Measurement of selective effect of insulin on glucose disposal from labeled glucose oral test minimal model

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The oral minimal model method can simultaneously measure insulin secretion, insulin action, and the rate of glucose appearance after meal or glucose ingestion (3, 6, 11, 12). In particular, the oral glucose minimal model (OMM) measures insulin sensitivity (S_I) as well as the rate of appearance of ingested glucose (R_a) (11, 12). One limitation of the S_I index is that it reflects glucose disposal only. This would be reminiscent of the insulin-modified tracer (Ra meal) and insulin-insulin reference tracer model (RM). We show that by comparing OMM S_I with the IVGTT S_I measured in the same individual, we can provide a reliable estimate of the S_I index.

METHODS

Data

The database consisted of 88 normal subjects [46 males and 42 females; age = 58 ± 2 yr (range 19–87); body wt = 77 ± 2 kg (range 53–129); BMI = 26.71 ± 0.1 kg/m^2 (range 20–35); fasting glucose = 92.07 ± 0.7 mg/dl (range 77.29–105.12)] who received both a triple-tracer mixed meal (10 kcal/kg: 45% carbohydrate, 15% protein, 40% fat) contained 1/2 g/kg glucose. The meal was labeled with [1-13C]glucose (G*), thus allowing us to derive the exogenous, i.e., coming from the meal, glucose (G_meal) as

\[
G_{meal} = G* \cdot \left[ 1 + \frac{1}{z_{meal}} \right] \tag{1}
\]

where z_meal is the tracer-to-tracer ratio in the meal. Plasma samples were collected at -120, -20, -10, 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 260, 280, 300, 360, and 420 min.

Figure 1, A–C, left, shows mean glucose, exogenous glucose, and insulin plasma concentration curves. Beginning at time 0, [6,6-2H_2]glucose was infused intravenously at a variable rate, mimicking Ra_meal [6,6-2H_2]glucose. Due to the use of the tracer-to-tracer clamp technique, provided a model-independent reference for the appearance rate of ingested glucose (R^{ref}_{a meal}) (4). R^{ref}_{a meal} was then used as a known input of a model of labeled glucose kinetics. This model, denoted as reference tracer model (RM), was identified from labeled meal data and yielded a reference measure of disposal insulin sensitivity, S_I^{ref}, OMM is validated by comparing OMM-based estimates of S_I and R_a meal to S_I^{ref} and R^{ref}_{a meal}, respectively. Validation is further strengthened by comparing OMM S_I with the IVGTT S_I measured in the same subjects with the traditional labeled minimal model.

Labeled mixed meal. The triple-tracer mixed meal (10 kcal/kg: 45% carbohydrate, 15% protein, 40% fat) contained 1±0.02 g/kg glucose. The meal was labeled with [1-13C]glucose (G*), thus allowing us to derive the exogenous, i.e., coming from the meal, glucose (G.meal) as

\[
G_{meal} = G* \cdot \left[ 1 + \frac{1}{z_{meal}} \right] \tag{1}
\]

where z_meal is the tracer-to-tracer ratio in the meal. Plasma samples were collected at -120, -20, -10, 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 260, 280, 300, 360, and 420 min.

Figure 1, A–C, left, shows mean glucose, exogenous glucose, and insulin plasma concentration curves. Beginning at time 0, [6,6-2H_2]glucose was infused intravenously at a variable rate, mimicking Ra_meal [6,6-2H_2]glucose. Due to the use of the tracer-to-tracer clamp technique, provided a model-independent reference for the appearance rate of ingested glucose (R^{ref}_{a meal}) (4). R^{ref}_{a meal} was then used as a known input of a model of labeled glucose kinetics. This model, denoted as reference tracer model (RM), was identified from labeled meal data and yielded a reference measure of disposal insulin sensitivity, S_I^{ref}, OMM is validated by comparing OMM-based estimates of S_I and R_a meal to S_I^{ref} and R^{ref}_{a meal}, respectively. Validation is further strengthened by comparing OMM S_I with the IVGTT S_I measured in the same subjects with the traditional labeled minimal model.
followed by a short insulin infusion of 0.02 U/kg between 20 and 25 min. Plasma samples were collected at 0, 2, 4, 6, 8, 10, 15, 20, 22, 25, 26, 28, 31, 35, 45, 60, 75, 90, 120, 180, and 240 min. Figure 1, A–C, right, shows mean glucose, exogenous glucose, and insulin plasma concentration.

Models

Oral minimal models. For the sake of clarity, a brief description of the “cold” (i.e., unlabeled) oral minimal models (11, 12) precedes the presentation of the new “hot” (i.e., labeled) OMM*. This is done not only because the two oral minimal models hinge on the same mono-compartmental structure of glucose kinetics, but also because they share the exogenous glucose input, i.e., Ra meal. Denoting by G the total plasma glucose concentration, the rate of glucose disappearance (Rd) and the net hepatic glucose balance (NHGB) (Fig. 3, left) model equation following Ref. 5 is

$$G(t) = \frac{-R_d(t) + NHGB(t) + R_{a\text{ meal}}(t)}{V}; \quad G(0) = G_0$$

where V is the distribution volume.

By assuming for Rd and NHGB the functional description proposed in Ref. 5, one obtains OMM:

$$G(t) = \begin{cases} \frac{-S_0 \cdot X(t) \cdot G(t) + S_0 \cdot G_b + R_{a\text{ meal}}(t)}{V} & G(0) = G_b \\ \frac{-p_2 \cdot X(t) + p_3 \cdot [1(t) - I_b]}{V} & X(0) = 0 \end{cases}$$

where S_0 is fractional (i.e., per unit distribution volume) glucose effectiveness measuring glucose ability per se to promote glucose disposal and inhibit NHGB, I is plasma insulin concentration, X is insulin action on glucose disposal and production, with p_2 and p_3 rate constants describing its dynamics and magnitude; b denotes basal values. R_{a\text{ meal}} is described as a piecewise-linear function with known break point t_i and unknown amplitude α_i:

$$R_{a\text{ meal}}(t) = \begin{cases} \alpha_{i-1} \frac{t - t_{i-1}}{t_i - t_{i-1}} & \text{per } t_{i-1} \leq t \leq t_i, \quad i = 1 \ldots 8 \\ 0 & \text{else} \end{cases}$$

with α denoting [α_1, α_2, \ldots, α_8]^T.
Labeled oral minimal model. OMM is unable to distinguish the individual contribution of glucose production and disposal. To overcome this limitation, a glucose tracer is administered orally and unlabeled glucose and plasma concentrations of the glucose tracer are measured. OMM* relies on \( G_{\text{meal}} \) (eq. 1), and model equation is

\[
S_1 \text{ is given by}
\]

\[
S_1 = \frac{p_1}{p_2} \cdot V (\text{dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \text{per } \mu \text{U/ml}) \quad (6)
\]

\[
\text{Labeled oral minimal model. OMM is unable to distinguish the individual contribution of glucose production and disposal. To overcome this limitation, a glucose tracer is administered orally and unlabeled glucose and plasma concentrations of the glucose tracer are measured. OMM* relies on } G_{\text{meal}} \text{ (eq. 1), and model equation is}
\]

\[
G_{\text{meal}}(t) = - \frac{R_{\text{d meal}}(t) + R_{\text{a meal}}(t)}{V_p} \cdot G_{\text{meal}}(0) = 0 \quad (7)
\]

where \( R_{\text{d meal}} \) is the \( R_d \) of \( G_{\text{meal}} \). By assuming for \( R_{\text{d meal}} \) the same functional description proposed previously (10) and for \( R_{\text{a meal}} \) the parametric description of Eq. 5, one obtains OMM* (Fig. 3, middle). Model equations are

\[
\begin{cases}
G_{\text{meal}}(t) = - [S_G^* + X^*(t)] \cdot G_{\text{meal}}(t) + \frac{R_{\text{a meal}}(t)}{V_p} \cdot G_{\text{meal}}(0) = 0 \\
X^*(t) = - p_2^* \cdot X^*(t) + p_3^* \cdot [I(t) - I_p] \\
X^*(0) = 0
\end{cases} \quad (8)
\]

where \( S_G^* \) is fractional (per unit distribution volume) glucose effectiveness measuring glucose ability per se to promote glucose disposal and \( X^* \) insulin action on glucose disposal with \( p_2^* \) and \( p_3^* \) rate constants describing its dynamics and magnitude, respectively. OMM* estimates \( R_{\text{a meal}} \) together with \( S_1 \) on glucose disposal, with \( S_1^* \) defined as:

\[
S_1^* = \frac{p_1^*}{p_2^*} \cdot V (\text{dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \text{per } \mu \text{U/ml}) \quad (9)
\]

Because OMM* and OMM share \( R_{\text{a meal}} \), the two models were identified simultaneously (see Identification).

Reference-labeled model. The validation of OMM* estimates of \( R_{\text{a meal}} \) and \( S_1^* \) was accomplished by using the following rationale. As mentioned in Data, during the labeled meal, two additional tracers were infused intravenously. In particular, \([\text{6}^{-3}\text{H}]\text{glucose}\) was infused to clamp the ratio between concentrations in plasma of \([\text{6}^{-3}\text{H}]\text{glucose}\) and ingested glucose tracer. This allowed us to derive reliable and virtually model-independent estimates of \( R_{\text{a meal}} \) (4). This estimate,

\[
\text{denoted } R_{\text{a meal}}^{\text{ref}}, \text{was used not only as a reference with which OMM* estimate of } R_{\text{a meal}} \text{ was compared but also as a known input of a model with the same structure of OMM* (Fig. 3, right):}
\]

\[
G_{\text{meal}}(t) = - \frac{R_{\text{d meal}}^{\text{ref}}(t) + R_{\text{a meal}}^{\text{ref}}(t)}{V'} \cdot G_{\text{meal}}(0) = 0 \quad (10)
\]

which becomes

\[
\begin{cases}
G_{\text{meal}}(t) = - [S_G^{\text{ref}} + X^{\text{ref}}(t)] \cdot G_{\text{meal}}(t) + \frac{R_{\text{a meal}}^{\text{ref}}(t)}{V'} \cdot G_{\text{meal}}(0) = 0 \\
X^{\text{ref}}(t) = p_1^{\text{ref}} \cdot X^{\text{ref}}(t) + p_2^{\text{ref}} \cdot [I(t) - I_p] \\
X^{\text{ref}}(0) = 0
\end{cases} \quad (11)
\]

By identifying this model, denoted as reference-labeled model (RM*), from \( G_{\text{meal}} \) and insulin data we were able to obtain reference values for OMM* parameters (indicated by \( ^{\text{ref}} \)), in particular for \( S_1^* \) (see Identification). Comparison between \( S_1^* \), estimated with RM*, and \( S_1^* \) provided by OMM*, allowed OMM* validation.

IVGTT* minimal models. IVGTT* data were interpreted with the classic single-compartment IVM (5) and with the labeled two-compartment minimal models (IVMM*) (14), thus obtaining an estimate of \( S_1 \) and \( S_1^* \) in the same subjects. This, in addition to providing a one-to-one comparison of IVGTT* \( S_1^* \) vs. OMM* \( S_1^* \), allows us to examine the relationship between \( S_1 \) and \( S_1^* \) in IVGTT and meal.

Identification

Identifiability. Because OMM* (like OMM) is a priori nonidentifiable, the a priori knowledge necessary for its identification was obtained from RM*. OMM* was thus identified by fixing \( V^* \) and \( S_1^* \) to the mean values obtained with RM*, i.e., \( V^* = V^{\text{ref}}, S_1^* = S_1^{\text{ref}} \). Mean values can safely be used because they are normally distributed (RESULTS). At variance with OMM, where a Bayesian prior on \( p_2 \) was needed to improve numerical identifiability, OMM* takes advantage of the fact that it shares \( R_{\text{a meal}} \) with OMM. The simultaneous identification of OMM* and OMM from two measurements (\( G_{\text{meal}} \) and \( G_{\text{meal}} \)) relaxes the necessity of using Bayesian priors for \( p_2 \) and \( p_3^{\text{ref}} \). A constraint (11, 12) was imposed to guarantee that the area under the estimated \( R_{\text{a meal}} \) equals the total amount of ingested glucose, \( D \), multiplied by the fraction that is actually absorbed, \( f \). Because \( f \) values estimated with RM* were not normally distributed (RESULTS), \( f \) was
fixed to the median of RM*: \( f = f^{\text{m-ref}} \). Finally, oral tracer measurements provided information as to when \( R_a^{\text{meal}} \) began to rise in each subject. If tracer concentration is zero up to time \( t_i \), and is different from zero at time \( t_i+1 \), then one can safely assume that \( R_a^{\text{meal}} \) is zero up to \( t_i \).

Parameter estimation. All models were numerically identified by nonlinear least squares (7, 9), as implemented in SAAM II [Simulation Analysis and Modeling software (2)]. Measurement error was assumed to be independent, gaussian, with zero mean and known constant standard deviation. Insulin concentration is the model-forcing function and is assumed to be known without error.

Statistical Analysis.

Data are presented as means ± SE. Two-sample comparisons were done by Wilcoxon signed rank test, and a Shapiro-Wilk test was used to verify whether parameters were normally distributed (significance level set to 5%). Pearson’s correlation was used to evaluate univariate correlation.

RESULTS

Parameter estimates of RM*, OMM*, and OMM (simultaneously identified), IVMM*, and IVMM are shown in Table 1 (means ± SE) with their precision. RM*, RM parameters were estimated with good precision, and their mean values were \( S_{0}^{\text{ref}} = 0.0118 \text{ min}^{-1} \), \( V^{\text{ref}} = 1.60 \text{ dl/kg} \), \( p_t^{\text{ref}} = 0.039 \text{ min}^{-1} \), and \( S_{0}^{\text{meas}} = 9.24 \times 10^{-4} \text{ dl/kg}^{-1} \text{min}^{-1} \) per \( \mu \text{U/ml} \). The fraction of ingested glucose that reaches plasma, calculated as the ratio between area under \( R_a^{\text{meal}} \) and ingested dose, was \( f^{\text{ref}} = 0.89 \). Parameters \( S_{0}^{\text{ref}} \), \( V^{\text{ref}} \) were normally distributed (\( P \) values not significant by Shapiro-Wilk test), although \( f^{\text{ref}} \) was not (median value \( f^{\text{m-ref}} = 0.90 \)).

OMM* and OMM. The mean profile of \( R_a^{\text{meal}} \) reconstructed by simultaneously identifying OMM* and OMM, is shown in Fig. 4. Of note, \( R_a^{\text{meal}} \) was virtually superimposable (data not shown) to that reconstructed by identifying OMM alone (11).

OMM* and OMM parameters were \( S_{1}^{*} = 9.64 \times 10^{-4} \text{ dl/kg}^{-1} \text{min}^{-1} \) per \( \mu \text{U/ml} \), \( p_z^{*} = 0.043 \text{ min}^{-1} \), \( S_{1} = 12.24 \times 10^{-4} \text{ dl/kg}^{-1} \text{min}^{-1} \) per \( \mu \text{U/ml} \), and \( p_x = 0.011 \text{ min}^{-1} \). This estimate of \( S_{1} \) was not significantly different from that obtained in Eq. II by fitting OMM to total glucose concentration alone, where we found \( S_{1} = 11.68 \times 10^{-4} \text{ dl/kg}^{-1} \text{min}^{-1} \) per \( \mu \text{U/ml} \). The correlation between the two sets of \( S_{1} \) estimates is very high: \( r = 0.96, P < 0.001 \).

IVMM* and IVMM. \( S_{1}^{*} \) and \( S_{1} \) were 11.59 and 6.91 \( \times 10^{-4} \text{ dl/kg}^{-1} \text{min}^{-1} \) per \( \mu \text{U/ml} \), respectively.

OMM* vs. RM*. A good agreement was found between OMM* \( R_a^{\text{meal}} \) and \( R_a^{\text{ref}} \) (Fig. 4). \( S_{1}^{*} \) provided by OMM* and \( S_{1}^{\text{ref}} \) were well correlated (\( r = 0.80, P < 0.0001 \); Fig. 4A), and their mean values were not significantly different (9.64 vs. \( 9.24 \times 10^{-4} \text{ dl/kg}^{-1} \text{min}^{-1} \) per \( \mu \text{U/ml} \)). To quantify how sensitive the OMM/OMM* estimate of \( S_{1} \) and \( S_{1}^{*} \) to the assumptions made on \( V, V^{*}, S_{G}, S_{G}^{*} \), and \( f \), we used multiple regression analysis between the percentage deviation of \( S_{1} \) from \( S_{1}^{*} \) and the percentage deviation of \( V, S_{G}, \) and \( f \) (\( V^{*}, S_{G}^{*}, f \)). We found that the percentage deviations of \( V, S_{G}^{*}, \) and \( f \) explain the deviation in \( S_{1} \) estimate (0.685, \( P < 0.0001 \)), although the deviation of \( V \) doesn’t contribute significantly to the regression. Conversely, the percentage deviations of \( f, S_{G}^{*}, \) and \( V^{*} \) explain the deviation in \( S_{1}^{*} \) estimate (0.747, \( P < 0.0001 \)).

OMM* vs. IVMM*. Correlation between IVMM* and OMM* \( S_{1}^{*} \) was significant (\( r = 0.67, P < 0.0001 \); Fig. 5B) but their values were significantly different (9.64 vs. 11.59 \( \times 10^{-4} \text{ dl/kg}^{-1} \text{min}^{-1} \) per \( \mu \text{U/ml} \)).

\( S_{1} \) vs. \( S_{1}^{*} \). The relationship between \( S_{1} \) and \( S_{1}^{*} \) is different during meal and IVGTT. With the oral minimal models, one had 12.24 vs. \( 9.64 \times 10^{-4} \text{ dl/kg}^{-1} \text{min}^{-1} \) per \( \mu \text{U/ml} \), with \( S_{1} > S_{1}^{*} \) in 81% of the subjects, although for the IVGTT \( S_{1} \) was lower than \( S_{1}^{*} \) on average (\( S_{1} = 6.91 \text{ vs. } S_{1}^{*} = 11.59 \times 10^{-4} \text{ dl/kg}^{-1} \text{min}^{-1} \) per \( \mu \text{U/ml} \)) and was in ~90% of the subjects.

DISCUSSION

The OMM method was developed to measure \( S_{1} \) under physiological conditions, e.g., a meal or OGTT (12). In addi-
However, from Fig. 5, differences exist at the individual level, thus indicating that OMM* is more robust in population rather than individual studies.

The addition of a tracer to the meal, besides allowing segregation of exogenous component, Gmeal, of plasma glucose concentration G, and thus estimation of insulin sensitivity on glucose disposal, also has beneficial effects on the numerical identifiability of OMM* (and OMM). In fact, OMM*, which is based on Gmeal data, shares Rmeal with OMM, which is based on G data. Thus, for its identification, the unlabeled plasma glucose concentration data G can also be exploited by simultaneously identifying OMM. By doing so, the number of available data doubles, although the number of parameters increases by only two (i.e., from 10 parameters with OMM* alone to 12 with both OMM* and OMM). The improvement in numerical identifiability allowed estimation of $p_2^{*}$ and $p_2$ in each individual without having to resort to Bayesian priors as in Refs. 11 and 12. However, parameters $V$, $V^*$, $S_G$, $S_G^*$, and $f$ still need to be fixed to population values derived from the reference model. To quantify how sensitive the OMM/OMM* estimate of $S_I$ and $S_I^*$ to the assumptions made on these parameters were, we investigated, by multiple regression analysis, the relationship between their percentage deviation and that of $S_I$ and $S_I^*$. We found that the percentage deviations of $f$ and $S_G$ explain the deviation in $S_I$ estimate (0.685, $P < 0.0001$), whereas the deviation of $V$ doesn’t contribute significantly to the regression. The percentage deviations of $f$, $S_G^*$, and $V^*$ from the fixed values explain the deviation in $S_I^*$ estimate (0.747, $P < 0.0001$).

It is of interest to compare $S_I^*$ with OMM* and IVGTT*. The two estimates showed a significant correlation, $r = 0.67, P < 0.0001$ (Fig. 5B), although the latter measure was significantly higher: 11.59 vs. $9.64 \times 10^{-4} \text{dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U/ml}$. This relation is of interest to compare $S_I^*$ with OMM* and IVGTT*. The two estimates showed a significant correlation, $r = 0.67, P < 0.0001$ (Fig. 5B), although the latter measure was significantly higher: 11.59 vs. $9.64 \times 10^{-4} \text{dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U/ml}$. This relationship between the cold and hot estimates of $S_I$ in IVGTT vs. meal is worth commenting on. The IVGTT results observed in the present 88 subjects are consistent with those previously reported in smaller-size studies (8, 10, 14). $S_I$ was lower than $S_I^*$ in ~90% of the subjects: $S_I = 6.91 \times 10^{-4}$ vs. $S_I^* = 11.59 \times 10^{-4} \text{dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U/ml}$. This relationship is clearly unphysiological, because $S_I$ measures the overall effect of insulin on both glucose disposal and production, whereas $S_I^*$ measures only the effect of insulin on disposal; by definition, $S_I$ should be equal to or greater than $S_I^*$. Possible reasons for this unexpected pattern have been discussed in Ref. 8. The relationship between $S_I$ and $S_I^*$ improves dramatically with the OMM, $S_I = 12.24 \times 10^{-4}$ vs. $S_I^* = 9.64 \times 10^{-4} \text{dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U/ml}$, with $S_I > S_I^*$ in 81% of the subjects. However, $S_I$ was still less than $S_I^*$ in 19% of the subjects. It is likely that the improved performance of the oral minimal models stems from the fact that the minimal model assumptions are more tenable during the gentle meal than during the massive IVGTT perturbation, particularly those concerning the description of how NHGB is controlled by glucose and insulin, which are embodied in both cold models. However, the finding that $S_I^*$ was greater than $S_I$ in 19% of the subjects also calls for a revision of this functional description for a meal perturbation.

A comment on the relationship between $p_2$ and $p_2^*$ in IVGTT and meal, as well as on the difference between intravenous and oral values, is also in order. During both IVGTT and meal, $p_2$
is approximately one-third of $p_2^*$ (Table 1). This means that insulin action on the liver has a slower dynamic than insulin action on glucose utilization, and, in all likelihood, the underlying assumption of the classic minimal model, that insulin action on glucose production has the same dynamics of insulin action on glucose utilization, is probably not entirely correct. Moreover, the difference found in $p_2$ and $p_2^*$ values during IVGTT and meal can be explained by considering the differences between the two tests. During IVGTT, glucose and insulin explore a wider range of values than during the meal. The approach also permits assessment of the overall effect of insulin on glucose production and disposal ($S_I$). Furthermore, when the labeled and unlabeled oral minimal models are combined with the oral C-peptide minimal model (6, 15), insulin secretion and β-cell function indexes can also be measured at the same time. However, although in the present study good performance of the method was observed in a wide range of glucose tolerance ($S_I$: 1.52 ± 30.40 $\times 10^{-4}$ dl·kg$^{-1}$·min$^{-1}$ per µU/ml), further studies in diabetic individuals with abnormalities in insulin secretion and action of various degrees of severity are needed to better define the domain of validity of the model. Finally, because insulin action appears to be dependent on the pattern of insulin (1, 13), future studies will be required to determine whether the ability of insulin to stimulate glucose uptake and suppress glucose production in the presence of the continuously changing insulin concentrations observed after a meal is the same as that observed in the presence of different insulin profiles (e.g., during a hyperinsulinemic clamp).

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