Modeling hepatic insulin sensitivity during a meal: validation against the euglycemic hyperinsulinemic clamp

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Dalla Man C, Piccinini F, Basu R, Basu A, Rizza RA, Cobelli C. Modeling hepatic insulin sensitivity during a meal: validation against the euglycemic hyperinsulinemic clamp. Am J Physiol Endocrinol Metab 304: E819–E825, 2013. First published February 26, 2013; doi:10.1152/ajpendo.00482.2012.—Recently, we proposed a model describing the suppression of endogenous glucose production (EGP) during a meal. It assumes that EGP suppression depends on glucose concentration and its rate of change and on delayed insulin action. Hepatic insulin sensitivity (SILmeal) can be derived from EGP model parameters. This model was shown to adequately describe EGP profiles measured with multiple tracer techniques; however, SILmeal has never been compared directly with its euglycemic hyperinsulinemic clamp counterpart (SILclamp). To do so, 62 subjects with different degrees of glucose tolerance underwent a triple-tracer mixed meal. Fifty-seven subjects also underwent a labeled (3-3H)glucose euglycemic hyperinsulinemic clamp. From the triple-tracer meal data, virtually model-independent estimates of EGP were obtained using the tracer-to-tracer clamp technique, and the EGP model was identified in each subject. Model fit was satisfactory, and SILmeal was estimated with good precision. Correlation between SILclamp and SILmeal was good (r = 0.72, P < 0.001); however, SILmeal was lower than SILclamp (4.60 ± 0.64 vs. 8.73 ± 1.07 10^−4 dl·kg^−1·min^−1 per μU/ml, P = 0.01). This difference may be due to different ranges of insulin explored during the two tests (ΔIclamp = 15.60 ± 1.61 vs. ΔImeal = 83.37 ± 10.71 μU/ml) as well as steady- vs. non-steady-state glucose and insulin profiles. In conclusion, the new EGP model provides an estimate of hepatic insulin sensitivity during a meal that is in good agreement with that derived in the same individuals with a hyperinsulinemic clamp. When used in conjunction with the minimal model, the approach potentially enables estimation of hepatic insulin sensitivity from a single-tracer labeled meal or oral glucose tolerance test.

endogenous glucose production; glucose kinetics; tracer; insulin resistance

THE LIVER IS ONE OF THE MOST IMPORTANT ORGANS responsible for glucose regulation. In healthy subjects, hepatic glucose production and utilization are modulated to maintain appropriate glucose levels despite external perturbations (e.g., meal or physical activities). Endogenous glucose production (EGP) after a meal is suppressed due to both glucose and insulin signaling. However, in pathological conditions such as diabetes, glucose and insulin are unable to appropriately suppress EGP (16), causing hyperglycemia and glucose intolerance. For instance, hepatic insulin resistance was shown to contribute to fasting hyperglycemia in impaired fasting glucose (IFG) (19), and this is at least in part due to impaired insulin-induced suppression of gluconeogenesis (8). The availability of a tool that enables assessment of the role of glucose (GEHmeal) and insulin (SI1meal) in the suppression of EGP under physiological conditions during a meal would enable a better understanding of the contribution of hepatic insulin resistance to glucose intolerance. Previously, we proposed a model that describes the suppression of EGP during a meal (14). This model assumes that suppression of EGP is proportional to the plasma glucose concentration and the glucose derivative, which is related to insulin concentration in the portal vein, as well as delayed insulin action. The model was tested successfully against EGP measured during a meal with a triple-tracer protocol and tracer/tracer ratio clamp method. However, the results of this model were not compared with those derived using a euglycemic hyperinsulinemic clamp, which is considered the gold standard for the measurement of hepatic insulin sensitivity. (5, 15). The euglycemic hyperinsulinemic clamp consists of an intravenous infusion of insulin that raises plasma insulin concentration to a constant value (higher than basal) and a concomitant infusion of dextrose (labeled with tracer) sufficient to maintain plasma glucose to basal level. This approach enables measurement of glucose production and utilization in the presence of constant insulin and glucose concentrations. Under these conditions, EGP (derived from the liver, kidney, and perhaps intestine) equals the difference between the tracer-determined rate of glucose appearance and the glucose infusion rate, which in turn enables assessment of hepatic insulin sensitivity (SI1clamp).

The purpose of the current experiments was to compare meal- (SI1meal) and clamp-derived (SI1clamp) estimates of hepatic insulin action measured in the same subjects on two separate occasions using both a triple-tracer mixed meal and a labeled euglycemic hyperinsulinemic clamp.

MATERIALS AND METHODS

Subjects and Protocol

The study was approved by the Mayo Institutional Review Board, and all subjects gave informed written consent prior to enrollment. Sixty-two Caucasian subjects [32 IFG and 30 normal fasting glucose (NFG); age 53 ± 1 yr, BMI 29.7 ± 0.6 kg/m²] were studied. All subjects were in good health and at stable weights and did not engage in vigorous physical exercise. Subjects were part of a larger study examining mechanisms of postprandial hyperglycemia, the results of which have been reported elsewhere (6–8).

As reported previously (6–8), subjects were studied on three occasions in random order when they underwent either a standard oral glucose tolerance test (OGTT), a triple-tracer mixed-meal test, or a euglycemic hyperinsulinemic clamp. The OGTT data will be used here only to classify subjects on the basis of their glucose tolerance: normal glucose tolerant (NGT; 2-h plasma glucose <7.8 mmol/l; n =
E280  HEPATIC INSULIN SENSITIVITY DURING A MEAL

25), impaired glucose tolerant (IGT; 2-h plasma glucose between 7.8 and 11.1 mmol/l; n = 37), and diabetes (2-h plasma glucose >11.1 mmol/l). The entire cohort can thus be divided into the following subgroups: IFG/diabetes mellitus (DM) (n = 9), IFG/IGT (n = 16), IFG/NGT (n = 7), NFG/IGT (n = 12), and NFG/NGT (n = 18). We refer to Bock et al. (7) for a complete description of the OGTT protocol, whereas labeled clamp and triple-tracer mixed-meal procedures are described below.

All subjects were instructed to follow a weight maintenance diet containing 55% carbohydrate, 30% fat, and 15% protein for 3 days before the study day. Subjects were admitted to the Mayo CTSA-CRU at 1700 on the evening before the study and ate a standard 10 kcal/kg meal (55% carbohydrate, 30% fat, and 15% protein) between 1830 and 1900. Subjects remained fasted overnight.

Triple-tracer mixed meal. An 18-gauge cannula was inserted at 0600 into a forearm vein for tracer infusions on the day of the study. Another 18-gauge cannula was inserted in a retrograde fashion into a dorsal hand vein of the opposite arm, and the hand was placed in a heated box (~55°C) to enable sampling of arterialized venous blood. A primed (12 mg/kg) continuous (0.12 mg·kg⁻¹·min⁻¹) infusion of [6,6-²H₂]glucose (MassTrace, Woburn, MA) was started at 0700. At time 0, i.e., 1000, subjects ingested a standard mixed meal within 15 min, which consisted of three scrambled eggs, 55 g of Canadian bacon (or 47 g of steak), and Jell-O containing 75 g of glucose that was enriched (to ~4%) with [1-¹³C]glucose, as described previously (3, 4).

Two additional tracers, [6,6-²H₂]glucose and [6-³H]glucose, were infused intravenously with the tracer-to-tracer clamp technique (3), i.e., at a variable rate mimicking EGP and [1-¹³C]glucose rate of appearance in plasma, respectively. Plasma samples were collected at the following times: 180, 178, 170, 140, 100, 30, 20, 10, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 280, 300, and 360 min. More details on protocol and measurements can be found in Ref. 6.

Labeled euglycemic hyperinsulinemic clamp. At 0600, an 18-gauge cannula was inserted in a forearm vein for tracer and hormone infusions. Another cannula was inserted in a retrograde fashion into a dorsal hand vein of the opposite arm, and the hand was placed in a heated box (~55°C) to enable sampling of arterialized venous blood. A primed (fasting plasma glucose 5.5 mmol·l⁻¹) continuous (0.12 µCi/min) infusion of [3-³H]glucose (New England Nuclear, Boston, MA) was started at 0700 and continued until the end of the study.

A constant infusion containing somatostatin (60 ng·kg⁻¹·min⁻¹), glucagon (0.65 µg·kg⁻¹·min⁻¹), and growth hormone (3 ng·kg⁻¹·min⁻¹) was started at 1000 (i.e., time 0) for 4 h. An infusion of insulin also started at a rate of 0.25 or 0.50 mU·kg⁻¹·min⁻¹ in the subjects receiving either a prandial (15 IFG and 13 NFG) or prandial (16 IFG and 15 NFG) insulin infusion, respectively. Dextrose (D50W) containing [3-³H]glucose was infused as necessary to maintain plasma glucose concentrations at ~5.0 mmol/l over the 4 h of the study, as described previously (15). In addition, the basal [3-³H]glucose infusion was adjusted downward beginning at time 0 in a manner mimicking the anticipated pattern of fall of EGP in an effort to further minimize the change in plasma [3-³H]glucose-specific activity. Arterialized venous blood samples were collected at regular intervals for measurement of glucose, tracer, and hormone concentrations. Only 57 among 62 subjects completed the clamp study.

EGP During the Meal

Virtually model-independent estimates of EGP were obtained by applying the two-compartment model (18) to the clamped tracer/tracer ratio (TTR; [6,6-²H₂]glucose/endogenous glucose) (3), using the following equation:

\[
\text{EGP}(t) = \frac{\text{INF}(t)}{\text{TTR}(t)} - \frac{V_1 \cdot G_{\text{end}}(t)}{\text{TTR}(t)} \cdot \frac{d\text{TTR}(t)}{dt} + k_{12} \left[ \frac{q_2(t)}{\text{TTR}(t)} - Q_2(t) \right] \tag{1}
\]

where \(G_{\text{end}}\) is the plasma concentration of endogenous glucose, \(V_1\) is the volume of distribution of the accessible pool, and \(k_{12}\) is the rate constant between the peripheral and the accessible compartment, fixed to 130 ml/kg and 0.07 min, respectively, according to previous studies in normal subjects (18); \(q_2\) and \(Q_2\) are the amounts of [6,6-²H₂]glucose and endogenous glucose in the peripheral compartment, which can be reconstructed by integrating model equations. From Eq. 1 it is evident that the estimate of EGP depends generally on the order of the chosen model (here a 2-compartment model) and parameter values. However, the extent of this dependence is minimized in the present study, since the intravenous tracer is infused so as to mimic the expected pattern of EGP and thus minimize TTR time variations (TTR clamp). When this condition is reached, at least approximately (see RESULTS), dTTR/dt is close to zero; a constant TTR is maintained in the system, i.e., \(q_2/TTR = Q_2\), and EGP being the ratio between the rate of infusion and TTR is influenced minimally by the choice of the model.

Hepatic Insulin Sensitivity From a Meal

EGP model. According to Dalla Man et al. (14), EGP can be described by the following model:

\[
\text{EGP}(t) = \text{EGP}_b - k_G \left[ \frac{G(t) - G_b}{X(t) - X_{\text{Der}}(t)} \right] = \text{EGP}(0) = \text{EGP}_b \tag{2}
\]

where EGP_b is basal endogenous glucose production and \(k_G\) is hepatic glucose effectiveness.

\(X^0\) is hepatic insulin action, defined as

\[
X^0(t) = \begin{cases} X(t) - k_1 \cdot X^0(t) + k_1 \cdot X^0(t) & \text{if } t > 0 \\ X(t) = k_1 \cdot X^0(t) + q_2(t) + q_2(t) \cdot (I(t) - I_0) & \text{if } t = 0 \end{cases} \tag{3}
\]

where \(k_1\) is the delay of hepatic insulin action with respect to plasma insulin and \(k_G\) a parameter governing its efficacy.

\(X_{\text{Der}}\) is a surrogate of portal insulin that anticipates insulin and glucose patterns and was demonstrated to significantly improve model ability to fit the rapid suppression of EGP occurring immediately after the meal:

\[
X_{\text{Der}}(t) = \begin{cases} \frac{dG(t)}{dt} & \text{if } \frac{dG(t)}{dt} \geq 0 \\ 0 & \text{if } \frac{dG(t)}{dt} < 0 \end{cases} \tag{4}
\]

where \(k_{G_{\text{Der}}} \) is a parameter governing the magnitude of glucose derivative control.

Hepatic insulin sensitivity. The index of hepatic insulin sensitivity (\(S_1^{\text{clamp}}\)) can be derived from model parameters as follows:

\[
S_1^{\text{clamp}} = \frac{\partial \text{EGP}}{\partial I} \bigg|_{I_s} \cdot \frac{1}{G_b} = \frac{k_1}{G_b} \tag{5}
\]

where the symbol \(I_s\) indicates that the derivative of EGP is calculated in steady state.

Hepatic Insulin Sensitivity from a Euglycemic Hyperinsulinemic Clamp

Steady-state formulas. Whenever glucose concentration is well clamped (i.e., glucose is maintained fairly constant) and glucose infusion rate (GIR), infused to maintain constant plasma glucose concentration, is at steady state, insulin sensitivity (\(S_1^{\text{clamp}}\)) can be
calculated from plasma glucose and insulin concentrations and glucose rate of infusion as follows (5, 15):

\[
SI_{\text{clamp}} = \frac{GIR_{\text{ss}}}{G_{\text{ss}} \cdot \Delta I}
\]

(6)

where GIR_{\text{ss}} (mg·kg\(^{-1}\)·min\(^{-1}\)) is the steady-state (average in the last 40 min) glucose infusion rate, G_{\text{ss}} the steady-state glucose concentration, and \(\Delta I\) is the difference between end test and basal insulin concentrations.

Insulin sensitivity on glucose disposal (SI_{\text{Dclamp}}) can be calculated from \([3-3H]\)glucose and plasma insulin concentrations and from rate of infusion of glucose tracer as follows (5):

\[
SI_{\text{Dclamp}} = \frac{GIR_{\text{ss}}}{G_{\text{ss}} \cdot \Delta I \cdot S_{\text{Ass}}}
\]

(7)

where GIR_{\text{ss}}* (Ci·kg\(^{-1}\)·min\(^{-1}\)) is the difference between steady-state (average in the last 40 min) tracer infusion rate at the end of the clamp period and during the tracer equilibration period, S_{\text{Ass}} is the end test-specific activity, i.e., \([3-3H]\)glucose/glucose, G_{\text{ss}} is the steady-state glucose concentration, and \(\Delta I\) is the difference between end test and basal plasma insulin concentration.

Hepatic insulin sensitivity (SI_{\text{Lclamp}}) can then be derived as

\[
SI_{\text{Lclamp}} = SI_{\text{clamp}} - SI_{\text{Dclamp}}
\]

(8)

Non-steady-state correction. Maintaining plasma glucose constant during the study and having constant GIR and GIR* at the end of the test is not an easy task, since the individual responses to insulin perturbation are variable. Thus, in some cases the steady-state formulas are not applicable, and glucose dynamics should be taken into account to accurately assess overall and disposal insulin sensitivity. To this purpose, we used the minimal model of glucose and tracer glucose kinetics (11), and SI_{\text{clamp}} and SI_{\text{Dclamp}} were derived from model parameters (see APPENDIX). Of note, whenever plasma glucose, tracer glucose, and their infusion rates are in steady state, model-derived SI_{\text{clamp}} and SI_{\text{Dclamp}} coincide with Eqs. 6 and 7, respectively.

Parameter Estimation

All models were identified numerically by nonlinear least squares (9, 10) implemented in Matlab version 7.7. Error of EGP time course was assumed to be independent, Gaussian, with zero mean and unknown constant standard deviation, estimated a posteriori. For the minimal model, error on glucose data was assumed to be independent, Gaussian, with zero mean and known standard deviation [coefficient of variation (CV) = 2% of the data]. For the labeled minimal model, measurement error on tracer glucose data was assumed to be independent, Gaussian, with zero mean and known standard deviation (CV = 5% of the data). Glucose and insulin concentrations, for the EGP model, and insulin concentration, for minimal model identification, are the model forcing functions, which are assumed to be known without error.

Statistical Analysis

Data are presented as means ± SE if not stated differently. Two sample comparisons were done by Wilcoxon signed-rank test, whereas differences among groups were assessed with Mann-Whitney rank sum test (significance level set to 5%). Pearson’s correlation was used to evaluate univariate correlation.

RESULTS

Labeled Mixed Meal

Plasma glucose and insulin concentrations measured during the labeled meal are shown in Fig. 1, top left and bottom left, respectively. The clamped TTR of [6,6-2H\(_2\)]glucose/endogenous glucose and EGP are shown in Fig. 1, top right and bottom right,

**Fig. 1.** Mean plasma glucose (top left) and insulin concentration (bottom left), tracer/tracee ratio (top right), and endogenous glucose production (bottom right) measured during the triple-tracer mixed meal.
respectively. TTR varies, as expected, in a limited range, thus confirming that the EGP estimate is virtually model independent.

The models (Eqs. 2–4) fit EGP data well (not shown) and provided precise estimates of the parameters in all 62 subjects: k0 = 0.0098 ± 0.0006 dL·kg⁻¹·min⁻¹ (CV = 18 ± 2%), kGR = 0.195 ± 0.0196 dL/kg/CV = 68 ± 26%, k1 = 0.0156 ± 0.0008 min/CV = 14 ± 2%, k2 = 0.037 ± 0.003 m·kg⁻¹·min⁻¹ per μU/ml/CV = 22 ± 4%, and S1Lclamp = 8.73 ± 1.07 10⁻⁴ dL·kg⁻¹·min⁻¹ per μU/ml/CV = 22 ± 6%.

Labeled Euglycemic Hyperinsulinemic Clamp

Plasma glucose and tracer glucose concentrations were maintained on average almost constant during the clamp (Fig. 2). However, this was not always the case at the single individual level. The above-described minimal models were thus identified to compensate the non-steady-state error. Among the 57 subjects that completed the study, only 39 could be analyzed here since 12 subjects had a GIR of 0 mg·kg⁻¹·min⁻¹ (very low insulin sensitivity) and six subjects had incomplete analyzable [3-3H]glucose samples. Non-steady-state correction was applied to all of the remaining subjects.

The minimal model performed satisfactorily in 27 of the 39 subjects (not shown) while providing imprecise parameter estimates (CV > 100%) in 12 subjects. On the other hand, the tracer minimal model performed satisfactory only in 24 of the 39 subjects (not shown), whereas it provided a bad data fit in 13 subjects and imprecise parameter estimates (CV > 100%) in two other subjects. In conclusion, hepatic insulin sensitivity could be estimated accurately only in 24 subjects (1 IFG/DM, 5 IFG/IGT, 3 IFG/NGT, 4 NFG/IGT, and 11 NFG/NGT; S1Lclamp = 8.73 ± 1.07 10⁻⁴ dL·kg⁻¹·min⁻¹ per μU/ml).

Comparisons

Hepatic insulin sensitivity estimated from clamp and meal has been compared in the 24 subjects for which clamp estimates were sufficiently accurate (Fig. 3). Correlation between S1Lclamp and S1Lmeal was good (r = 0.72, P < 0.0001), with S1Lmeal being lower than S1Lclamp (4.60 ± 0.64 vs. 8.73 ± 0.71 10⁻⁴ dL·kg⁻¹·min⁻¹ per μU/ml, P < 0.01). It is noteworthy that the correlation improved to 0.80, P < 0.001, if the NFG group was considered (n = 15), whereas correlation was lower in the IFG group (r = 0.56, P = 0.11), with the nonsignificant P value likely due to the limited sample size.

Hepatic Insulin Sensitivity in Prediabetes

We also assessed the adequacy of the EGP model to distinguish among classes of subjects with different degrees of glucose tolerance. As reported in Fig. 4, S1Lmeal was significantly lower in IFG compared with NFG/NGT (3.37 ± 0.41 vs. 5.34 ± 0.71 10⁻⁴ dL·kg⁻¹·min⁻¹ per μU/ml, P < 0.05). Moreover, S1Lmeal was significantly lower in NFG/IGT, IFG/IGT, and IFG/DM compared with both NFG/NGT and IFG/NGT (3.37 ± 0.56, 3.27 ± 0.50, and 2.08 ± 0.20 vs. 5.34 ± 0.71 and 5.24 ± 1.26 10⁻⁴ dL·kg⁻¹·min⁻¹ per μU/ml, respectively, P < 0.05).

DISCUSSION

Recently, we proposed a model that describes glucose and insulin control on EGP during a meal (14). This model also provides an estimate of hepatic insulin sensitivity, an important index that quantifies the ability of insulin to suppress glucose production during a meal.

The objective of the current experiments was to compare this model-based estimate of hepatic insulin sensitivity during a meal with that obtained from the labeled euglycemic hyperinsulinemic clamp. At variance with the labeled euglycemic hyperinsulinemic clamp method, in which hepatic insulin sensitivity is derived as a difference between whole body and disposal indices, the current model derives...
Hepatic insulin sensitivity from the measurement of EGP profile obtained with the triple-tracer technique and an EGP structural model.

Model-independent estimates of EGP were derived in 62 subjects with different degrees of glucose tolerance from triple-tracer meal data using the tracer-to-tracer clamp technique. We confirmed that this EGP model provides an excellent description of the EGP profiles as well as a precise estimate of hepatic insulin sensitivity.

The triple-tracer meal approach is less labor intensive, safer, and less dependent upon investigator skill than the euglycemic hyperinsulinemic clamp method. Therefore, it is reassuring that our results show a good correlation between the indices provided by the two methods (r = 0.72, P < 0.0001), even if they differ in absolute values (S ILmeal vs. S ILclamp). Right: correlation plot. EGP, endogenous glucose production.

The difference in the absolute values between the two methods could be explained in part by the different ranges of insulin explored during the two tests (ΔI clamp 15.60 ± 1.61 vs. ΔI meal 83.37 ± 10.71 μU/ml, P < 0.00001). In fact, it is likely that the liver inhibits glucose production more effectively at lower than at higher insulin levels. For instance, the results reported by Bock et al. (8) support the hypothesis that the hepatic response to insulin is nonlinear. Bock et al. (8) found that an increase in plasma insulin of 31 pmol/l (from 48 to 79) in IFG and 33 pmol/l (from 27 to 60) in NFG resulted in a decrease in the rate of EGP of 5 (from 15.2 to 10) and 8 (from 15.3 to 7.3) μmol·kg⁻¹·min⁻¹, respectively; on the other hand, an increase in plasma insulin of 99 pmol/l (from 48 to 147) in IFG and 107 pmol/l (from 27 to 134) in NFG (>3-fold) resulted in a decrease in the rate of EGP of 9.1 (from 15.2 to 6.1) and 11.4 (from 15.3 to 3.9) μmol·kg⁻¹·min⁻¹, respectively (<2-fold).

To gain greater insight into this possibility, we also tested the performance of a nonlinear model assuming a saturation of insulin action at higher insulin concentrations. However, the resultant model was too complex and unable to provide a precise estimate of parameters and was thus discarded. In addition, as we discussed earlier (13), it is interesting to speculate that the different experimental conditions (constant glucose and insulin concentrations during a clamp vs. continuously changing concentrations following meal ingestion) may play a role in the ability of insulin to turn off glucose production.

Of note, hepatic insulin sensitivity estimated with the meal EGP model discriminates among different classes of glucose tolerance and provides results that are concordant to those obtained using the euglycemic hyperinsulinemic clamp approach (6). S ILmeal was significantly lower in IFG compared with NFG/NGT group and significantly lower in NFG/IGT, IFG/IGT, and IFG/DM compared with both NFG/NGT and IFG/NGT. This concordance is reassuring since it supports the use of the EGP model to assess hepatic insulin action under a variety of physiological and pathophysiological conditions.

Correction of non-steady-state error in the clamp experiment with the minimal model was needed to enable comparison meal vs. clamp indices, since glucose and [6-3H]glucose concentrations were not always constant during the clamp and, in some cases, the minimal model was needed to enable comparison meal vs. clamp indices.
cases, GIR did not reach the steady state. In fact, overall $S_I$ from clamp data with the minimal model and the steady-state formulas were well correlated ($r = 0.85$, $P < 0.001$) and similar on average (model = 24.11 ± 2.53 vs. clamp = 23.27 ± 2.64 $10^{-4}$ dl·kg$^{-1}$·min$^{-1}$ per µU/ml); however, they differed at the individual level, in large part due to the quality of the clamp. For example, if a “good” clamp condition was obtained, e.g., in subject no. 22, the two methods gave virtually identical results (model = 23.43 vs. clamp = 23.77 $10^{-4}$ dl·kg$^{-1}$·min$^{-1}$ per µU/ml). On the other hand, if the plasma glucose concentration was not stable or if the GIR did not reach steady state, e.g., in subject no. 4, then the two methods provided discordant results (model = 37.22 vs. formula = 13.35 $10^{-4}$ dl·kg$^{-1}$·min$^{-1}$ per µU/ml). Similarly, for the effects of insulin on glucose disposal, correlation between model and steady-state clamp results was $r = 0.92$, $P < 0.001$, and estimates were not different on average (model = 16.70 ± 1.96 vs. clamp = 14.82 ± 1.93 $10^{-4}$ dl·kg$^{-1}$·min$^{-1}$ per µU/ml). However, results varied at the individual level. Furthermore, when $S_{IL}^{clamp}$ is calculated as the difference between overall and disposal index, the non-steady-state error is amplified and correlation between model and steady-state formulas deteriorated ($r = 0.47$, $P = 0.021$). Therefore, we conclude that use of $S_{IL}^{clamp}$, which in turn enables a more robust validation of $S_{IL}^{meal}$.

Other indices of hepatic insulin sensitivity have been proposed in the literature. For instance, Matsuda and DeFronzo (17) defined hepatic insulin resistance index as the product between EGPb and skeletal muscle insulin resistance estimated with the euglycemic hyperinsulinemic clamp (2). However, to the best of our knowledge, these authors did not compare it with hepatic insulin sensitivity estimated during the clamp. The results obtained in this data set confirm what we found previously (14), i.e., that the inverse of the surrogates indices correlate poorly with $S_{IL}^{meal}$ [$r = 0.35$, $P = 0.09$, not significant, for $1/(FPI \times EGP)$ and $r = 0.46$, $P = 0.024$, for $1/(AUC_G \times AUC_I)$]. This result is not surprising. As discussed previously (14), the Matsuda and DeFronzo (17) index, by neglecting the change in EGP that occurs when insulin and glucose concentrations change over time, is an index of basal hepatic insulin resistance and cannot distinguish subjects who have similar basal insulin action but differ in the ability of glucose and/or insulin to suppress glucose production. On the other hand, the critical assumption under the Abdul-Ghani et al. (1) index is that, during the initial 30 min after food ingestion, both glucose rate of appearance and glucose utilization are approximately zero. In addition, the correlation between $1/(AUC_G \times AUC_I)$ and $S_{IL}^{clamp}$ was negligible ($r = 0.22$, $P = 0.3$ not significant). In contrast to Matsuda and DeFronzo (17), we also did not find a good correlation between $1/(FPI \times EGP)$ and $S_{IL}^{clamp}$ ($r = 0.25$, $P = 0.2$, not significant). A possible explanation is that in Matsuda and DeFronzo (17), comparison was performed using disposal and not hepatic insulin sensitivity index derived from clamp. As a matter of fact, our results show a correlation between $1/(FPI \times EGP)$ and $S_{IL}^{clamp}$ ($r = 0.48$, $P = 0.017$).

The current study suffers from several limitations. The comparison between meal and clamp insulin sensitivity indices could be performed only in 24 of the 62 subjects, due mainly to the difficulty in obtaining accurate clamp data and clamp conditions. However, even in presence of a relatively limited sample size, our results show a good and statistically significant correlation between the hepatic insulin sensitivities estimated with the two tests. A second limitation of this study is that a repeatability analysis was not part of the protocol design. Future studies will be required to address this issue.

Finally, our method, as well as the labeled euglycemic clamp, is unable to distinguish the hepatic from the renal source of glucose since EGP represents overall endogenous glucose production. In conclusion, the triple-tracer meal approach used in conjunction with a new EGP model provides indices of hepatic insulin action that are concordant with those measured in the same individuals using the euglycemic hyperinsulinemic clamp method. This provides a method that can now assess hepatic glucose effectiveness as well as hepatic insulin action following meal ingestion. It also provides the basis for future studies directed at incorporating this new model into the oral minimal models (13) to estimate $S_{IL}^{meal}$ and EGP directly during a glucose tolerance test, potentially using a single glucose tracer. If so, this will make the proposed method appealing for clinical trials instead of the laborious labeled euglycemic hyperinsulinemic clamp method.

**APPENDIX**

The equations for the two-compartment glucose minimal model (11) are

\[
\begin{align*}
G_1(t) & = -[p_1 + X(t)] \cdot G_1(t) + p_1 \cdot G_b + k_{12} \cdot G_2(t) + \frac{GIR(t)}{V} \\
G_2(t) & = k_{21} \cdot G_1(t) - k_{12} \cdot G_2(t) \\
X(t) & = -p_2 \cdot X(t) + p_3 \cdot [I(t) - I_b]
\end{align*}
\]

where $G_1$ and $G_2$ are plasma glucose concentrations in compartments 1 and 2, respectively, 1 is plasma insulin concentration, suffix “b” denotes basal values, $X$ is the insulin action on glucose production and disposal, $V$ is the distribution volume, $k_{12}$ and $k_{21}$ are the transfer coefficients between the two compartments, and $p_1$, $p_2$, and $p_3$ are the model parameters. Specifically, $p_1$ is the fractional (i.e., per unit distribution volume) glucose effectiveness, which measures the ability of glucose per se to promote glucose disposal and inhibit glucose
production, $p_2$ is the rate constant describing the dynamics of insulin action, and $p_3$ is the parameter governing the magnitude of insulin action. Overall insulin sensitivity is given by

$$S^{\text{lamp}}_1 = \frac{p_3}{p_2} \cdot V$$

(A2)

where $G^*_1$ and $G^*_2$ are $[3^{-3}H]$glucose concentrations in compartments 1 and 2, respectively, $V$ is plasma insulin concentration, $X^*$ is the insulin action on glucose disposal, $V^*$ is the distribution volume, $k^*_1$ and $k^*_2$ are the transfer coefficients between the two compartments, and $p_1^*$, $p_2^*$, and $p_3^*$ are the model parameters. Specifically, $p_1^*$ is the fractional (i.e., per unit distribution volume) disposal glucose effectiveness that measures the ability of glucose per se to promote glucose disposal, $p_2^*$ is the rate constant describing the dynamics of insulin action on glucose disposal, and $p_3^*$ is the parameter governing its magnitude.

Disposal insulin sensitivity is given by

$$S^{\text{Delamp}}_1 = \frac{p_3^*}{p_2^*} \cdot V^*$$

(A4)

Of note, if $G(t) = G_{ss}$ and GIR$(t) = GIR_{ss}$, Eqs. A2 and 6 coincide. Similarly, the equations of the two-compartment glucose tracer minimal model are

$$S^{\text{lamp}}_1 = \frac{p_3}{p_2} \cdot V$$

(A3)

where $G^*_1$ and $G^*_2$ are $[3^{-3}H]$glucose concentrations in compartments 1 and 2, respectively, $V$ is plasma insulin concentration, $X^*$ is the insulin action on glucose disposal, $V^*$ is the distribution volume, $k^*_1$ and $k^*_2$ are the transfer coefficients between the two compartments, and $p_1^*$, $p_2^*$, and $p_3^*$ are the model parameters. Specifically, $p_1^*$ is the fractional (i.e., per unit distribution volume) disposal glucose effectiveness that measures the ability of glucose per se to promote glucose disposal, $p_2^*$ is the rate constant describing the dynamics of insulin action on glucose disposal, and $p_3^*$ is the parameter governing its magnitude.

Disposal insulin sensitivity is given by

$$S^{\text{Delamp}}_1 = \frac{p_3^*}{p_2^*} \cdot V^*$$

(A4)

Of note, if $G(t) = G_{ss}$ and GIR$(t) = GIR_{ss}$, Eqs. A2 and 6 coincide. Similarly, the equations of the two-compartment glucose tracer minimal model are

$$S^{\text{lamp}}_1 = \frac{p_3}{p_2} \cdot V$$

(A3)