Integrated model of hepatic and peripheral glucose regulation for estimation of endogenous glucose production during the hot IVGTT

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Krudys, Kevin M., Michael G. Dodds, Stephanie M. Nissen, and Paolo Vicini. Integrated model of hepatic and peripheral glucose regulation for estimation of endogenous glucose production during the hot IVGTT. Am J Physiol Endocrinol Metab 288: E1038–E1046, 2005.—We developed a new model to describe endogenous glucose kinetics during a labeled (hot) intravenous glucose tolerance test (IVGTT) to derive a time profile of endogenous glucose production (EGP). We reanalyzed data from a previously published study (P. Vicini, I. J. Zachwieja, K. E. Yarasheski, D. M. Bier, A. Caumo, and C. Cobelli. Am J Physiol Endocrinol Metab 276: E285–E294, 1999), in which insulin-modified [6,6-2H2]glucose-labeled IVGTTs (0.33 g/kg glucose) were performed in 10 normal subjects. In addition, a second tracer ([U-13C]glucose) was infused in a variable rate to clamp the endogenous glucose tracer-to-tracer ratio (TTR). Our new model describing endogenous glucose kinetics was incorporated into the two-compartment minimal-model structure. The model gave estimates of glucose effectiveness [1.54 ± 0.31 (SE) mL·kg−1·min−1], insulin sensitivity (37.74 ± 5.23 104 dL·kg−1·min−1·μU−1·ml), and a new parameter describing the sensitivity of EGP to the inhibitory effect of insulin (IC50 = 0.0195 ± 0.0046 min−1). The model additionally provided an estimate of the time course of EGP showing almost immediate inhibition, followed by a secondary inhibitory effect caused by infusion of insulin, and a large overshoot as EGP returns to its basal value. Our estimates show very good agreement with those obtained via deconvolution and the model-independent TTR clamp technique. These results suggest that the new integrated model can serve as a simple one-step approach to obtain metabolic indexes while also providing a parametric description of EGP.

hot minimal model; intravenous glucose tolerance test; mathematical modeling; insulin sensitivity

AS THE BODY’S MAIN SOURCE of endogenous glucose production (EGP), the liver plays a crucial role in maintaining normal glucose homeostasis. In response to a glucose challenge, insulin is secreted, and the resultant hyperglycemia and hyperinsulinemia conspire to suppress hepatic glucose production and stimulate glucose uptake in splanchnic and peripheral tissues. During the course of disease progression, however, the liver may become resistant to the suppressive effects of glucose and insulin on glucose production. The resulting excess production acts to maintain the chronic hyperglycemic state observed in type 2 diabetics. It is therefore of great interest to accurately assess the dynamic regulation of EGP in response to a glucose perturbation.

The dynamic picture emerging from glucose and insulin’s time course after an intravenous glucose tolerance test (IVGTT) has allowed remarkable and unique insights into the pathophysiology of glucose homeostasis (8), especially when coupled with the parametric information available when the data are analyzed with the minimal model of glucose disappearance (9). Over the years, the metabolic indexes of glucose effectiveness (3) and insulin sensitivity (38) estimated via the minimal model have been subjected to experimental validation against equivalent experimental designs, and insulin sensitivity has been found to be an informative predictor of cardiovascular risk (27) and to be strongly related to genetic factors (10). New protocols that modify the standard IVGTT to facilitate minimal-model parameter estimation have also been proposed, such as an infusion of insulin (22) or tolbutamide administration (7) and variations thereof (39, 40).

Among other modifications of the standard IVGTT, the addition of a tracer of glucose to the glucose bolus was proposed as a way to dissect the respective roles of liver and periphery in glucose dynamics (14). This augmented information allowed clarification of the impact of some of the underlying assumptions of the original minimal model, in particular the single-compartment approximation for glucose distribution (15), which does not allow a plausible estimate of EGP in the early portion of the test, and seems to impact the estimate of glucose effectiveness more severely than does insulin sensitivity (19). Although the original minimal model remains a very powerful and extremely useful tool for the individual assessment of metabolic indexes, these conclusions show novel experimental and modeling directions for enhancing our understanding of the regulation of glucose dynamics.

The labeled (hereafter hot) IVGTT interpreted with the two-compartment minimal model of glucose kinetics (43) has been shown to be a valuable research method to estimate metabolic indexes such as insulin sensitivity (SI) and glucose effectiveness (Sg). Furthermore, by using the two-compartment model structure as the impulse response of the glucose system, a physiologically plausible profile of EGP can be obtained via deconvolution (44). Recently, the deconvolution approach has been validated with the tracer-to-tracee ratio (TTR) clamp technique, which uses a model-independent method to estimate EGP under the constraint that glucose specific activity is held constant throughout the IVGTT (45). Although the use of a deconvolution technique minimizes the role of the model used to represent the glucose system response (35), neither one of these techniques provides a mechanistic description of liver glucose production, nor of its regulation by glucose and insulin. Moreover, deconvolution can be computationally demanding and requires nontrivial choices of a regularization parameter and virtual grid (44). The TTR clamp...
MODELING ENDOGENOUS GLUCOSE PRODUCTION DURING THE IVGTT

The aim of this study is to build a description of EGP during the hot IVGTT into the two-compartment minimal model of glucose kinetics (43). The new model attempts to take into account autoregulation of hepatic glucose production by hyperglycemia (29, 36) as well as the indirect action of insulin to inhibit hepatic glucose production (1). In addition to a physiologically plausible estimate of the time course of EGP during the IVGTT, the new model provides the index IC50, reflecting the sensitivity of suppression of EGP to insulin. To test the performance of the new model, we reanalyzed data from a previous study (45) of labeled IVGTTs performed in healthy subjects and compared the resulting estimates of EGP with those obtained from deconvolution and the TTR clamp technique.

EXPERIMENTAL DESIGN AND METHODS

Database

The data analyzed in this study were obtained from 10 stable-labeled IVGTTs performed in healthy young adults, as previously reported by Vicini et al. (45) For further details on study design, protocol, and measurements, we refer the reader to Ref. 45. Briefly, 0.33 g/kg glucose, labeled with [6,6-2H2]glucose, was rapidly administered as a bolus at time 120 min, after a 2-h (0–120 min) primed (7 mg/kg) constant intravenous infusion (70 μg·kg⁻¹·min⁻¹) of [U-13C]glucose used to assess basal EGP. The amount of labeled glucose in the dose was 10% of the unlabeled. The IVGTT was modified with a 5-min infusion of regular human insulin (0.03 U/kg) starting at time 20 min of the IVGTT. Throughout the IVGTT, [U-13C]glucose was continuously infused in a stepwise fashion according to the anticipated pattern of EGP to maintain a constant TTR. Blood samples were taken during the [U-13C]glucose primed infusion at 0, 2, 3, 5, 8, 10, 15, 20, 30, 45, 60, 75, 90, 100, 110, and 120 min and during the IVGTT at 122, 123, 124, 125, 126, 128, 130, 132, 134, 136, 139, 142, 144, 146, 148, 150, 155, 160, 170, 175, 180, 190, 200, 220, 240, 260, 280, 300, 330, and 360 min. Each sample was assayed for glucose and insulin, as well as the complete mass spectra for [U-13C]glucose and [6,6-2H2]glucose.

The TTR (the stable label equivalent of specific activity) in the accessible pool and is expressed as the sum of a constant component of glucose disposal; G(t), cold glucose concentration

\[
dx(t) = -p_1[x(t) - s_0(t - t_1)] \quad x(0) = 0 \quad (1c)
\]

\[
g(t) = \frac{q_1(t)}{V_1} \quad (1d)
\]

where \(q_1\) and \(q_2\) are glucose tracer masses (mg/kg for a stable-label IVGTT) in the accessible and slowly equilibrating compartments, respectively, \(x(t) = k_1 \cdot I'(t)\) is insulin action (min⁻¹) with \(I'(t)\) denoting the concentration of insulin in a compartment remote from plasma (μU/ml), \(g(t)\) is plasma hot glucose concentration (mg/dl), \(R_{a,0}\) is the constant component of glucose disposal accounting for the inhibition of glucose clearance by glucose itself, assumed to be 1 mg·kg⁻¹·min⁻¹ (11), \(V_1\) is the volume of the accessible pool (ml/kg), \(G(t)\) is the glucose concentration in the accessible pool (mg/dl), \(d\) is the hot glucose dose (mg/kg), \(k(I)\) and \(I_0\) are plasma insulin and basal insulin concentrations (μU/ml), respectively, \(k_0\), \(k_1\), \(k_2\), and \(k_02\) are constant rate parameters (min⁻¹), and \(p_2 = k_0\) (min⁻¹) and \(s_0 = k_0k_2k_0\) (ml·μU⁻¹·min⁻¹) are insulin action parameters.

Insulin-independent glucose disposal is assumed to occur in the accessible pool and is expressed as the sum of a constant component and a dynamic component proportional to glucose mass, given by

\[
k_p + \frac{R_{a,0}}{G(V_1)\cdot I(t)}
\]

where insulin-independent glucose disposal, occurring in the slowly equilibrating compartment, is controlled by insulin in a compartment remote from plasma, and is described by

\[
k_{02} + x(t)
\]

To ensure a priori identifiability, it is assumed that insulin-independent glucose disposal is three times insulin-dependent glucose disposal in the basal steady state (17), yielding the constraint

\[
k_p + \frac{R_{a,0}}{G(V_1)\cdot I(t)} = \frac{3k_1k_02}{k_02 + k_12}
\]

where \(G_b\) is basal glucose concentration (mg/dl). The resulting set of uniquely identifiable parameters is thus: \(V_1, k_{21}, k_{12}, k_{02}, p_2, \) and \(s_0-\)

Two-Compartment Hot Minimal Model

The two-compartment hot minimal model (13, 43), as shown in

Fig. 1. Two-compartment minimal model. \(q_1\) and \(q_2\), hot glucose masses in the accessible and slowly equilibrating compartments, respectively; \(I'\), insulin concentration in a compartment remote from plasma; \(k_0\), kinetic rate parameters; \(k_0\), \(k_0\), and \(k_0\) insulin action rate parameters; \(k_0\), the proportionality constant; \(d\), hot glucose dose; \(V_1\), volume of the accessible compartment; \(R_{a,0}\), the constant component of glucose disposal; \(G(t)\), cold glucose concentration in the accessible compartment; \(g\), hot glucose concentration in the accessible compartment. Plasma insulin refers to insulin concentration in plasma above basal. See Eqs. 1a–1d in the text for further model parameterization details.
The two-compartment hot minimal model further provides estimates of model-derived parameters of interest, such as $S_{G}^*$, plasma clearance rate (PCR), and $S_{G}^*$. For derivations of model-derived parameters, we refer to Ref. 43. $S_{G}^*$ (dl·kg$^{-1}$·min$^{-1}$) reflects the ability of glucose per se to stimulate glucose disposal and is given by

$$S_{G}^* = V_{1} \left( k_{p} + \frac{k_{21}k_{02}}{k_{02} + k_{12}} \right)$$

The steady-state PCR (ml·kg$^{-1}$·min$^{-1}$) is defined as

$$PCR = V_{1} \left( k_{p} + \frac{R_{d}}{V_{G}} + \frac{k_{21}k_{02}}{k_{02} + k_{12}} \right)$$

and insulin sensitivity, $S_{G}^*$ (ml·kg$^{-1}$·min$^{-1}$·U$^{-1}$·ml), or the ability of insulin to enhance the glucose stimulation of glucose disposal is

$$S_{G}^* = V_{1} s_{u} \frac{k_{21}k_{12}}{(k_{02} + k_{12})^2}$$

Two-Compartment Minimal Model With EGP

To describe endogenous glucose kinetics during the IVGTT, a model for liver glucose metabolism was incorporated into the two-compartment hot minimal model framework. We chose to use the indirect response modeling paradigm often used in pharmacodynamics (41) to describe the control of insulin and glucose on hepatic glucose production. Such models are useful in situations when the response to an input is controlled by the production or loss of a mediator variable. The amount of releasable glucose in a hypothetical liver compartment was described by a combination of indirect response modeling paradigm often used in pharmacodynamics (41) to describe the control of insulin and glucose on hepatic glucose production. All other parameters are equivalent to those of the 2-compartment minimal model described in Eqs. 1a–1d, since exogenous and endogenous glucose kinetics should be indistinguishable once they reach the plasma compartment.

The new expression for glucose production was thus incorporated into the two-compartment minimal model framework, resulting in the two-compartment minimal model with EGP (Fig. 2). The complete set of model equations is, therefore,

$$\frac{dG_{1}(t)}{dt} = k_{in} \times [1 - H_{1}(t)] - k_{out} \times [1 - H_{1}(t)] \cdot G_{1}(t) \quad (8)$$

where $G_{1}$ is the amount of available glucose in the liver (mg/kg), $k_{in}$ (mg·kg$^{-1}$·min$^{-1}$) is the apparent zero-order rate constant of hepatic glucose formation, and $k_{out}$ (min$^{-1}$) is the first-order rate constant describing the loss of hepatic glucose into the systemic circulation. $H_{1}(t)$ and $H_{2}(t)$ are inhibitory functions reflecting the ability of glucose and remote insulin, respectively, to inhibit glucose production. The inhibition of EGP attributable to elevated glucose concentration was modeled as

$$H_{1}(t) = \frac{G(t)}{G_{h} + G(t)} \quad (9)$$

where the location of inhibition is glucose formation ($k_{in}$), reflecting the ability of glucose to inhibit its own formation through glycogenolysis (29, 36). For simplicity, we assumed glucose inhibition of glucose production is half-maximal under basal conditions. Insulin’s inhibitory effect on EGP is represented by

$$H_{2}(t) = \frac{x(t)}{IC_{50} + x(t)} \quad (10)$$

where IC$_{50}$ (min$^{-1}$) is the insulin action producing 50% of maximum inhibition of glucose production. Here, we assumed insulin acts in a remote compartment to inhibit the rate at which glucose is released from the liver. Because insulin has been shown to act by both direct and indirect mechanisms (1, 5, 24, 25), the term $H_{2}(t)$ reflects a combination of both effects. At steady-state, $G_{1}(t) = G_{h}$ and $x(t) = 0$, giving the initial condition:

$$G_{1}(0) = \frac{k_{in}}{2 \times k_{out}} \quad (11)$$

The set of parameters distinctive of the two-compartment minimal model with EGP model is therefore: $k_{in}$, $k_{out}$, and IC$_{50}$. The remaining parameter values ($V_{1}$, $k_{21}$, $k_{12}$, $k_{02}$, $p_{2}$, and $s_{u}$) are assumed to be equivalent to those of the two-compartment minimal model described in Eqs. 1a–1d, since exogenous and endogenous glucose kinetics should be indistinguishable once they reach the plasma compartment.

The new expression for glucose production was thus incorporated into the two-compartment minimal model framework, resulting in the two-compartment minimal model with EGP (Fig. 2). The complete set of model equations is, therefore,

$$\frac{dG_{1}(t)}{dt} = k_{in} \times [1 - H_{1}(t)] - k_{out} \times [1 - H_{1}(t)] \cdot G_{1}(t) \quad (12a)$$

$$\frac{dE_{G_{1}}(t)}{dt} = \left[ \frac{k_{1} + k_{out}}{V_{G}} + k_{21} \right] E_{G_{1}}(t) + k_{12}E_{G_{2}}(t) + k_{out} \times [1 - H_{2}(t)] \cdot G_{1}(t) \quad (12b)$$

$$\frac{dE_{G_{2}}(t)}{dt} = k_{21}E_{G_{1}}(t) - [k_{02} + x(t) + k_{12}]E_{G_{2}}(t) \quad (12c)$$

$$\frac{dx(t)}{dt} = -p_{2}[x(t) - s_{u}[1(t) - k_{1}]] \quad x(0) = 0 \quad (12d)$$

$$G_{1}(t) = \frac{EG_{1}(t)}{V_{1}} \quad (12e)$$

where $EG_{1}$ and $EG_{2}$ now represent endogenous glucose masses (mg/kg) in the accessible and slowly equilibrating compartments, respectively, and $G_{1}(t)$ is the endogenous glucose concentration (mg/dl). Note the accessible pool and slowly equilibrating compartment now have nonzero initial conditions, since endogenous glucose is present at steady state. Last, the models allow the prediction of total glucose concentration as the algebraic sum of the exogenous and endogenous components:

$$G(t) = G_{1}(t) + g(t) = \frac{EG_{1}(t)}{V_{1}} + \frac{q(t)}{V_{1}} = \frac{EG_{1}(t) + q(t)}{V_{1}} \quad (13)$$

In the modeling, we have neglected the presence of the [U-13C]glucose tracer for simplicity and because normally the hot IVGTT is
carried out with only one tracer. The amount of [U-13C]glucose is small by comparison with the other species, and including it in the model did not change the results appreciably (data not shown).

Estimation of Hepatic Glucose Production

Radziuk’s two-compartment model. EGP(t) was also estimated using non-steady-state theory by means of the TTR clamp and Radziuk’s two-compartment model with one time-varying loss from the accessible pool (37). Although the TTR clamp technique is experimentally laborious and rarely used in practice, non-steady-state theory predicts that, as long as the TTR is held constant, a reliable estimate of EGP can be obtained regardless of the appropriateness of the model used to describe non-steady-state glucose kinetics (i.e., Steele’s one-compartment model or Radziuk’s two-compartment model will perform similarly; see Ref. 45). Therefore, although the TTR clamp technique does require a model, it is in a sense model-independent, in that model error is minimized by the protocol. Briefly, in Ref. 45, two-compartment model parameters were individually identified from data obtained from the 2-h (0–120 min) primed, constant infusion of [U-13C]glucose. To limit the impact of experimental noise in the calculations, both endogenous glucose and tracer-to-tracee data were smoothed with a three-point moving average. EGP(t) can then be related to the TTR, endogenous glucose concentration, and model parameters through an algebraic equation. For further details on EGP(t) estimation with this method, we refer the reader to Refs. 16 and 45.

Deconvolution. The deconvolution approach to EGP(t) estimation assumes the same model structure described by Eqs. 1a–1d but reconstruits the time course of EGP by using a nonparametric deconvolution algorithm. Briefly, the integral equation relating the input (EGP) to the output, endogenous glucose concentration (Gi) is given by:

\[ G_i(t) = \int_0^t h(t,\tau)E(t)E(t)\,d\tau + G_0 \]  

(14)

where \( h(t,\tau) \) (kg/dl) is the time-varying impulse response of the glucose system as determined by the two-compartment minimal model identified from [6,6-2H2]glucose data. A recently developed stochastic deconvolution algorithm was used in Ref. 45 to invert the integral equation, thereby resulting in a time-continuous estimate of EGP(t). For further details of the algorithm and examples of its application to similar problems, we refer to Refs 30, 33, 34, 44, and 45.

Two-compartment minimal model with glucose production. The model framework proposed in Eqs. 12a–12e was used to model endogenous glucose data. The input to this model is therefore the total exogenous glucose dose. The two-compartment minimal model with EGP (Eqs. 12a–12e) was used to model endogenous glucose data. The input to this model is thus the EGP (Eq. 15). Total glucose measurements were described by the summation of the two models’ predictions (Eq. 13). The combined input to this model is therefore given by both endogenous (hepatic) and non-steady-state glucose kinetics (i.e., Steele’s one-compartment model or Radziuk’s two-compartment model will perform similarly; see Ref. 45). Therefore, although the TTR clamp technique does require a model, it is in a sense model-independent, in that model error is minimized by the protocol. Briefly, in Ref. 45, two-compartment model parameters were individually identified from data obtained from the 2-h (0–120 min) primed, constant infusion of [U-13C]glucose. To limit the impact of experimental noise in the calculations, both endogenous glucose and tracer-to-tracee data were smoothed with a three-point moving average. EGP(t) can then be related to the TTR, endogenous glucose concentration, and model parameters through an algebraic equation. For further details on EGP(t) estimation with this method, we refer the reader to Refs. 16 and 45.

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RESULTS

Model fits to endogenous and exogenous glucose data sets were acceptable in all subjects. Plots of weighted residuals (means ± SD) are shown in Fig. 3 and show no systematic deviations. Individual parameter estimates of the two-compartment model with EGP are summarized in Table 1, along with their precisions. Mean values ± SE of parameters specific to the glucose production model are: \( k_{\text{in}} = 5.22 ± 0.55 \) ml·kg⁻¹·min⁻¹, \( k_{\text{out}} = 0.133 ± 0.044 \) min⁻¹ and IC50 = 0.0195 ± 0.0046 min⁻¹. Precisions for the glucose production parameters were elevated relative to minimal model parameters, but were still satisfactory. Table 2 contains estimates of the model-derived metabolic indexes (SG*, PCR, and SI*).

EGP Estimation

Each individual’s estimated time course of EGP, as calculated by Radziuk’s two-compartment model, deconvolution, and the two-compartment minimal model with EGP, is reproduced in Fig. 4. In all three cases, almost complete inhibition of EGP was observed during the first 5–20 min of the IVGTT. Because of experimental errors in clamping the TTR in the early portion of the test, estimates obtained with the two-compartment model of Radziuk occasionally gave rise to unphysiological oscillations in the rate of EGP, often resulting in negative values. The two-compartment minimal model with EGP, however, produced physiologically plausible estimates of EGP immediately after the glucose injection. Furthermore, the bimodal pattern of EGP during the first 40 min of the IVGTT suggests the new method most clearly reflects the expected effect of the exogenous insulin infusion (140–145 min of the experimental protocol) on inhibition of EGP. The timing of this secondary effect is delayed relative to the insulin infusion, reflecting insulin’s indirect effect and its ability to suppress EGP from a compartment remote from plasma. Following this period, all three models predict an overshoot as EGP returns to pretest levels. Interindividual variability in both the timing and magnitude of the resumption of EGP is evident in Fig. 4. Examination of the terminal phase of the curves shows the deconvolution estimate consistently underestimates basal EGP relative to the two-compartment method of Radziuk, as mentioned in Ref. 45, whereas our new method provides a basal estimate in good agreement with Radziuk. In general, all three
methods are in close accordance throughout the duration of the IVGTT.

**EGP Inhibition**

The mean profiles of the inhibitory terms $H_1(t)$ and $H_2(t)$ (Fig. 5) were examined in an attempt to detect differences between the time courses of glucose and insulin inhibition on EGP. One can see from Eq. 9 that the glucose inhibitory term, $H_1(t)$, is equal to 0.5 in the basal state, since half-maximal inhibition is assumed at fasting glucose concentration. At the beginning of the experiment, $H_1(t)$ exhibits a rapid, although relatively small, increase over its baseline value, reflecting the effect of the glucose bolus. As plasma glucose levels fall below basal $\sim 60$ min after the glucose challenge, $H_1(t)$ actually works to increase the influx of glucose in the liver compartment, suggesting glucose may be responsible in our model for the overshoot of EGP as it returns to baseline. The time course of $H_2(t)$ is zero at baseline, since insulin action is assumed zero at basal insulin levels. Again, the profile is biphasic because of the effects of the exogenous insulin infusion on suppression of EGP. Temporal and functional distinctions between the two inhibitory terms were not easily discernible, since both $H_1(t)$ and $H_2(t)$ return to baseline roughly 120 min after the glucose bolus. However, insulin appears to be a more potent inhibitor of EGP in our model, since $H_2(t)$ values reached a maximum of 80–90% on average above baseline, whereas glucose exerted a smaller effect (10–20% above baseline) on glucose production.

**DISCUSSION**

The two-compartment minimal model of glucose kinetics was proposed primarily to overcome the fact that the one-compartment model provides an unphysiological time course of hepatic glucose production during a labeled IVGTT (13). However, to obtain a physiologically plausible time course of EGP, the two-compartment model must first be identified and subsequently fed to a deconvolution algorithm to serve as a description of the impulse response of the glucose system. In this work, the two-compartment minimal model is extended to include a mechanistic description, based on pharmacodynamic concepts, of hepatic glucose production and its control by glucose and insulin. In this manner, we identify two-compartment minimal model parameters while simultaneously providing a physiologically plausible reconstruction of the time course of EGP. The EGP estimates obtained from the new model compare very well with independent estimates obtained from deconvolution and the model-independent TTR clamp technique.

Indirect response models are often used in pharmaceutical applications in cases where the time course of the pharmacodynamic response of a drug is delayed with respect to the time course of the drug’s concentration in plasma. This characteristic makes this type of model compatible with the delayed, indirect fashion in which insulin has been postulated to inhibit EGP. According to the “single gateway hypothesis,” the remote insulin’s site of action is the adipose tissue, where insulin acts to suppress lypolysis, resulting in a reduction in plasma free fatty acids and subsequent inhibition of EGP (8). Insulin, however, is also believed to act directly by activation of glycogen synthase and inhibition of glycogen phosphorylase, as well as suppression of key gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (5, 24, 25). Although the predictive performance of our model suggests an indirect mechanism is primarily responsible for EGP inhibition, the inhibitory term describing insulin’s effect on EGP, $H_2(t)$, can only be viewed as a composite of the different possible mechanisms we outlined.

Previous work suggests EGP is suppressed slowly with respect to the time course of plasma glucose concentration (45), suggesting a delayed signal is responsible for EGP inhibition by glucose as well. Given this delay, and the fact that EGP is thought to be negatively correlated with plasma glucose concentrations (9), we included a glucose-dependent inhibitory term in our indirect response modeling framework. We postulate that this inhibitory term acts on the rate at which glucose is made available in the liver and is thus modulated by the constant $k_m$. This inhibition most likely represents the ability of hyperglycemia to inhibit glucogenolysis, mainly through the glycogen phosphorylase pathway (36). Although others have suggested that glucose’s suppressive effect is negligible during the IVGTT (26), its inclusion in our model greatly improved model performance. Furthermore, during the IVGTT, one-third of the effect of glucose on net glucose disappearance is the
result of suppression of hepatic glucose output (2), so exclusion of this term would likely result in an incomplete description of EGP. Because we include endogenous glucose, which acts both to stimulate glucose disposal and inhibit glucose production, in the estimation of $S_G^2$, it is in fact imperative that we include a separate description of glucose’s effect on EGP, since $S_G^2$ only describes the ability of glucose to stimulate glucose disposal. Some concerns still remain regarding the estimation of glucose effectiveness, especially when comparing subjects with varying glucose tolerances (23) or under the conditions regarding this parameter and the best tactic to deter-

### Table 1. Estimated metabolic parameters

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>$V_1, \text{dl/kg}$</th>
<th>$k_{21, \text{min}^{-1}}$</th>
<th>$k_{12, \text{min}^{-1}}$</th>
<th>$k_{01, \text{min}^{-1}}$</th>
<th>$p_{21, \text{min}^{-1}}$</th>
<th>$s_k, \text{ml/min}^{-1} \mu U^{-1}$</th>
<th>$k_{16}, \text{mg/kg}^{-1} \text{min}^{-1}$</th>
<th>$k_{20, \text{min}^{-1}}$</th>
<th>$IC_{50}, \text{min}^{-1}$</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>2.11 (3)</td>
<td>0.025 (10)</td>
<td>0.057 (50)</td>
<td>0.0115 (40)</td>
<td>0.3779 (52)</td>
<td>0.0106 (42)</td>
<td>5.45 (5)</td>
<td>0.103 (35)</td>
<td>0.0211 (62)</td>
</tr>
<tr>
<td>2</td>
<td>1.63 (2)</td>
<td>0.042 (6)</td>
<td>0.021 (18)</td>
<td>0.0018 (12)</td>
<td>0.1056 (13)</td>
<td>0.0013 (9)</td>
<td>4.78 (4)</td>
<td>0.043 (30)</td>
<td>0.0016 (32)</td>
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<td>3</td>
<td>2.35 (3)</td>
<td>0.015 (25)</td>
<td>0.004 (10)</td>
<td>0.0043 (9)</td>
<td>0.1543 (15)</td>
<td>0.0029 (15)</td>
<td>4.06 (3)</td>
<td>0.027 (30)</td>
<td>0.0223 (30)</td>
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<td>0.0015 (7)</td>
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<td>0.055 (45)</td>
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<td>0.0081 (21)</td>
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<td>0.255 (41)</td>
<td>0.0080 (35)</td>
<td>0.1265 (15)</td>
<td>0.0053 (48)</td>
<td>3.39 (5)</td>
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<td>0.1637 (20)</td>
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<td>0.141 (52)</td>
<td>0.0143 (34)</td>
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<td>0.0242 (49)</td>
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<td>0.0643 (10)</td>
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</table>

Mean±SE 1.91±0.92 0.066±0.012 0.106±0.024 0.0606±0.00012 0.1307±0.0297 0.0393±0.0010 5.22±0.55 0.133±0.044 0.1995±0.0046

Estimated parameters from the kinetic models describing [6,6-3H]glucose, endogenous glucose, and total glucose concentrations. Precisions of the parameter estimates are shown in parentheses and are expressed as %CV. $V_1$, volume of the accessible pool; $k_{ij}$, kinetic rate parameters; $p_{21}$, parameter value = $k_{01}$; $s_k$, parameter value = $k_{01}/k_{20}$, where $k_{01}$, $k_{20}$, and $k_{16}$ are insulin action rate parameters; $k_{16}$, rate constant of hepatic glucose formation; $k_{20}$, kinetic rate of hepatic glucose secretion; $IC_{50}$, insulin action producing half-maximal inhibition of glucose production.

when its use is extended to estimating EGP, by relaxing the assumption that the same insulin signal acts on the liver and the peripheral tissues (14). This assumption was tested by including separate descriptions of insulin’s action on the liver and the periphery. In general, we found close agreement between the two profiles, with no significant delay between them (data not shown). Our results agree with a previous study aimed at partitioning insulin action into its effects on glucose distribution, glucose disposal, and EGP (26). In the present study, inclusion of a separate description of insulin action on the liver, however, resulted in poor model convergence and parameter accuracy with no improvement in the Akaike Information Criterion and was therefore not included in the final model.

Our model provides a new index, $IC_{50}$, which serves as an estimate of insulin sensitivity of suppression of EGP. Specifically, this parameter is defined as the amount of insulin action required to inhibit EGP by 50%, with lower values reflecting greater sensitivity. Recently, a comparison of clamp studies and oral glucose tolerance tests performed in the same subjects suggested that suppression of EGP by insulin is the single most important determinant of glucose tolerance (6). It would therefore be of interest to extend our studies to glucose-intolerant subjects and validate our results against independent methods to determine whether estimation of $IC_{50}$ in the new model can be an inexpensive and powerful predictor of glucose tolerance in the nonsteady state.

With our new model, we were able to obtain time courses of EGP in good agreement with deconvolution and TTR clamp estimates. Vicini et al. (45) have expressed concerns that the deconvolution estimate consistently underestimates basal glucose clearance in relation to the TTR estimate. The new model, however, appears to provide better agreement with the model-independent method, as seen in the terminal portions of the curves in Fig. 3. This result may seem puzzling at first, considering both deconvolution and our new method use the same two-compartment minimal-model structure. The new method, however, identifies two-compartment minimal parameters from hot glucose, endogenous glucose, and total glucose concentrations, as opposed to the deconvolution approach, which uses only hot glucose data for model identification. The information provided by the additional data sets likely drives the estimation to a more suitable estimate of basal EGP.

### Table 2. Estimated metabolic indexes

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>PCR, ml/kg^{-1}min^{-1}</th>
<th>$S_C^2$, \times 10^4 \text{ dl/kg}^{-1} \text{ min}^{-1} \mu U^{-1} \text{ ml}^{-1}$</th>
<th>$S_C^2$, ml/kg^{-1}min^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.45 (5)</td>
<td>66.6 (19)</td>
<td>2.29 (8)</td>
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<tr>
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<td>35.4 (8)</td>
<td>1.01 (12)</td>
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<tr>
<td>3</td>
<td>2.43 (5)</td>
<td>38.6 (13)</td>
<td>1.13 (11)</td>
</tr>
<tr>
<td>4</td>
<td>1.63 (5)</td>
<td>18.9 (5)</td>
<td>0.51 (15)</td>
</tr>
<tr>
<td>5</td>
<td>4.36 (4)</td>
<td>61.7 (14)</td>
<td>3.20 (5)</td>
</tr>
<tr>
<td>6</td>
<td>1.54 (11)</td>
<td>24.6 (20)</td>
<td>0.41 (32)</td>
</tr>
<tr>
<td>7</td>
<td>3.98 (8)</td>
<td>47.0 (29)</td>
<td>2.87 (11)</td>
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<tr>
<td>8</td>
<td>3.02 (6)</td>
<td>36.0 (22)</td>
<td>1.77 (10)</td>
</tr>
<tr>
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<td>2.14 (6)</td>
<td>30.4 (8)</td>
<td>0.75 (18)</td>
</tr>
<tr>
<td>10</td>
<td>2.59 (6)</td>
<td>18.2 (7)</td>
<td>1.39 (11)</td>
</tr>
</tbody>
</table>

Mean±SE 2.73±0.30 37.74±5.23 1.54±0.31

Estimated metabolic indexes from the kinetic models describing [6,6-3H]glucose, endogenous glucose, and total glucose concentrations. Precisions of the parameter estimates are shown in parentheses and are expressed as %CV. PCR, plasma clearance rate; $S_C^2$, insulin sensitivity; $S_C^2$, glucose effectiveness.
Although the use of all three data sets in our estimation may at first seem redundant because of the fact that the outputs relate linearly, we found the practice actually improves model identification greatly, since model parameters appear nonlinearly in the outputs and model behavior should be consistent between hot, cold, and endogenous glucose. Two-compartment minimal-model parameters, however, were not significantly different (2-tailed paired t-test, \( P < 0.05 \)) from those originally estimated for these subjects using only hot glucose data (Table 2 and Ref. 45).

Fig. 4. Estimations of endogenous glucose production in all 10 subjects obtained from Radziuk’s 2-compartment model (dotted line), deconvolution (broken line), and 2-compartment minimal model with endogenous glucose production (solid line).

Fig. 5. Mean time courses \((n = 10)\) of the inhibitory functions \(H_1(t)\) and \(H_2(t)\) responsible for the suppression of endogenous glucose production during the hot intravenous glucose tolerance test resulting from increased levels of glucose and insulin, respectively. In the basal state, \(H_1(t) = 0.5\) and \(H_2(t) = 0\). Error bars are SDs.
A modified version of the two-compartment hot minimal model has recently been proposed (42) to obviate some inconsistencies arising with standard IVGTTs when the R_{a0} = 1 mg kg\(^{-1}\) min\(^{-1}\) assumption is used. Specifically, the modified model constrains the constant fractional component of insulin-independent glucose disposal, k_{p}, to be positive (Eq. 2). Although we report the results obtained with the model in Ref. 43, the value of k_{p} was always positive. We also repeated the calculations with the modified model and found no significant differences.

We have integrated a model of EGP and its control by insulin and glucose into the two-compartment minimal model framework. The new model allows for EGP reconstruction and parameter estimation from hot IVGTT data in a single step. The time course of EGP estimated with the new model is in good agreement with that obtained from deconvolution and the TTR clamp technique. Our new model also provides estimates of metabolic indexes of insulin sensitivity and glucose effectiveness, as well as a new index reflecting the sensitivity of suppression of EGP to insulin. Thus this method can potentially serve as a simpler alternative to deconvolution or the suppression of EGP to insulin. Therefore, this method can potentially provide a simpler alternative to deconvolution or the suppression of EGP to insulin. Thus, this method can potentially serve as a simpler alternative to deconvolution or the suppression of EGP to insulin.

**REFERENCES**


