

**Gianna Toffolo, Rita Basu, Chiara Dalla Man, Robert Rizza and Claudio Cobelli**

*Am J Physiol Endocrinol Metab* 291:800-806, 2006. First published May 23, 2006;

doi:10.1152/ajpendo.00461.2005

**You might find this additional information useful...**

---

This article cites 12 articles, 7 of which you can access free at:

<http://ajpendo.physiology.org/cgi/content/full/291/4/E800#BIBL>

This article has been cited by 7 other HighWire hosted articles, the first 5 are:

**Application of Isotopic Techniques Using Constant Specific Activity or Enrichment to the Study of Carbohydrate Metabolism**

A. Vella and R. A. Rizza

*Diabetes*, October 1, 2009; 58 (10): 2168-2174.

[Full Text] [PDF]

**Effects of Type 2 Diabetes on Insulin Secretion, Insulin Action, Glucose Effectiveness, and Postprandial Glucose Metabolism**

A. Basu, C. Dalla Man, R. Basu, G. Toffolo, C. Cobelli and R. A. Rizza

*Diabetes Care*, May 1, 2009; 32 (5): 866-872.

[Abstract] [Full Text] [PDF]

**Prandial Insulin and the Systemic Appearance of Meal-Derived Glucose in People With Type 1 Diabetes**

A. Vella, P. Shah, A. Basu and R. A. Rizza

*Diabetes Care*, November 1, 2008; 31 (11): 2230-2231.

[Full Text] [PDF]

**Pioglitazone Decreases Fasting and Postprandial Endogenous Glucose Production in Proportion to Decrease in Hepatic Triglyceride Content**

B. Ravikumar, J. Gerrard, C. Dalla Man, M. J. Firbank, A. Lane, P. T. English, C. Cobelli and R. Taylor

*Diabetes*, September 1, 2008; 57 (9): 2288-2295.

[Abstract] [Full Text] [PDF]

**Calculating glucose fluxes during meal tolerance test: a new computational approach**

R. Hovorka, H. Jayatilake, E. Rogatsky, V. Tomuta, T. Hovorka and D. T. Stein

*Am J Physiol Endocrinol Metab*, August 1, 2007; 293 (2): E610-E619.

[Abstract] [Full Text] [PDF]

Updated information and services including high-resolution figures, can be found at:

<http://ajpendo.physiology.org/cgi/content/full/291/4/E800>

Additional material and information about *AJP - Endocrinology and Metabolism* can be found at:

<http://www.the-aps.org/publications/ajpendo>

---

This information is current as of November 23, 2009 .

## Assessment of postprandial glucose metabolism: conventional dual- vs. triple-tracer method

Gianna Toffolo,<sup>1</sup> Rita Basu,<sup>2</sup> Chiara Dalla Man,<sup>1</sup> Robert Rizza,<sup>2</sup> and Claudio Cobelli<sup>1</sup>

<sup>1</sup>Department of Information Engineering, University of Padua, Padua, Italy; and <sup>2</sup>Department of Internal Medicine, Division of Endocrinology, Diabetes, Metabolism and Nutrition, Mayo Clinic and Foundation, Rochester, Minnesota

Submitted 21 September 2005; accepted in final form 17 May 2006

**Toffolo, Gianna, Rita Basu, Chiara Dalla Man, Robert Rizza, and Claudio Cobelli.** Assessment of postprandial glucose metabolism: conventional dual- vs. triple-tracer method. *Am J Physiol Endocrinol Metab* 291: E800–E806, 2006. First published May 23, 2006; doi:10.1152/ajpendo.00461.2005.—The dual-tracer method has been used conventionally for assessment of postprandial fluxes, i.e., appearance in plasma of ingested glucose ( $R_{a\text{ meal}}$ ), endogenous glucose production (EGP), and disposal ( $R_d$ ). To quantify the magnitude of errors affecting the calculations and their dependence on model assumptions, this method was assessed and compared with the triple-tracer method, which provides model-independent estimates. For this purpose, the dual-tracer protocol was performed twice in eight normal subjects, with [ $1\text{-}^{13}\text{C}$ ]glucose to trace ingested glucose and [ $6,6\text{-}^2\text{H}_2$ ]glucose constantly infused. A third tracer, [ $6\text{-}^3\text{H}$ ]glucose, was infused at variable rates to render the calculation of  $R_{a\text{ meal}}$  and EGP virtually model independent. The dual-tracer method analyzed with a one-compartment model performed poorly, since  $R_{a\text{ meal}}$  peak was significantly lower and delayed compared with triple-tracer reference, resulting in a significantly lower estimation of the amount of absorbed glucose ( $9,036 \pm 558$  vs.  $11,316 \pm 823$   $\mu\text{mol/kg}$ ,  $P = 0.0117$ ). EGP showed a paradoxical pattern, with an initial overshoot followed by a rapid decay to negative values, resulting in a significant underestimation of EGP suppression ( $57 \pm 3$  vs.  $65 \pm 4\%$ ,  $P = 0.0117$ ). A two-compartment model performed better but did not overcome the limitations of the dual-tracer approach, since the amount of absorbed glucose was still significantly underestimated ( $10,231 \pm 661$  vs.  $12,169 \pm 838$   $\mu\text{mol/kg}$ ,  $P = 0.0117$ ) and EGP still showed a paradoxical behavior.  $R_d$ , estimated from  $R_{a\text{ meal}}$  and EGP, was significantly underestimated with the dual-tracer method, irrespective of adopted model. We conclude that three suitably infused tracers are required for accurate assessment of postprandial  $R_{a\text{ meal}}$ , EGP, and  $R_d$ . nonsteady state; turnover; meal; kinetics; compartmental models

THE ABILITY OF AN INDIVIDUAL to dispose of an oral glucose load is the result of three different processes: appearance in plasma of ingested glucose ( $R_{a\text{ meal}}$ ), endogenous glucose production (EGP), and glucose disposal ( $R_d$ ). A dual-tracer method has been used largely to assess these fluxes. This method employs one tracer mixed with ingested glucose and a second tracer infused intravenously and calculates the systemic appearance rate of both ingested tracer and total (i.e., ingested and endogenously produced) glucose.  $R_{a\text{ meal}}$  is then calculated by multiplying the rate of appearance of ingested tracer by specific activity (or tracer-to-tracee ratio if a stable tracer is used) of glucose or carbohydrate contained in the meal. EGP is subsequently calculated by subtracting  $R_{a\text{ meal}}$  from total glucose  $R_a$ . Finally, glucose  $R_d$  is calculated by subtracting rate of change

of plasma glucose mass from total  $R_a$ . As extensively discussed in a number of papers (1, 2, 6, 8), the marked tracer nonsteady state that occurs with the dual-tracer method introduces errors and renders the calculation of  $R_{a\text{ meal}}$ , EGP, and  $R_d$  dependent on both the model used in the calculation (e.g., one or two compartments) and its parameters (e.g., the volume of distribution).

To circumvent these problems, a triple-tracer method (2) was proposed. This method used, in addition to the oral tracer (e.g., [ $1\text{-}^{13}\text{C}$ ]glucose), two intravenous tracers (e.g., [ $6\text{-}^3\text{H}$ ]glucose and [ $6,6\text{-}^2\text{H}_2$ ]glucose) infused in patterns that minimize the change in their plasma ratio to oral tracer and endogenous glucose, respectively, thus minimizing non-steady-state errors and rendering the calculation of turnover essentially model independent. As a result, very similar patterns of  $R_{a\text{ meal}}$ , EGP, and  $R_d$  were estimated using three different models: a steady-state formula, a single-compartment model (1-CM) (11, 12), and a two-compartment (9) model (2-CM). In contrast, when the three models were adopted to interpret data using only two of the three tracers, as is done with the dual-tracer method,  $R_{a\text{ meal}}$ , EGP, and  $R_d$  were highly dependent on the model chosen and differed substantially among models. However, in these experiments, all of the intravenous tracer infusion rates were varied in time. This resulted in a more pronounced change in the tracer-to-tracee ratios than would normally have been observed with the conventional dual-tracer method, where the intravenous tracer infusion rate is kept constant. Therefore, although those studies provided both the theoretical and experimental rationales for the use of the triple-tracer method, they were unable to quantify the magnitude of the errors that occur with the conventional dual-tracer method and the extent to which these errors impact the estimation of individual components of postprandial glucose metabolism, i.e.,  $R_{a\text{ meal}}$ , EGP, and  $R_d$ .

The present studies, therefore, were undertaken to compare estimates of  $R_{a\text{ meal}}$ , EGP, and  $R_d$  simultaneously measured in the same individual using the triple- and conventional dual-tracer methods. We did so to quantify the effect of non-steady-state errors and of model assumptions on calculation of postprandial fluxes. Subjects were studied on two separate days. The conventional dual-tracer protocol was used on both occasions. In addition, on one occasion a third tracer was infused in a pattern that mimicked the anticipated  $R_a$  of the meal, whereas on the other occasion the third tracer was infused in a pattern that mimicked the anticipated  $R_a$  of EGP, thereby enabling essentially model-independent measurements of these fluxes.

Address for reprint requests and other correspondence: Claudio Cobelli, Dept. of Information Engineering, Via Gradenigo 6/a, 35131 Padua, Italy (e-mail: cobelli@dei.unipd.it).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Results obtained with the conventional dual-tracer method were compared with reference profiles obtained with the triple-tracer method to determine whether the magnitude of the errors observed with the dual-tracer method warrants the added complexity and cost of the triple-tracer method.

## METHODS

**Subjects.** After approval from the Mayo Institutional Review Board, eight healthy subjects (4 women and 4 men, mean age  $44 \pm 2$  yr, height  $172 \pm 4$  cm, weight  $85 \pm 8$  kg, body mass index  $29.7 \pm 2.3$  kg/m<sup>2</sup>, lean body mass  $48 \pm 5$  kg) gave informed written consent to participate in the study. All subjects were in good health and did not participate in regular vigorous physical activity (defined as  $>30$  min of aerobic exercise/day for more than 3 days/wk).

Net insulin action and the effects of insulin on glucose disposal assessed by the oral glucose minimal models (4, 5) varied from  $4.9$  to  $29.1 \cdot 10^{-4}$  dl $\cdot$ kg<sup>-1</sup> $\cdot$ min<sup>-1</sup> per  $\mu$ U/ml and  $1.5$  to  $17 \cdot 10^{-4}$  dl $\cdot$ kg<sup>-1</sup> $\cdot$ min<sup>-1</sup> per  $\mu$ U/ml, respectively, indicating that subjects with a wide range of insulin sensitivity were studied.

**Experimental design** All studies were conducted at the Mayo Clinic General Research Center.

Mixed meals (10 kcal/kg, 45% carbohydrate, 15% protein, 40% fat) consisted of scrambled eggs, Canadian bacon, 100 ml of water, and Jell-O containing [1-<sup>13</sup>C]glucose (Cambridge Isotope Laboratories, Andover, MA), which were consumed within 15 min (7). The beaker containing the Jell-O was rinsed twice with 20 ml of water, and the rinse solution was consumed. To prepare the Jell-O, 1.2 g/kg body wt

dextrose was dissolved in 200 ml of water by gentle heating. After being cooled to room temperature, sufficient [1-<sup>13</sup>C]glucose was added to achieve an enrichment of  $\sim 4\%$ . Following thorough mixing, an aliquot was removed for analysis of [1-<sup>13</sup>C]glucose enrichment by GC-MS. The dextrose solution containing the [1-<sup>13</sup>C]glucose then was gently warmed, and 5 g of sugar-free gelatin (Knox unflavored gelatin; Nabisco, East Hanover, NJ) and 1 g of sugar-free orange flavored Kool-Aid (Kraft General Foods, White Plains, NY) were added. The mixture was allowed to solidify overnight in a refrigerator.

Subjects ingested the mixed meal labeled with [1-<sup>13</sup>C]glucose tracer on both occasions at *time 0* (Fig. 1). On each occasion, a continuous infusion of [6,6-<sup>2</sup>H<sub>2</sub>]glucose (11.84 mg/kg bolus, 0.1184 mg/kg continuous infusion; Cambridge Isotope Laboratories, Andover, MA) was started 180 min before the meal and continued for 360 min. On one occasion, [6-<sup>3</sup>H]glucose (PerkinElmer Life Sciences, Boston, MA) was infused in a manner designed to mimic the anticipated pattern of appearance of the [1-<sup>13</sup>C]glucose contained in the meal, whereas on the other occasion [6-<sup>3</sup>H]glucose was infused in a manner anticipated to mimic the pattern of change of EGP, as suggested by previous studies (2). Blood was sampled from the arterialized venous site at  $-30, -20, -10, 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 260, 280, 300,$  and 360 min.

**Analytical techniques.** Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at  $-20^{\circ}\text{C}$  until they were assayed. Plasma glucose concentration was measured using a glucose oxidase method (YSI, Yellow Springs, OH). Plasma insulin concentration was measured using a chemiluminescence assay, with reagents obtained from Beckman Coulter (Access Assay; Beckman Coulter, Chaska,

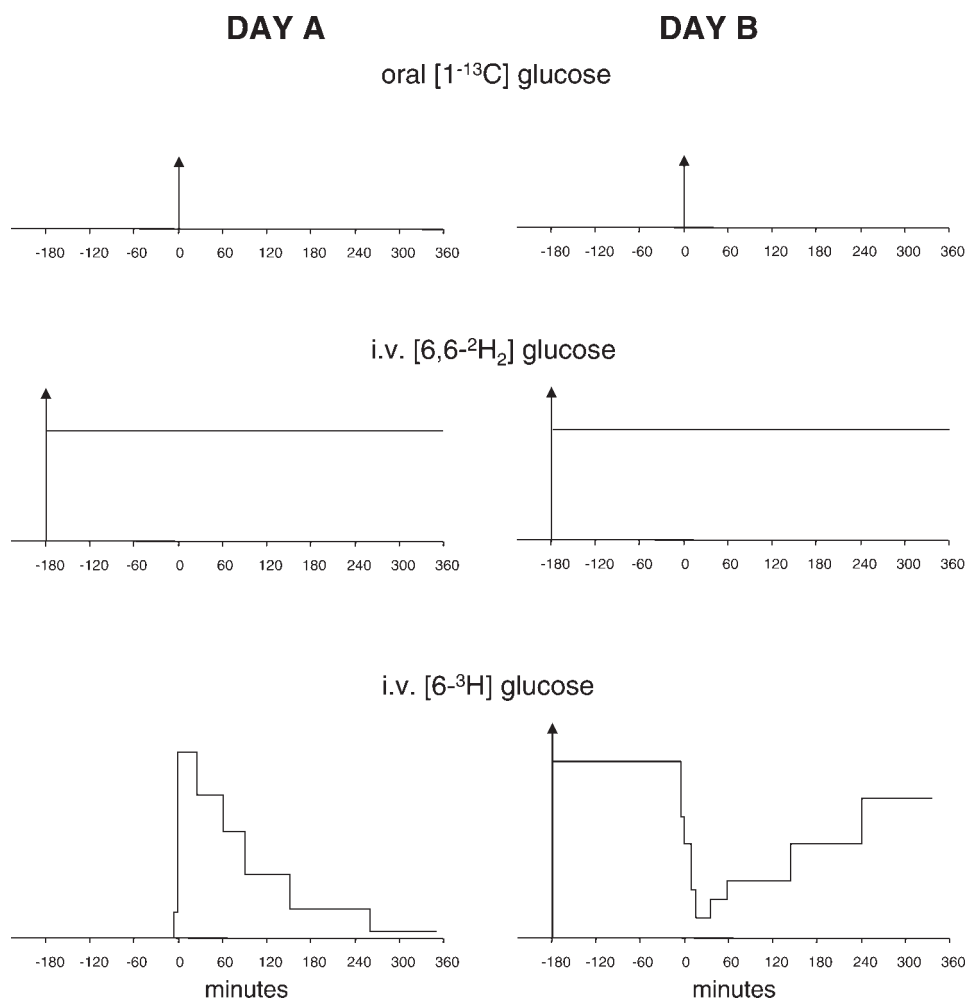


Fig. 1. Study design. On both *days A* and *B*, a mixed meal containing [1-<sup>13</sup>C]glucose was ingested at *time 0*, and a primed continuous infusion of [6,6-<sup>2</sup>H<sub>2</sub>]glucose, started at *time -180*, was continued until the end of the experiment. On *day A*, an additional variable intravenous infusion of [6-<sup>3</sup>H]glucose was started at *time 0*, mimicking the anticipated pattern of appearance of the ingested glucose. On *day B*, an additive primed continuous infusion of [6-<sup>3</sup>H]glucose was started from *time -180* until *time 0* and then varied to mimic the anticipated pattern of change of EGP.

MN). Body composition was measured using dual-energy X-ray absorptiometry (DPX scanner; Lunar, Madison, WI). Plasma [6-<sup>3</sup>H]glucose specific activity was measured by liquid scintillation, counting as previously described (10). Plasma enrichment of [1-<sup>13</sup>C]glucose and [6,6-<sup>2</sup>H<sub>2</sub>]glucose was measured using GC-MS (Thermoquest, San Jose, CA) to simultaneously monitor the C<sub>1-2</sub> and C<sub>3-6</sub> fragments, as described by Beylot et al. (3). Pilot experiments in five subjects established that, under the conditions of the present experiment, enrichment of <sup>13</sup>C due to carbon cycling is below the limit of detection with this technique.

**Calculation.** R<sub>a meal</sub>, EGP, and R<sub>d</sub> were calculated, using either the triple- or dual-tracer methods, with two different models of glucose kinetics: a 1-CM and a 2-CM (2). Briefly, the 1-CM assumes that the unknown and time-varying R<sub>a</sub> and R<sub>d</sub> take place in the accessible compartment, which has a volume equal to a fraction of the total distribution volume. 2-CM assumes time-varying R<sub>a</sub> and R<sub>d</sub> from the accessible compartment and constant-rate parameters between the accessible and the remote compartments. Below, we present the general formulas, which were particularized for the triple- and dual-tracer methods.

**General formulas.** Both the 1-CM and 2-CM allowed the estimation of the (unknown) R<sub>a</sub> in plasma of the tracee from the known rate of infusion of the tracer and measurements of tracer and tracee concentration in plasma (see Appendix A in Ref. 2 for details). According to the 1-CM,

$$R_a = \frac{\text{inf}}{\text{TTR}} - \frac{p \times V \times C}{\text{TTR}} \times \frac{d(\text{TTR})}{dt} \quad (1)$$

where inf is the tracer infusion rate, TTR is the tracer-to-tracee ratio in plasma, C is the tracee plasma concentration, V is the volume of distribution (fixed in all subjects to 200 ml/kg bw), and p is the pool fraction (fixed to 0.65).

According to the 2-CM,

$$R_a = \frac{\text{inf}}{\text{TTR}} - \frac{V_1 \times C}{\text{TTR}} \times \frac{d(\text{TTR})}{dt} + k_{12} \left( \frac{q_2}{\text{TTR}} - Q_2 \right) \quad (2)$$

where V<sub>1</sub> is the volume of distribution of the accessible pool and k<sub>12</sub> 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 (9). q<sub>2</sub> and Q<sub>2</sub> are the amounts of tracer and tracee, respectively, in the peripheral compartment to be evaluated by integrating model equations.

As detailed in the following paragraph, the triple- and dual-tracer methods use Eqs. 1 and 2 to calculate R<sub>a meal</sub> and EGP by adopting as tracer and tracee different glucose variables, namely natural glucose (G<sub>nat</sub>, μmol/ml), i.e., glucose at natural composition coming from ingested glucose and from EGP; endogenous glucose (G<sub>end</sub>, μmol/ml) derived from EGP only; ingested tracer (G<sub>13C</sub>, μmol/ml); and infused tracers (G<sub>2H</sub>, μmol/ml and G<sub>3H</sub>, dpm/ml). The concentrations of these variables were derived from their measurements by using the procedure outlined in (2).

**Triple-tracer R<sub>a meal</sub> and EGP.** On day A, [6-<sup>3</sup>H]glucose was infused to mimic the R<sub>a</sub> of [1-<sup>13</sup>C]glucose contained in the meal (referred to as R<sub>a 13C</sub>; Fig. 1, bottom left). R<sub>a 13C</sub> was calculated by the 1-CM (Eq. 1) or 2-CM (Eq. 2), assuming [6-<sup>3</sup>H]glucose as tracer and [1-<sup>13</sup>C]glucose as tracee. R<sub>a meal</sub> was then calculated by multiplying R<sub>a 13C</sub> by the ratio of total glucose to [1-<sup>13</sup>C]glucose in the meal, expressed as a function of the ratio between [1-<sup>13</sup>C]glucose and natural glucose measured in the meal (TTR<sub>meal</sub>).

$$R_{a \text{ meal}} = R_{a 13C} \left( \frac{G_{\text{nat}} + G_{13C}}{G_{13C}} \right)_{\text{meal}} = R_{a 13C} \left( \frac{1}{\text{TTR}_{\text{meal}}} + 1 \right) \quad (3)$$

An index was defined to quantify the total amount, Q<sub>meal</sub>, of ingested glucose that reaches the systemic circulation in the 6-h duration of the study, equal to the area under the R<sub>a meal</sub> curve.

$$Q_{\text{meal}} = \text{AUC}(R_a) = \int_0^{360} R_{a \text{ meal}}(t) dt \quad (4)$$

On day B, [6-<sup>3</sup>H]glucose was infused to mimic the R<sub>a</sub> of endogenously produced glucose (Fig. 1, bottom right). EGP was derived by the 1-CM (Eq. 1) or 2-CM (Eq. 2), assuming [6-<sup>3</sup>H]glucose as tracer and G<sub>end</sub> as tracee. Suppression of EGP (EGPS) was calculated by normalizing the area below basal to the basal area over the 6-h duration of the study

$$\text{EGPS} = \frac{\int_0^{360} (\text{EGP}(t) - \text{EGP}_b) dt}{\text{EGP}_b \times 360} \times 100 \quad (5)$$

**Dual-tracer R<sub>a meal</sub> and EGP.** Because [6,6-<sup>2</sup>H<sub>2</sub>]glucose tracer is infused at a constant rate on both days A and B (Fig. 1, middle), dual-tracer calculations were performed twice. R<sub>a 13C</sub> was calculated using the 1-CM (Eq. 1) or 2-CM (Eq. 2) with [6,6-<sup>2</sup>H<sub>2</sub>]glucose as tracer and [1-<sup>13</sup>C]glucose as tracee. R<sub>a meal</sub> and Q<sub>meal</sub> were calculated for the triple-tracer method using Eqs. 3 and 4, respectively. To calculate EGP, the total R<sub>a</sub> of natural glucose was first calculated by the 1-CM or 2-CM (Eq. 1), with [6,6-<sup>2</sup>H<sub>2</sub>]glucose as tracer and natural glucose as tracee, and then R<sub>a meal</sub> was subtracted from it. EGPS was calculated using Eq. 5.

**Glucose R<sub>d</sub>.** Once R<sub>a meal</sub> and EGP were available, with either the triple- or the dual-tracer method, the postprandial profile of glucose disappearance was calculated with the 1-CM and 2-CM, respectively.

$$R_d = (R_{a \text{ meal}} + \text{EGP} + F_{2H}) - pV \frac{dG}{dt} \quad (6)$$

$$R_d = (R_{a \text{ meal}} + \text{EGP} + F_{2H}) - V_1 \frac{dG}{dt} - k_{21} V_1 G + k_{12} Q_2 \quad (7)$$

where F<sub>2H</sub> is the infusion rate of [6,6-<sup>2</sup>H<sub>2</sub>]glucose tracer, G is glucose concentration in the accessible compartments, k<sub>21</sub> and k<sub>12</sub> are constant-rate parameters between the peripheral and accessible compartments (assumed equal to 0.05 and 0.07 min, respectively), and Q<sub>2</sub> is the amount of glucose in the peripheral compartment. Dual-tracer calculation of R<sub>d</sub> was performed twice on days A and B. With the triple-tracer method, R<sub>a meal</sub> estimated on day A and EGP estimated on day B were used in Eqs. 6 and 7, with G calculated as its average profile on the 2 days. An index quantifies the increase of R<sub>d</sub> (R<sub>d i</sub>) by normalizing the area above basal to the basal area over the 6-h duration of the study.

$$R_{d i} = \frac{\int_0^{360} (R_d(t) - R_{d b}) dt}{R_{d b} \times 360} \times 100 \quad (8)$$

**Data analysis.** All data are expressed as means ± SE. Postprandial fluxes are expressed as micromoles per kilogram per minute of lean body mass. Values obtained from -30 to 0 min (i.e., prior to the meal) were considered as basal.

The time derivatives of the tracer-to-tracee ratios and areas under curves have been calculated as in Ref. 2.

Wilcoxon's signed rank test was used to determine the statistical significance of differences. A P value <0.05 was considered to be statistically significant.

## RESULTS

**Plasma concentrations.** Plasma glucose, insulin, and glucose tracer concentrations are shown in Fig. 2. As is evident, the concentration of glucose, insulin,  $[1-^{13}\text{C}]$ glucose, and  $[6,6-^2\text{H}_2]$ glucose were virtually identical on the 2 study days, indicating a high degree of reproducibility of the experimental conditions, whereas  $[6-^3\text{H}]$ glucose concentration exhibited different patterns resulting from different infusion profiles. On *day A*, the intravenous infusion of  $[6-^3\text{H}]$ glucose was varied so that it mimicked the anticipated pattern of change of  $R_{a\text{ meal}}$ , resulting in a gradual but smooth fall in the ratio of plasma  $[6-^3\text{H}]$ - and  $[1-^{13}\text{C}]$ glucose from  $\sim 12,000$  down to  $\sim 6,000$  dpm/ $\mu\text{mol}$  over the 6 h of study (Fig. 3, *top left*). On the other hand, on *day B*, the intravenous infusion  $[6-^3\text{H}]$ glucose was varied so that it mimicked the anticipated pattern of change of EGP, resulting in a slow increase of the ratio of  $[6-^3\text{H}]$ glucose to endogenous glucose from  $\sim 400$  to  $600$  dpm/ $\mu\text{mol}$  (Fig. 3, *bottom left*). In contrast, because  $[6,6-^2\text{H}_2]$ glucose was infused intravenously at a constant rate, the ratio of  $[6,6-^2\text{H}_2]$ glucose to

$[1-^{13}\text{C}]$ glucose (used to calculate meal appearance with the dual method) and the ratio of  $[6,6-^2\text{H}_2]$ glucose to unlabeled glucose (used to calculate total glucose appearance with the dual method) rapidly and markedly fell after meal ingestion (Fig. 3, *right*).

**$R_{a\text{ meal}}$ .** Figure 4 shows estimates of glucose fluxes with the dual-tracer vs. the reference triple-tracer method obtained with the 1-CM (*left*) and 2-CM (*right*) models.  $R_{a\text{ meal}}$  (Fig. 4, *left*) measured with the dual-tracer method, and the 1-CM peaked later ( $37.5 \pm 1.6$  vs.  $25 \pm 1.9$  min,  $P = 0.012$ ) and at a lower value ( $58.4 \pm 5.5$  vs.  $82.5 \pm 8.7$   $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ,  $P = 0.0117$ ) than with the triple-tracer method. With the dual-tracer method and 2-CM, the time to  $R_{a\text{ meal}}$  peak was delayed ( $40 \pm 1.9$  vs.  $25 \pm 1.9$  min,  $P = 0.0117$ ), but the peak values were comparable [ $81.5 \pm 7.7$  vs.  $79.9 \pm 8.1$   $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , not significant (NS)]. The area under meal appearance was lower with the dual-tracer method, by 20% with the 1-CM ( $9,036 \pm 558$  vs.  $11,316 \pm 823$   $\mu\text{mol}/\text{kg}$ ,  $P = 0.0117$ ) and by 16% with 2-CM ( $10,231 \pm 661$  vs.  $12,169 \pm 838$   $\mu\text{mol}/\text{kg}$ ,  $P = 0.0117$ ). Compared with the dose of ingested glucose, we found that there were no differences between glucose dose and the total amount of ingested glucose that reaches the systemic circulation ( $Q_{\text{meal}}$ ) estimated with the triple-tracer technique both with 1-CM and 2-CM (dose =  $11,952 \pm 989$  vs.  $Q_{\text{meal}}$  (1-CM) =  $11,316 \pm 823$ ,  $Q_{\text{meal}}$  (2-CM) =  $12,169 \pm 838$   $\mu\text{mol}/\text{kg}$ ,  $P = 0.5754$ ), whereas  $Q_{\text{meal}}$  estimated with the dual-tracer method was significantly lower [ $Q_{\text{meal}}$  (1-CM) =  $9,036 \pm 559$ ,  $Q_{\text{meal}}$  (2-CM) =  $10,231 \pm 662$   $\mu\text{mol}/\text{kg}$ ,  $P = 0.0117$ ].

**EGP.** The pattern of EGP (Fig. 4, *middle*) was different with the dual and the triple-tracer approaches, particularly during the first 60 min after meal ingestion with both the 1-CM and 2-CM. The dual method indicated an initial paradoxical increase, resulting in an overestimation of EGP compared with the triple-tracer method during the initial 40 min that reaches significance at 20 min with the 1-CM. From 40 to 60 min, EGP with the dual-tracer method reaches negative values in all but one subject, resulting in a significantly negative average nadir with both the 1-CM ( $-2.5 \pm 1$ ,  $P = 0.041$ ) and 2-CM ( $-6.0 \pm 1.3$ ,  $P = 0.0026$ ). This represents an underestimation of the reference EGP derived with the triple-tracer method, which took on negative values in only a limited number of subjects, resulting in an average nadir not significantly different from zero. The difference in EGP estimated with the dual- and triple-tracer method was more evident with the 1-CM, and thus EGPS was lower than the triple-tracer method when the dual-tracer method was employed with the 1-CM ( $57 \pm 3$  vs.  $65 \pm 4\%$ ,  $P = 0.0117$ ) but not with 2-CM ( $63 \pm 4$  vs.  $65 \pm 4\%$ , NS).

**Glucose disposal.** The postprandial pattern of  $R_d$  also differed when calculated with the dual- and triple-tracer methods. Compared with the triple-tracer method,  $R_d$  was underestimated with the dual-tracer method, regardless of whether the data were analyzed using the 1-CM or 2-CM (Fig. 4, *bottom*). In particular, the postprandial peak in  $R_d$  was underestimated with the dual-tracer method with both the 1-CM (*day A*:  $52.4 \pm 4.5$ ; *day B*:  $55.3 \pm 4.2$  vs.  $76.3 \pm 8.2\%$ ,  $P = 0.0117$ ) and 2-CM (*day A*:  $64.7 \pm 6.3$  vs.  $79.4 \pm 8.9\%$ ,  $P = 0.0357$ ; *day B*:  $66.8 \pm 5.0$  vs.  $79.4 \pm 8.9\%$ ,  $P = 0.0499$ ). In addition, the postprandial area of  $R_d$  above basal normalized to the area under basal  $R_d$

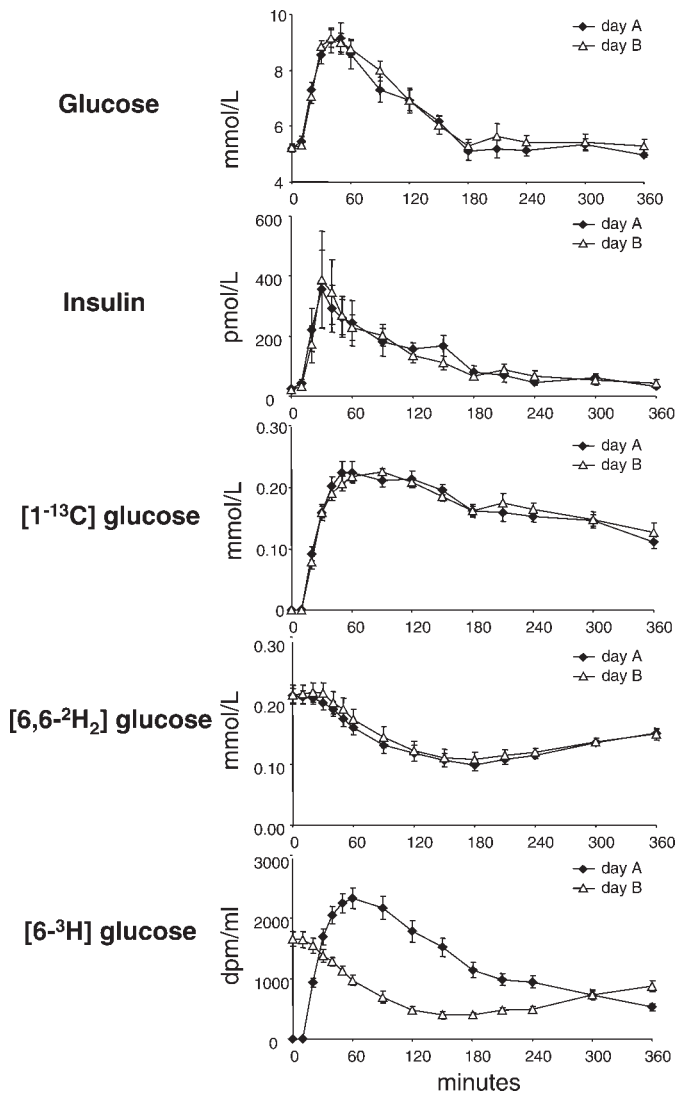


Fig. 2. Mean plasma glucose, insulin,  $[1-^{13}\text{C}]$ glucose,  $[6,6-^2\text{H}_2]$ glucose, and  $[6-^3\text{H}]$ glucose concentrations. Vertical bars represent SE.

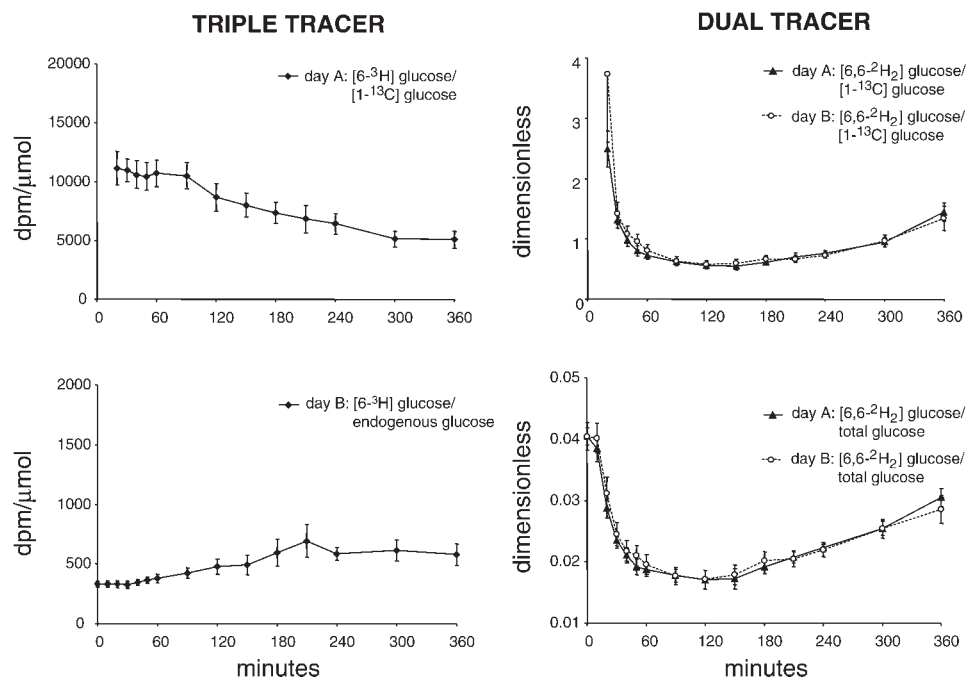


Fig. 3. Tracer-to-tracee ratios used in the calculation of the triple- (left) and the dual-tracer (right) methods. Vertical bars represent SE.

( $R_{d1}$ ) was underestimated with both the 1-CM (*day A*:  $109 \pm 7$ ; *day B*:  $110 \pm 7$  vs.  $148 \pm 19\%$ ,  $P = 0.0117$ ) and 2-CM ( $128 \pm 9$  and  $124 \pm 8$  vs.  $158 \pm 20\%$ ,  $P = 0.0173$ ).

## DISCUSSION

The present data confirm that use of the dual-tracer method is accompanied by marked changes in plasma tracer-to-tracee ratios following glucose ingestion and that these changes can, to a large extent, be minimized by a triple-tracer method (2). These data further indicate that the nonsteady state associated with the dual-tracer method results in substantial error in the estimation of both rate and pattern of postprandial glucose fluxes, i.e.,  $R_{a\text{ meal}}$ , EGP, and  $R_d$ , compared with reference profiles of the triple-tracer method. Taken together, these data suggest that three tracers with appropriate infusion profiles are required for simultaneous assessment of these three postprandial fluxes.

The conventional dual-tracer method uses two tracers, one ingested and the other intravenously infused at a constant rate, to estimate  $R_{a\text{ meal}}$ , total glucose appearance (the difference equaling EGP), and  $R_d$  by using either the 1-CM or 2-CM. The estimated  $R_{a\text{ meal}}$  and EGP are dependent on both the structure and parameter values of the model used to derive them. The 1-CM has been used with a wide range of values for the distribution volume, ranging from 130 to 230 ml/kg (6). The 2-CM assumes a time-varying loss from the accessible pool and constant exchange parameters between the accessible and peripheral pools, likely providing a more realistic representation of glucose kinetics. The problem is that there has been no "gold" standard to determine which method yields the most accurate estimate of postprandial glucose turnover. Livesey et al. (6) reported that a 1-CM with a distribution volume equal to 230 ml/kg provides  $R_{a\text{ meal}}$  and EGP profiles similar to those derived from a 2-CM. However, as shown in the present studies, this is not a proof of model validity, since both the 1-CM and 2-CM are affected by non-steady-state errors.

The triple-tracer method was proposed in an effort to minimize these errors; it requires a more complex experimental setting since it uses three tracers, one ingested and two intravenously infused at variable rates, but provides an almost model-independent assessment of  $R_{a\text{ meal}}$  and EGP, i.e., not dependent on the model to derive them, either 1-CM and 2-CM, or on the value of parameters used in the models.

In the present study, eight subjects, characterized by a wide range of insulin sensitivity, were studied with a 2-day protocol designed to perform simultaneously dual- and triple-tracer studies. The conventional dual-tracer method (i.e., constant intravenous tracer infusion) was accompanied by the use of a third tracer infused to optimize estimation of either  $R_{a\text{ meal}}$  (*day A*) or EGP (*day B*). As is evident in Fig. 3, the plasma ratio of  $[6\text{-}^3\text{H}]\text{glucose}$  to  $[1\text{-}^{13}\text{C}]\text{glucose}$  on *day A* and to endogenous glucose on *day B* were not perfectly constant. Obtaining perfectly constant TTRs is impossible, because this would require exact knowledge of the time course of  $R_{a\text{ meal}}$  and EGP. In practice, only an approximate knowledge is necessary (2) to adjust the tracer infusion rates in a manner that substantially reduces the variation in TTRs, thereby markedly reducing the error introduced by the nonsteady state. Data were analyzed by using the 1-CM and 2-CM with parameters fixed to population values. The impact of model structure and nonindividual parameter values on estimated fluxes is minimized by the triple-tracer method (2), which can thus be used as a reference against which the ability of the dual-tracer method to provide estimates of postprandial fluxes can be evaluated. As expected, the dual-tracer method resulted in marked non-steady-state conditions, with very pronounced initial TTR declines, which rendered  $R_{a\text{ meal}}$  and EGP estimation model dependent.  $R_{a\text{ meal}}$  (Fig. 4, top) was markedly lower with 1-CM compared with 2-CM, and EGP showed a paradoxical pattern with both models (Fig. 4, middle), i.e., an initial increase above basal, more pronounced with the 1-CM, and a rapid decline to even negative values, more pronounced with the 2-CM.

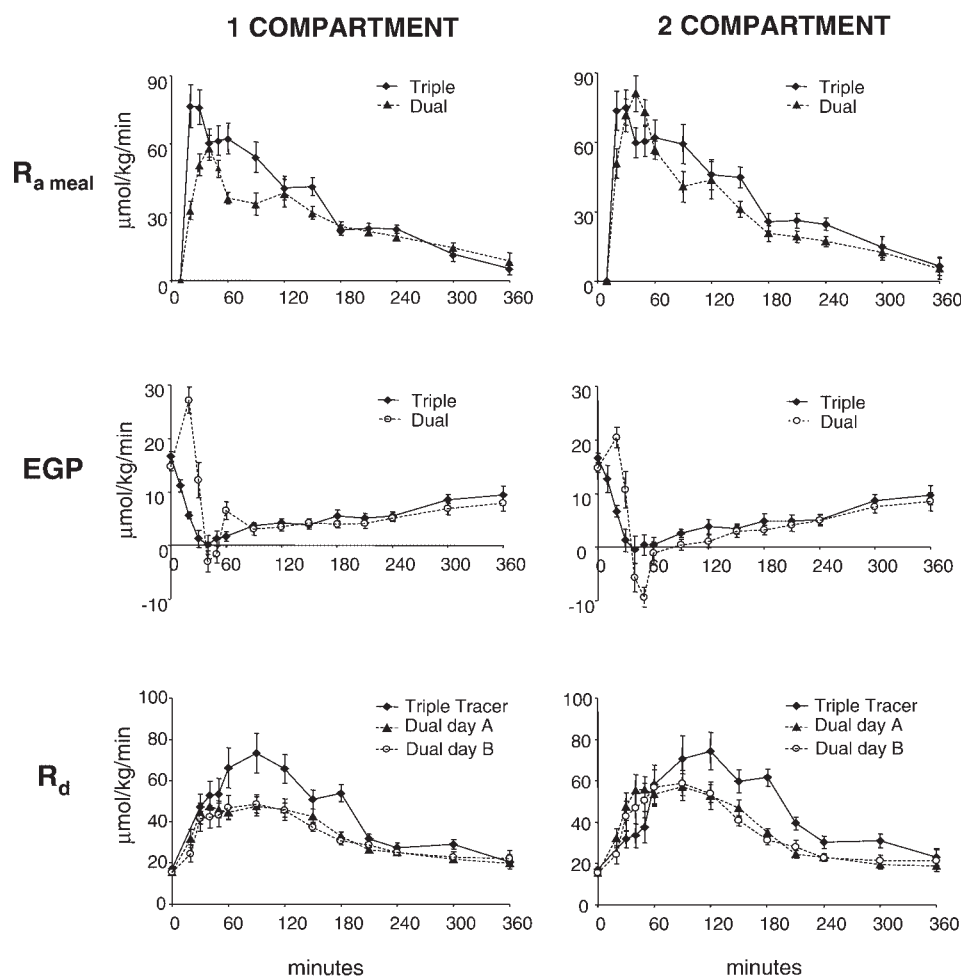


Fig. 4. Rate of appearance of ingested glucose ( $R_{a \text{ meal}}$ ) on day A (top), endogenous glucose production (EGP) on day B (middle), and rate of glucose disappearance ( $R_d$ ; bottom) with the triple- vs. dual-tracer method with one-compartment (left) and two-compartment (right) models. Vertical bars represent SE.

A comparison of the dual-tracer fluxes with the triple-tracer reference fluxes (Fig. 4) confirmed previous findings (2). The dual-tracer and 1-CM performed poorly, with  $R_{a \text{ meal}}$  peak significantly lower and delayed compared with the triple-tracer reference, resulting in a significantly lower estimation of the total amount of ingested glucose, and EGP initially overestimated (0- to 40-min period following glucose ingestion) with respect to the triple reference, resulting in a significant underestimation of EGP suppression. The 2-CM performed better than the 1-CM, particularly for  $R_{a \text{ meal}}$ , which peaked at a similar value, even if it was somewhat delayed. The total amount of ingested glucose was still significantly underestimated compared with reference, although to a minor extent compared with the 1-CM. The dual-tracer method and the 2-CM still provided a paradoxical pattern of EGP, but with a correct estimate of the total suppression index, since initial overestimation of EGP (0- to 40-min period following glucose ingestion) was compensated by late underestimation (40–120 min).

$R_d$  also can be calculated once  $R_{a \text{ meal}}$  and EGP are known. Both dual and triple-tracer methods can provide an estimate of  $R_d$  from mass balance equation of the accessible compartment, i.e., from rate of change of glucose mass. Estimation of  $R_d$  is thus sensitive to the model structure and parameter values with both dual and triple-tracer approaches, but the triple tracer has the advantage of a more accurate measure of total glucose

appearance (i.e.,  $R_{a \text{ meal}} + \text{EGP}$ ) than does the conventional dual tracer. In the present studies,  $R_d$  peak and above-basal increase estimated with either the 1-CM or 2-CM were similar with the triple- but not with the dual-tracer method, thus confirming that dependence of  $R_d$  on model structure is less critical with the triple-tracer method. Assuming the triple tracer as a reference (Fig. 4), the dual-tracer approach using either the 1-CM or 2-CM significantly underestimated both  $R_d$  peak and its overall increase above basal.

These results indicate that, although triple-tracer flux estimates are almost model independent, those derived with the dual-tracer method are dependent on the model used to derive them. However, it is important to point out that all of these results were derived by fixing some model parameters to specific values. With the 1-CM, calculations required to fix the product of the total distribution volume ( $V$ ) by an operational pool fraction ( $p$ ). We used for  $pV$  a value equal to 130 ml/kg, but we have previously shown (2) that, although the value chosen for  $pV$  has a marked effect on fluxes calculated with the dual-tracer method, the triple-tracer calculations are essentially independent of the assumed volume of distribution. For instance, as pointed out by Livesey et al. (6), the  $R_{a \text{ meal}}$  measured with the dual-tracer method increased as the volume assumed for  $pV$  was increased. However, no single  $pV$  value appeared to be adequate for deriving glucose fluxes with the dual-tracer method, since three different time-varying  $pV$  pro-

files would be required to derive  $R_{a\text{ meal}}$ , EGP, and  $R_d$  equal to the reference values derived with the triple-tracer method. With the 2-CM, exchange rate parameters  $k_{12}$  and  $k_{21}$  and the volume of distribution of the accessible pool need to be fixed. Ideally, these parameters should be determined separately in each individual, but this would require an additional tracer experiment. Therefore, we fixed them to values derived from previous studies in normal subjects (9). As is evident from inspection of 2-CM equations, the influence of model parameters on estimated fluxes is more pronounced when the tracer-to-tracee ratio varies in time, as with the dual-tracer method. However, the extent to which use of individual vs. population rate parameters improves the performance of the 2-CM is difficult to predict.

In conclusion, our results show that changes in tracer-to-tracee ratios that occur with the conventional dual-tracer method preclude accurate estimation of postprandial fluxes  $R_{a\text{ meal}}$ , EGP, and  $R_d$ . The use of the 2-CM with the dual-tracer approach reduces but does not completely avoid these errors. The dual-tracer method underestimates  $R_{a\text{ meal}}$  and  $R_d$  and inaccurately assesses the degree and pattern of suppression of EGP. Of concern, the size of these errors differs depending on the degree of tracer-to-tracee non-steady state, making comparison of fluxes in individuals with different degrees of glucose tolerance (e.g., diabetic vs. nondiabetic subjects) problematic. Although it is experimentally more complex, the triple-tracer method minimizes non-steady-state errors, thereby enabling more accurate assessment of postprandial fluxes  $R_{a\text{ meal}}$ , EGP, and  $R_d$ . However, if the purpose of the experiment is to assess  $R_{a\text{ meal}}$  alone or EGP alone, then only two tracers are required, i.e., the oral tracer plus a second intravenous tracer properly infused to optimize estimation of  $R_{a\text{ meal}}$  in the former case and EGP in the latter.

#### ACKNOWLEDGMENTS

We thank R. Rood, B. Dicke, L. Heins, J. Feehan, and B. Norby for technical assistance and the staff of the Mayo General Clinical Research Center for assistance in performing the studies.

#### GRANTS

This study was supported by the National Institutes of Health (DK-29953 and RR-00585), a Novo Nordisk research infrastructure grant, the Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Italy, and the Mayo Foundation. R. Basu was supported by an American Diabetes Association Mentor-based fellowship.

#### REFERENCES

1. **Allsop JR, Wolfe RR, and Burke JF.** The reliability of rates of glucose appearance in vivo calculated from constant tracer infusions. *Biochem* 172: 407–416, 1978.
2. **Basu R, Di Camillo B, Toffolo G, Basu A, Shah P, Vella A, Rizza R, and Cobelli C.** Use of a novel triple-tracer approach to assess postprandial glucose metabolism. *Am J Physiol Endocrinol Metab* 284: E55–E69, 2003.
3. **Beylot M, Previs SF, David F, and Brunengraber H.** Determination of the  $^{13}\text{C}$ -labeling pattern of glucose by gas chromatography-mass spectrometry. *Anal Biochem* 212: 526–531, 1993.
4. **Dalla Man C, Caumo A, and Cobelli C.** The oral glucose minimal model: estimation of insulin sensitivity from a meal test. *IEEE Trans Biomed Eng* 49: 419–429, 2002.
5. **Dalla Man C, Caumo A, Basu R, Rizza R, Toffolo G, and Cobelli C.** Measurement of selective effect of insulin on glucose disposal from labeled glucose oral test minimal model. *Am J Physiol Endocrinol Metab* 289: E909–E914, 2005.
6. **Livesey JH, Wilson PDG, Dainty JR, Brown JC, Faulks RM, Roe MA, Newman TA, Eagles J, Mellon FA, and Greenwood RH.** Simultaneous time-varying systemic appearance of oral and hepatic glucose in adults monitored with stable isotopes. *Am J Physiol Endocrinol Metab* 275: E717–E728, 1998.
7. **McMahon M, Marsh H, and Rizza R.** Comparison of the pattern of postprandial carbohydrate metabolism after ingestion of a glucose drink or a mixed meal. *J Clin Endocrinol Metab* 68: 647–653, 1989.
8. **Mari A, Wahren J, DeFronzo R, and Ferrannini E.** Glucose absorption and production following oral glucose: comparison of compartmental and arteriovenous-difference methods. *Metabolism* 43: 1419–1425, 1994.
9. **Radziuk J, Norwich KH, and Vranic M.** Experimental validation of measurements of glucose turnover in nonsteady state. *Am J Physiol Endocrinol Metab Gastrointest Physiol* 234: E84–E93, 1978.
10. **Rizza RA, Mandarino LJ, and Gerich JE.** Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol Endocrinol Metab* 240: E630–E639, 1981.
11. **Steele R, Wall J, DeBodo R, and Altszuler N.** Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol* 187: 15–24, 1956.
12. **Steele R, Bjerknes C, Rathgeb I, and Altszuler N.** Glucose uptake and production during the oral glucose tolerance test. *Diabetes* 17: 415–421, 1968.