Robert A. Rizza,[1] Gianna Toffolo,[2] and Claudio Cobelli[2]

Accurate Measurement of Postprandial Glucose Turnover: Why Is It Difficult and How Can It Be Done (Relatively) Simply?

Diabetes 2016;65:1133–1145 | DOI: 10.2337/db15-1166

Fasting hyperglycemia occurs when an excessive rate of endogenous glucose production (EGP) is not accompanied by an adequate compensatory increase in the rate of glucose disappearance (R_d). The situation following food ingestion is more complex as the amount of glucose that reaches the circulation for disposal is a function of the systemic rate of appearance of the ingested glucose (referred to as the rate of meal appearance [Ra_{meal}]), the pattern and degree of suppression of EGP, and the rapidity of stimulation of the R_d. In an effort to measure these processes, Steele et al. proposed what has come to be referred to as the dual-tracer method in which the ingested glucose is labeled with one tracer while a second tracer is infused intravenously at a constant rate. Unfortunately, subsequent studies have shown that although this approach is technically simple, the marked changes in plasma specific activity or the tracer-to-tracee ratio, if stable tracers are used, introduce a substantial error in the calculation of Ra_{meal}, EGP, and R_d, thereby leading to incorrect and at times misleading results. This Perspective discusses the causes of these so-called "nonsteady-state" errors and how they can be avoided by the use of the triple-tracer approach.

WHY IS IT IMPORTANT TO ACCURATELY MEASURE POSTPRANDIAL GLUCOSE TURNOVER?

The long-term goal in the treatment of people with diabetes is to enable them to live long, productive, and enjoyable lives free of the acute and chronic complications of diabetes. To do so, the premise is that both the pattern of metabolism and the concentration of glucose, fat, amino acids, and other substrates and hormones need to be maintained as close to normal as possible. Diabetes is characterized by fasting and postprandial hyperglycemia (1). Both become progressively more severe as the disease evolves. Glucose concentrations are determined by the balance between the amount of glucose entering and leaving the systemic circulation. Following an overnight fast, glucose is released into the systemic circulation primarily by the liver with a smaller contribution coming from the kidneys and perhaps the intestine. Glucose leaves the systemic circulation by insulin-dependent uptake in tissues such as muscle and insulin-independent uptake in tissues such as the brain.

The situation becomes more complex following food ingestion. Carbohydrate contained in a meal is absorbed by the intestine into the portal vein. A portion of the absorbed glucose is extracted by the liver (and perhaps intestines) and stored as glycogen via direct or indirect pathways or is degraded to three carbon precursors. The remainder transverses the liver and enters the systemic circulation (referred to in this Perspective as the rate of meal glucose appearance [Ra_{meal}]). In people without diabetes, the increase in glucose is accompanied by a rapid increase in insulin and suppression of glucagon secretion (1). However, insulin secretion is decreased and delayed and glucagon does not suppress appropriately in people with type 2 diabetes. The coordinated changes in glucose, insulin, and glucagon in individuals without diabetes result in a prompt reduction in endogenous glucose production (EGP), thereby further decreasing the total amount of glucose that reaches the systemic circulation

[1]Division of Endocrinology, Metabolism, Diabetes, Nutrition, and Internal Medicine, Department of Internal Medicine, Mayo Clinic, Rochester, MN
[2]Department of Information Engineering, University of Padova, Padova, Italy
Corresponding author: Robert A. Rizza, rizza.robert@mayo.edu.

Received 1 September 2015 and accepted 25 February 2016.

R.A.R., G.T., and C.C. contributed equally to the manuscript.

© 2016 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered.

(referred to as the rate of glucose appearance [R_a]) for disposal (referred to as rate of glucose disappearance [R_d]). Therefore, coordinated changes in the Ra_{meal}, EGP, and glucose disposal limit the postprandial glycemic excursion in people without diabetes. In contrast, the lack of appropriate regulation of these fluxes causes postprandial hyperglycemia in people with diabetes. Understanding how therapies, used either alone or in combination, for the treatment of people with diabetes affect these parameters presumably will enhance both their safety and effectiveness.

WHY IS MEASUREMENT OF R_a, Ra_{meal}, EGP, AND R_d DIFFICULT IN THE POSTPRANDIAL STATE?

Following an overnight fast, the rate of glucose entering the circulation equals the rate of glucose leaving the circulation and therefore the system is at steady state. Under these conditions, glucose turnover readily can be estimated by infusing a glucose tracer at a constant rate (inf) and then measuring the specific activity (when using a radioactive tracer) or the tracer-to-tracee ratio (ttr) derivable from the enrichment when stable isotopes are used (2). For simplicity's sake, we will use ttr throughout this Perspective; however, the same principles apply when specific activity is used to calculate turnover. At steady state, $R_a = R_d =$ inf/ttr. Following food ingestion, the estimation of R_a and R_d becomes difficult because they are no longer equal and both are changing over time, resulting in tracer/tracee nonsteady state. In addition, R_a now equals the sum of Ra_{meal} and EGP, which also are changing with time in a discordant manner.

The presence of rapid changes in the plasma ttr necessitates the postulation of a structural model of the glucose system capable of describing how the system functions under nonsteady-state conditions. This includes specifying which parameters are time-varying as well as how they change during the nonsteady state. The usual approach is to start by trying to obtain an accurate estimate of Ra(t) (i.e., R_a at a given time point) and then using the mass balance equation (i.e., the change in glucose mass equals R_a minus R_d) to calculate Rd(t). In addition, the model (and the experimental conditions, as will be discussed in the following section) also has to enable the partition of Ra(t) into Ra_{meal}(t) and EGP(t). Moreover, in the absence of tracer/tracee steady state, results are highly dependent on the model assumed (see next section). Therefore, different models yield different estimates of Ra(t), Rd(t), Ra_{meal}(t), and EGP(t). In theory, the accuracy of the estimate of these parameters can be improved by postulating increasingly complex models (e.g., those that account for differences in the rates of equilibration of glucose and onset of action of insulin in the liver, muscle, and various other tissues in people with or without diabetes). However, the increased complexity of the model has to be balanced against increased difficulty in accurately identifying model parameters. Maintenance of tracer/tracee steady state by an appropriate tracer experiment design enables model-independent measurement of Ra(t), Ra_{meal}(t), EGP(t), and Rd(t), thereby avoiding these problems.

WHAT NONSTEADY-STATE MODELS HAVE BEEN USED TO MEASURE POSTPRANDIAL GLUCOSE TURNOVER?

Steele's One-Compartment Nonsteady-State Model

Steele et al. (3) pioneered the use of tracers to assess the pattern of postprandial glucose turnover. They did so by infusing glucose labeled with [U-$^{14}C_6$]glucose into the duodenum of dogs along with an intravenous infusion of [6-^{14}C]glucose to trace the systemic R_a of both the unlabeled glucose and the intraduodenally infused [U-$^{14}C_6$]glucose. They then divided the R_a of [U-$^{14}C_6$]glucose by the specific activity of the intraduodenally infused glucose to determine Ra_{meal} in mg/kg/min. The authors calculated EGP by subtracting Ra_{meal} from R_a and calculated R_d by subtracting the change in glucose mass over a given time interval from R_a. Figure 1 shows the typical change in plasma ttr that occurs when this experimental approach is used in humans (4). In the experiments shown in Fig. 1, [1-^{13}C]glucose was ingested at time 0 and [6,6-$^2H_2$]glucose was infused intravenously (IV tracer) at a constant rate beginning at −180 min. The vertical arrow in the top left panel of Fig. 1 indicates meal ingestion, and the vertical arrow and horizontal line in the bottom left panel of Fig. 1 indicate the prime continuous infusion of the systemic tracer. As is evident, there is a large fall in both the IV tracer–to–oral tracee ratio (used to calculate Ra_{meal}) and the IV ttr (used to calculate R_a) during the first 60–90 min, followed by a gradual rise thereafter. Steele et al. (5) astutely realized that specific activity measured in the plasma does not represent that present in the liver, interstitial fluid, and other compartments. They explored several ways to circumvent this problem, including the use of a nonhomogenous compartment model. For the sake of simplicity, they settled on the nonhomogenous single one-compartment model labeled as tracee model (Fig. 3). They did so because "it becomes necessary to make such an arbitrary selection when it is desired to calculate from the data in which rapid changes in plasma glucose concentration or specific activity are taking place as for example after insulin injection. We have chosen a value one half of the total glucose pool for this purpose…" (6). Over the succeeding years, the rapidly equilibrating pool has been expressed as pV (commonly referred to as the "effective" volume), where p is the correction factor and V is the total body volume of distribution of glucose. The size of p has been debated and, even more problematically, has been shown to change over time and to be dependent on the prevailing insulin concentration (7,8).

Nevertheless, Steele's one-compartment "dual-tracer" method became widely accepted and has been used by a large number of investigators (including the authors) to assess postprandial glucose turnover. As shown in Fig. 1, this method yielded what at first appeared to be reasonable results; namely, a rapid rate of appearance of the ingested glucose, suppression of EGP, and stimulation of R_d. However, there were some troubling observations, including what was commonly referred to as a "paradoxical" increase in EGP immediately after meal ingestion.

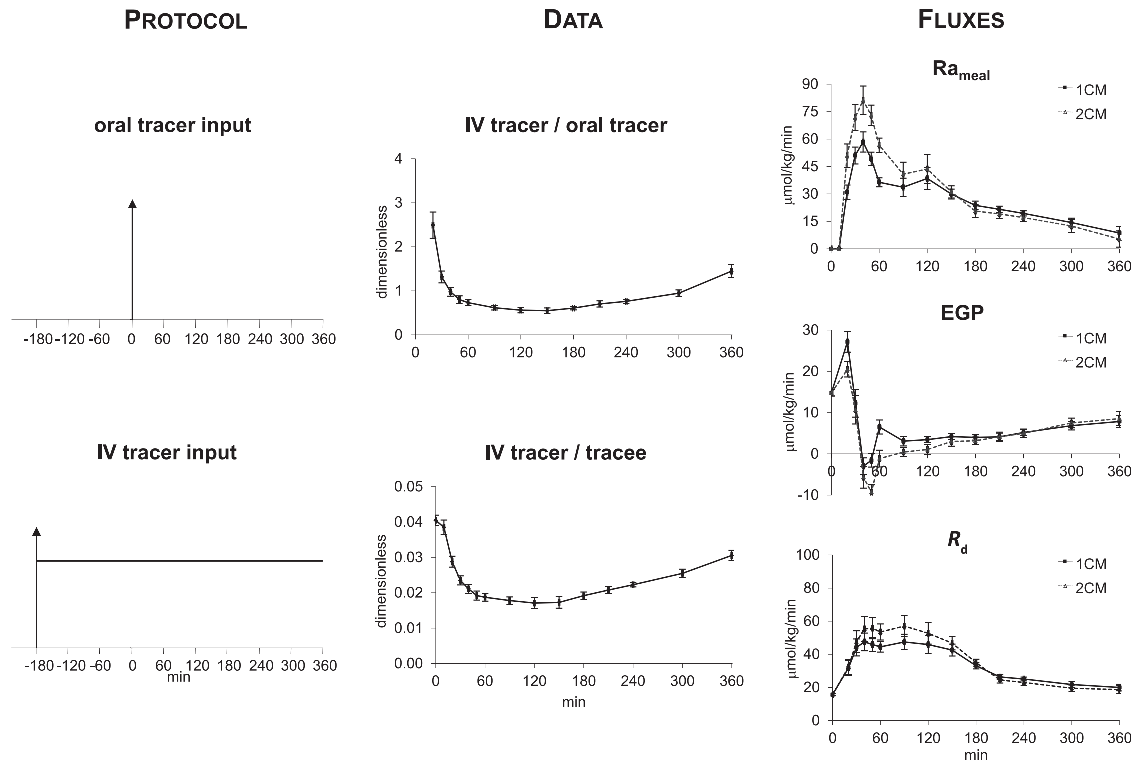


Figure 1—The dual-tracer approach was used in eight healthy subjects. The ttrs (dimensionless) are shown in the middle panels and the Ra_{meal}, EGP, and R_d are shown in the left panels. Rates were calculated using either Steele's one-compartment model (1CM) (3,5,6) or Radziuk's two-compartment model (2CM) (10) following the ingestion of a mixed meal containing [1-^{13}C]glucose (oral tracer) at time 0 (arrow). An intravenous infusion of [6,6-$^{2}H_2$]glucose (IV tracer) was started at −180 min and infused at a constant rate until 360 min. The vertical arrow in the top left panel indicates meal ingestion and the vertical arrow and horizontal line in the bottom left panel indicate the prime continuous infusion of the systemic tracer. Tracee represents unlabeled glucose (4).

The increase in EGP was called paradoxical because it occurred at a time when glucose and insulin concentrations were rising and glucagon concentrations were falling. Even more disturbing, "negative" rates of EGP often were observed (Fig. 1). Finegood et al. (9) subsequently demonstrated that "negative" rates of EGP were observed during hyperinsulinemic-euglycemic clamps when the tracer-determined R_a was lower than the glucose infusion rate at a given time point. These were due to the inadequacy of Steele's one-compartment method during nonsteady-state conditions (Fig. 2). These investigators established that the fall in plasma glucose specific activity that occurred during the first 90 min after the start of a clamp when unlabeled glucose was infused (Fig. 2, top panel) resulted in a marked underestimation of the actual R_a, generating biologically implausible "negative" rates of EGP (Fig. 2, bottom panel). This error resolved after the first 2 h when plasma glucose specific activity again approached steady state. This error did not occur if the infused glucose contained the tracer in amounts that approximated what was present in plasma before the start of the clamp and therefore did not perturb plasma glucose specific activity (9). This enabled glucose turnover to be measured under tracer/tracee steady-state conditions (i.e., model independent).

A comparison of Figs. 1 and 2 shows that the fall in plasma glucose specific activity during the first 2 h following carbohydrate ingestion are as marked as those observed by Finegood et al. (9) during a hyperinsulinemic glucose clamp when the so-called Hot GINF method (tracer is added to the glucose infusate in amounts sufficient to minimize the change in plasma ttr) is not used. Although the Hot GINF method now is universally accepted as the standard for measurement of glucose turnover during a euglycemic clamp, it has not been as widely appreciated that the same nonsteady-state errors occur when the dual-tracer method is used to measure glucose turnover following glucose ingestion. When Steele's one-compartment model (3) is used to calculate turnover, the rapid fall in the plasma IV tracer/oral tracer ratio used to calculate Ra_{meal} that occurs during the first 2 h after food ingestion results in marked underestimation of Ra_{meal}. Similarly, the rapid fall in the IV ttr used to calculate R_a results in a marked underestimation of R_a. These errors introduce an even greater error in EGP, as it is calculated by subtracting a large incorrect number (Ra_{meal}) from an equally large incorrect number (R_a). Even worse, when EGP is calculated in this manner, it is not only inaccurate but also very imprecise as it contains the imprecision of the two large numbers (R_a and Ra_{meal}). Furthermore, R_d is

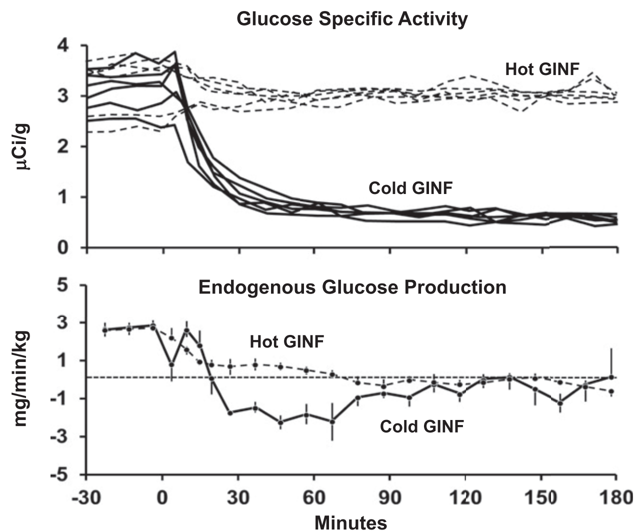


Figure 2—Plasma glucose specific activity observed during a hyperinsulinemic-euglycemic clamp when unlabeled glucose alone (Cold GINF, solid line) or glucose containing [3-^{3}H]glucose in amounts sufficient to minimize the change in plasma glucose specific activity (Hot GINF, dotted line) is shown in the upper panel. Rates of EGP observed in dogs when plasma glucose specific activity was permitted to fall (Cold GINF) or when changes in plasma specific activity were minimized (Hot GINF) are shown in the lower panel (9).

incorrect as it is calculated by subtracting the change in glucose mass from an erroneous R_a. The initial "paradoxical" increase in EGP and the subsequent "negative" rates of EGP are due to unequal errors that occur during the rapid fall in the ttr used to calculate Ra_{meal} and R_a. These aberrant and biologically implausible patterns are not observed when EGP is measured with the model-independent triple-tracer approach (Fig. 5).

Radziuk's Two-Compartment Nonsteady-State Model

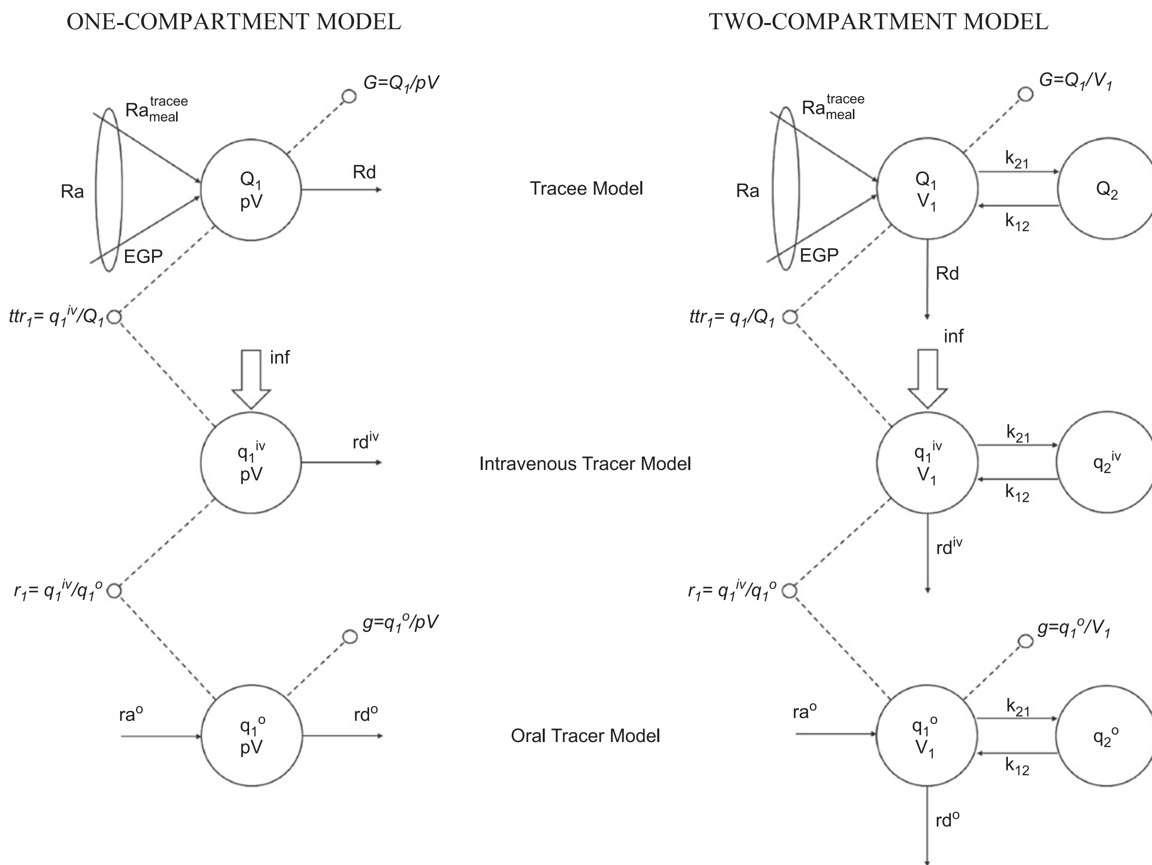
As noted above, Steele and colleagues (6) also explored the use of other models, as they recognized that a one-compartment nonhomogenous model is a compromise. Nevertheless, they rejected those models because of their complexity. However, because of the obvious problems with a one-compartment model, other investigators have explored the use of multicompartmental models. Perhaps the most commonly used one has been the two-compartment model proposed by Radziuk (10) that postulates both an accessible and a slowly exchanging nonaccessible compartment (Fig. 3).

As shown in Fig. 1, the postprandial pattern of change in Ra_{meal}, EGP, and R_d calculated with Radziuk's two-compartment model (10) are similar to those calculated with the more commonly used Steele's one-compartment model. However, the early "paradoxical" increase in EGP is less evident, and the peak rates of Ra_{meal} and R_d are higher than when calculated with Steele's one-compartment model. Also, EGP transiently is somewhat more "negative" when calculated with the two-compartment rather than the one-compartment model. Although data that subsequently have become available have shown that insulin-stimulated glucose uptake in peripheral tissues varies with time and differs from the rate at which insulin suppresses EGP, both of which differ in people with or without diabetes (11,12), the assumption of a single homogeneous remote compartment was a reasonable simplifying assumption at the time of the development of Radziuk's two-compartment model (10). However, as with the one-compartment model, this assumption, as well as assumptions regarding the size of the initial volume of distribution and of the rate constants governing the exchange between compartments, is not required if turnover can be measured in the presence of tracer/tracee steady state. The structure, equations, and underlying assumptions of Steele's one-compartment and Radziuk's two-compartment models are summarized in Fig. 3.

Measurement of Postprandial Glucose Turnover in the Presence of Tracer/Tracee Steady State: The Triple-Tracer Method

Nonsteady-state experiments such as those performed by Finegood et al. (9) indicate that the accuracy of the estimation of glucose turnover can be enhanced if a tracer is infused in a manner that minimizes the change in the plasma ttr of the parameter of interest. As discussed above, the Hot GINF method does this during a clamp by ensuring that ttr of the infused glucose approximates what is present in the plasma before the initiation of the clamp (9). Nonsteady-state tracer theory (13,14) anticipated this experimental finding by introducing the ttr clamp technique.

The structure and underlying assumptions of the tracer-to-tracee glucose clamp method is shown in Fig. 4. The triple-tracer method (13,14) uses the ttr clamp to keep the appropriate plasma ttrs constant following glucose ingestion. However, instead of infusing labeled glucose at a constant rate, it varies the intravenous infusion rates of two different tracers in a manner that mimics the anticipated changes in systemic rate of appearance of ingested tracer and of unlabeled glucose released by EGP (Fig. 5). As the pattern of appearance of ingested glucose differs dramatically from that of postprandial suppression of EGP, two intravenous tracers are needed: one to trace the appearance of the label contained in the ingested glucose and one to trace the rate of release of unlabeled glucose due to EGP. It is called the triple-tracer method to distinguish it from Steele's one-compartment model and Radziuk's two-compartment model (or dual-tracer) approach described above. In order to minimize the change in plasma ttrs, this method requires a priori general knowledge of the temporal pattern of change of Ra_{meal} and EGP. Such knowledge can be relatively easily gained by conducting a few pilot studies that start with tracer infusion profiles that are anticipated to mimic postprandial changes in Ra_{meal} and EGP and then modifying them (if necessary) so as to minimize the change in plasma ttr. Sample infusion profiles (which the authors would be delighted to provide upon request) and the resultant ttrs are shown in Fig. 5. In these early experiments, the plasma Ra_{meal} ttr drifted up and the EGP ttr drifted down (these variances can be reduced with experience); however, point-to-point changes during a given time interval were small.



Steele's One-Compartment Model

The one-compartment model underlying Steele's dual-tracer approach is shown in the left panel above for the tracee, i.e., the unlabeled glucose, as well as for the IV and oral tracers (3,5,6). The tracee and the two tracers distribute in the accessible compartment, including plasma and other spaces in rapid equilibrium with it, with a volume equal to a fraction of the total distribution volume. The unknown and time varying R_a and R_d of tracee take place in the accessible compartment and are estimated from the known intravenous infusion rate of tracer and measurements of tracer and tracee concentrations in plasma, according to the following formulas (see Appendix A of ref. 36 for details):

$$Ra(t) = \frac{inf}{ttr_1(t)} - \frac{p \cdot V \cdot G(t)}{ttr_1(t)} \dot{ttr}_1(t) \quad \text{(Eq. 1)}$$

$$Rd(t) = Ra(t) - p \cdot V \cdot \dot{G}(t) \quad \text{(Eq. 2)}$$

where inf denotes the constant infusion rate of the IV tracer; ttr_1, the plasma ttr; G, the plasma tracee concentration; p, the pool fraction fixed to 0.65; and V, the glucose volume of distribution fixed to 200 mL/kg body weight. The dot indicates derivative with respect to time.

During a meal, the tracee comes from both the meal and the endogenous production, and Ra(t) given by Eq. 1 includes the contribution of both sources, denoted as Ra_{meal}^{tracee} and EGP. Thus, the use of the IV tracer alone does not enable the partition of R_a into these two components. To accomplish this, a second tracer is added to the meal to trace the appearance in plasma of glucose from exogenous source. The rate of appearance of ingested tracer, ra^o, is calculated using an equation similar to Eq. 1 by considering the IV tracer as "tracer" and the oral tracer as "tracee":

$$ra^o(t) = \frac{inf}{r_1(t)} - \frac{p \cdot V \cdot g(t)}{r_1(t)} \dot{r}_1(t) \quad \text{(Eq. 3)}$$

where g is the oral tracer concentration and r_1 is the ratio between IV and oral tracer concentration, both measured in plasma.

Ra_{meal}^{tracee} then is calculated by scaling ra^o based on the prevalence of tracer in the meal, expressed in terms of the ttr measured in a meal sample (ttr_{meal}):

$$Ra_{meal}^{tracee}(t) = ra^o(t) \left[\frac{1}{ttr_{meal}}\right] \quad \text{(Eq. 4)}$$

Figure 3—The dual-tracer approach.

Ra_{meal}^{tracee} then is subtracted from R_a to calculate EGP:

$$EGP(t) = Ra(t) - Ra_{meal}^{tracee}(t) \quad \text{(Eq. 5)}$$

If a nonweightless mass tracer is used, as is invariably the case with stable isotope tracers, ra^o is added to Ra_{meal}^{tracee} to quantify the systemic R_a of ingested (tracer+tracee) glucose:

$$Ra_{meal}(t) = Ra_{meal}^{tracee}(t) + ra^o(t) = ra^o(t)\left[\frac{1}{ttr_{meal}} + 1\right] \quad \text{(Eq. 6)}$$

Similarly, the R_d from all sources, either endogenous production or meal or IV tracer is calculated by the following equation:

$$Rd_{tot}(t) = EGP(t) + Ra_{meal}(t) + inf - p \cdot V \cdot \dot{G}_{tot}(t) \quad \text{(Eq. 7)}$$

where G_{tot} represents the total (tracee+tracers) concentration measured in plasma. The inf is negligible if a massless (radioactive) tracer is infused. In this case, G_{tot} virtually coincides with G.

Radziuk's Two-Compartment Model

The two-compartment model proposed by Radziuk (10) is shown in the right panel above. It uses diffusion-convection-reaction equations to describe tracer/tracee equilibration between an accessible compartment (plasma) and a remote compartment. This model also assumes a time-varying R_a and R_d from the accessible compartment and constant rate parameters between the accessible and the remote compartment.

The unknown and time-varying rate of R_a and R_d of tracee are estimated from the known intravenous infusion rate of the tracer and measurements of tracer and tracee concentrations in plasma, according to the following formulas (see Appendix A of ref. 36 for details):

$$Ra(t) = \frac{inf}{ttr_1(t)} - \frac{V_1 \cdot G(t)}{ttr_1(t)} \cdot \dot{ttr}_1(t) + k_{12}\left(\frac{q_2^{iv}(t)}{ttr_1(t)} - Q_2(t)\right) \quad \text{(Eq. 8)}$$

$$Rd(t) = Ra(t) - V_1 \cdot \dot{G}(t) - k_{21}V_1G(t) + k_{12}Q_2(t) \quad \text{(Eq. 9)}$$

where V_1 is the volume of distribution of the accessible pool and k_{21} and k_{12} are the rate constants between the peripheral and the accessible compartment fixed to 130 mL/kg, 0.05 and 0.07 min^{-1}, respectively, according to previous studies in normal subjects. q_2^{iv} and Q_2 are the amounts of IV tracer and tracee in the peripheral compartment to be evaluated by integrating the two-compartment model equations.

As with the one-compartment model, R_a given by Eq. 8 equals the sum of Ra_{meal}^{tracee} and EGP. Following the same rationale described above for the one-compartment model, ra^o is first calculated using the oral tracer concentration g and the ratio between IV and oral tracer concentrations r_1:

$$ra^o(t) = \frac{inf}{r_1(t)} - \frac{V_1 \cdot g(t)}{r_1(t)} \cdot \dot{r}_1(t) + k_{12}\left(\frac{q_2^{iv}(t)}{r_1(t)} - q_2^o(t)\right) \quad \text{(Eq. 10)}$$

where q_2^o is the amounts of oral tracer in the peripheral compartment to be evaluated by integrating model equations. Ra_{meal}^{tracee}, EGP, and Ra_{meal} are then calculated as for the one-compartment model (Eqs. 4, 5, and 6), while the R_d coming from all sources, either endogenous production or meal or IV tracer is now expressed as:

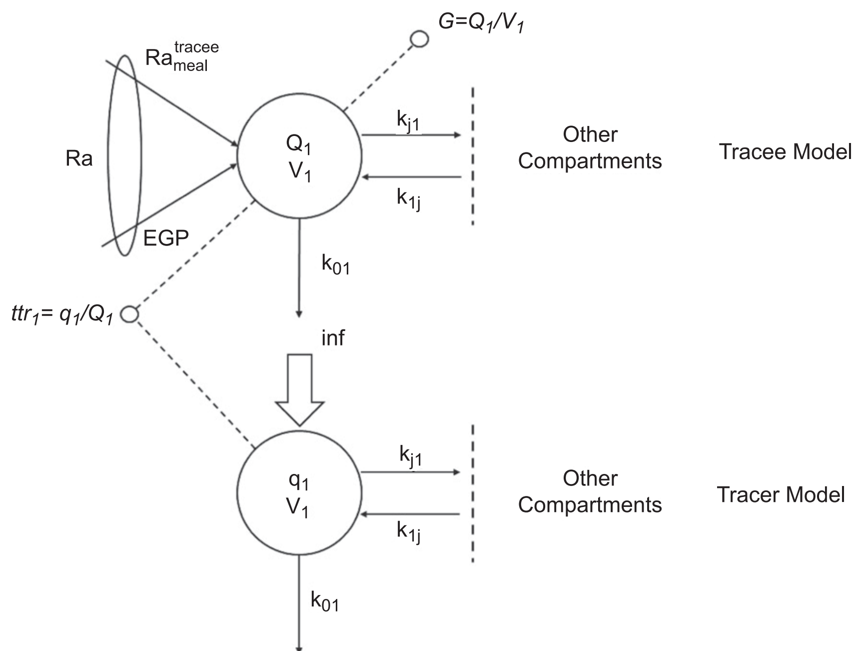
$$Rd_{tot}(t) = EGP(t) + Ra_{meal}(t) + inf - V_1 \cdot \dot{G}_{tot}(t) - k_{21}V_1G_{tot}(t) + k_{12}Q_{2tot}(t) \quad \text{(Eq. 11)}$$

where G_{tot} represents the total glucose concentration (tracee+tracers) measured in plasma, and Q_{2tot} is the total amount of glucose in the peripheral compartment to be evaluated by integrating the two-compartment model equations. As with the one-compartment model, inf is negligible if a massless (radioactive) tracer is infused.

Figure 3—*Continued*.

Previous theoretical (13,14) and experimental (9,15) studies have shown that changes of this magnitude introduce minimal error into the calculation of glucose turnover. This is evident by the fact that Ra_{meal}, EGP, and R_d are essentially the same when turnover measured with the triple-tracer method was calculated using either Steele's one-compartment or Radziuk's two-compartment model. Figure 6 shows a comparison of results measured with the triple-tracer approach, which essentially is a model-independent method to those obtained in the same individuals at the same time using the conventional dual-tracer approach calculated either by using Steele's model of nonhomogenous one-compartment (upper panels) or Radziuk's two-compartment model (lower panels). As is evident, the dual approach calculated with Steele's one-compartment model underestimates the triple-tracer Ra_{meal} profile during the first 180 min, then provides concordant estimates thereafter at a time when the plasma ttr once again is approximating steady state (Fig. 6, left upper panel). The degree of over- and underestimation of Ra_{meal} is lower with the two- than with the one-compartment model (Fig. 6, left lower panel). In contrast to the paradoxical increase in EGP that is observed with the dual-tracer approach (again less with the two- than with the one-compartment

THE TRACER-TO-TRACEE GLUCOSE CLAMP



Let us consider a general compartmental model for which we assume a system made up of *n* compartments—at variance with Steele's one-compartment (3,5,6) and Radziuk's two-compartment model (10). Assume that the tracee system is in steady state and a tracer experiment is carried out—typically a primed continuous infusion—until the tracer reaches a steady state throughout the system. This is represented by denoting the steady-state ttr as ttr_{1ss}. Let us now suppose that a tracee perturbation, e.g., due to a meal, pushes the system out of the steady state and denotes that with Ra(t) and the sum of $Ra_{meal}(t)$ and EGP(t), which will change in time.

For a general compartmental model describing tracee and tracer dynamics, it has been shown by Cobelli et al. (14) that:

$$Ra(t) = \frac{inf(t)}{ttr_1(t)} - \frac{V_1 G(t)}{ttr_1(t)} \dot{ttr}_1(t) - \sum_{i=2}^{n} \left(1 - \frac{ttr_i(t)}{ttr_1(t)}\right) k_{1i}\, Q_i(t) \qquad \text{(Eq. 1)}$$

where ttr_i is the trr in the ith compartment and $\dot{ttr}_1(t)$ denotes the derivative of $ttr_1(t)$. The key aspect of this equation is that if the tracer administration is adjusted so as to induce no changes in $ttr_1(t)$ over time, i.e. $ttr_1(t) = ttr_{1ss}$, the time derivative of $ttr_1(t)$ in the accessible pool is zero, the ttr equilibrates in all compartments, i.e., $ttr_i(t) = ttr_{1ss}$, and the contributions of the second and third term in Eq. 1 become null. As a result, Ra(t) is given by:

$$Ra(t) = \frac{inf(t)}{ttr_1(t)} = \frac{inf(t)}{ttr_{1ss}} \qquad \text{(Eq. 2)}$$

which becomes model independent because it only hinges on what can be measured in the accessible pool. In other words, theory indicates that the change in the ttr should be prevented by infusing the tracer in such a way as to follow the changes of Ra(t). If the tracer infusion rate, inf(t), is adjusted so that $inf(t) = Ra(t) \cdot ttr_{1SS}$, $ttr_1(t)$ remains equal to ttr_{1SS} throughout the experiment.

This means that inf(t) must have the same shape of Ra(t), with the proportionality factor between the two being the desired target value ttr_{1SS}. Of note is that the resultant Eq. 2 is similar to the one used to calculate steady-state R_a, but here both inf(t) and Ra(t) change in time.

In practice, maintaining plasma $ttr_1(t)$ absolutely constant is impossible. Nevertheless, it is useful for the investigator to try to clamp $ttr_1(t)$ at a constant level by changing the tracer infusion rate in a suitable way because a reduction in the rate of change of $ttr_1(t)$ will provide an estimate of Ra(t) much less dependent on the validity of the model used with respect to a constant tracer infusion.

What are the possible approaches to clamp $ttr_1(t)$? One approach is to develop a closed-loop tracer administration scheme that results in ongoing adjustment of the tracer infusion rate in a manner that maintains plasma ttr constant. However, this requires near-instant measurement of plasma tracer and tracee concentrations. Such methods currently are not readily available. Therefore, an open-loop approach to clamp $ttr_1(t)$ has to be adopted. This can be accomplished by infusing inf(t) in a profile that has a shape that approximates that of the unknown Ra(t).

This may sound like circular reasoning because adjusting the tracer infusion rate requires a priori knowledge of Ra(t), which is exactly what one is trying to determine. However, usually some information about the behavior of Ra(t) during the nonsteady state is available. This a priori knowledge can be used to design a tentative format of tracer administration for the first trial (educated guess). This guess can be verified later by measuring $ttr_1(t)$ and adjusting the infusion profile in subsequent experiments if $ttr_1(t)$ is not constant. This procedure can be repeated a few times until a satisfactory format is obtained. In our experience, a few iterations are sufficient to achieve acceptable results.

Figure 4—The tracer-to-tracee glucose clamp.

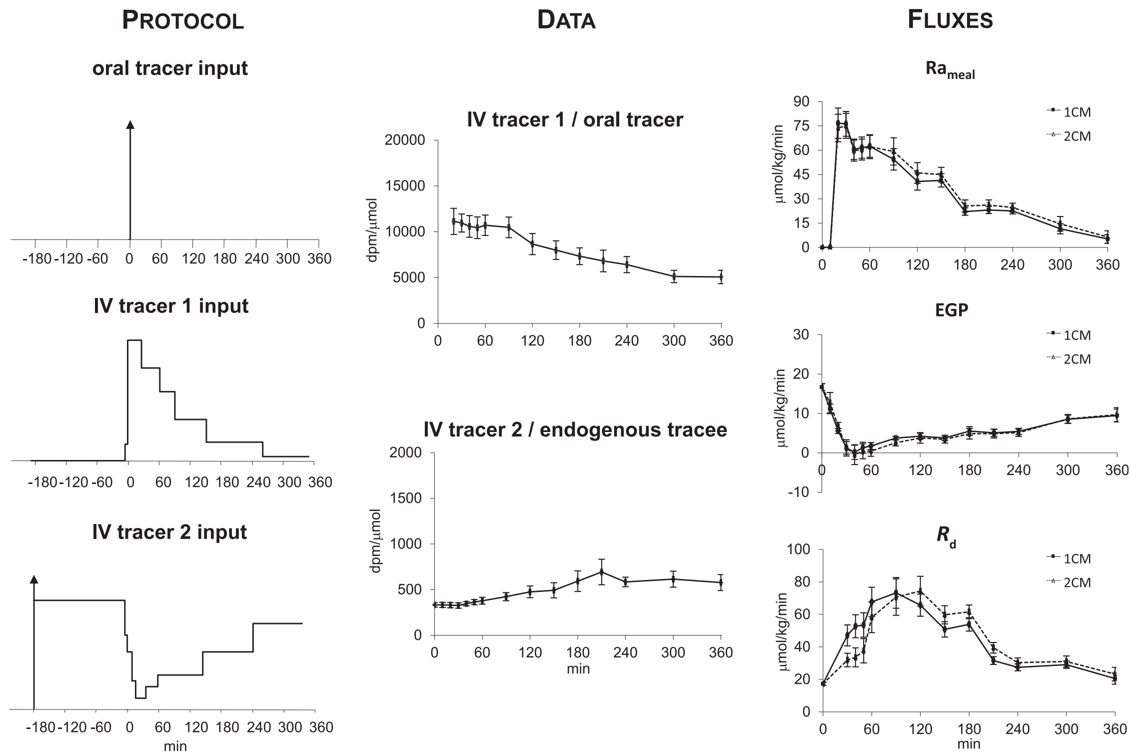


Figure 5—The dual- and triple-tracer approaches were simultaneously used in eight healthy subjects (4). The ttrs, $\mathrm{Ra_{meal}}$, EGP, and R_d were calculated using either Steele's one-compartment model (1CM) or Radziuk's two-compartment model (2CM) following the ingestion of a mixed meal containing [1-^{13}C]glucose (oral tracer) at time 0 (arrow). In order to compare the triple- versus dual-tracer approach in the same subjects, the dual-tracer protocol shown in Fig. 1 was performed on two separate occasions and a third tracer ([6-^{13}H]glucose) was infused at a variable rate to mimic the anticipated changes in $\mathrm{Ra_{meal}}$ on one occasion (as percent of the total meal tracer infused: −180 to 3 min: 0%; 3–8 min: 1%; 8–35 min: 24%; 35–70 min: 24%; 70–100 min: 15%; 100–160 min: 18%; 160–270 min: 15%; 270–360 min: 3%) and on the other occasion to mimic the anticipated changes in EGP (as percent of the baseline EGP tracer: −180 to 3 min: 100%; 3–8 min: 70%; 8–18 min: 55%; 18–25 min: 30%; 25–45 min: 15%; 45–70 min: 25%; 70–160 min: 35%; 160–260 min: 55%; 260–360 min: 80%).

model), the triple-tracer method shows that EGP smoothly falls (as would be anticipated during a time when glucose and insulin concentrations are rising) during the first 30–60 min and then slowly rises thereafter (Fig. 6, middle panels). Once again, as rate of change in the plasma ttr slows from 2 to 3 h onward during the dual-tracer method, the dual- and triple-tracer methods provide concordant estimates of EGP. R_d measured with either the one- or two-compartment dual-tracer models, whether assessed as peak or total postprandial increment, is lower than actual R_d measured with the triple-tracer approach (Fig. 6, right panels).

The rationale underlying the calculation of $\mathrm{Ra_{meal}}$, EGP, and R_d with the triple-tracer approach when using either a one- or two-compartment model is described in Fig. 7.

DISCUSSION

No method is perfect. The triple-tracer method has advantages and disadvantages when compared with the dual-tracer approach (Table 1). The triple-tracer approach is more complicated than the dual-tracer approach. Assuming the goal is to measure $\mathrm{Ra_{meal}}$, EGP, and R_d, the triple-tracer method requires three tracers, whereas the dual-tracer method uses only two tracers. In addition, the IV tracer infusion rates need to be varied with the triple-tracer approach, whereas the single IV tracer infusion is kept constant with the dual-tracer approach. The need to vary the tracer infusion rates with time seems complicated, but it is not. Relatively inexpensive pumps can be readily programmed to infuse the tracers in the profiles shown in Fig. 5. However, it is essential to conduct pilot studies in order to optimize the tracer infusions so as to keep both the plasma oral and endogenous ttrs constant.

As there is no "gold standard" to compare results with the oral triple-tracer approach, Haidar et al. (16) infused [U-^{13}C]glucose plus 20% dextrose intravenously in a profile mimicking that which would be anticipated to be observed after the ingestion of a carbohydrate-containing meal and traced its rate of appearance using [U-^{13}C;1,2,3,4,5,6,6-^{2}H$_7$]glucose also infused intravenously in a pattern anticipated to mimic the "meal" intravenous infusion. Unfortunately, rather than maintaining the plasma "meal" ttr constant, it increased by approximately 300% during the first 60 min (see Fig. 3*B* in ref. 16), resulting in an overestimate of the $\mathrm{Ra_{meal}}$ over that interval (see Fig. 5*B* in ref. 16). Although considerably less

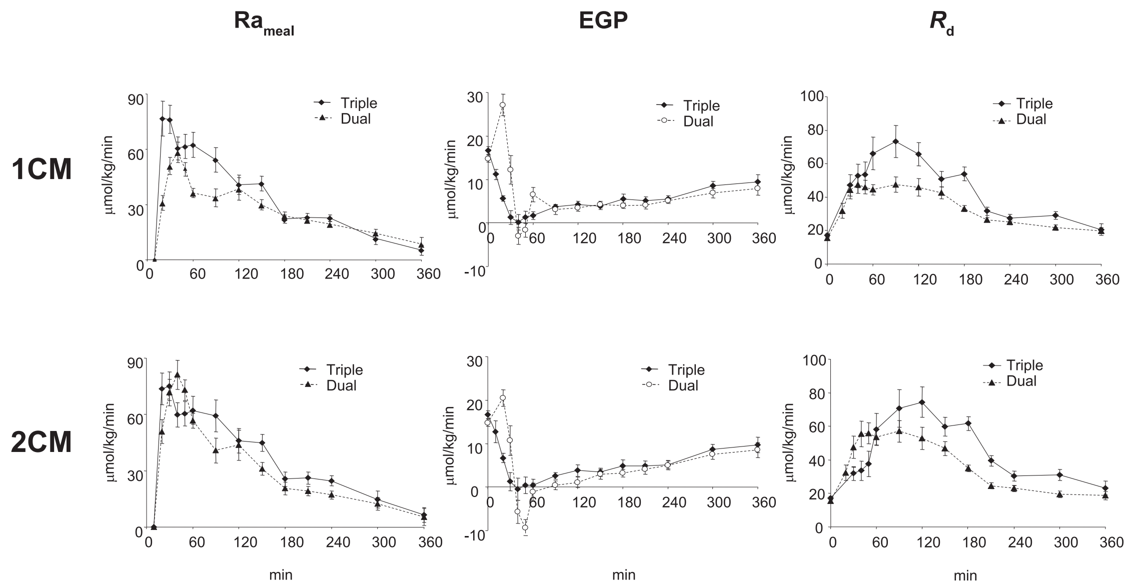


Figure 6—Ra_{meal}, EGP, and R_d determined using either the dual-tracer (dual) or triple-tracer (triple) method when calculated with either Steele's one-compartment model (1CM) or Radziuk's two-compartment model (2CM) (4).

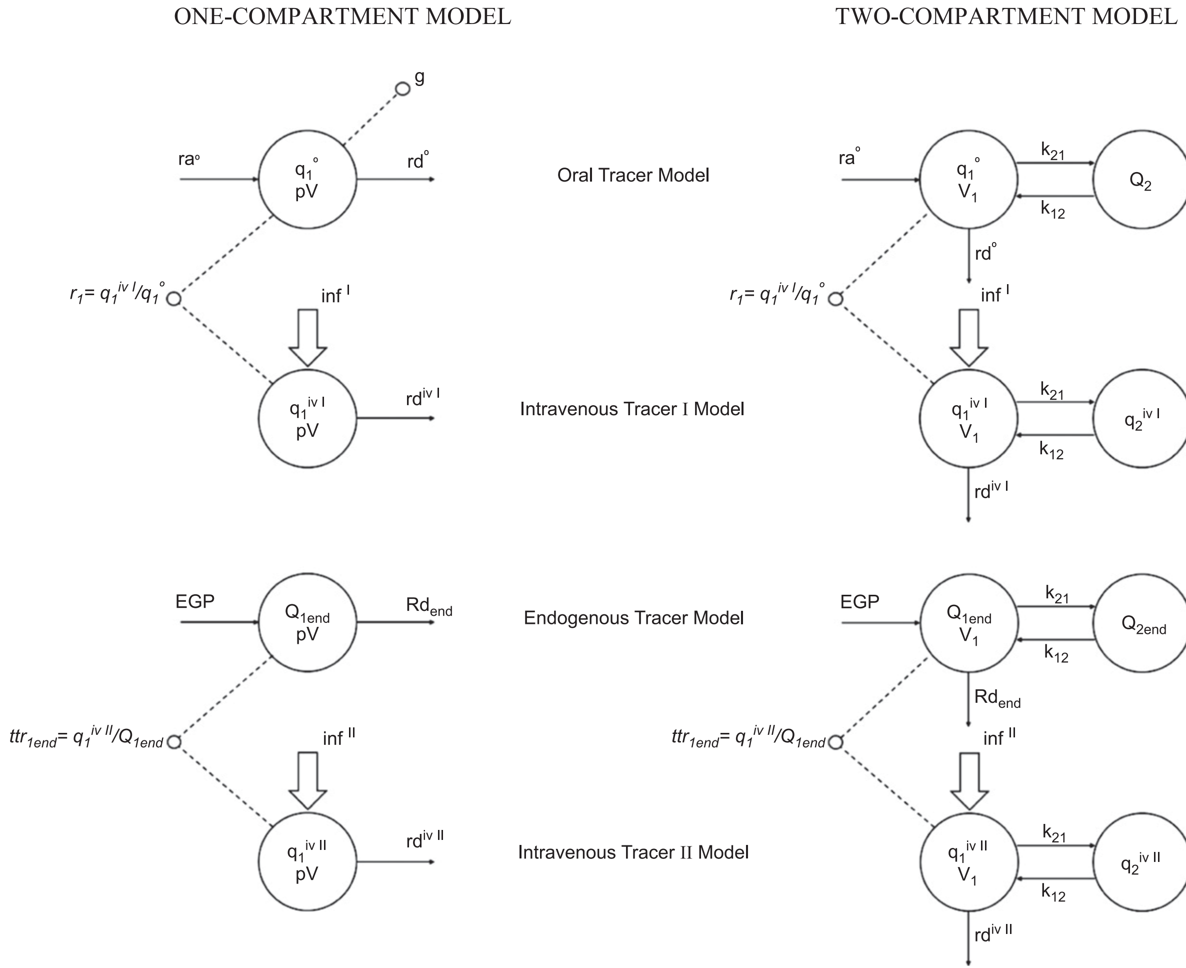
than the approximately 600% fall in the "meal" plasma ttr observed in the same interval using the dual-tracer approach, it still contrasts with the relatively flat ttr that can be achieved after conducting a few pilot experiments (Fig. 5, middle panels). However, Haidar et al. (16) did an excellent job of maintaining the plasma endogenous ttr constant by varying the intravenous infusion of [6,6$^2H_2$]glucose, which enabled an accurate estimation of EGP.

Although optimizing the tracer infusions may seem daunting, in practice, it is not. Over the past 12 years, we and others have successfully conducted over 600 triple-tracer studies in more than 300 subjects, including children (17); young and older adults (18–23); subjects with prediabetes (24), type 2 diabetes (25–29), and type 1 diabetes in the presence or absence of exercise or agents that delay gastric emptying (18,30–32); and obese subjects before and after vagal blockade (33) or Roux-en-Y gastric bypass (32).

Three tracers and analyzing three specific activities or enrichments cost more than two tracers and analyzing two specific activities or enrichments. The incremental cost of tracers (~\$200 vs. ~\$100) is relatively small. The cost of analysis of a third ttr is greater, the extent of which depends on the site of analysis (e.g., commercial laboratory vs. academic center). However, the increment in cost to use the triple-tracer approach needs to be considered in light of the cost of recruiting, studying, and measuring various hormones and substrates, particularly as the dual-tracer method yields inaccurate and at times misleading information. EGP is measured directly with the triple-tracer approach rather than by subtracting a large inaccurate and imprecise number from another large inaccurate and imprecise number as is done with the dual-tracer approach. However, R_a is not measured directly with the triple-tracer approach, so it must be calculated as the sum of its component parts (Ra_{meal} and EGP). This is rarely a problem as generally Ra_{meal} and EGP are the parameters of interest. Although the triple-tracer approach minimizes the impact of tracer/tracee nonsteady state on the calculation of Ra_{meal} and EGP and therefore R_a, a model of the glucose space still is required to accurately calculate R_d. The same limitation applies to the dual-tracer method; however, in this instance additional uncertainty is introduced as R_a is incorrect. R_d measures the rate of disappearance of glucose from the glucose space. Therefore, if glucose concentration exceeds the renal threshold, as commonly occurs after food ingestion in people with type 2 diabetes, R_d is influenced by both tissue glucose uptake and urinary glucose excretion.

Care needs to be taken to be sure that the ingested carbohydrate is uniformly labeled with the tracer; otherwise, the rate of appearance of the ingested tracer will not reflect the rate of appearance of the concurrently ingested carbohydrate. The splanchnic catheterization technique, alone or in combination with a hyperglycemic clamp, can measure net splanchnic balance after the ingestion of a meal (34,35). However, these methods are invasive and must be used in combination with meal and systemic tracers to determine the relative contribution of changes in Ra_{meal} and EGP to changes in net splanchnic balance. Radioactive tracers should not be used in certain settings (e.g., when studying children) and cannot be used in some countries. Fortunately, sophisticated mass spectrometry techniques permit the use of the triple-tracer approach with three stable tracers, e.g., [6,6-$^2H_2$]glucose, [1-^{13}C]glucose, and [U-^{13}C]glucose (17). However, care needs to be taken to correct for carbon recycling if [6-^{13}C]glucose

THE TRIPLE-TRACER APPROACH



One-compartment model

The triple-tracer method was developed to permit the accurate estimation of postprandial fluxes by adopting the clamped tracer/tracee approach. In addition to the oral tracer, two tracers are infused intravenously in patterns that mimic the expected time courses of Ra_{meal} and EGP. A schematic of the one-compartment models for endogenous glucose and for the three tracers is shown in the left panel above. The first IV tracer minimizes change in the ratio between its plasma concentration and that of the oral tracer, thus improving the estimate of ra^o, calculated according to the following equation:

$$ra^o(t) = \frac{inf^I(t)}{r_1(t)} - \frac{p \cdot V \cdot g(t)}{r_1(t)} \dot{r}_1(t) \qquad \text{(Eq. 1)}$$

where inf^I denotes the infusion rate of the first IV tracer; r_1, the ratio between IV and oral tracer concentrations, and g, the oral tracer concentration, both measured in plasma. Ra_{meal}^{tracee} and Ra_{meal} are then calculated from ra^0 as for the dual-tracer approach (Eq. 4 and Eq. 6 of Fig. 3).

The second IV tracer is infused in a manner that minimizes the change in ratio between its plasma concentration and the glucose concentration (G_{end}) derived from EGP. G_{end} is calculated by subtracting the contribution of the tracee, i.e. unlabeled glucose derived from the meal, from total tracee, i.e., unlabeled glucose concentration:

$$G_{end}(t) = G(t) - g(t)\left(\frac{1}{ttr_{meal}}\right) \qquad \text{(Eq. 2)}$$

where G is the plasma concentration of tracee glucose; g, the concentration measured in plasma of the oral tracer; and ttr_{meal}, the ttr of the ingested glucose.

EGP is then calculated as:

$$EGP(t) = \frac{inf^{II}(t)}{ttr_{1end}(t)} - \frac{p \cdot V \cdot G_{end}(t)}{ttr_{1end}(t)} \dot{ttr}_{1end}(t) \qquad \text{(Eq. 3)}$$

Figure 7—The triple-tracer approach.

where inf^{II} denotes the infusion rate of the second IV tracer and ttr_{1end} denotes the ratio between the second IV tracer and endogenous glucose.

The R_d coming from all sources, either endogenous production or meal or IV tracer is calculated as:

$$Rd_{tot}(t) = EGP(t) + Ra_{meal}(t) + inf^{I}(t) + inf^{II}(t) - p \cdot V \cdot \dot{G}_{tot}(t) \quad \text{(Eq. 4)}$$

where G_{tot} represents the total (tracee+tracers) glucose concentration measured in plasma.

inf^{I} and inf^{II} are negligible when massless (radioactive) tracers are used. In this case, G_{tot} virtually coincides with G.

Two-compartment model

A schematic of two-compartment models underlying the triple-tracer approach is shown in the right panel above. The rationale outlined above for the one-compartment model can be extended to the two-compartment configuration, by simply substituting the equations for ra^{o}, EGP, and Rd_{tot}, with the following ones:

$$ra^{o}(t) = \frac{inf^{I}(t)}{r_1(t)} - \frac{V_1 \cdot g(t)}{r_1(t)} \cdot \dot{r}_1(t) + k_{12}\left(\frac{q_2^{ivI}(t)}{r_1(t)} - q_2^{o}(t)\right) \quad \text{(Eq. 5)}$$

$$EGP(t) = \frac{inf^{II}(t)}{ttr_{1end}(t)} - \frac{V_1 \cdot G_{end}(t)}{ttr_{1end}(t)} \dot{ttr}_{1end}(t) + k_{12}\left(\frac{q_2^{ivII}(t)}{ttr_{1end}(t)} - Q_{2end}(t)\right) \quad \text{(Eq. 6)}$$

$$Rd_{tot}(t) = EGP(t) + Ra_{meal}(t) + inf^{I}(t) + inf^{II}(t) - V_1 \cdot \dot{G}_{tot}(t) - k_{21} V_1 G_{tot}(t) + k_{12} Q_{2tot}(t) \quad \text{(Eq. 7)}$$

where q_2^{o}, Q_{2end}, and Q_{2tot} are the amounts in the peripheral compartment of the oral glucose tracer, of the glucose from endogenous production, and of the total (i.e., tracee+tracers) glucose to be evaluated by integrating the two-compartment model equations.

Figure 7—*Continued*.

or [1-^{13}C]glucose is used as a tracer. But only two tracers are required if the goal is to measure only Ra_{meal} or only EGP.

THE PATH FORWARD

High-quality and sophisticated genotyping is most productive when combined with high-quality and sophisticated phenotyping and vice versa. In addition, as the causes of postprandial hyperglycemia in people with type 2 diabetes are complex and are likely to differ in different populations, development and validation of novel therapies require an in-depth understanding of the mechanisms responsible for altered postprandial glucose metabolism. The use of the triple-tracer approach enables simultaneous measurement of the R_a of the ingested glucose (and splanchnic glucose uptake obtained by subtracting area under

Table 1—Advantages and disadvantages of dual- and triple-tracer methods

	Advantages	Disadvantages
Dual-tracer method	• Requires only two tracers • Requires only one tracer infusion pump • Less expensive than the triple-tracer approach	• Gives an inaccurate assessment of the rates of EGP, Ra_{meal}, and R_d, with the error being the largest during the first 1–2 h after meal ingestion
Triple-tracer method	• Provides more accurate assessment of the rates of EGP, Ra_{meal}, and glucose disposal following ingestion of a carbohydrate-containing meal	• Requires three tracers • Requires programmable tracer infusion pumps • Costs approximately 33% more than the dual-tracer method • Requires analysis of the enrichment/specific activities of three tracers • Requires EGP to be calculated directly rather than by subtracting Ra_{meal} from total R_a • Requires a few pilot studies to confirm that the tracer infusion profiles are appropriate

the curve of Ra_{meal} from the amount of glucose ingested), EGP, and R_d. In addition, if insulin and C-peptide are measured, insulin secretion and insulin action can be concurrently assessed using the oral minimal model (22).

The triple-tracer approach builds on original insight of Steele et al. (3) that by labeling ingested glucose and by infusing a systemic tracer, postprandial R_a can be partitioned between that which is coming from the ingested glucose and that which is due to EGP. Steele and colleagues (3,5,6) also realized that the resultant tracer/tracee nonsteady state required a fudge factor (an "effective" volume of distribution) as specific activity or enrichment in the accessible (plasma) pool does not reflect that in distant metabolically active tissues. Unfortunately, subsequent data have shown that the use of an "effective" volume of distribution introduces indeterminate errors that are both time and context dependent (7,8). The use of the triple-tracer approach minimizes such errors. Although this method may seem complicated, in practice, it generally is not. As noted earlier, we and others have used the triple-tracer approach to study the pattern of postprandial glucose metabolism in a large number of subjects with a variety of metabolic conditions in diverse settings (17–33). Upon request, these authors would be pleased to provide the tracer infusion profiles used in those studies. However, we strongly recommend that a few pilot experiments still need to be performed to refine the meal and endogenous tracer infusion profiles for the particular condition being studied. Once done, subsequent experiments are relatively straightforward and become simpler with experience. Care still needs to be taken to optimize the tracer infusion profiles when new disease states are to be studied (e.g., extreme insulin resistance) or the composition of the test meals differs substantially (e.g., complex carbohydrates vs. readily absorbable mono- or disaccharides). Of note, it is essential in the latter situation that the complex carbohydrates and disaccharides are uniformly labeled; otherwise, the systemic rate of appearance of the oral tracer will not reflect the rate of degradation and absorption of the ingested carbohydrate.

The dual-tracer method requires starting an IV tracer infusion several hours before meal ingestion. The pump then infuses tracer at a constant rate throughout the experiment. The triple-tracer method also requires starting a tracer infusion several hours before meal ingestion. The pump then infuses tracer at a constant rate until time 0 (when the meal is to be ingested) and then automatically changes the infusion rate to mimic the anticipated pattern of change of EGP. A second intravenous tracer is started when the meal is ingested that infuses a second tracer in a profile (easily programmed) that is anticipated to mimic the rate of appearance of the ingested glucose. In the opinion of the authors, the value of obtaining an accurate and reproducible assessment of Ra_{meal}, the postprandial pattern of suppression of EGP, and the postprandial pattern of stimulation of R_d more than offsets the cost of a second tracer and a second pump.

Acknowledgments. The authors would like to thank Drs. Ananda Basu, Rita Basu, and Adrian Vella, of the Mayo Clinic, and Dr. Chiara Dalla Man, of the University of Padova, for their thoughtful suggestions and support.

Funding. This work was supported by the National Institutes of Health (DK29953 and DK82396); the Ministero dell'Universitá e della Ricerca Scientifica e Tecnologica, Italy; and the Mayo Clinic.

References

1. Butler PC, Rizza RA. Contribution to postprandial hyperglycemia and effect on initial splanchnic glucose clearance of hepatic glucose cycling in glucose-intolerant or NIDDM patients. Diabetes 1991;40:73–81
2. Cobelli C, Toffolo G, Foster DM. Tracer-to-tracee ratio for analysis of stable isotope tracer data: link with radioactive kinetic formalism. Am J Physiol 1992;262:E968–E975
3. Steele R, Bjerknes C, Rathgeb I, Altszuler N. Glucose uptake and production during the oral glucose tolerance test. Diabetes 1968;17:415–421
4. Toffolo G, Basu R, Dalla Man C, Rizza R, Cobelli C. Assessment of post-prandial glucose metabolism: conventional dual- vs. triple-tracer method. Am J Physiol Endocrinol Metab 2006;291:E800–E806
5. Steele R, Wall JS, De Bodo RC, Altszuler N. Measurement of size and turnover rate of body glucose pool by the isotope dilution method. Am J Physiol 1956;187:15–24
6. De Bodo RC, Steele R, Altszuler N, Dunn A, Bishop JS. On the hormonal regulation of carbohydrate metabolism; studies with C^{14} glucose. Renal Prog Horm Res 1963;19:443–483
7. Butler PC, Caumo A, Zerman A, O'Brien PC, Cobelli C, Rizza RA. Methods for assessment of the rate of onset and offset of insulin action during nonsteady state in humans. Am J Physiol 1993;264:E548–E560
8. Allsop JR, Wolfe RR, Burke JF. The realiability of rates of glucose appearance in vivo calculated from constant tracer infusions. Biochem J 1978;172:407–416
9. Finegood DT, Bergman RN, Vranic M. Modeling error and apparent isotope discrimination confound estimation of endogenous glucose production during euglycemic glucose clamps. Diabetes 1988;37:1025–1034
10. Radziuk J. An integral equation approach to measuring turnover in nonsteady compartmental and distributed systems. Bull Math Biol 1976;38:679–693
11. Yang YJ, Hope ID, Ader M, Bergman RN. Insulin transport across capillaries is rate limiting for insulin action in dogs. J Clin Invest 1989;84:1620–1628
12. Turk D, Alzaid A, Dinneen S, Nair KS, Rizza R. The effects of non-insulin-dependent diabetes mellitus on the kinetics of onset of insulin action in hepatic and extrahepatic tissues. J Clin Invest 1995;95:755–762
13. Norwich KH. Measuring rates of appearance in systems which are not in steady state. Can J Physiol Pharmacol 1973;51:91–101
14. Cobelli C, Mari A, Ferrannini E. Non-steady state: error analysis of Steele's model and developments for glucose kinetics. Am J Physiol 1987;252:E679–E689
15. Hother-Nielsen O, Henriksen JE, Staehr P, Beck-Nielsen H. Labelled glucose infusate technique in clamp studies. Is precise matching of glucose specific activity important? Endocrinol Metab (Seoul) 1995;2:275–287
16. Haidar A, Elleri D, Allen JM, et al. Validity of triple- and dual-tracer techniques to estimate glucose appearance. Am J Physiol Endocrinol Metab 2012;302:E1493–E1501
17. Toffolo G, Dalla Man C, Cobelli C, Sunehag AL. Glucose fluxes during OGTT in adolescents assessed by a stable isotope triple tracer method. J Pediatr Endocrinol Metab 2008;21:31–45

18. Hinshaw L, Schiavon M, Mallad A, et al. Effects of delayed gastric emptying on postprandial glucose kinetics, insulin sensitivity, and β-cell function. Am J Physiol Endocrinol Metab 2014;307:E494–E502

19. Basu R, Dalla Man C, Campioni M, et al. Effects of age and sex on postprandial glucose metabolism: differences in glucose turnover, insulin secretion, insulin action, and hepatic insulin extraction. Diabetes 2006;55:2001–2014

20. Basu R, Dalla Man C, Campioni M, et al. Two years of treatment with dehydroepiandrosterone does not improve insulin secretion, insulin action, or postprandial glucose turnover in elderly men or women. Diabetes 2007;56:753–766

21. Basu R, Dalla Man C, Campioni M, et al. Effect of 2 years of testosterone replacement on insulin secretion, insulin action, glucose effectiveness, hepatic insulin clearance, and postprandial glucose turnover in elderly men. Diabetes Care 2007;30:1972–1978

22. Basu R, Breda E, Oberg AL, et al. Mechanisms of the age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action, and clearance. Diabetes 2003;52:1738–1748

23. Saad A, Dalla Man C, Nandy DK, et al. Diurnal pattern to insulin secretion and insulin action in healthy individuals. Diabetes 2012;61:2691–2700

24. Bock G, Dalla Man C, Micheletto F, et al. The effect of DPP-4 inhibition with sitagliptin on incretin secretion and on fasting and postprandial glucose turnover in subjects with impaired fasting glucose. Clin Endocrinol (Oxf) 2010; 73:189–196

25. Basu A, Dalla Man C, Basu R, Toffolo G, Cobelli C, Rizza RA. Effects of type 2 diabetes on insulin secretion, insulin action, glucose effectiveness, and postprandial glucose metabolism. Diabetes Care 2009;32:866–872

26. Sathananthan M, Shah M, Edens KL, et al. Six and 12 weeks of caloric restriction increases β cell function and lowers fasting and postprandial glucose concentrations in people with type 2 diabetes. J Nutr 2015;145: 2046–2051

27. Smushkin G, Sathananthan M, Piccinini F, et al. The effect of a bile acid sequestrant on glucose metabolism in subjects with type 2 diabetes. Diabetes 2013;62:1094–1101

28. Vella A, Bock G, Giesler PD, et al. The effect of dipeptidyl peptidase-4 inhibition on gastric volume, satiation and enteroendocrine secretion in type 2 diabetes: a double-blind, placebo-controlled crossover study. Clin Endocrinol (Oxf) 2008;69:737–744

29. Dalla Man C, Bock G, Giesler PD, et al. Dipeptidyl peptidase-4 inhibition by vildagliptin and the effect on insulin secretion and action in response to meal ingestion in type 2 diabetes. Diabetes Care 2009;32:14–18

30. Hinshaw L, Dalla Man C, Nandy DK, et al. Diurnal pattern of insulin action in type 1 diabetes: implications for a closed-loop system. Diabetes 2013;62:2223–2229

31. Mallad A, Hinshaw L, Schiavon M, et al. Exercise effects on postprandial glucose metabolism in type 1 diabetes: a triple-tracer approach. Am J Physiol Endocrinol Metab 2015;308:E1106–E1115

32. Shah M, Law JH, Micheletto F, et al. Contribution of endogenous glucagon-like peptide 1 to glucose metabolism after Roux-en-Y gastric bypass. Diabetes 2014;63:483–493

33. Sathananthan M, Ikramuddin S, Swain JM, et al. The effect of vagal nerve blockade using electrical impulses on glucose metabolism in nondiabetic subjects. Diabetes Metab Syndr Obes 2014;7:305–312

34. Felig P, Wahren J, Hendler R. Influence of maturity-onset diabetes on splanchnic glucose balance after oral glucose ingestion. Diabetes 1978;27:121–126

35. Ludvik B, Nolan JJ, Roberts A, et al. Evidence for decreased splanchnic glucose uptake after oral glucose administration in non-insulin-dependent diabetes mellitus. J Clin Invest 1997;100:2354–2361

36. Basu R, Di Camillo B, Toffolo G, et al. Use of a novel triple-tracer approach to assess postprandial glucose metabolism. Am J Physiol Endocrinol Metab 2003;284:E55–E69