

# Minimal model estimation of glucose absorption and insulin sensitivity from oral test: validation with a tracer method

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**Dalla Man, Chiara, Andrea Caumo, Rita Basu, Robert Rizza, Gianna Toffolo, and Claudio Cobelli.** Minimal model estimation of glucose absorption and insulin sensitivity from oral test: validation with a tracer method. *Am J Physiol Endocrinol Metab* 287: E637–E643, 2004. First published May 11, 2004; 10.1152/ajpendo.00319.2003.—Measuring insulin sensitivity during the physiological milieu of oral glucose perturbation, e.g., a meal or an oral glucose tolerance test, would be extremely valuable but difficult since the rate of appearance of absorbed glucose is unknown. The reference method is a tracer two-step one: first, the rate of appearance of glucose ( $R_{a\text{ meal}}^{\text{ref}}$ ) is reconstructed by employing the tracer-to-tracee ratio clamp technique with two tracers and a model of non-steady-state glucose kinetics; next, this  $R_{a\text{ meal}}^{\text{ref}}$  is used as the known input of a model describing insulin action on glucose kinetics to estimate insulin sensitivity ( $S_I^{\text{ref}}$ ). Recently, a nontracer method based on the oral minimal model (OMM) has been proposed to estimate simultaneously the above quantities, denoted  $R_{a\text{ meal}}$  and  $S_I$ , respectively, from plasma glucose and insulin concentrations measured after an oral glucose perturbation. This last method has obvious advantages over the tracer method, but its domain of validity has never been assessed against a reference method. It is thus important to establish whether or not the “nontracer”  $R_{a\text{ meal}}$  and  $S_I$  compare well with the “tracer”  $R_{a\text{ meal}}^{\text{ref}}$  and  $S_I^{\text{ref}}$ . We do this comparison on a database of 88 subjects, and it is very satisfactory:  $R_{a\text{ meal}}$  profiles agree well with the  $R_{a\text{ meal}}^{\text{ref}}$  and correlation of  $S_I^{\text{ref}}$  with  $S_I$  is  $r = 0.86$  ( $P < 0.0001$ ). We conclude that OMM candidates as a reliable tool to measure both the rate of glucose absorption and insulin sensitivity from oral glucose tests without employing tracers.

glucose kinetics; rate of appearance of glucose; tracer; meal; insulin resistance

INSULIN SENSITIVITY ( $S_I$ ), i.e., the ability of insulin to enhance glucose utilization and inhibit glucose production, is an important parameter to assess the efficiency of the glucose regulatory system. This index, useful not only for diagnosis but also for assessing the efficacy of a given therapy, is usually measured using methods involving an intravenous administration of glucose and/or insulin, such as the glucose clamp technique (10) or the intravenous glucose tolerance test (IVGTT) interpreted with the minimal model (5). However, both of these techniques realize experimentally a “nonphysiological milieu” since neither the elevated insulin-basal glucose condition of the clamp technique nor the rapid glucose and insulin perturbations of an IVGTT reflect the condition of daily living. Therefore, it is highly desirable to have a method able to quantify insulin sensitivity in a normal life “physiological milieu,” e.g., during a meal.

Unfortunately, the estimation of insulin sensitivity after an oral glucose perturbation is not an easy task, the major obstacle being that the rate of appearance of glucose absorption is unknown. The most reliable, probably the reference method for estimating insulin sensitivity during a meal, is a two-step procedure. First, the rate of appearance of absorbed glucose is reconstructed as accurately as possible with a tracer method. A reliable method uses a tracer-to-tracee clamp technique in conjunction with a model of glucose kinetics in nonsteady state (14). Two tracers are necessary, one given intravenously, mimicking the rate of appearance of glucose absorption, and one together with the meal. Once the rate of appearance of absorbed glucose is known, to estimate insulin sensitivity one has to explain the meal plasma glucose measurements with a model describing insulin action on glucose production and disposal. This model already exists and is the classic single-compartment minimal model. In fact, when glucose and insulin change smoothly as during an oral test, the single-compartment description of glucose kinetics has proven to be sufficiently accurate (2).

In summary, the state-of-the-art method for the estimation of insulin sensitivity during an oral test entails the use of a two-tracer protocol in conjunction with a model of non-steady-state glucose kinetics to obtain the rate of appearance of glucose absorption ( $R_{a\text{ meal}}^{\text{ref}}$ ), and then the use of the minimal model to extract insulin sensitivity ( $S_I^{\text{ref}}$ ) from plasma glucose and insulin concentrations. Since this is an expensive and labor-intensive procedure, an alternative approach capable of reducing the cost and the effort is required to favor a widespread application of the oral test for the estimation of insulin sensitivity. Recently, a nontracer method based on a new model, the oral minimal model (OMM), has been proposed to estimate insulin sensitivity ( $S_I$ ) together with the glucose rate of appearance ( $R_{a\text{ meal}}$ ) from plasma glucose and insulin concentrations measured after a meal or an oral glucose tolerance test (OGTT; see Ref. 9). The method has obvious advantages over the reference tracer method discussed above, but its performance in recovering both insulin sensitivity and rate of appearance of glucose absorption has never been compared with other methods, albeit some reassuring indirect validation results are available: in two studies (3, 9) insulin sensitivity was compared with that obtained from IVGTT performed in the same subjects, and correlation was satisfactory. It is thus important to establish whether or not the “nontracer”  $R_{a\text{ meal}}$  and  $S_I$  provided by OMM compare well with the “tracer”  $R_{a\text{ meal}}^{\text{ref}}$  and  $S_I^{\text{ref}}$  provided by the reference method.

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This is precisely the aim of the present contribution. We do this comparison on a database consisting of 88 subjects who underwent the triple-tracer meal protocol described (4).

#### DATABASE AND PROTOCOL

The database consists of 88 normal subjects (46 males and 42 females; age =  $58 \pm 2$  yr, body weight =  $77 \pm 2$  kg) who received a triple-tracer mixed meal (10 kcal/kg, 45% carbohydrate, 15% protein, and 40% fat) containing  $1 \pm 0.02$  g/kg glucose. The meal was labeled with [ $^{13}\text{C}$ ]glucose (in the following *tracer I*) to segregate the exogenous, i.e., coming from the meal, glucose ( $G_{\text{ex}}$ ) from the endogenous one ( $G_{\text{end}}$ ). Two additional tracers {[ $6,6\text{-}^2\text{H}_2$ ]glucose (*tracer II*) and [ $6\text{-}^3\text{H}$ ]glucose, (*tracer III*)} were infused intravenously at variable rates, mimicking the endogenous glucose production (EGP) and  $R_{\text{a meal}}$ , respectively; by this way, the change in the plasma tracer-to-tracee ratios *tracer II*/ $G_{\text{end}}$  and *tracer III*/ $G_{\text{ex}}$  was minimized, and Steele equation provided an essentially model-independent estimate of EGP and  $R_{\text{a meal}}$  (4). As far as the present contribution is concerned, one only needs two of the three tracers, i.e., the one labeling the meal (*tracer I*) and the one mimicking  $R_{\text{a meal}}$  (*tracer III*).

Plasma samples were collected at times ( $t$ ) -120, -30, -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 shows the mean glucose and insulin plasma concentration curves.

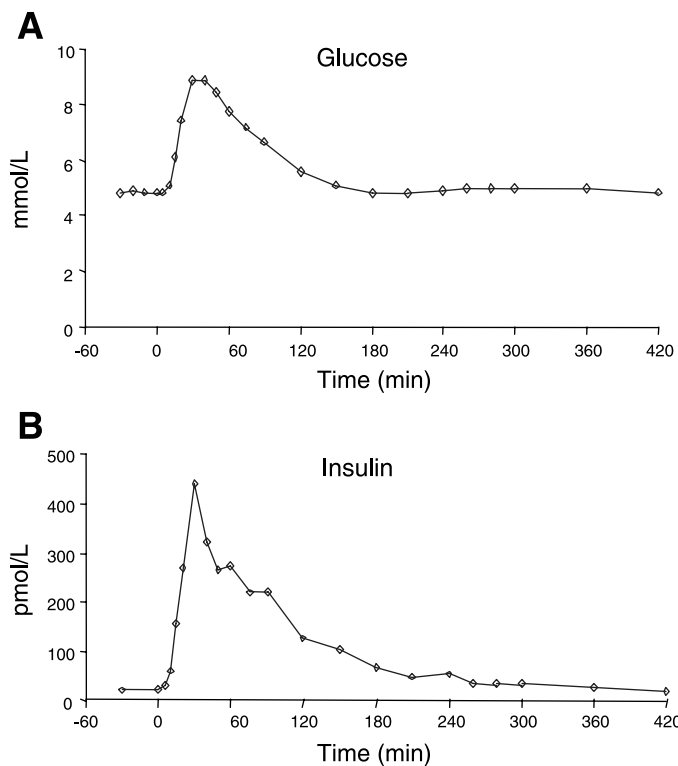


Fig. 1. Concentration of plasma glucose and insulin during a meal in normal subjects (mean,  $n = 88$ ).

#### METHODS

##### Tracer Method

**Reconstruction of  $R_{\text{a meal}}^{\text{ref}}$ .** An accurate estimation of  $R_{\text{a meal}}^{\text{ref}}$  was obtained by applying the single-compartment model of Steele et al. (14) to the clamped tracer-to-tracee ratio ( $\text{TTR} = \text{tracer III}/G_{\text{ex}}$ ):

$$R_{\text{a meal}}^{\text{ref}}(t) = \frac{F(t)}{\text{TTR}(t)} - p \cdot V \cdot \frac{G_{\text{ex}}(t)}{\text{TTR}(t)} \cdot \frac{d\text{TTR}}{dt} \quad (1)$$

where  $F(t)$  is the pump infusion profile of *tracer III*,  $V$  is the distribution volume of glucose,  $p$  is the pool fraction, and  $G_{\text{ex}}$  is the concentration in plasma of the oral ingested glucose.

**Estimation of  $S_1^{\text{ref}}$ .** By knowing  $R_{\text{a meal}}^{\text{ref}}$ , it was possible to identify the classic minimal model in each subject by using  $R_{\text{a meal}}^{\text{ref}}$  as the known exogenous input. The model is described by

$$\begin{cases} \dot{G}(t) = -[S_G^{\text{ref}} + X(t)] \cdot G(t) + S_G^{\text{ref}} \cdot G_b + \frac{R_{\text{a meal}}^{\text{ref}}(t)}{V^{\text{ref}}}; & G(0) = G_b \\ \dot{X}(t) = -p_2^{\text{ref}} \cdot X(t) + p_3^{\text{ref}} \cdot [I(t) - I_b]; & X(0) = 0 \end{cases} \quad (2)$$

where  $G$  is plasma glucose concentration,  $I$  is plasma insulin concentration, suffix “b” denotes basal values,  $X$  is insulin action,  $V$  is the distribution volume, and  $S_G$ ,  $p_2$ , and  $p_3$  are model parameters (super-script “ref” stands for “reference”). Specifically,  $S_G$  is the fractional (i.e., per unit distribution volume) glucose effectiveness measuring glucose ability, per se, to promote glucose disposal and inhibit glucose production;  $p_2$  is the rate constant describing the dynamics of insulin action;  $p_3$  is the parameter governing the magnitude of insulin action. The insulin sensitivity index is given by (5):

$$S_1^{\text{ref}} = \frac{p_3^{\text{ref}}}{p_2^{\text{ref}}} \cdot V^{\text{ref}} (\text{dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}) \quad (3)$$

##### Nontracer Method

A new model has been recently developed to estimate insulin sensitivity together with the glucose rate of appearance during an oral test (9). It couples the single-compartment minimal model with a parametric description of the glucose rate of appearance:

$$\begin{cases} \dot{G}(t) = -[S_G + X(t)] \cdot G(t) + S_G \cdot G_b + \frac{R_{\text{a meal}}(t)}{V}; & G(0) = G_b \\ \dot{X}(t) = -p_2 \cdot X(t) + p_3 \cdot [I(t) - I_b]; & X(0) = 0 \end{cases} \quad (4)$$

The parametric description of  $R_{\text{a meal}}$  proposed (9) is a piecewise-linear function with known break point  $t_i$  and unknown amplitude  $\alpha_i$ :

$$R_{\text{a meal}}(t) = \begin{cases} \alpha_{i-1} + \frac{\alpha_i - \alpha_{i-1}}{t_i - t_{i-1}} \cdot (t - t_{i-1}) & \text{per } t_{i-1} \leq t \leq t_i \quad i = 1 \dots 8 \\ 0 & \text{otherwise} \end{cases} \quad (5)$$

$S_1$  was estimated as

$$S_1 = \frac{p_3}{p_2} \cdot V (\text{dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}) \quad (6)$$

##### Parameter Estimation

**Tracer method.** To estimate  $R_{\text{a meal}}^{\text{ref}}$ , we used the single-compartment model of Steele et al. (14). Because the tracer-to-tracee ratio was well clamped, a two-compartment model gave virtually the same results (4). The tracer-to-tracee ratio profile was smoothed using WINSTODEC (13), which also provides the first derivative of the

smoothed signal. Finally, the minimal model of glucose kinetics was numerically identified by nonlinear least squares (7, 8), as implemented in SAAM II (Simulation Analysis and Modeling software; see Ref. 1). Measurement error on glucose data was assumed to be independent, Gaussian, with zero mean and known SD [coefficient of variation (CV) = 2%]. Insulin concentration is the model-forcing function and was assumed to be known without error. The model provided estimates of  $S_G^{\text{ref}}$ ,  $V^{\text{ref}}$ ,  $p_2^{\text{ref}}$ , and  $S_I^{\text{ref}}$ . Knowledge of  $R_{a\text{ meal}}^{\text{ref}}$  allowed us to calculate  $f^{\text{ref}}$  [the fraction of the ingested dose (D) that is actually absorbed] as

$$f^{\text{ref}} = \frac{\int_0^{\infty} R_{a\text{ meal}}^{\text{ref}} dt}{D} \quad (7)$$

**Nontracer method.** OMM was numerically identified with the same strategy described above. However, OMM identification requires a number of assumptions that were discussed in detail (9). Briefly, to ensure its a priori identifiability, one has to assume values for V and  $S_G$ . Here, we fixed them to the median obtained with the tracer method, i.e.,  $V = V^{\text{m-ref}}$ ,  $S_G = S_G^{\text{m-ref}}$  (median was preferred to mean values since parameters are not normally distributed, see RESULTS). To improve numerical identifiability of the remaining parameters,  $p_2$ ,  $p_3$ , and  $\alpha_i$  ( $i = 1 \dots 8$ ), a Gaussian bayesian prior was considered on the square root of  $p_2^{\text{ref}}$  ( $\text{SQRP}_2^{\text{ref}}$ ), which is normally distributed (see RESULTS):  $\text{SQRP}_2 = \text{mean SQRP}_2^{\text{ref}}$ ,  $\text{SD} = 10\%$ . Finally, a constraint was imposed to guarantee that the area under the estimated  $R_{a\text{ meal}}$  equals the total amount of ingested glucose, D, multiplied by the fraction of the ingested dose that is actually absorbed, f (fixed to the median of reference values  $f = f^{\text{m-ref}}$ ): this constraint provides an additional relationship among the unknown parameters  $\alpha_i$ , thus reducing the number of unknowns by one. In summary, OMM provides estimates of  $S_I$ ,  $p_2$ , and  $\alpha_i$  (i.e., the  $R_{a\text{ meal}}$  profile).

### Statistical Analysis

Data are presented as means  $\pm$  SE. Two-sample comparisons were done by Wilcoxon signed-rank test, and Shapiro-Wilk test was used to verify if parameters are normally distributed (significance level set to 5%). Pearson's correlation was used to evaluate univariate correlation. To investigate how sensitive the OMM estimate of  $S_I$  was to the assumptions on V,  $S_G$ , f, and  $p_2$ , we investigated the relationship between the percent deviation of  $S_I$ ,  $\Delta S_I\%$ , and the percent deviation of the various parameters that were fixed (namely  $\Delta V\%$ ,  $\Delta S_G\%$ ,  $\Delta f\%$ , and  $\Delta p_2\%$ ) using regression analysis. The percent deviation of each parameter was calculated as the difference between the reference and OMM parameter value divided by the reference. We used single-regression analysis to determine the association of  $\Delta S_I\%$  with  $\Delta V\%$ ,  $\Delta S_G\%$ ,  $\Delta f\%$ , and  $\Delta p_2\%$  separately. Next, we used forward and backward stepwise multiple regression (using F ratio-to-remove = 4 and F ratio-to-enter = 3.996) to assess the relevance of the fixed parameters as predictors of the error affecting  $S_I$ .

## RESULTS

### Tracer Method

$R_{a\text{ meal}}^{\text{ref}}$  obtained using the tracer-to-tracee ratio clamp technique is shown in Fig. 2A.

The fit of the minimal model (Eq. 2) was satisfactory, since, as indicated in Fig. 3A, average weighted residuals did not show systematic deviations from zero. Reference parameters of glucose kinetics were estimated with good precision:  $S_G^{\text{ref}} = 0.031 \pm 0.002 \text{ min}^{-1}$  (CV = 12  $\pm$  1%; mean  $\pm$  SE);  $V^{\text{ref}} = 1.50 \pm 0.05 \text{ dl/kg}$  (CV = 4  $\pm$  1%);  $p_2^{\text{ref}} = 0.013 \pm 0.001$

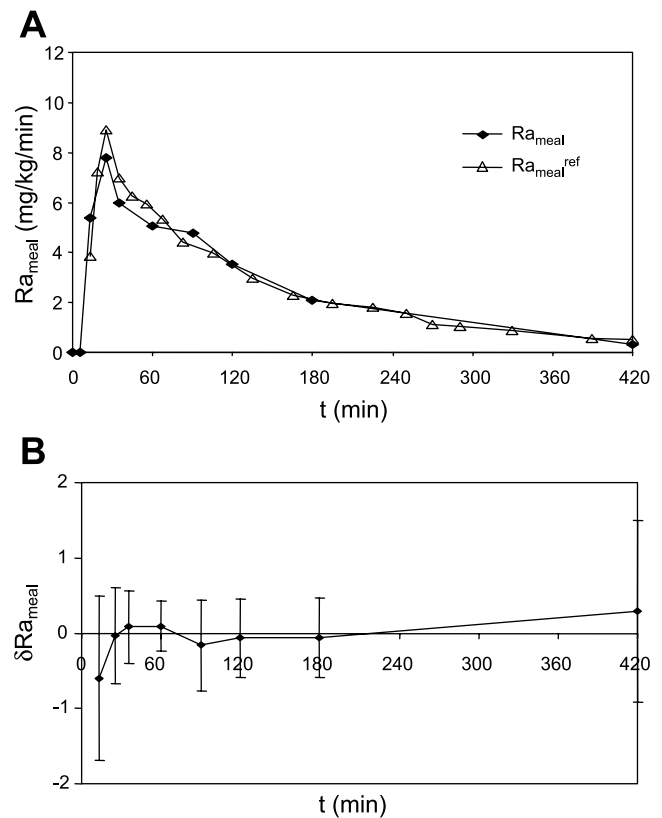


Fig. 2. A: comparison between the time course of the rate of meal absorption ( $R_{a\text{ meal}}$ ) predicted by the oral minimal model (OMM) and the reference  $R_{a\text{ meal}}$  ( $R_{a\text{ meal}}^{\text{ref}}$ ) reconstructed with the tracer method (mean,  $n = 88$ ).  $t$ , Time. B: deviation of OMM  $R_{a\text{ meal}}$  from  $R_{a\text{ meal}}^{\text{ref}}$ ,  $\delta R_{a\text{ meal}} = (R_{a\text{ meal}}^{\text{ref}} - R_{a\text{ meal}})/R_{a\text{ meal}}^{\text{ref}}$  (vertical bars represent SD).

$\text{min}^{-1}$  (CV = 11  $\pm$  1%);  $S_I^{\text{ref}} = 11.55 \pm 0.68 \times 10^{-4} \text{ dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}$  (CV = 5  $\pm$  1%). The fraction of ingested glucose that reaches plasma, calculated as the ratio between the area under  $R_{a\text{ meal}}^{\text{ref}}$  and the ingested dose, was  $f^{\text{ref}} = 0.89 \pm 0.02$ . Because the kinetic parameters were not normally distributed ( $P < 0.01$  by Shapiro-Wilk test; Fig. 4), median values were different from the mean ones:  $S_G^{\text{m-ref}} = 0.025 \text{ min}^{-1}$ ;  $V^{\text{m-ref}} = 1.45 \text{ dl/kg}$ ;  $p_2^{\text{m-ref}} = 0.012 \text{ min}^{-1}$ ;  $S_I^{\text{m-ref}} = 10.35 \times 10^{-4} \text{ dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}$ ;  $f^{\text{m-ref}} = 0.9$ . As anticipated in *Parameter Estimation*,  $\text{SQRP}_2^{\text{ref}}$  was normally distributed ( $P = 0.35$  by Shapiro-Wilk test), whereas  $p_2^{\text{ref}}$  was not ( $\text{SQRP}_2^{\text{ref}} = 0.11 \pm 0.004 \text{ min}^{-1/2}$ ).

### Nontracer Method

The mean profile of  $R_{a\text{ meal}}$  provided by the model is shown in Fig. 2A. The fit of OMM was satisfactory (Fig. 3B). Insulin sensitivity was estimated with good precision:  $S_I = 11.68 \pm 0.73 \times 10^{-4} \text{ dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}$ , CV = 7  $\pm$  0.3%; parameters  $\alpha_i$  were also estimated with good precision, the less precise parameter being  $\alpha_8$ , which is expected to be very close to zero:  $\alpha_1 = 5.36 \pm 0.33 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (CV = 12  $\pm$  2%),  $\alpha_2 = 7.78 \pm 0.24 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (CV = 10  $\pm$  0.4%),  $\alpha_3 = 6.00 \pm 0.28 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (CV = 27  $\pm$  9%),  $\alpha_4 = 5.05 \pm 0.22 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (CV = 11  $\pm$  0.5%),  $\alpha_5 = 4.77 \pm 0.28 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (CV = 30  $\pm$  15%),  $\alpha_6 = 3.52 \pm 0.19 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (CV = 12  $\pm$  2%),  $\alpha_7 = 2.09 \pm 0.09 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$

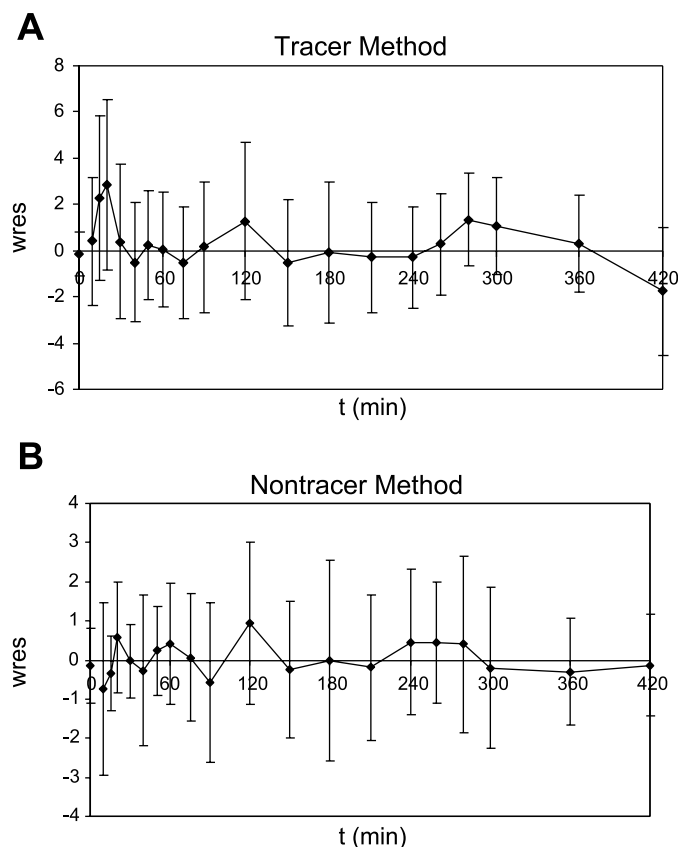


Fig. 3. Weighted residuals (wres; vertical bars represent SD) of the minimal model of glucose kinetics forced with the tracer method  $R_{a\text{ meal}}^{\text{ref}}$  (A) and of the nontracer OMM, which reconstructs  $R_{a\text{ meal}}$  (B).

$\text{kg}^{-1} \cdot \text{min}^{-1}$  ( $\text{CV} = 6 \pm 0.5\%$ ), and  $\alpha_8 = 0.34 \pm 0.05 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $\text{CV} = 42 \pm 4\%$ ). The bayesian estimate of  $p_2$  gives  $p_2 = 0.011 \pm 0.0005 \text{ min}^{-1}$  ( $\text{CV} = 15 \pm 0.7\%$ ).

### Comparison

A good agreement was found between the OMM  $R_{a\text{ meal}}$  and  $R_{a\text{ meal}}^{\text{ref}}$  profiles (mean curves are shown in Fig. 2A). We calculated the relative deviation of OMM  $R_{a\text{ meal}}$  estimates from the reference  $R_{a\text{ meal}}^{\text{ref}}$  in each breakpoint of the piecewise-linear function as:

$$\delta R_{a\text{ meal}} = \frac{R_{a\text{ meal}}^{\text{ref}} - R_{a\text{ meal}}}{R_{a\text{ meal}}^{\text{ref}}} \quad (8)$$

The temporal profile of the mean  $\delta R_{a\text{ meal}}$  is shown in Fig. 2B and does not present systematic deviation from zero. Moreover,  $S_I$  provided by OMM and  $S_I^{\text{ref}}$  were not significantly different ( $S_I = 11.68 \pm 0.73 \times 10^{-4} \text{ dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}$ ,  $S_I^{\text{ref}} = 11.55 \pm 0.68 \times 10^{-4} \text{ dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}$ ) and well correlated ( $r = 0.86$ ,  $P < 0.0001$ ; Fig. 5). To quantify the agreement between OMM and reference  $S_I$  in each individual, we calculated the absolute percentage deviation between the two measurements as:

$$|\Delta S_I| = \frac{|S_I^{\text{ref}} - S_I|}{S_I^{\text{ref}}} \quad (9)$$

It was found that  $|\Delta S_I| = 29 \pm 3\%$  on average (range 0.42–160%). Finally, to assess the influence of model parameters  $S_G$ ,  $f$ ,  $p_2$ , and  $V$  on individual  $S_I$  estimates, a multivariate regression was performed of  $\Delta S_I$  vs.  $\Delta S_G$ ,  $\Delta f$ ,  $\Delta p_2$ , and  $\Delta V$  covariates. Results indicate that  $\Delta S_G$ ,  $\Delta f$ , and  $\Delta p_2$  contribute to  $\Delta S_I$  variance ( $r = 0.752$ ,  $P < 0.0001$ ), whereas  $\Delta V$  does not. We conclude that the OMM is quite robust and most applicable in population studies, but it is less accurate in estimating the individual values of  $S_I$ .

### DISCUSSION

Insulin sensitivity measures the ability of insulin to inhibit glucose production and enhance glucose utilization. It is used in clinical and epidemiological studies to quantify insulin resistance as a risk factor for pathological conditions, such as obesity and hypertension, and to assess the efficacy of a given therapy. Insulin sensitivity is usually estimated using intravenous administration of glucose and/or insulin, such as the glucose clamp technique (10) or the IVGTT interpreted with the minimal model (5). However, both of these techniques measure insulin sensitivity by experimentally creating a non-physiological milieu, and it would be important to be able to measure this parameter in a normal-life physiological milieu, e.g., during a meal.

Recently, a new OMM has been proposed that is able to estimate insulin sensitivity  $S_I$  in a given individual from plasma glucose and insulin concentration measured after an oral glucose perturbation, by simultaneously reconstructing also the rate of appearance of absorbed glucose ( $R_{a\text{ meal}}$ ). In this study, we have validated the OMM against what can be considered today the state-of-art reference method for estimating insulin sensitivity during a meal or an OGTT. The reference method relies on tracers and comprises the following two steps: first, a two-tracer protocol is used in conjunction with a model of non-steady-state glucose kinetics to obtain the rate of glucose absorption; second, the calculated rate of glucose absorption is used in conjunction with the classic minimal model to measure insulin sensitivity from the measured plasma glucose and insulin concentrations.

To test the nontracer OMM against the tracer method, we analyzed with both methods 88 subjects who underwent the triple-tracer meal protocol described (4). The comparison between the  $R_{a\text{ meal}}$  profile obtained with the OMM and  $R_{a\text{ meal}}^{\text{ref}}$  indicates a good agreement between the two (Fig. 2). The temporal plot of the deviations of OMM  $R_{a\text{ meal}}$  estimates from the reference in each breakpoint of the piecewise-linear function does not present systematic deviation from zero. However, some differences between the reference profile and OMM prediction can be seen at the individual level. A likely explanation for these discrepancies is that OMM requires us to fix some parameters. In particular, the use of a population value for the fraction of absorbed dose ( $f$ ) forces the area under the curve of  $R_{a\text{ meal}}$  to take on a value that is correct on average but differs slightly at the individual level. The comparison between insulin sensitivity indexes obtained by OMM and the reference model indicates an excellent agreement in average between the estimates of insulin sensitivity ( $S_I^{\text{ref}} = 11.55 \pm 1 \times 10^{-4} \text{ dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}$ ,  $S_I = 11.68 \pm 2 \times 10^{-4} \text{ dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}$ ).



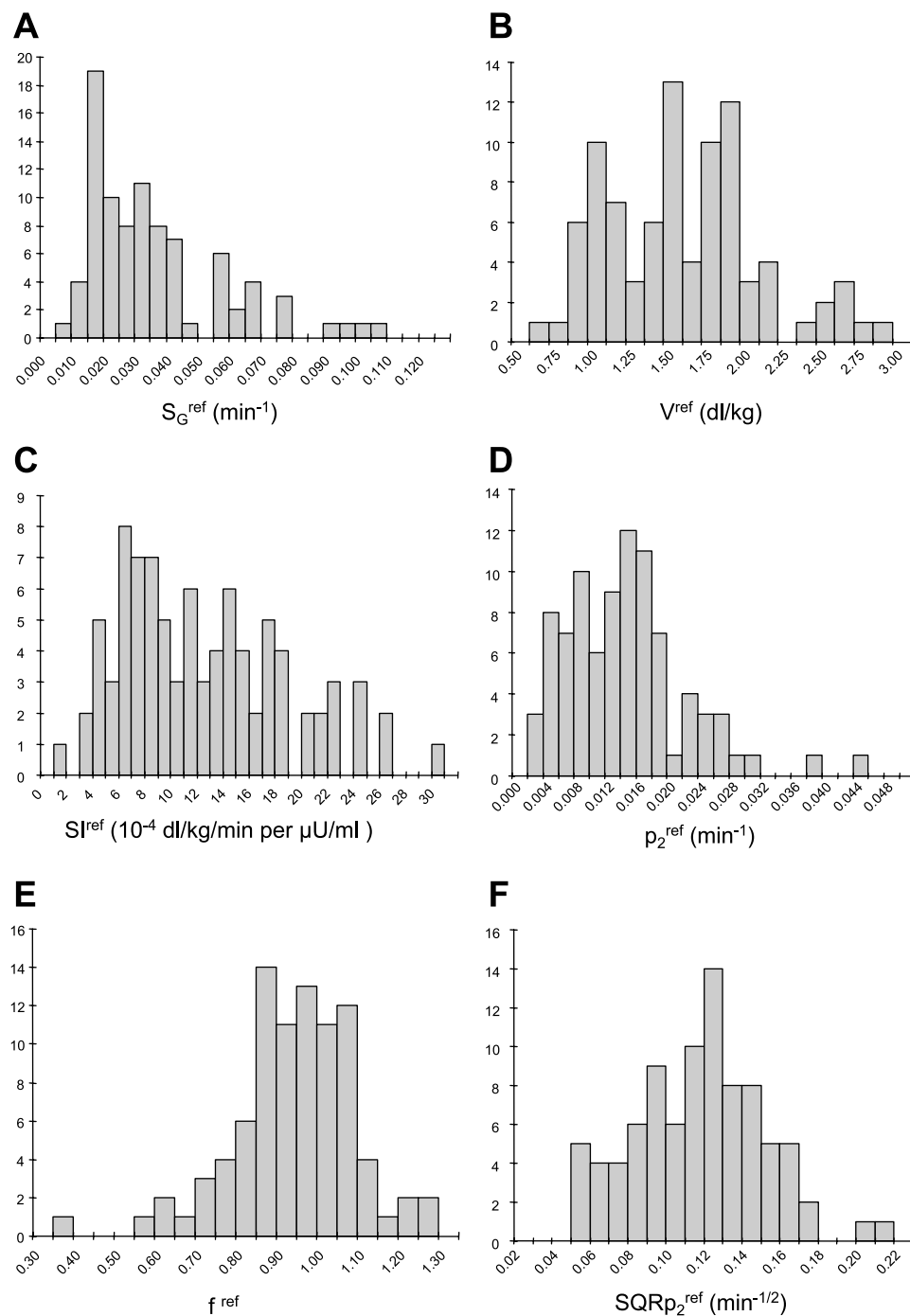


Fig. 4. Distributions of reference model parameter estimates ( $\text{SQR}p_2$  denotes square root of  $p_2$ ) obtained from the tracer method.  $S_G^{\text{ref}}$ , reference glucose effectiveness;  $V^{\text{ref}}$ , reference distribution volume;  $SI^{\text{ref}}$ , reference sensitivity index;  $p_2^{\text{ref}}$ , reference rate constant of insulin action;  $f^{\text{ref}}$ , reference fraction of ingested glucose that is absorbed.

$\text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}$ ); correlation between the two indexes is satisfactory ( $r = 0.86$ ,  $P < 0.0001$ ; Fig. 5).

The ability of the OMM to provide results that are in good agreement with those provided by the more complex and expensive tracer method hinge on a simple but effective parametric modeling of the rate of glucose absorption and on the assumption that some model parameters such as  $V$ ,  $S_G$ , and  $f$  equal their population values. In addition, the model uses a bayesian prior on  $p_2$  to improve the numerical identification properties of the model. The novelty of the present study is that the reference model provides values of kinetic parameters

during a meal. The large number of subjects studied in the present experiments permitted the distributions to be determined for all parameters. As is evident in Fig. 4, kinetic parameters were not normally distributed (as confirmed by the Shapiro-Wilk normality test,  $P < 0.01$ ). Therefore, the median rather than mean values ( $V^{\text{m-ref}} = 1.45 \text{ dl/kg}$ ,  $S_G^{\text{m-ref}} = 0.025 \text{ min}^{-1}$ ,  $f^{\text{m-ref}} = 0.90$ ) were used as reference for OMM to better describe nonnormality of the distributions. SAAM II software can only provide Gaussian priors for the Bayesian estimation. Because  $\text{SQR}p_2$ , but not  $p_2$ , is normally distributed ( $P = 0.35$  and  $P < 0.0001$ , respectively, by Shapiro-Wilk test),

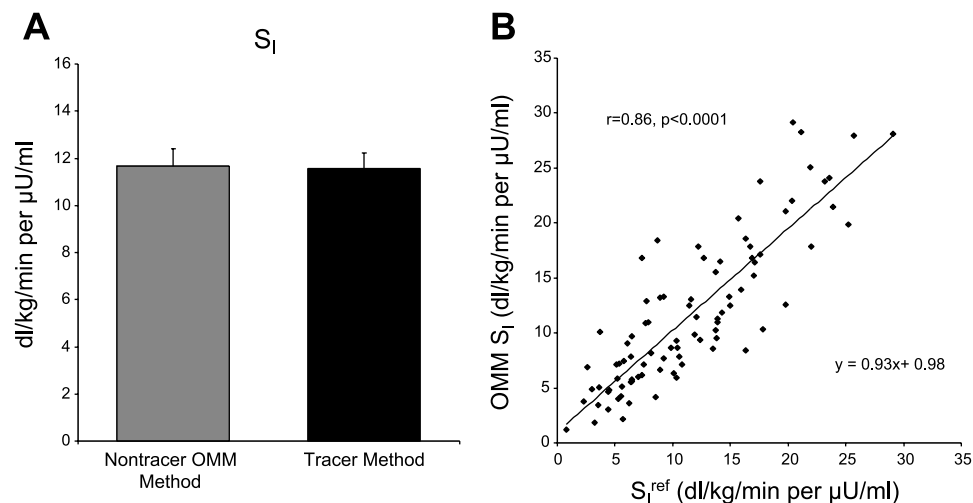


Fig. 5. A: comparison between  $S_1$  estimated from the OMM and  $S_1^{\text{ref}}$ . B: correlation plot.

the model was parameterized to make  $\text{SQR}p_2$  explicit and use the Gaussian prior on it (Fig. 4). With the use of these reference values, OMM provided an accurate estimate of insulin sensitivity not significantly different from  $S_1^{\text{ref}}$  ( $S_1 = 11.68 \pm 0.73$  vs.  $S_1^{\text{ref}} = 11.55 \pm 0.68 \times 10^{-4} \text{ dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}$ ) also when insulin sensitivity is expressed per unit of distribution volume ( $S_1 = 8.05 \pm 0.50$  vs.  $S_1^{\text{ref}} = 8.56 \pm 0.60 \times 10^{-4} \text{ min} \cdot \mu\text{U}^{-1} \cdot \text{ml}$ ). In previous studies (3, 9), mean population values derived from IVGTT and clamp studies were used [ $V = 1.7 \text{ dl/kg}$ ;  $S_G = 0.014 \text{ min}^{-1}$ ,  $p_2 = 0.03 \text{ min}^{-1}$ ,  $f = 0.86$  (4)]. As seen above, mean values of parameters during a meal are different from those of the IVGTT; in particular, volume of distribution and  $p_2$  are lower ( $V^{\text{ref}} = 1.50 \text{ dl/kg}$ ,  $p_2^{\text{ref}} = 0.013 \text{ min}^{-1}$ ), whereas glucose effectiveness is higher ( $S_G^{\text{ref}} = 0.031 \text{ min}^{-1}$ ). However, the OMM method is quite robust on average: if intravenous values of kinetic parameters are used for OMM identification, insulin sensitivity ( $S_1^{\text{iv}} = 11.82 \pm 0.66 \times 10^{-4} \text{ dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}$ ; see Ref. 3) is not statistically different from  $S_1^{\text{ref}}$  (insulin sensitivity per unit of distribution volume is different,  $S_1^{\text{iv}} = 6.95 \pm 0.39 \times 10^{-4} \text{ min} \cdot \mu\text{U}^{-1} \cdot \text{ml}$  vs.  $S_1^{\text{ref}} = 8.56 \pm 0.60 \times 10^{-4} \text{ min} \cdot \mu\text{U}^{-1} \cdot \text{ml}$ ,  $P = 0.0005$  because of differences in the distribution volume) and correlation between OMM and the reference model remains very good ( $r = 0.83$ ,  $P < 0.001$ ).

The necessity of using some population a priori knowledge for OMM numerical identification can affect  $S_1$  estimates in the single individual: the absolute percentage deviation between reference and OMM measurements is  $|\Delta S_1| = 29 \pm 3\%$  on average (range 0.42–160%). The discrepancy between the estimates of insulin sensitivity obtained with the reference model and with the OMM arises, at least in part, from the assumptions that parameters such as  $V$ ,  $S_G$ ,  $f$ , and  $p_2$  take on the same value in all subjects ( $p_2$  is constrained to a Bayesian prior). To investigate how sensitive the OMM estimate of  $S_1$  was to the assumptions made on  $V$ ,  $S_G$ ,  $f$ , and  $p_2$ , we investigated the relationship existing between the percent deviation of  $S_1$ ,  $\Delta S_1\%$ , and the percent deviation on the various parameters that were fixed (namely  $\Delta V\%$ ,  $\Delta S_G\%$ ,  $\Delta f\%$ , and  $\Delta p_2\%$ ) using multiple-regression analysis. We found that the percentage deviation of  $f$ ,  $S_G$ , and  $p_2$  from the fixed values explains the

deviation in  $S_1$  estimate ( $0.752$ ,  $P < 0.0001$ ), whereas the deviation of  $V$  does not give a significant contribution to the regression. We conclude that the OMM is quite robust and well applicable in population studies, although it is less accurate in estimating the individual values of  $S_1$ .

Until now, it has been possible to compare the  $S_1$  provided by OMM with the analogous index obtained from the IVGTT (3, 9). Although  $S_1$  values estimated during IVGTT were 40% lower than OMM ones, they correlated well with each other ( $r = 0.62$ ,  $P < 0.001$ ). Whereas these results were reassuring, one source of concern was that the experimental conditions during the IVGTT were markedly different from those during the oral test both in terms of the route of glucose administration and in the pattern of change of plasma glucose and insulin concentrations. Also, in the previous study (3, 9), the IVGTT studies and the oral tests were not performed on the same days, adding a potential source of variability to the comparison. Thus the present validation of the OMM estimates of  $R_{a \text{ meal}}$  and  $S_1$  against an independent “oral reference technique” adds credibility to the OMM and provides the necessary prerequisite for a more general validation of this novel method against the hyperinsulinemic-euglycemic clamp: this important issue may be addressed in a separate study, but the available preliminary results (6) are reassuring.

Finally, it is also of interest to compare OMM and reference  $S_1$  with other indexes, such as the Homeostasis Model Assessment (HOMA) (15), the Quantitative Insulin Sensitivity Check Index (QUICKI) (11), and the insulin sensitivity index (ISI) of Matsuda-De Fronzo (12), frequently used to measure insulin sensitivity during an OGTT or meal. Correlation between  $S_1^{\text{OMM}}$  and ISI index is  $r = 0.63$ , whereas correlation with HOMA or QUICKI is much lower ( $r = 0.43$  and  $0.46$ , respectively). This was expected because HOMA and QUICKI indexes are derived from glucose and insulin concentration in the basal state, whereas OMM and ISI indexes are both estimated using the 420-min data. Similar results were found if  $S_1^{\text{ref}}$  was compared with the three indexes (HOMA:  $r = 0.43$ ; QUICKI:  $r = 0.44$ ; ISI:  $r = 0.59$ ).

In conclusion, we have validated the nontracer OMM method by comparing its  $R_{a \text{ meal}}$  and  $S_1$  measurements with

those obtained by the tracer reference method. Our results indicate that the OMM provides an accurate estimation of both the  $R_{a\text{ meal}}$  profile and insulin sensitivity. Thus the OMM candidates as a simple, cost-effective, and reliable tool to measure both the rate of glucose absorption and insulin sensitivity from oral glucose tests without employing tracers.

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