tracer kinetics

review

Estimating the fractional synthetic rate of plasma apolipoproteins and lipids from stable isotope data

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Abstract The use of isotopic tracer studies to quantitate parameters characterizing apolipoprotein metabolism is enjoying a resurgence. This is due in large part to the availability of a number of stable isotopes and methods to measure them accurately in small quantities. Most experimental protocols in which stable isotopes are used call for endogenous labeling of the apolipoprotein of interest by an infusion of a labeled amino acid. Unlike the radioactively labeled amino acid counterpart in which turnover studies have traditionally been carried out for 72 hours to 14 days, the duration of the stable isotope experiment is normally less than 24 hours. This has contributed to some problems related to estimating the kinetic parameters because simplistic formulas whose underlying assumptions are not applicable to the lipoprotein system under study are often invoked. This is particularly true for the fractional synthetic rate (FSR). The purpose of this review is to address some of these problems. We derive the formula commonly used to estimate the FSR. In so doing, the underlying assumptions are carefully delineated. We then discuss several ways in which the formula is applied. Finally, we discuss the implications of these assumptions when the formula is applied to specific lipoprotein systems. **—Foster, D. M., P. H. R. Barrett, G. Toffolo, W. F. Beltz, and C. Cobelli.** Estimating the fractional synthetic rate of plasma apolipoproteins and lipids from stable isotope data. *J. Lipid Res.* 1993. **34:** 2193–2205.

Supplementary key words tracer • isotope • kinetics • fractional catabolic rate • fractional synthetic rate

In vivo turnover studies have contributed significantly to our understanding of the physiology and pathophysiology of lipoprotein lipid and apolipoprotein metabolism. Whereas many researchers have concentrated recently on the structural information available through molecular biology and genetic studies, the use of tracers to study lipoprotein kinetics as a means of understanding lipoprotein metabolism is experiencing a resurgence. This is due in large part to two facts: *i)* there is a need to investigate the functional characteristics of the plasma lipoproteins in concert with their structure, and *ii)* stable isotope tracers can now be used because methods to accurately quantitate small amounts are more readily available. Two additional advantages of using stable isotopes are: *i)* there is no radiation exposure to the patients, and *ii)* there are no problems similar to those in disposing of radionuclides. Schaefer, Rader, and Brewer (1) have recently reviewed the use of stable isotopes in lipoprotein kinetic studies. Gaw, Packard, and Shepherd (2) have reviewed VLDL and LDL turnovers for both radioactive and stable isotopes. What is missing in these reviews is a critical assessment of the methods currently in use or available to interpret stable isotope kinetic data. The purpose of this review is to provide this assessment.

To date, all stable isotope turnover studies in which the kinetics of lipoproteins have been investigated rely on endogenous labeling procedures. Either a bolus (3), or a constant or primed, constant infusion [(4–19) for apolipoproteins, (20–28) for plasma lipids, and (29–31) for hepatic lipogenesis] of a stable isotopically labeled precursor serves as the source of the isotope whose incorporation is followed in the lipoprotein of interest. With the exception of the studies reported in some articles (8, 13, 14), the kinetic parameter of interest, the fractional synthetic rate (FSR), has been estimated using a formula that is a hybrid of the traditional noncompartmental and compartmental methodologies because a specific compartmental assumption must be made on the link between the precursor and product system (32–36). One study (19) used a combination of the two methodologies; this approach was critiqued by Barrett et al. (37).

We will concentrate in this review on this formula and the implications of its application. First, we will derive the formula for a general isotopic tracer experiment assuming the accessible pools (those pools that are available for test input and measurement) for both the precursor and

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; FSR, fractional synthetic rate; FCR, fractional catabolic rate.

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product are homogeneous. This will highlight the underlying assumptions, assumptions that must be compatible with the system to which the formula is being applied. Some examples illustrating key points will be given. Second, we will discuss the formula applied to the situation where the accessible precursor pool is homogenous but the accessible product is heterogeneous. Whereas the formula derived in the first situation is theoretically still valid, we will see that heterogeneity makes the application of the formula and the interpretation of the FSR difficult. Third, we will discuss the reverse case, i.e., when the accessible precursor is heterogeneous and the accessible product pool is homogeneous. In this case, the validity of the formula is restricted to very specific situations. We will then turn to the work of Cobelli, Toffolo, and Foster (38) to review some related issues including measurement variables in stable isotope tracer studies. Finally, some issues related to experimental design and data analysis will be reviewed.

DERIVATION OF THE FORMULA FOR THE FSR

Case 1: Homogeneous precursor and product pools

The fractional synthetic rate is defined as the rate of incorporation of a precursor into a product per unit of product mass. The usual formula to calculate this parameter from a stable isotope tracer experiment is

$$\text{FSR} = \frac{\text{initial rate of change in product enrichment}}{\text{initial precursor enrichment}}. \quad \textit{Eq. 1)}$$

This formula was first applied to experiments in which an amino acid was enriched with a stable isotope and infused as a precursor for the apolipoprotein of interest (7). Several others have used this or variants of this formula as reviewed in reference 1.

We begin by deriving the FSR formula from first principles using the precursor-product model shown in **Fig. 1.** This figure also summarizes the notation to be used in the derivation.

The assumptions required to derive the formula are given below. 1. The tracee system remains in a steady state during the experiment; 2. the accessible precursor pool A and accessible product pool B are each described by a single compartment which may interact with a complex network of other compartments; 3. the accessible precursor pool A is the immediate precursor to the accessible product pool B; and 4. there is no tracer in the product pool B at time zero. One sees immediately why this is a hybrid noncompartmental and compartmental model: while it is not

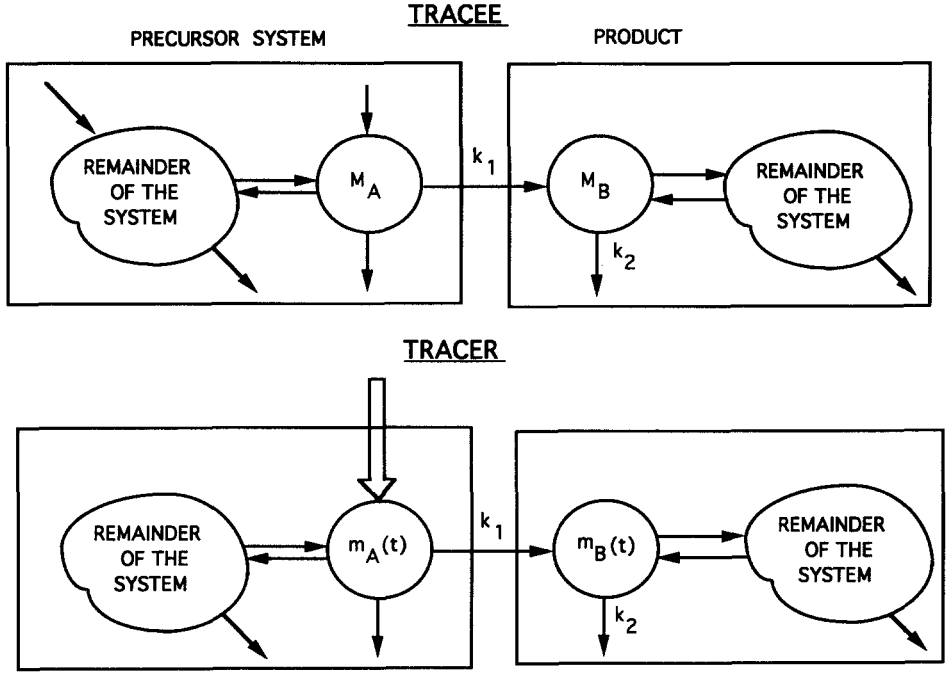

Fig. 1. The model describing the precursor(A)–product(B) system for the tracee (top panel) and tracer (bottom panel). The arrows between pools A and B and the "remainder of the system" describe the exchange processes in this part of the system; the arrows into and out of these respective components represent de novo production and irreversible loss. The rate constant quantitating the fraction of precursor converted to product is k_1 (time^{-1}). The rate constant quantitating the fraction of product lost from the B is k_2 (time^{-1}). The steady-state masses of precursor A and product B tracee are M_A and $M_B(t)$, respectively. The tracer system is shown in the bottom panel. Tracer masses at time t are $m_A(t)$ and m_B, respectively. Tracer-labeled precursor introduced into the system is indicated by the open arrow into the precursor pool.

necessary to postulate a specific multicompartmental structure for either the precursor or product system, the accessible precursor compartment must immediately precede the accessible product compartment.

For this system, we can write the mass balance equation describing $m_B(t)$, the tracer mass in B, as

$$\frac{dm_B(t)}{dt} = k_1 m_A(t) - k_2 m_B(t) + f(t) \qquad \text{Eq. 2)}$$

where $m_A(t)$ is the mass of tracer in A; k_1, and k_2 are, respectively, the rate constants describing transfer from A to B and irreversible loss from B; $f(t)$ is a function describing the net exchange of tracer mass in B with the "remainder" of the product system.

The assumption of no tracer mass in the product at time zero means $m_B(0) = f(0) = 0$; this allows us to write the following equation from equation 2:

$$\frac{dm_B(0)}{dt} = k_1 m_A(0) \qquad \text{Eq. 3)}$$

Writing

$$\frac{dm_B(0)}{dt} = m'_B(0)$$

one can solve equation 3 for k_1:

$$k_1 = \frac{m'_B(0)}{m_A(0)} \qquad \text{Eq. 4)}$$

Notice it is the presence of $m_A(0)$ in the denominator of equation 4 that requires the use of a priming dose. If one does not use the priming dose, $m_A(0) = 0$ and equation 4 is not defined. In addition, only $m_A(0)$ is required; there is no assumption that $m_A(0)$ be at a plateau value. This means that equation 4 is valid for experiments in which tracer is introduced into A either as a bolus or a primed, constant infusion.

Multiplying equation 4 by the ratio between M_A and M_B, the steady state tracee masses in A and B, respectively, we obtain the following expression for the FSR:

$$FSR = k_1 \frac{M_A}{M_B} = \frac{m'_B(0)/M_B}{m_A(0)/M_A} \qquad \text{Eq. 5)}$$

There is, however, one more assumption that leads to a convenient relationship between the FSR and the fractional clearance or catabolic rate (FCR). This assumption is that A is the sole precursor to B, i.e., $k_1 M_A$ is the production rate of B. As the system is in the steady state, the production rate of B must equal its disposal. The disposal rate of B can be expressed as the product of the FCR and the product tracee mass M_B:

$$k_1 \cdot M_A = \text{FCR} \cdot M_B \qquad \text{Eq. 6)}$$

and thus

$$\text{FSR} = \frac{k_1 M_A}{M_B} = \text{FCR} \qquad \text{Eq. 7)}$$

Equation 7 shows the relationship between the FSR and FCR when A is the sole precursor to B. For systems where product losses are in the accessible pool only, the FCR coincides with k_2. In general, the FCR is greater than k_2 as it also includes losses from nonaccessible pools.

To use equation 5, note that the ratio of the tracer to tracee mass in both the precursor and product are required. Following the notation used by Cobelli et al. (38), we will define these variables as $z_A(t)$ and $z_B(t)$:

$$z_A(t) = \frac{m_A(t)}{M_A} \qquad z_B(t) = \frac{m_B(t)}{M_B} \qquad \text{Eq. 8)}$$

Using this variable, we can now write equation 5

$$\text{FSR} = \frac{z'_B(0)}{z_A(0)} \qquad \text{Eq. 9)}$$

where $z'_B(0) = \frac{m'_B(0)}{M_B}$. This is essentially the same as equation 1 except that the tracer to tracee ratio is used in the expression instead of enrichment; we will say more about this later.

Application of equation 9: validity of the assumptions. The validity of the assumptions can be discussed first in the context of the model shown in **Fig. 2.** In this model, a single pool precursor system drives a single pool product system. We will adopt a simpler notation for the precursor and product pools (A and B) for convenience. The circles labeled A and B represent either the tracee mass M_A and M_B (assumed to be in a steady state) or the tracer mass $m_A(t)$ and $m_B(t)$. The fractional turnover rates of each are described by the rate constants k_1 and k_2. The experiment consists of a primed, constant infusion of precursor able to instantaneously attain a plateau value z_A for the precursor tracer to tracee ratio. The product tracer to tracee ratio $z_B(t)$ rises monoexponentially

$$z_B(t) = z_A(1 - e^{-\alpha t}) \quad t > 0 \qquad \text{Eq. 10)}$$

where the plateau value is z_A since B receives all tracer and tracee from A (equivalent tracer supply), and the rate constant α is equal to k_2. It is important to note that there is no delay in the appearance of tracer in the product; this

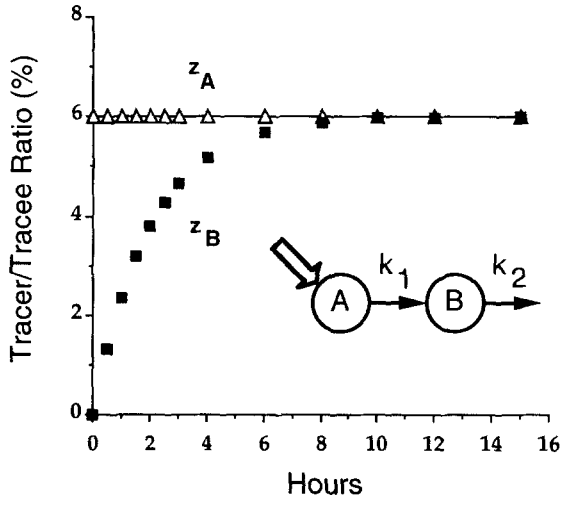

Fig. 2. The tracer to tracee ratio (%) for a system in which a single pool precursor (A) directly feeds a product pool (B); there is no other loss from pool A. A primed, constant infusion of precursor (open triangles) results in a rise of product (solid squares) to a plateau value; the rate constants are $k_1 = k_2 = 0.5$ h^{-1}. The steady state masses of A and B are equal and are normalized for convenience to 1.

is revealed by the early samples. In this example, $k_1 = k_2 = 0.5$ h^{-1}, the mass M_A of A is assumed to be 1, $m_A = 0.06$ and $z_B(t) = 0.06(1 - e^{-0.5t})$. From equation 7, the FSR is equal to FCR = k_2. This can be estimated as the rate constant α of the monoexponential equation 10 fitted to the z_B data. It is easy to verify that α provides a model based measurement of the ratio $\frac{z'_B(0)}{z_A(0)}$ of equation 9.

We will now use the above example as a template to illustrate the impact of the assumptions of equation 9 to its applicability in the interpretation of lipoprotein kinetic data. We assume the "data" are noise-free; adding random error to the simulated data only complicates the situation needlessly and diverts our attention from the primary points to be made. First we will discuss the affect of assuming that the initial increase in product enrichment is approximated by a straight line. Then we will give an example illustrating that the lipoprotein assembly and secretion process results in a delay between the precursor pool (plasma amino acid) and the appearance of label in the product (plasma lipoprotein). Such a delay can be seen in some of the stable isotope tracer data reported in the literature (5, 7).

Table 1 summarizes the data (% tracer to tracee ratio) from time zero to 4 h. The column, labeled slope, is the slope of the line joining the origin with the respective datum. If this is taken as an approximation of $z'_B(0)$ in equation 9, then an FSR (h^{-1}) can be calculated; this is summarized in the last column of Table 1. Given that the true FSR is 0.5 h^{-1}, it is clear that even if the data were

TABLE 1.

Time	Data	Slope	FSR
h			
0	0		
0.5	1.33	2.66	0.44
1.0	2.36	2.36	0.39
1.5	3.17	2.11	0.35
2.0	3.79	1.90	0.32
3.0	4.66	1.55	0.26
4.0	5.19	1.30	0.22

"perfect," by 30 min this approximation produces an error greater than 10%, and that this error becomes larger the later the first datum is collected.

Other approximations of $z'_B(0)$ have been obtained by performing linear regression on the first few data. If one does this with the data given in Table 1, then performing a linear regression through the origin for the data from 0–1 h to 0–4 h will produce a range of FSR values of 0.44 h^{-1} to 0.22 h^{-1}.

Finally, it is easy to see that if one does not force the regression line through the origin, even more serious underestimations of the FSR will occur. Thus FSR values reported in the literature using this method to estimate $z'_B(0)$ should be regarded as underestimates.

The effect of a delay in the appearance of product. **Fig. 3** shows the single pool precursor-product system shown in Fig. 2 under the same experimental conditions, but with a delay of 0.5 h before tracer appears in the product; one should notice the delay in appearance of tracer in B. The expression for $z(t)$ must be modified to take the delay into account:

$$z_B(t) = z_A(1 - e^{-\alpha(t-\tau)}) \quad t \geq \tau \qquad Eq.\ 11)$$

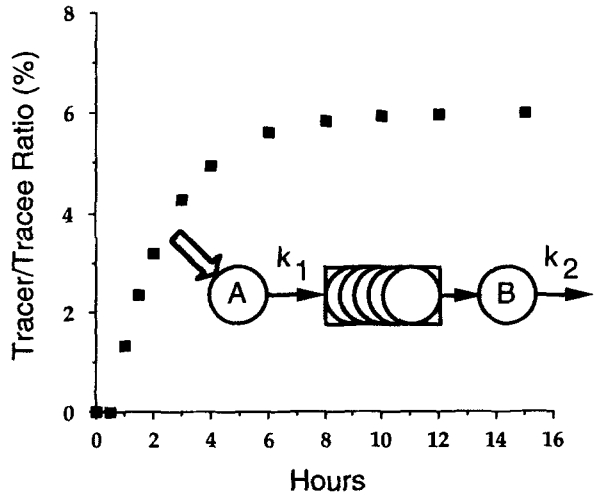

Fig. 3. The tracer to tracee ratio (%) for a system in which there is a single pool precursor (A) feeding a product pool (B) after a 0.5 h delay. A primed, constant infusion of precursor results in a delayed rise of product (solid squares) to a plateau value; the rate constants $k_1 = k_2 = 0.5$. The steady state masses of A and B are equal and for convenience normalized to 1.

where z_A is the plateau value, $\alpha = k_2$ as before, and τ is the time of the delay. As with the previous example, $k_1 = k_2 = 0.5$ h^{-1}. The mass of A is 1 and as equation 6 still holds, the mass of B is also 1. From the definition, the FSR is expressed as $k_1 \frac{M_A}{M_B}$ (cf equation 5), and it is still equal to FCR $= k_2$. Therefore, the FSR does not change, but remains at 0.5 h^{-1}.

It is clear, however, that one cannot use equation 9 to estimate the FSR since $z'_B(0) = 0$. In the presence of the delay τ, equation 9 must be modified

$$\text{FSR} = \frac{z'_B(\tau)}{z_A(\tau)} \qquad \textit{Eq. 12)}$$

Therefore, one has to estimate first the delay τ from the z_B data, and then evaluate $z'_B(t)$ and $z_A(t)$ at time τ. It is easy to verify from equation 12 for this example that, since $z'_B(\tau) = z_A \cdot \alpha$, α provides a model-based measurement of the right hand side of equation 12.

On the other hand, what values for the FSR will be estimated from these data using the various methods reported in the literature? In other words, how does the delay compound the estimates described above when no delay was considered? Two common methods are summarized in Figs. 4 and 5. As above, the "data" are assumed to be noise-free; adding random error to the simulated data only complicates the situation needlessly and diverts our attention from the primary points to be made.

The first, illustrated in **Fig. 4,** neglects the effect of the delay on the data by determining an estimate of "$z'_B(0)$" as the slope of the line connecting the origin with the first datum. "Forcing" the data through the origin is what neglects the delay. The slope, then, depends upon the time of the first datum. In the figure, we illustrate four possibilities which, using equation 9 with $z_A(0) = 6\%$, gives an

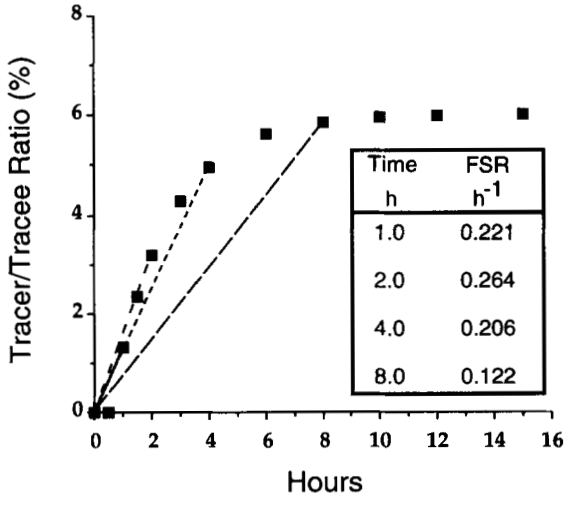

Fig. 4. The FSR for the data shown in Fig. 3 as estimated by calculating the initial slope ($z'_B(0)$) by connecting time zero with different options for the first datum. The true FSR is 0.5 h^{-1}.

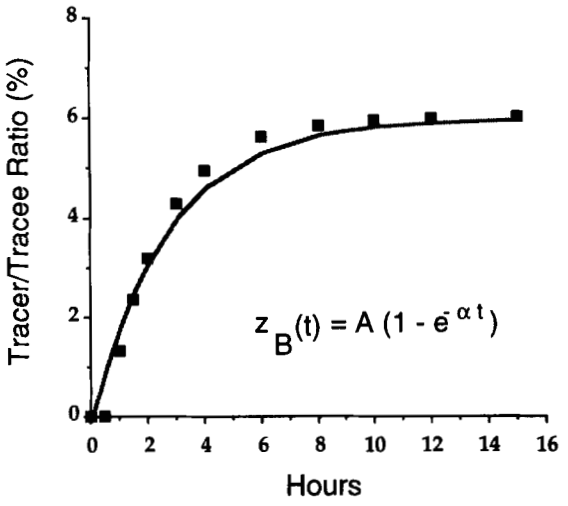

Fig. 5. The data shown in Fig. 3 fit with the monoexponential function $z_B(t) = A(1 - e^{-\alpha t})$.

FSR ranging from 0.122 h^{-1} to 0.264 h^{-1}; all are less than the true value of 0.5 h^{-1}. A variation of the method is linear regression performed on the first few data points (untransformed), again forcing the line through the origin. The combination of the curvilinear rise to the plateau and when the data are actually collected makes this methodology equally unreliable and inaccurate.

The second method (**Fig. 5**) assumes that the tracer to tracee ratio in the product can be described by the monoexponential function equation 10. This neglects the delay and forces the "predicted" data through the origin. The parameter α is then used as before to estimate the FSR. In this example, an estimate of α of 0.37 h^{-1} is obtained, 26% less than the true value of 0.5 h^{-1}.

This example shows that neglecting the delay τ can cause significant errors in estimating the FSR. In addition to that, it also demonstrates the use of exponential fitting to implement the FSR formulas. Obviously, equation 10 is correct only when both the model and the experimental conditions are the same as in the example, i.e., the kinetics of B are monocompartmental, A is the sole precursor of B, and z_A is constant during the experiment. In the general case, a more complex function will describe the rise of $z_B(t)$ to the plateau value. Fitting the proper function to the z_B data allows one to derive a model-based estimate of the FSR as expressed by equation 9 or equation 12.

A negative FSR. In references 22–26, changes in the FSR during an experiment, including negative values, were discussed. In essence, equation 9 was applied at consecutive 4-h intervals. One sees immediately from the derivation of equation 4 that this application is not correct. If $t \neq 0$, k_1 is not given by equation 4 but becomes

$$k_1 = \frac{m'_B(t) + k_2 m_B(t) - f(t)}{m_A(t)} \qquad \textit{Eq. 13)}$$

Note that when $t = 0$, this expression reduces to that given in equation 4 from which equation 9, the FSR formula, has been derived. However, if equation 9 is inadvertently used to calculate the FSR at $t \neq 0$, one has

$$\text{FSR} = \frac{z'_B(t)}{z_A(t)} = \left(k_1 - \frac{k_2 m_B(t) - f(t)}{m_A(t)}\right)\frac{M_A}{M_B} \qquad \text{Eq. 14)}$$

Since $\frac{M_A}{M_B} > 0$, it is clear a negative FSR will result when $\frac{k_2 m_B(t) - f(t)}{m_A(t)} > k_1$. The point is that this shows that it is invalid to estimate $z'_B(0)$ using data at time other than zero.

Besides accounting for the negative values for the FSR, equation 14 can also account for the changes in the FSR observed in references 22-26, since the multiple dosing tracer administration format used in those studies (22-26) creates perturbations that will alter the time dependence of $m_A(t)$ and $m_B(t)$.

Case 2: Homogeneous precursor and heterogeneous product pools

The heterogeneity of the plasma lipoproteins is another factor that complicates the currently used methods to estimate the FSR. Rather than deal with the general theory as we did in the previous section, we will consider as an example the situation described in **Fig. 6.** Here we have a single precursor pool A feeding into two product pools B and C; the measured tracer and tracee masses in the product is the sum of the tracer and tracee in the two product pools, respectively.

The formula for FSR. While the definition of the FSR remains unchanged, the expression given in equation 5 must be modified to include the fact that there are two kinetically distinct product pools. The expression for the FSR is

$$\text{FSR} = \frac{(k_B + k_C)M_A}{M_B + M_C} \qquad \text{Eq. 15)}$$

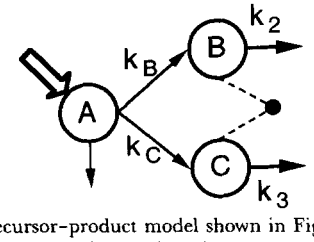

Fig. 6. The precursor-product model shown in Fig. 2 is expanded to show a product system consisting of two homogeneous pools; total tracer measured is the sum of the tracer in the two pools (indicated by the dotted line and bullet). The rate constants describing the transfer of material from A to B and A to C are k_B and k_C, respectively; the rate constants describing the loss from pools B and C are k_2 and k_3, respectively.

The counterpart of equation 9 is derived in the Appendix; it is

$$\text{FSR} = \frac{m'_B(0) + m'_C(0)}{M_B + M_C} = \frac{z'_{B+C}(0)}{z_A(0)} \qquad \text{Eq. 16)}$$

For the model given in Fig. 6, the counterpart of equation 10 is

$$z_{B+C}(t) = \frac{m_B(t) + m_C(t)}{M_B + M_C} = A_1(1 - e^{-\alpha_1 t}) + A_2(1 - e^{-\alpha_2 t}) \qquad \text{Eq. 17)}$$

where

$$A_1 = z_A \frac{k_B/k_2}{k_B/k_2 + k_C/k_3} \qquad A_2 = z_A \frac{k_C/k_3}{k_B/k_2 + k_C/k_3}$$

The plateau value of z_{B+C} is $A_1 + A_2$ which still coincides with z_A, and the exponentials α_1 and α_2 are equal to k_2 and k_3. From equation 17, it is clear that $z_{B+C}(t)$ depends upon the individual terms k_B and k_C, and not on $k_B + k_C$. Therefore, it can change if some of these parameters change. Conversely, the FSR may not change even if some parameters do change as it is related to the derivative at time zero.

Example: effect of a heterogeneous product on estimating the FSR. While equation 16 is a counterpart to equation 9 for this case, what kinds of problems can one encounter as a result of the heterogeneity of the product? The following example illustrates the fact that the FSR is very difficult to interpret when heterogeneity is involved.

Consider the following example using the model shown in Fig. 6. Two sets of data are shown in **Fig. 7A** representing the tracer to tracee ratio in the product B+C simulated in two hypothetical studies where the only change is the amount of material flowing out of pools B and C; the rate constants describing this movement are k_2 and k_3. In this example, the mass of A is assumed to be 1, z_A is at the plateau value 6%, and in both cases, $k_B = 0.3\ \text{h}^{-1}$ and $k_C = 0.2\ \text{h}^{-1}$.

In the upper curve, $k_2 = k_3 = 0.5\ \text{h}^{-1}$. To calculate the masses of B and C, one can solve a variation of equation 6:

$$k_B M_A = 0.3 = k_2 M_B = 0.5\ M_B \quad k_C M_A = 0.2 = k_3 M_C = 0.5\ M_C$$

where M_A, M_B, and M_C are the tracee masses of A, B, and C, respectively. This gives $M_B = 0.6$ and $M_C = 0.4$, so $M_B + M_C = 1$. By equation 15, the FSR equals $0.5\ \text{h}^{-1}$. In the lower curve, $k_2 = 1\ \text{h}^{-1}$ and $k_3 = 0.29\ \text{h}^{-1}$. Again, to calculate the masses of B and C, one solves:

$$k_B M_A = 0.3 = k_2 M_B = M_B \quad k_C M_A = 0.2 = k_3 M_C = 0.29\ M_C$$

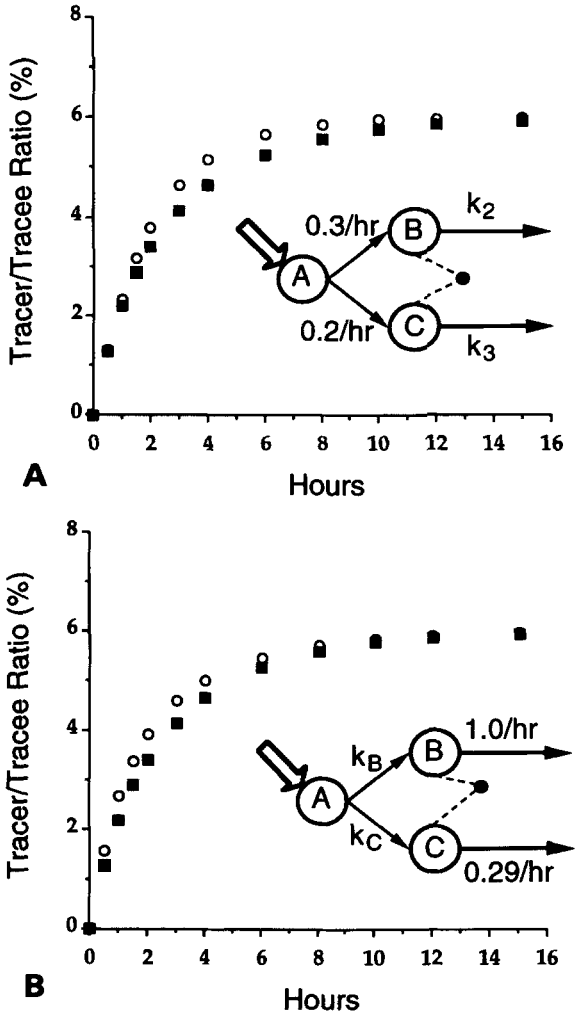

Fig. 7. A: Two simulated sets of data using the model shown in Fig. 6. In both cases, k_B = 0.3 h^{-1} and k_C = 0.2 h^{-1}, and the mass of A equals 1. For the upper curve, k_2 = k_3 = 0.5 h^{-1}; for the lower curve, k_2 = 1 h^{-1} and k_3 = 0.29 h^{-1}. B: Two simulated sets of data using the model shown in Fig. 6. In both cases, k_2 = 1.0 h^{-1} and k_3 = 0.29 h^{-1}, and the mass of A equals 1. For the upper curve, k_B = 0.30 h^{-1} and k_C = 0.203 h^{-1}; for the lower curve, k_B = 0.50 h^{-1} and k_C = 0.145 h^{-1}.

to obtain M_B = 0.3 and M_C = 0.7, so again $M_B + M_C$ = 1, and the FSR equals 0.5 h^{-1}. Whereas these curves are clearly different, they have the same derivative at time zero and thus the FSR does not change.

What would happen if one were unaware of the heterogeneity issue? If one were to use equation 10 to estimate the FSR, one would estimate 0.47 h^{-1} and 0.35 h^{-1}, respectively, for the FSR of the upper and lower curves, thereby concluding that the difference between them is due to a change in the FSR. One would reach the same erroneous conclusion using the method illustrated in Fig. 4.

In Fig. 7B, two curves are shown where k_2 and k_3 do not change; the change is due to changes in k_B and k_C. Note that after the first hour, both sets of data in Fig. 7A and 7B are essentially the same since, due to the equivalent tracer supply, the tracer to tracee ratio in the whole system tends towards z_A. Following the same line of reasoning in the above discussion, one can calculate the masses M_B and M_C equal to 0.3 and 0.7, respectively, for the upper curve; for the lower curve, both masses are equal to 0.5. From this, the FSR, determined using equation 10, for the upper curve is 0.58 h^{-1} and 0.5 h^{-1} for the lower. Although this example demonstrates an apparent change in the FCR (FSR) it is really changes in the secretory process that account for the effect on FSR.

Notice in this example there is no delay in the appearance of tracer in the product. We did not include this because we wanted to focus only on the heterogeneity issue. Clearly a delay similar to that illustrated in Fig. 3 would only compound the errors. These examples demonstrate that in interpreting lipid and lipoprotein tracer data one must be aware of the effect that heterogeneity can have on estimating metabolic parameters of interest.

Case 3: Heterogeneous precursor and homogeneous product pools

We now consider as an example the situation described in **Fig. 8.** Here we have the two precursor pools A and B feeding a single product pool C; the measured tracer and tracee in the product is $z_C(t)$. This situation would arise in the case where, for example, there are both liver and intestinal sources of an apolipoprotein of interest.

As above, while the definitions of the FSR and FCR remain unchanged, the expressions given in equation 5 and equation 7 must be modified to include the fact that there are two kinetically distinct precursor pools. The expression for the FSR is

$$\text{FSR} = \frac{k_A M_A + k_B M_B}{M_C} \qquad \textit{Eq. 18)}$$

and the expression for the FCR is

$$\text{FCR} = k_3 \qquad \textit{Eq. 19)}$$

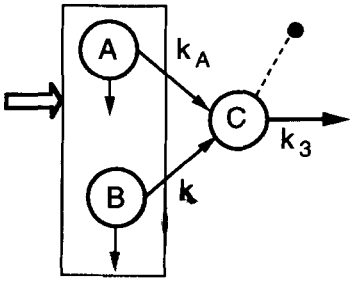

Fig. 8. The precursor–product model shown in Fig. 2 is expanded to show a precursor system consisting of two homogeneous pools and a single product pool. The rate constants describing the transfer of material from A to C and B to C are k_A and k_B, respectively; the rate constant describing the loss from pool C is k_3.

As before, the FSR and FCR coincide since $k_AM_A + k_BM_B = k_3M_C$.

In this case, the counterpart of equation 9 is

$$FSR = \frac{z'_C(0)}{\frac{R_A}{(R_A+R_B)} z_A(0) + \frac{R_B}{(R_A+R_B)} z_B(0)} \quad Eq.\ 20)$$

where $R_A = k_AM_A$ and $R_B = k_BM_B$. The derivation of this equation is shown in the Appendix.

In the previous section we saw that despite heterogeneity of the product, an FSR could be estimated; the problem in this case was in the interpretation of the FSR. In contrast, in the case of heterogeneous precursor pools, it is impossible to estimate an FSR without a knowledge of both $z_A(0)$ and $z_B(0)$ and the relative contribution of each pool to the product. The point is that with heterogeneity of the precursor pool, one cannot interpret the FSR with any degree of certainty. As an aside, the value of $z_C(\infty)$ evaluated at infinity can be used as the denominator in equation 20. Although, from a practical point of view this is not a feasible approach.

MEASUREMENT VARIABLES

To apply equation 9, one must have a measure of the tracer to tracee mass ratio $z(t)$. As discussed by Cobelli et al. (38), for the radioactive tracer, specific activity can be used. However, for stable isotope tracers, the situation is not so clear; $z(t)$ can either be measured directly or must be calculated from other measurement variables.

For the stable isotope experiment, there are a number of other measurement variables of which the isotope ratio $r(t)$ and enrichment $e(t)$ are the most common. Unlike the radioactive isotope case, there is no easy way to write an expression for $z(t)$ from these variables. Equation 1 is the analog of equation 9, but written in terms of enrichment rather than $z(t)$. The question now arises as to the consequences of using equation 1 instead of equation 9; i.e., can one use enrichment in place of $z(t)$ in equation 9?

To answer this question, one must know the relationship between enrichment $e(t)$ and $z(t)$. Usually enrichment is defined:

$$e(t) = \frac{M^s + m^s(t)}{M^a + m^a(t) + M^s + m^s(t)} - \frac{M^s}{M^a + M^s} \quad Eq.\ 21)$$

where M^a and M^s are the masses of the most (superscript a) and least (superscript s) abundant species in the tracee respectively $m^a(t)$ and $m^s(t)$ are the same for the tracer.

Let e_I be the enrichment of the infusate. As pointed out by Cobelli et al. (38), the relationship between $z(t)$ and enrichment $e(t)$ is

$$z(t) = \frac{e(t)}{e_I - e(t)} \quad Eq.\ 22)$$

Clearly, one cannot substitute enrichment for $z(t)$. However, the question can be asked: to what extent does equation 1 approximate equation 9?

To answer this question, we look first at the relationship between $\frac{de_B(0)}{dt}$ and $\frac{dz_B(0)}{dt}$. Since by assumption $e_B(0) = 0$, one can differentiate equation 22 to calculate

$$z'_B(0) = \frac{dz_B(0)}{dt} = \frac{1}{e_I} \cdot \frac{de_B(0)}{dt} \quad Eq.\ 23)$$

Next, from equation 22, one can write

$$z_A(0) = \frac{e_A(0)}{e_I - e_A(0)} \quad Eq.\ 24)$$

Combining equations 23 and 24,

$$\frac{z'_B(0)}{z_A(0)} = \frac{e_I - e_A(0)}{e_I} \cdot \frac{e'_B(0)}{e_A(0)} \quad Eq.\ 25)$$

Therefore, since $\frac{e_I - e_A(0)}{e_I} < 1$, the use of enrichment instead of the tracer to tracee ratio $z(t)$ in equation 9 will overestimate the FSR.

A measure of the error can be obtained by knowing the magnitude of $\frac{e_I - e_A(0)}{e_I}$. This expression is always less than one because $e_A(0) \neq 0$, as the protocol calls for a primed, constant infusion of isotope. The extent to which this is less than one depends, then, on the enrichment in the infusate, e_I, and $e_A(0)$ which depends on the size of the priming dose. This will be variable from experiment to experiment.

What differences exist practically between $z(t)$ and $e(t)$? In **Fig. 9,** we show a set of data using the ideal model of Fig. 2 where the enrichment of the infusate $e_I(0)$ is 99%. The tracer to tracee ratio, $z(t)$, as the theory predicts will always be greater than enrichment. In this particular situation in which equation 9 is used to calculate the FSR, the difference in using $z(t)$ and $e(t)$ is 8%; 0.50 h^{-1} versus 0.54 h^{-1}. The size of the difference depends upon the amount of enrichment; the greater the enrichment, the larger the error. In our experience, we have observed differences in the two ranging from 2% to 15%.

In closing this section, it should be pointed out that there is a specific situation where equation 1 is correct. As discussed by Toffolo, Foster, and Cobelli (36), this situation occurs when the total, i.e., tracer plus tracee (instead

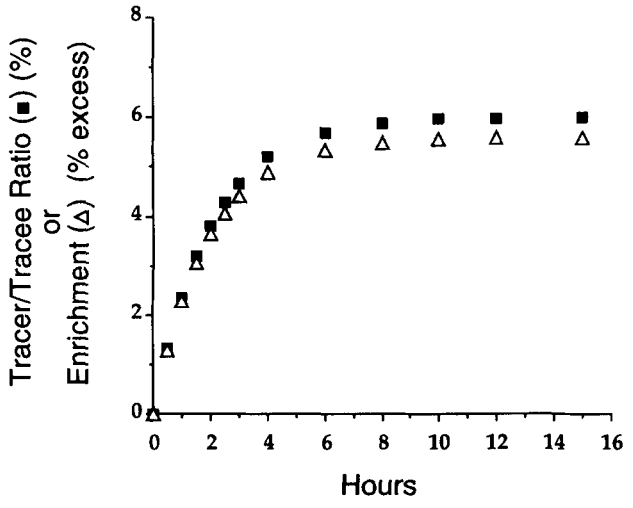

Fig. 9. A set of data derived from the model shown in Fig. 2 plotted in terms of the tracer to tracee ratio (squares) and enrichment (triangles).

of tracee), fluxes remain constant and equal to the value prior to the tracer experiment.

DISCUSSION

Tracer kinetic studies are a crucial tool to aid in our understanding of the physiology and pathophysiology of lipoprotein metabolism. As knowledge of the physicochemical properties of the plasma lipoproteins increases, so does the complexity of the metabolic characteristics. When one designs new kinetic studies to take advantage of this information and new techniques such as stable isotope methodologies, one must also be aware of what has been learned in previous studies. In particular, one should be aware of the different kinetic analysis methodologies, how these have been used in the interpretation of isotope tracer studies, and how this information can be used to design stable isotope kinetic experiments. Thus while this review has focused on the derivation and application of equation 9, there are other related issues that should be addressed in planning stable isotope experiments.

Data analysis methodologies

In this review we have focused on one formula to estimate the FSR. However, in designing stable isotope kinetic studies, one needs to be aware of other methodologies that are available, and to assess their applicability to a particular experimental design or set of experimental data. In part, the methodology chosen depends upon the information required from the data.

As we have seen, in most stable isotope experiments, equation 9 is used to estimate the FSR, which in turn is used to estimate production rates. On the other hand, for the radioactive tracer, the kinetic parameter most frequently estimated from the tracer data is the fractional catabolic rate (FCR) (32, 33). This parameter can be estimated using noncompartmental analysis or compartmental methods such as the so-called Matthews analysis (32, 39). As we have seen, for models based on assumptions 1-4 given earlier, the FCR and FSR must be the same, telling us that the quantitation of a physiological event does not depend upon the label used, i.e., stable or radioactive isotope.

There are some misconceptions, however, about the applicability of the various methodologies. For example, when Schaefer et al. (1) discuss Patterson et al. (3), they remark that a major disadvantage of introducing the labeled precursor using a single bolus method is that it requires multicompartmental analysis in order to determine the kinetic parameters, and in addition, tracer recycling makes the interpretation of the terminal portion of the decay curve difficult to interpret. We note in the derivation of equation 9 that the precursor can be introduced either as a bolus or a primed, constant infusion; what is necessary is that $m_A(0) \neq 0$. Hence, their first point on tracer input is not correct. The implication of the second point is that tracer recycling is a problem only of the bolus; in fact, it is a problem for both exogenous and endogenous labeling. To date, no one has worked out a satisfactory solution to the recycling issue; Venkatakrishnan (40) has presented some preliminary work on apoB and apoA-I using a leucine model proposed by Cobelli et al. (41).

Schaefer et al. (1) remark later that the results of Parhofer et al. (13) suggest that apoB kinetic parameters are independent of the method of administration of tracer as long as the data are analyzed by compartmental analysis. They (1) go on to say that the primed, constant infusion permits a more simplified analysis. The implication is that the system kinetics somehow depend upon the method of introducing the tracer into the system, or in some cases, whether a radioactive or stable isotope tracer is used. This is clearly not the case.

Lipoprotein secretion and plasma residence time

A knowledge of the apolipoprotein and lipid secretion and plasma residence times plays an important role in designing tracer experiments. Secretion time here refers to the time at which tracer label from a precursor appears in the lipoprotein particle of interest; this affects the nature of the delay discussed earlier in this review, and hence the use of equation 9 to estimate the FSR. In fact, one notes in the derivation of equation 9 that A must be the immediate precursor of B; a delay in the appearance of B can be interpreted as A in plasma not being the immediate precursor. The immediate precursor probably is, in fact, a nonaccessible pool of tRNA. Problems associated with using amino acid precursors in general and labeling the hepatic protein synthetic precursor pool have recently been discussed by Reeds et al. (15).

On the other hand, if other analytical methodologies are to be used, a knowledge of the plasma residence time is important in order to determine how long the study should be conducted. The buoyant, triglyceride-rich lipoproteins—chylomicrons and VLDL—have plasma residence times of a few hours at most. The more dense LDL and HDL particles, and their associated apolipoproteins and lipids, have plasma residence times of a few days. The short term studies frequently encountered in stable isotope studies force one to use formulas such as equation 9 since the information necessary to describe additional metabolic complexities cannot be obtained from the data.

Studies in which the lipoprotein of interest is labeled endogenously using a radioactive tracer have taken this information into account. Examples are provided for apoB (42–46) in VLDL, IDL, and LDL, and for triglycerides (47). Depending upon the plasma lipoprotein being studied, the duration of turnover studies varied from 48–72 h to over 10 days. A knowledge of the system behavior from radioactive isotopic studies was taken into account in designing the experiments reported in references 8 and 14.

In designing stable isotope experiments in which equation 9 is to be used to interpret the data, many investigators infuse the labeled amino acid for a period of up to 15–20 h. If one is willing to accept the assumptions upon which equation 9 is based, then an estimate of the FSR can be obtained from the information at $t = 0$ for particles in which the label is rapidly incorporated. Hence frequent, early samples are required, and in some cases the experiment could terminate before 12–18 h.

Two specific problems arise, however, when one tries to estimate the FSR of apolipoproteins or lipids associated with the slowly turning over LDL or HDL. One is to define the precursor (A in Fig. 1), and the other is created by the delay in appearance of tracer in these lipoproteins.

Consider, for example, estimating the FSR of apoB in VLDL and LDL using a labeled amino acid. One first uses the information from the labeled amino acid and VLDL apoB at time zero to estimate the FSR of VLDL apoB. The question is how to estimate the FSR of LDL apoB assuming VLDL apoB as the precursor. Recognizing that there is a delay in the appearance of label in LDL apoB, one could select the time $t = t^*$ at which *i)* the tracer to tracee ratio in VLDL is non-zero, and *ii)* tracer is just beginning to appear in LDL; and then modify equation 9 to estimate the FSR. In this case, if A were VLDL apoB and B were LDL apoB, the FSR could be given by $\frac{z'_B(t^*)}{z_A(t^*)}$.

In this approach, which still relies on the validity of the assumption upon which equation 9 is based, a plateau value for VLDL apoB is not needed. What is needed is the time at which tracer begins to appear in LDL apoB, and VLDL and LDL apoB data to use in calculating the appropriate terms in equation 9. On the other hand, some have taken the plateau value of VLDL apoB as a zero time precursor value, and calculated the initial rate of change of LDL apoB or HDL apoA at time zero using the methods discussed earlier in this review. One can see that this approach is not correct, and hence values reported for LDL and HDL stable isotope kinetics using this approach should be interpreted with caution.

Tracer kinetic experimental design

In this review, we have focused on data analysis issues, an understanding of which should help researchers plan and design kinetic studies using stable isotopes. We should emphasize that while we have concentrated on stable isotope kinetic data, some of these issues apply equally to the radioactive data situation. For example, the problems discussed in terms of heterogeneity in this review have their counterparts in the radioactive experiment; some of these have been discussed by Berman (32, 33).

We have also pointed out that the point of designing kinetic studies is to elucidate details of metabolic events not available from other methodologies. These designs must include both data generation and data analysis. If one knows a priori the method of analysis to be used, this can often help in the experimental design by indicating when and where to sample. This is especially useful if a model of the system already exists.

The apoB cascade to describe VLDL apoB metabolism for both endogenous and exogenous studies is frequently used [see Berman (32)]. This model arose from simpler ones as more complex data became available. Investigators with a knowledge of this model know that *i)* frequent early samples must be taken, and *ii)* the study should last from 36 to 72 h with later samples taken at infrequent intervals. With the infrequent and short sampling schedule often used in stable isotope experiments, especially those starting sampling after 2 h, one is forced to use methodologies such as equation 9.

For LDL and HDL turnover studies, one knows from the residence time calculations that the studies should last up to 2–3 weeks. However, it is also known how kinetic heterogeneity affects the interpretation of the data. The situation for LDL is reviewed in reference (32) and discussed more recently by Foster et al. (48); the latter also contains a detailed description of how to interpret a non-constant urine-to-plasmas ratio. Heterogeneity of HDL is discussed by Zech et al. (49). From these studies, it is clear that the short term turnover studies of these lipoproteins using stable isotopes cannot possibly reveal the complexity of the system already known.

What council can be given to those contemplating designing a lipoprotein kinetic study? This is a difficult question to answer as it depends upon many factors. From

the data analysis point of view, these are well described by Berman (50) who stated that the choice of a model to interpret the data is dependent upon the information content in the data themselves and the information desired by the investigator. If one designs an experiment and is planning to use equation 9, for example, then one knows a priori that the underlying assumptions are probably not justified, and that the parameter estimates should be interpreted with caution. On the other hand, if one designs a study and is planning to use the so-called Matthews analysis (32), then heterogeneity cannot be accounted for and, as illustrated in this review, the kinetic parameter must again be interpreted with caution. If one is performing an LDL turnover study and collecting urine data, then the use of a model that simultaneously incorporates plasma and urine data will reveal a more complex system.

It is beyond the scope of this review to go into detail, but methods are being developed to subfractionate the lipoproteins operationally defined on the basis of buoyant density. For both radioactive and stable isotope studies, such subfractionation can aid in accounting for heterogeneity.

In conclusion, one must appreciate that in designing turnover studies, experimental data generation and model development to interpret the data go hand-in-hand. Models are presented as state-of-the-art only. They incorporate into their structure what is known and hypothesized about a system, and are compatible with the available data. Often structures are present in a model that cannot be explained on the basis of known information. These aspects of the model need to be challenged by further experimentation, the results of which often force a change in the model structure.

Modeling is thus a dynamic process as we probe more deeply into lipoprotein metabolism. The more complex models that are developed also can be used to justify situations where simpler models can be used, or to illustrate the kinds of problems that may ensue when the simpler models are chosen. This observation has been illustrated in this review.

APPENDIX

The focus of this review deals with the design and interpretation of lipid and lipoprotein tracer kinetic studies using stable isotopes. However, we do not want to give the impression that some of the situations we described in the review are unique only to these isotopes. The primary case in point relates to how lipoprotein heterogeneity affects the design and interpretation of lipid and lipoprotein turnover studies in general.

In this appendix, we will discuss the formula for the fractional synthetic rate used in the text for the homogeneous precursor and heterogeneous product, and heterogeneous precursor and homogeneous product.

Homogeneous precursor and heterogeneous product

We will discuss here the specific example given in Fig. 6 rather than the general theory. The formula for the FSR extended to this case is given in equation 15. By extending equation 2 the expression for the fractional catabolic rate can also be obtained:

$$\text{FCR} = \frac{k_2 M_B + k_3 M_C}{M_B + M_C} \qquad \textit{Eq. A-1)}$$

As before, since A is the sole precursor of B and C, the FSR and FCR are equal.

To derive equation 15, paralleling the derivation of equation 9, we write the mass balance equation for the tracer in the product

$$\frac{d(m_B(t) + m_C(t))}{dt} = (k_B + k_C)m_A(t) - k_2 m_B(t) - k_3 m_C(t) \qquad \textit{Eq. A-2}$$

and, assuming $m_B(0) = m_C(0) = 0$,

$$m'_B(0) + m'_C(0) = (k_B + k_C)m_A(0) \qquad \textit{Eq. A-3)}$$

Solving equation A-3 for $k_B + k_C$ and substituting into equation 15, one arrives at equation 16.

Heterogeneous precursor and homogeneous product

As above, rather than go into the general theory, we will focus as an example on the model shown in Fig. 8. As discussed in the text, while there is an expression for the FSR and FCR, equations 18 and 19, respectively, the counterpart to equation 9 is significantly more complex.

To see this, first derive the mass balance equation for the tracer in the product

$$\frac{dm_C(t)}{dt} = k_A m_A(t) + k_B m_B(t) - k_3 m_C(t) \qquad \textit{Eq. A-4)}$$

and as before assuming $m_C(0) = 0$,

$$m'_C(0) = k_A m_A(0) + k_B m_B(0) \qquad \textit{Eq. A-5)}$$

For convenience, we write $R_A = k_A M_A$ and $R_B = k_B M_B$. One can then rewrite equation A-5

$$m'_C(0) = R_A z_A(0) + R_B z_B(0) \qquad \textit{Eq. A-6)}$$

Dividing both sides of equation A-6 by $R_A + R_B$ and then solving for $R_A + R_B$, one can show that the numerator in equation 18 is

$$k_A M_A + k_B M_B = \frac{m'_C(0)}{\frac{R_A}{(R_A + R_B)} z_A(0) + \frac{R_B}{(R_A + R_B)} z_B(0)} \qquad \textit{Eq. A-7)}$$

From equation 18

$$\text{FSR} = \frac{z'_C(0)}{\frac{R_A}{(R_A + R_B)} z_A(0) + \frac{R_B}{(R_A + R_B)} z_B(0)} \qquad \textit{Eq. A-8)}$$

It is clear from equation A-8 that the FSR is not equal to the change in $z_C(t)$ at time zero, $z'_C(0)$, divided by the precursor A+B tracer to tracee ratio at time zero. The precursor tracer to tracee ratio z_{A+B} at time zero is $\frac{m_A(0) + m_B(0)}{M_A + M_B}$, while the denominator of equation A-8 is a weighted average of the tracer to tracee ratios of the individual precursor pools. The FSR equals $\frac{z'_C(0)}{z_{A+B}(0)}$ only in two special situations: when $k_A = k_B$ or $z_A = z_B$. Thus, apart from these situations, equation 9 cannot be used to estimate the FSR.

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