of EGP that would be observed when using ordinary least squares, as deconvolution is an ill-conditioned problem. An appropriate choice of the weighting factor eliminates the spurious EGP oscillations, while fitting the glucose concentrations within the expected experimental error.

Statistical analysis. All fluxes were normalized per square meter of body surface area (BSA), which was calculated according to the equation of Gehan and George, as reported in Bailey and Briars (1). Differences between groups were compared by the Mann-Whitney U-test. Associations between variables were first tested by the Spearman rank correlation test; when statistically significant ($P < 0.05$), linear regression was used to obtain further information.

RESULTS

The results of one case (subject 1) are presented in Fig. 2. After the initial washout curve of the [6,6-$^2$H$_2$]glucose prime, the tracer concentration reached a steady value at the end of the basal period, fluctuated at the beginning of the insulin infusion, and then stabilized during the remainder of the study. The solid line of the top panel shows the time course of the tracer concentration as predicted by the model, i.e., following the estimation of the parameters of glucose kinetics and insulin action in this subject. The small deviation of the predicted from the experimental data indicates that the model described accurately the individual characteristics of insulin action. In response to the low- and high-dose insulin infusions (i.e., 20 and 200 mU·min$^{-1}$·m$^{-2}$), plasma insulin rapidly reached stable plateaus at ~55 and 500 µU/ml, respectively. Although slower, the exogenous glucose infusion also reached quasi-stable rates during the last 20–30 min of each insulin step, averaging 867 and 2,671 µmol·min$^{-1}$·m$^{-2}$, respectively. The insulin plot, in addition to the curve representing the experimental plasma insulin concentrations, shows the delayed plasma insulin concentration as predicted by the model. This delay is also evident when comparing the time course of glucose clearance to the plasma insulin concentration profile. Whole body glucose clearance is shown as estimated by use of the Michaelis-Menten model with the disturbance term (solid line) and the time-varying E(t) approach (broken line curve). The visual comparison between these two curves gives an idea of how accurate the hypothesis is that the insulin dose-response curve follows Michaelis-Menten kinetics. Figure 2 also depicts the time course of EGP, which, in this subject, was stable in the basal period, rapidly halved in response to
The low-dose insulin infusion, and was completely suppressed by the high-dose insulin infusion.

The mean values of the model parameters in the basal state were: \( E_b \), 0.023 ± 0.002; \( \lambda_1 \), 5.6 ± 1.6 min\(^{-1} \); \( \lambda_2 \), 0.38 ± 0.04 min\(^{-1} \); \( \lambda_3 \), 0.040 ± 0.007 min\(^{-1} \); \( w_1 \), 0.45 ± 0.04; \( w_2 \), 0.48 ± 0.04. On average, the coefficients of variation of these parameters, as given by the least squares algorithm, were: <1% for \( E_b \), 22% for \( \lambda_1 \), 60% for \( \lambda_3 \), and 30% for \( w_1 \) and \( w_2 \). This indicates that the model parameters in the basal state are reasonably well defined, although the estimate of the fastest exponential of the periphery impulse response (\( \lambda_3 \)) was less precise. During the non-steady-state period, the coefficients of variation of the Michaelis-Menten and the delay parameter \( \alpha \) were <1% on average. On average, the disturbance term \( E_d(t) \) was not different from zero at all time instants from 0 to 60 min. The accuracy of model-predicted tracer concentrations in the whole study group can also be appreciated from Fig. 3. On average, the model-predicted curve matched the experimental data closely. Bars with twice the SE of the model residuals (i.e., the difference between the measured and the predicted tracer concentration) crossed the zero line at virtually all time points. This indicates that residuals were not different from zero throughout. Furthermore, the average values of the standard deviation of the model residual in the basal period and during the non-steady-state periods were virtually identical (basal SD = 3.1, low-insulin SD = 2.6, high-insulin SD = 3.8 µmol/l). These values correspond to a coefficient of variation of ~3%, which is of the same magnitude as the expected error of the plasma tracer concentration measurement. This indicates that the model error was largely explained by the experimental error.

Figure 4 shows the time course of plasma glucose clearance (separately for lean and obese subjects) as predicted by the model with (solid line) or without (broken line) the Michaelis-Menten assumption. In this calculation, the disturbance term \( E_d(t) \) (see Methods) was not included in individual calculation. Error bars are SE shown at 10-min intervals. Basal EGP averaged 303 ± 65 µmol·min\(^{-1} \)·m\(^{-2} \) in the seven subjects; the low-insulin infusion suppressed this flux more in lean than in obese subjects (78 ± 7 vs. 44 ± 7%, \( P < 0.05 \)), whereas the high-insulin infusion suppressed EGP to a similar extent in both [85 ± 19% vs. 83 ± 8%, \( P = \text{not significant (NS)} \)].

The individual values of plasma glucose and insulin concentrations, glucose clearance, and the model-derived parameters are given Table 2 for lean and obese subjects, whereas the individual Michaelis-Menten functions are plotted in Fig. 5. The EC\(_{50} \) was lower (\( P < 0.04 \)) in lean than in obese subjects. In the latter, a blunted response to insulin stimulation was particularly pronounced at physiological insulin values. Despite the small number of subjects studied, indexes of insulin sensitivity, such as ISI or the glucose clearance at a plasma insulin of 100 µU/ml, were clearly correlated with basal insulin concentration (\( I_b \)), body mass index (BMI), and waist-to-hip ratio (WHR), as expected. For instance, ISI was inversely correlated with \( I_b \) (\( r = 0.87, P < 0.02 \)), BMI (\( r = 0.80, P < 0.05 \)) and WHR (\( r = 0.86, P < 0.02 \)) (all correlations are performed on logarithmic transformed values). The correlation of ISI with \( I_b \), BMI, and WHR was due to EC\(_{50} \) (r values of 0.6–0.8, \( P < 0.05 \)), because CI\(_{\text{max}} \) was not correlated with these variables (\( P = \text{not significant} \)).

Moreover, in the non-obese subjects, the \( t_{1/2} \) and ISI were reciprocally related to one another (\( r = 0.92, P < 0.05 \)), indicating that the higher the insulin sensitivity, the longer the delay in insulin action. In accordance with this prediction, when measured at the presumed steady state (i.e., after 80 min of each insulin infusion), glucose clearance underestimated the true steady-state clearance.
clearance (as calculated by the model) by an amount that was directly related to $t^{1/2}$ ($r = 0.72, P = 0.02$).

DISCUSSION

The present study was prompted by the need for a single test providing a more complete description of the whole-body response to insulin stimulation. By combining a circulatory (non-compartmental) model of glucose kinetics with a simple model of insulin action (exponential delay), we were able to make use of non-steady-state data to determine in vivo the full dose response of insulin in the form of a Michaelis-Menten function. This approach has several advantages. First, it shortens the duration of the test, even in comparison with the single-day sequential clamp protocol. In our experiment, insulin stimulation lasted 3.5 h, whereas 6 h are required to create three hyperinsulinemic steady states, i.e., the minimum necessary to define a Michaelis-Menten curve. By using only the steady-state data of the present experiments, the generation of a Michaelis-Menten function failed in two insulin-resistant subjects, yielding negative EC50 values (results not shown).

Second, the test provides additional parameters (Table 2) that may be of interest in the study of the pathophysiology of glucose homeostasis. Third, the test is not strictly dependent on the experimental design, provided that large enough insulin gradients are created and quasi-steady-state periods are included. These advantages rely on the accuracy of the model of glucose kinetics and insulin action. The three main assumptions involved in the circulatory model of glucose kinetics (constant cardiac output, constant glucose mean transit time, and characteristics of heart and lung kinetics) appear to be rather robust, as recently discussed (14). In brief, whereas fractional clearance and mean transit time would be strongly affected by errors in cardiac output, volume and clearance (and thus the insulin sensitivity index), are less sensitive, and this dependence is progressively lost as steady state is approached. The heart and lung kinetics, being indirectly dependent on the insulin sensitivity index, are also less sensitive.

These assumptions are even less important in the circulatory model of glucose kinetics, because the contribution of this block to overall glucose kinetics is small and short-lived. Although experimental data are scarce, the insulin sensitivity index, estimated from the initial slope of the dose-response curve (see METHODS for details), is expressed as $ml \cdot min^{-1} \cdot m^{-2} \cdot l^{-1} \cdot mU$.

Table 2. Steady-state and model-derived metabolic parameters

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Glc, mM</th>
<th>Ins, mU/l</th>
<th>Cl, ml/min</th>
<th>Vol, ml/kg</th>
<th>$t_1/2$, min</th>
<th>EC50, mU/l</th>
<th>Clmax, ml/min</th>
<th>Cl100, ml/min</th>
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<td>71 ± 8</td>
<td>5.12 ± 0.38</td>
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<td>6.88</td>
<td>538</td>
<td>305</td>
</tr>
<tr>
<td>8</td>
<td>5.30</td>
<td>15</td>
<td>49</td>
<td>5.10</td>
<td>52</td>
<td>60</td>
<td>4.89</td>
<td>480</td>
<td>340</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>5.96 ± 0.44</td>
<td>23 ± 5</td>
<td>46 ± 10</td>
<td>5.88 ± 0.47</td>
<td>71 ± 10</td>
<td>58 ± 3</td>
<td>5.88 ± 0.57</td>
<td>534 ± 30</td>
<td>375 ± 15</td>
</tr>
</tbody>
</table>

Glc, plasma glucose concentration; Ins, plasma insulin concentration; Cl, glucose clearance; Vol, glucose distribution volume; $t_1/2$, delay between insulin appearance in plasma and insulin metabolic action; EC50, plasma insulin concentration inducing half-maximal response; Clmax, maximal glucose clearance; Cl100, predicted glucose clearance at a plasma insulin concentration of 100 mU/l; ISI, insulin sensitivity index, estimated from the initial slope of the dose-response curve.
the assumption that insulin enhances whole body glucose disposal by increasing fractional extraction without affecting transit times is supported by direct evidence (15). Furthermore, with a protocol that minimizes the changes in glucose specific activity, the calculation of glucose fluxes is almost model independent. More critical is the model of insulin action. The monoexponential delay used to represent the lag phase of insulin action has been successfully employed in other models (6, 13). The Michaelis-Menten function has been previously shown to suitably approximate the experimental dose-response data. In our experiments, this model successfully fit the observed tracer concentration profile during the entire test. As shown in Fig. 3, the mean of the differences between the predicted and the measured tracer concentrations (i.e., the residuals) at each time point was not different from zero. Furthermore, on average, the standard deviation of the residuals of the basal period (when the model error is presumably negligible) was virtually identical to that of the non-steady-state period and similar to the expected measurement error. This indicates that the deviation of the model-predicted tracer concentrations from the measured values is due mainly to measurement and not to modeling error. Another finding supporting the model of insulin action is that the time course of glucose clearance predicted by the model and that calculated without assuming a specific model of insulin action [time-varying E(t)] approach were very similar (Fig. 4).

The only data that were unable to fit properly in the plain Michaelis-Menten model in some subjects were at the start of the insulin infusion (0–60 min). From the analysis with the time-varying approach, we know that this failure is due to an irregular onset of insulin action during this phase. This is why the disturbance term (see METHODS) was used only in those first 60 min. Although there could be some physiological explanation for this transient perturbation (in some subjects, standing up to void), other reasons support the conclusion that neither the presence of irregularities nor the disturbance term invalidates the Michaelis-Menten model. First, the bulk of the experimental information for determining the parameters of the Michaelis-Menten model comes from the two quasi-steady-state periods (for both E_max and E_50) and from the rise in glucose clearance at the beginning of the high-insulin period (for α); during these periods, the disturbance is clearly irrelevant. In other words, the data points in the initial 60-min period, in which the disturbance is present, do not affect the estimation of E_max and E_50. Second, although the disturbance term was included in all subjects for uniformity of analysis, in some of them (e.g., the subject of Fig. 2) it could be omitted without a significant loss of fit, i.e., the plain Michaelis-Menten model was an adequate representation of the system in some cases. Third, on average, the disturbance term was not different from zero, and the time course of glucose clearance as predicted by the Michaelis-Menten model without the disturbance was very similar to that calculated by the time-varying fractional extraction approach (Fig. 4). This indicates that the deviations of the Michaelis-Menten model from the reality were not systematic and that even without E(t) this model is on average a good predictor of glucose clearance.

Although to minimize the errors in the estimation of the dose-response curves we have used an elaborate model of glucose kinetics, simplifications, such as the use of Steele’s model, could also be satisfactory, particularly with an experimental design that reduces the modeling error by minimizing the changes in specific activity. However, the use of Steele’s model would still require simulation of nonlinear differential equations and data fitting, as with the circulatory model, because of the coupling with the model of insulin action. Therefore, the potential loss of accuracy of the simplified models would not be accompanied by a significant increase in simplicity.

The physiological significance of the current results is obviously limited by the small number of subjects studied; nevertheless, comparison with existing information is possible. In agreement with previous studies, in our subjects obesity was associated with a twofold higher E_50 and similar C_max. In absolute terms, the estimates of C_max and E_50 generated by our model are higher than those previously reported by other laboratories (2, 7, 16, 17). However, already at the end of the second step of insulin infusion, we measured a mean steady-state (i.e., model-independent) glucose clearance rate of 480 ml·min⁻¹·m⁻² (Table 2), a value similar to previous steady-state estimates of C_max obtained at much higher plasma insulin concentrations. Similarly, Groop et al. (5) reported values of whole body glucose clearance of ~400 ml·min⁻¹·m⁻² in normal subjects at a plasma insulin concentration of 200 µU/ml above baseline. Therefore, differences in the estimated maximal insulin-stimulated glucose clearance may reflect the variability inherent in small groups of study subjects. Alternatively, because in our data the t½ of insulin action in normal subjects was longer when insulin was higher, it is possible that true C_max may have been underestimated in previous studies. Available estimates of E_50 vary widely (2, 7, 16, 17). This variability is, at least in part, related to experimental problems such as the use of protocols not optimized with regard to changes in glucose specific activity, actual attainment of steady state, or number of insulin steps. By modeling non-steady-state data, our approach makes use of a continuum of increasing glucose uptake values from basal to near-maximal and not just the few values that would be generated by the standard steady-state approach.

Of interest is the finding that the t½ of insulin action was not different between obese and lean subjects, although they had clearly different indexes of insulin sensitivity (Table 2). In contrast, previous analyses of insulin dose-response curves in lean and obese subjects have led to the conclusion that the half-time of insulin activation is prolonged in the obese (16). Although we cannot deny that our small study groups are not a representative sample of the respective populations, the discrepancy could be due to the method used by
Prager et al. (16) to estimate the $t_{1/2}$ of insulin action. When glucose uptake functions are expressed as a percentage of maximal glucose uptake, the closer glucose uptake is driven to saturation, the shorter is its apparent $t_{1/2}$. Because in obese subjects glucose uptake saturates at higher insulin concentrations than in lean subjects (higher $EC_{50}$), at the same insulin concentration, glucose uptake is less saturated in the obese than in the lean. Thus linear scaling of a nonlinear (saturable) process artificially shortens the $t_{1/2}$ of insulin-sensitive subjects more than that of insulin-resistant individuals. In contrast, our estimate of $t_{1/2}$ is obtained directly by fitting a model of insulin action to the data, thereby providing an unbiased estimate of insulin’s half-time of activation.

In conclusion, the test here presented exploits a more elaborate representation of insulin sensitivity than the standard euglycemic insulin clamp to characterize the full dose-response curve of insulin action in a single session of relatively short duration. It has the potential to prove useful in the clinical investigation of disorders of carbohydrate tolerance.

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