Use of Stable Isotopically Labeled Tracers for Studies of Metabolic Kinetics: An Overview

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Stable isotopically labeled tracers offer a reliable and safe alternative to the use of radioactive tracers for studies of metabolic kinetics. This overview examines some of the principles and technical issues regarding mass spectrometry instrumentation, and reviews some of the approaches used in the application of stable isotopically labeled tracers to studies of protein, lipid, and carbohydrate metabolic kinetics.

INTEREST IN APPLICATIONS of stable isotopically labeled tracers to studies of metabolic kinetics has witnessed tremendous growth in recent years. This trend is likely to continue into the future, as applications using radioactive tracers come under closer scrutiny due to concern over waste disposal and radiation exposure for human subjects. Studies with stable isotopically labeled tracers were once limited to specialized research centers, due to the high cost and sophistication of mass spectrometry instruments necessary to quantify stable isotopic enrichment. However, recent improvements in instrument design and performance, as well as computer software for instrument control and data acquisition and analysis, have increased the sensitivity and reliability of stable isotopic enrichment measurements while making the instruments easier to operate and less expensive to purchase. As a result, mass spectrometric analysis may be available to individual investigators. This article provides an overivew of issues regarding instrumentation and mathematical analysis related to the use of stable isotopically labeled tracers for investigations of metabolic kinetics. Interested readers are directed to more complete resources.[1]

BACKGROUND

Virtually any metabolic process involving protein, lipid, or carbohydrate metabolism can be studied using stable isotopes of carbon (^{13}C), hydrogen (^{2}H, deuterium), nitrogen (^{15}N), or oxygen (^{18}O). Strictly speaking, the term "isotope" refers to all forms of a given element containing different numbers of neutrons (ie, ^{12}C and ^{13}C are both stable isotopes of carbon); for metabolic tracer studies, the term "stable isotope" is often loosely applied to mean a nonradioactive isotope that is less abundant than the most abundant naturally occurring isotope. The stable isotopes of greatest use for metabolic tracer kinetics studies are ^{2}H, ^{13}C, ^{15}N, and ^{18}O (Table 1).

For a metabolic study, a tracer in which one or more atoms in the structure is replaced with one of these stable isotopes is administered. It is a misnomer to refer to such a labeled molecule as a stable isotope, since the term isotope refers to elements rather than molecules. Molecules that differ by the number or arrangement of isotopically labeled positions are referred to as "isotopomers." Thus, ^{13}C-leucine is a stable isotopically labeled isotopomer of leucine, not a stable isotope of leucine.

INSTRUMENTATION

There are two types of mass spectrometers commonly used to measure stable isotopic enrichments for studies of metabolic kinetics, the gas isotope ratio mass spectrometer (GIRMS) and the gas chromatograph/mass spectrometer (GC/MS) (Table 2). Applications using the GIRMS are limited in scope, since this instrument can only analyze gases. Analysis of biological molecules by the GIRMS is difficult, since these molecules must first be purified, combusted, and, when necessary, reduced to produce labeled CO_2, H_2, or N_2 for analysis. The principal advantage of the GIRMS is the ability to measure extremely low enrichments above natural abundance; major disadvantages include the limitation of working with gases only, difficulties in processing complex biological molecules, and a relatively large sample requirement for analysis.

The GC/MS can analyze complex biological mixtures and has a lower sample requirement for analysis compared with the GIRMS (Table 2). The minimum detectable enrichment is typically a few orders of magnitude higher for the GC/MS compared with the GIRMS, although extremely low enrichments approaching the limits of the GIRMS can be measured under special conditions if a highly substituted tracer is used such as $^{2}H_5$-phenylalanine.[2] The principals of operation of the GC/MS will be examined in greater detail, since this instrument is more versatile for diverse applications in analyses of amino acid, lipid, and carbohydrate metabolism.

A GC/MS is shown schematically in Fig 1. Samples are injected into a GC for separation. Samples must first be derivatized to make them volatile so they can be separated by the GC. A wide variety of derivatization reactions and chromatographic separation columns are available, such that desired compounds can be separated for analysis from complex mixtures. The effluent of the GC passes through an interface and into the MS consisting of three essential elements: an ionization source, an analyzer for separating ions, and a detector. The MS is kept under high vacuum, and may be controlled by a data-acquisition system.

Several methods for ionization are used in the GC/MS, the most basic of which is electron impact ionization (Fig 2A).

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Table 1. Selected Stable Isotope Tracers Used in Metabolic Studies

Element	Stable Isotope	Atom% Natural Abundance
H	1	99.985
	2	0.015
C	12	98.89
	13	1.11
N	14	99.63
	15	0.37
O	16	99.76
	17	0.037
	18	0.204

Molecules in the GC effluent pass through a stream of electrons in transit between a filament and a target anode. During a collision with an electron, an electron may be ejected from the molecule, resulting in a positively charged ion. This cation is repelled out of the source by a repeller plate that is held at positive potential, and passes through a series of electromagnetic lenses that focus the ion beam as it passes into the analyzer. GC/MS instruments most commonly use quadrupole MSs, although magnetic-sector or ion-trap analyzers are also available. The two pairs of opposing rods of the quadrupole (Fig 2B) carry either a constant DC or a radio-frequency AC potential. An ion resonates at a specific frequency dependent on its *m/z*. For a given electromagnetic field within the quadrupole, only an ion of one particular *m/z* will resonate at a frequency such that it will pass through to the detector, typically an electron multiplier. Ions with higher or lower *m/z* resonate at different frequencies and are absorbed by the rods. The electromagnetic field within the quadrupole and hence its tuning frequency can be changed on a millisecond time scale, such that a spectrum of ion intensities over a selected *m/z* range can be rapidly scanned.

The ionization process usually imparts excess energy to a molecular ion. The excess energy is dissipated by fragmentation processes. The sensitivity of the analysis may be reduced because of this fragmentation, since the labeled atom(s) in the molecule may be distributed between multiple fragments. However, for some studies this fragmentation can be particularly advantageous. For example, the two nitrogen atoms in glutamine are derived from different metabolic processes (the amino N results from amination of α-ketoglutarate to form glutamate, predominately in liver, and the amide N results from amination of glutamate, predominantely in muscle tissue). As illustrated in the electron impact ionization spectrum of a *tert*-butyldimethylsilyl (tBDMS) derivative of glutamine (Fig 3), both the amide and amino N atoms are found in fragments of *m/z* 413 or 545 (these fragments appear at *m/z* 414 and 546 for both amino- and amide-^{15}N-glutamine), whereas a fragment with *m/z* 258 contains the amino N (*m/z* 259 for 2-[^{15}N]-glutamine) but not the amide N (5-[^{15}N]-glutamine retains the *m/z* 258 fragment). Through appropriate choice of ions to monitor and data-reduction procedures, ^{15}N enrichment in both positions can be deduced. This procedure allowed us to quantify the relative labeling of the different nitrogen atoms of plasma glutamine when [^{15}N]-ammonia was administered.[3] This study

Table 2. Comparison of MS Instruments

Parameter	GIRMS	GC/MS
Type of sample	Pure gases (H_2, CO_2, N_2)	Complex biological mixtures (derivatized)
Amount of sample per analysis (nmol)	10-100	0.01-1
Range of enrichment (atom%)	0.0001-1	0.1-100

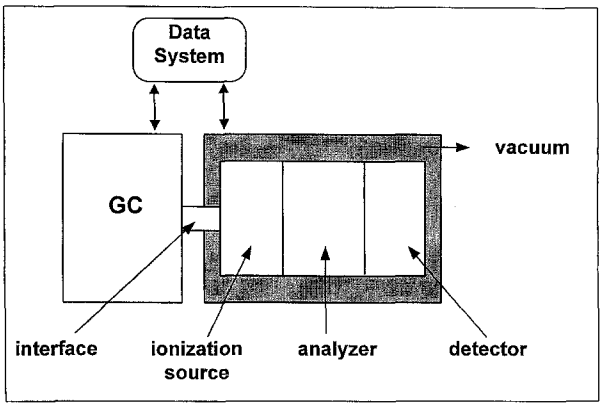

Fig 1. Configuration of a GC/MS.

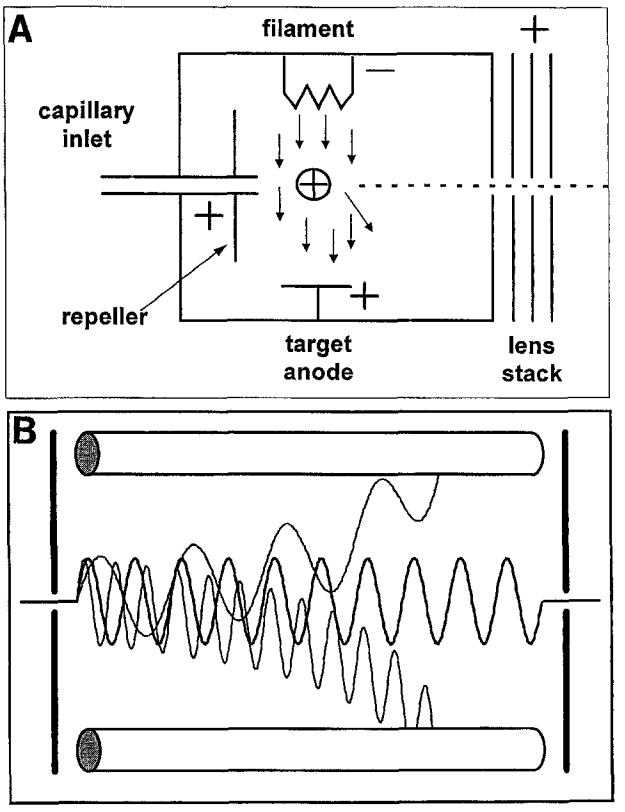

Fig 2. Operation of a GC/MS. (A) Schematic diagram of an electron impact ionization source. Molecules from a GC effluent are ionized, repelled out of the source body, and focused by a stack of lenses before entering the MS analyzer. (B) Schematic diagram of a quadrupole MS analyzer. Two of 4 rods of a quadrupole MS are illustrated; the other 2 rods would be above and below the plane of the figure. The analyzer is tuned to allow only ions of a single *m/z* to pass through to the detector.

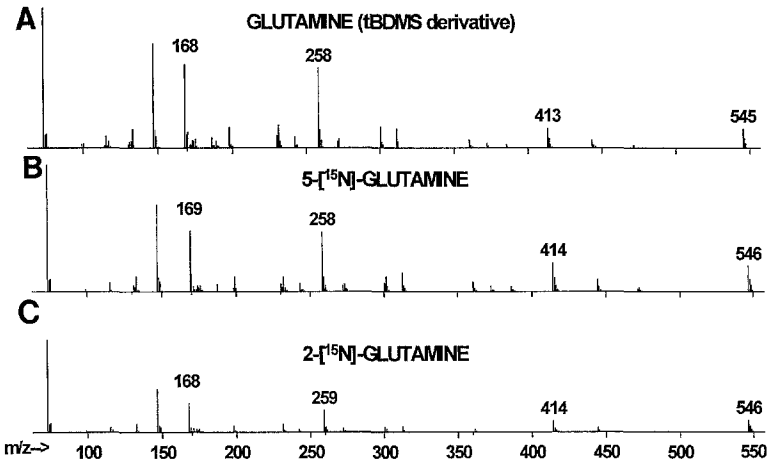

Fig 3. Electron impact ionization spectrum of glutamine derivatized with 4 +BDMS derivatization groups. (A) Natural-abundance sample; (B) amide-labeled ^{15}N-glutamine; (C) amino-labeled ^{15}N-glutamine.

demonstrated that more than 90% of the label appearing in glutamine was incorporated into the amide N following intravenous administration of [^{15}N]-ammonia (muscle clearance of ammonia predominated), whereas the label was almost evenly divided between amide and amino N positions following oral administration of [^{15}N]-ammonia (with a greater contribution from hepatic ammonia clearance).[3] This potential to selectively monitor enrichment of the same labeled element on different fragments of a given molecule is not generally possible when radioisotopically labeled tracers are used.

Measurements of isotopic enrichment by the GC/MS typically acquire data by selected ion monitoring (SIM) rather than by a full-scan acquisition. In this procedure, individual ions are selected to be monitored, in contrast to monitoring a full-sweeping scan (Fig 4). Figure 4A illustrates a full-scan acquisition of tBDMS derivatives of plasma amino acids. The total ion chromatogram (sum of ion intensities over the entire scanned spectrum) reveals the presence of multiple components separated by the GC. Figure 4B and C illustrates the selectivity of SIM using ions selective for leucine and isoleucine (*m/z* 200) or urea (*m/z* 231); from the complex mixture, only those individual components are found when their characteristic ions are monitored. SIM not only increases selectivity but also provides a large increase in sensitivity (signal to noise ratio) for measurement of isotopic enrichment, since the detector only monitors ions of interest rather than extraneous ions.

For isotopic enrichment measurement by a SIM GC/MS, ions of the natural and isotopically labeled fragment of the molecule of interest are monitored. For the example in Fig 4, [$^{15}N_2$]-urea enrichment is measured by monitoring ions of *m/z* 231 and 233. Isotopic enrichment is determined from the ratio of appropriate peak areas. The tracer to tracee ratio is typically determined by correlation of the peak area ratios against those obtained for a set of standards of known isotopic enrichment. The tracer to tracee ratio may also be obtained directly from the peak area ratios, although it may be necessary to apply a number of correction factors to this analysis.[1,4] There are numerous other ways in which isotopic enrichment can be expressed, but the tracer to tracee molar ratio is usually preferred for studies of metabolic kinetics.[1,4,5]

A third type of instrument used for metabolic kinetics applications provides a combustion interface between a GC and a GIRMS.[6-8] These instruments have only recently become available commercially, and have not yet been extensively used for tracer kinetics applications. This instrument combines the resolution capabilities of a GC with the accuracy and precision of a GIRMS, and is capable of precise and accurate measurements of very low isotopic enrichments on picogram to nanogram quantities of sample. There are three major disadvantages to this instrument. First, it lacks the selectivity intrinsic to a GC/MS with SIM; the enrichment of any gas (eg, $^{13}CO_2$) produced from an enriched sample eluting from the GC will be diluted by unlabeled co-eluting compounds, whereas it is usually possible to selectively monitor a single compound of

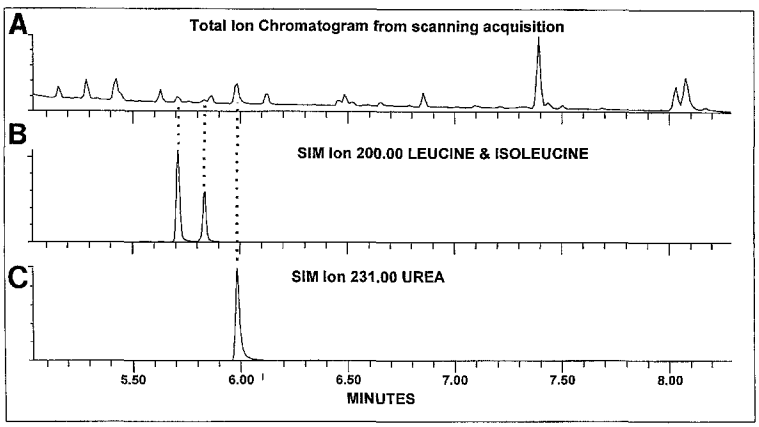

Fig 4. SIM. Plasma amino acids derivatized with tBDMS were separated on a DB-17 capillary column. (A) Total-ion chromatogram from a scanning acquisition; (B) and (C) a second injection that was monitored only at *m/z* 200 between 5.50 and 5.90 minutes (selective for leucine and isoleucine) and at *m/z* 231 between 5.90 and 6.30 minutes (selective for urea).

interest by SIM GC/MS even if the peak eluting from the GC is not pure. Second, the enrichment of the gas produced will be diluted by unlabeled atoms in the derivatizing reagent. (Since it is generally necessary to derivatize samples to increase volatility for GC separation, $^{13}CO_2$ produced from the sample will be diluted by ^{12}C from the derivative group.) Third, it is currently only practical to measure ^{13}C enrichment by this approach, although progress is being made to enable applications with ^{2}H or ^{15}N isotopes.

KINETIC ANALYSIS

Applications of stable isotopically labeled tracers for metabolic kinetics studies can be broadly classified into two categories: those that examine the kinetics of the tracer itself without metabolic transformations (autogenic kinetics) and those that involve conversion of the tracer as a precursor into one or more products (heterogenic kinetics). Clinical protocols for such studies may involve application of the tracer as a single bolus or as a continuous infusion with or without a priming dose to effect a rapid equilibration of isotopic enrichment. Tracer kinetics can be evaluated by a variety of compartmental or noncompartmental approaches. These topics are extensively covered by Wolfe.[1]

Rate of Appearance

The simplest autogenic tracer kinetics application is the determination of the rate of appearance (Ra) into plasma of a given substance, as outlined in Fig 5. A homogenous freely mixing pool at metabolic steady state has equal rates of input and output for a given unlabeled tracee substance (eg, glucose); for the point of illustration, assume this rate is 20 μmol/kg/min. A tracer form of the tracee substance containing one or more stable isotopes is given as a constant infusion at a known infusion rate (eg, 6,6-[$^{2}H_2$]-glucose); for illustration, assume this rate is 5 μmol/kg/min. The rate of tracer output will equal the rate of tracer input once the tracer is infused for sufficient time to achieve an isotopic equilibrium (Fig 5). At this point, the tracer to tracee ratio of material leaving the pool is identical to that within the pool, and is equal to the tracer to tracee ratio of material entering the pool. For the example given, the tracer to tracee ratio is 5:20, or 0.25. The Ra of the unlabeled tracee substance is obtained from the relation, Ra = tracer input rate (μmol/kg/min)/plateau tracer to tracee ratio. It is usually not desirable to use such a high rate of tracer infusion relative to the Ra as shown in this illustration, since this would violate the principle that the tracer should insignificantly perturb the tracee pool size.[1,4]

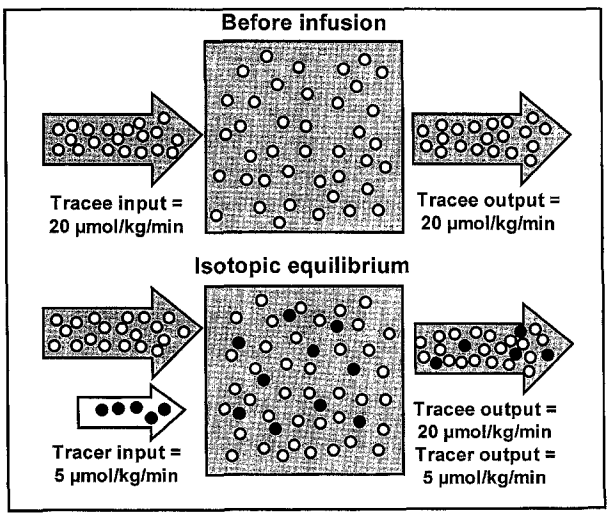

Fig 5. Determination of Ra. Schematic diagram shows input of unlabeled tracee and labeled tracer into a freely mixing pool. At isotopic equilibrium, the rate of tracer output equals the rate of tracer input.

Note that this determination of Ra is dependent only on the enrichment of the isotopic plateau, and is independent of the time course required to achieve an isotopic equilibrium plateau. A very long period of infusion is required if a pool has a very slow rate of turnover. A large dose of the tracer can be given as a single bolus before the constant infusion of tracer to accelerate this process. The ideal size of the priming dose relative to the infusion rate (units of time) should equal the pool size (moles per kilogram) divided by the pool Ra (moles per kilogram per unit of time).[1] This necessitates some a priori knowledge of the system, but appropriate priming doses can be achieved empirically through successive approximations in pilot studies if no prior knowledge of the system kinetics is available. It is possible to achieve an isotopic enrichment plateau in less than 1 hour with some metabolic systems using a primed constant infusion protocol.

The principal advantages of the constant infusion approach to measure Ra are its simplicity and relative brevity when a priming dose is used. A major limitation for some applications is the necessity for an isotopic enrichment plateau. There can be numerous limitations to achieving an isotopic enrichment plateau. For example, the metabolic pool may have a rate of turnover that is too slow to achieve a plateau in a time that is convenient for a clinical infusion protocol, or, as is usually the case, the tracee studied may exchange with other pools that have a rate of turnover that is slower than that of the accessible pool (eg, plasma) into which the tracer is introduced. In principle, a total system Ra (as opposed to a plasma Ra) can be measured if a true isotopic plateau can be achieved throughout the system by allowing the tracer to equilibrate with all pools with slow turnover rates; in practice, it may not be possible to maintain a subject at a metabolic steady state for sufficient duration for this to be achieved, thereby violating a different principle of the procedure. Nevertheless, the primed constant infusion to estimate plasma Ra has been an extremely useful approach to measure the metabolic kinetics of many substances of interest, including amino acids, urea, fatty acids, glycerol, and glucose, under a wide range of physiologic conditions.

Another advantage of the primed infusion approach is the potential ability to monitor rapid changes in Ra when the physiology of the system is acutely perturbed. For example, isotopic plateaus for plasma glycerol and palmitate can be achieved 1 hour following a primed continuous infusion of appropriate tracers into plasma (Fig 6A). A rapid increase in plasma Ra for both substances can be observed with administration of epinephrine, which stimulates lipolysis, as evidenced by rapid decreases in tracer to tracee ratios for both glycerol and palmitate (Fig 6A) accompanied by increases in plasma concen-

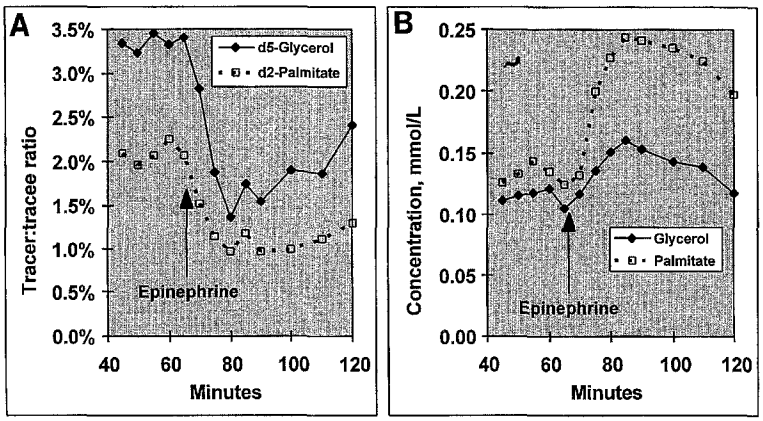

Fig 6. Rapid changes in Ra resulting from an acute physiologic perturbation. An isotopic equilibrium steady state in plasma glycerol and palmitate is established with a primed continuous infusion of $^2H_5$-glycerol and $^2H_2$-palmitate. After 60 minutes, the rate of lipolysis is stimulated by administration of epinephrine. (A) Time course of isotopic enrichment changes; (B) time course of plasma concentration changes.

trations (Fig 6B). Mathematical algorithms have been devised to quantify the change in Ra resulting from such non–steady-state metabolic kinetics.[1]

Fractional Synthetic Rate

One of the simplest heterogenic tracer applications is the determination of the fractional synthetic rate (FSR) of a product following infusion of a tracer form of a precursor that is incorporated into that product. This approach has been extensively used in studies of protein synthesis (especially muscle and plasma proteins), but in principle, the approach can be used for any metabolic process involving a direct precursor-product relationship.

The principle of this approach is outlined in Fig 7. It is assumed that the product (eg, muscle protein) comprises a single homogeneous pool that is turning over with a single-order rate constant k (units of time^{-1}). If the pool is at metabolic steady state, its FSR will equal its fractional turnover rate k. A precursor tracer is provided as a primed constant infusion (eg, a labeled amino acid in plasma). Enrichment of a homogeneous product pool will increase monoexponentially as a function of time (E_t) to asymptotically approach the precursor pool enrichment. The initial slope of this increase to plateau curve is the product ($E_p \cdot k$). Thus, the FSR is determined by dividing the initial rate of change of product enrichment by the precursor enrichment. In its simplest form, it is necessary that the precursor pool enrichment (E_p) be held constant from the beginning of the study using a primed constant infusion protocol, although mathematical treatments have been developed to derive the FSR when precursor pool enrichment is variable with time or the system is not at metabolic steady state.[9,10]

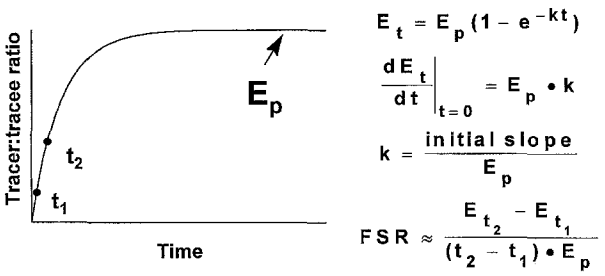

Fig 7. Determination of the FSR. Increase to plateau enrichment of an idealized single-pool system is shown. The FSR is derived from the initial slope, which can be closely approximated by measuring the change in enrichment over 2 time points, t_1 and t_2.

The accuracy of the FSR approach depends on accurate knowledge of the precursor pool enrichment. This offers a considerable challenge, since the precursor pool is often not accessible for analysis and there will usually be isotopic dilution between enrichment in the accessible pool where tracer is administered (eg, plasma) and the intracellular precursor pool at the site of synthesis (eg, hepatically derived plasma proteins). Several solutions to the precursor pool problem have been used, although no general solution applicable to all studies is evident. The most direct solution is to measure enrichment of the amino acyl–charged tRNA isolated from the tissue of study, since this is by definition the immediate precursor pool used for protein synthesis. This approach can be applied, with considerable analytical difficulty, to accessible tissues such as muscle,[11] but it cannot be applied to inaccessible tissues in human studies. Another direct approach is to infuse the tracer for sufficient duration so that the product achieves an isotopic enrichment plateau that must therefore equate with enrichment of the precursor pool. One of the best examples of the approach is very–low-density lipoprotein apolipoprotein B-100 (VLDL–apoB-100). This protein can achieve an isotopic enrichment plateau in less than 12 hours following a primed constant infusion of amino acid, by virtue of its rapid turnover rate (approximately 10 pools $\cdot$ d^{-1}) and the fact that it does not exchange with the nonplasma space. The FSR of VLDL–apoB-100 can be obtained solely from its initial rate of increase and the ultimate plateau of apoB-100 enrichment without reference to any free amino acid pool enrichment information.[12-15]

Several indirect solutions that substitute a surrogate for the actual precursor pool enrichment have been used. A total intracellular pool enrichment may be measured (eg, muscle tissue homogenate free amino acid pool), but this will necessarily reflect a composite of all intracellular pools that may be metabolically compartmentalized in vivo and will also include residual extracellular pools in the tissue (eg, blood and interstitial fluid) during sample processing.

A second indirect approach that has been used specifically for hepatically derived proteins is to use a glycine tracer and measure the enrichment of urinary hippurate. (Formation of hippurate by conjugation of glycine with benzoic acid occurs in hepatic mitochondria; hippurate is excreted into plasma and

eliminated in urine.) Although this approach does measure enrichment of a genuine intrahepatic glycine pool, it does not measure the true precursor glycine pool used at the site of protein synthesis. Thus, there is approximately a 20% difference in the isotopic enrichment of hippurate glycine and the plateau enrichment of VLDL–apoB-100 (which is exclusively hepatically derived) both in humans in vivo[12] and in rat liver single-pass perfusion studies.[16]

A third indirect approach is to measure the enrichment of a plasma metabolite of the tracer that is derived exclusively in the tissue of interest. This "reciprocal pool" approach has been used extensively in studies of muscle protein synthesis, where, for example, a tracer of leucine is infused and enrichment of its keto acid analog (α-ketoisocaproic acid) in the plasma is assumed to be in equilibrium with intramuscular leucine enrichment. (Transamination between branched-chain amino acids and their keto acid analogs occurs exclusively in muscle tissue.) It has recently been suggested that a reversal of this infusion protocol may be more accurate, ie, to infuse an isotopically labeled keto acid and measure enrichment of the amino acid following transamination and isotopic equilibration.[17]

A final indirect solution has been to apply a "flooding dose" of unlabeled precursor in conjunction with the tracer administered to the accessible pool (eg, leucine given as a plasma bolus).[9] This approach assumes that isotopic enrichment of the plasma pool will remain relatively constant if a sufficiently large quantity of tracee plus tracer is given, and that this "flood" will swamp the enrichment of the intracellular precursor pool. However, recent studies have demonstrated that this approach may not adequately regulate precursor enrichment in human subjects,[9] and evidence has been presented that the flooding dose itself may alter the rate of protein synthesis.[18,19]

Mass Isotopomer Distribution Analysis

There has been a large increase in applications to measure synthesis rates by mass isotopomer distribution analysis (MIDA) in recent years. This approach provides a mathematical model to resolve both the precursor pool enrichment and rate of product synthesis without direct measurement of precursor enrichment, but is limited in scope to applications where the product is a polymer containing multiple units of a given precursor molecule.

The principle of MIDA is shown schematically in Fig 8. The tracer is initially distributed through a precursor pool that is held at constant enrichment throughout the experimental period, usually by use of a primed constant infusion protocol. A product of this precursor pool is produced, which contains n units of the precursor (a prime example is the incorporation of acetate precursors into fatty acids). Monomer subunits are randomly selected from this precursor pool when the product is synthesized. A probability function (F) based on binomial expansion predicts the distribution of newly formed product molecules that will contain from 0 to n enriched monomers (Fig 8):

$$F(M+1) = \begin{bmatrix} n \\ 1 \end{bmatrix} \cdot (p)^1 \cdot (1-p)^{(n-1)}$$

$$= \frac{(n)!}{(n-1)!(1)!} \cdot (p)^1 \cdot (1-p)^{(n-1)}$$

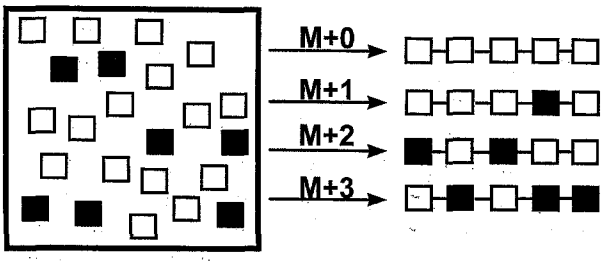

Fig 8. MIDA. Precursor subunits to be incorporated into a product are randomly taken from a pool that has been isotopically labeled (■). The probabilities of incorporating a given number of labeled precursors into the product are determined by the precursor pool enrichment and the binomial expansion formula.

$$F(M+2) = \begin{bmatrix} n \\ 2 \end{bmatrix} \cdot (p)^2 \cdot (1-p)^{(n-2)}$$

$$= \frac{(n)!}{(n-2)!(2)!} \cdot (p)^2 \cdot (1-p)^{(n-2)}$$

$$F(M+3) = \begin{bmatrix} n \\ 3 \end{bmatrix} \cdot (p)^3 \cdot (1-p)^{(n-3)}$$

$$= \frac{(n)!}{(n-3)!(3)!} \cdot (p)^3 \cdot (1-p)^{(n-3)}$$

For these equations, n is the number of monomer subunits in the product molecule, p is the probability that a given precursor subunit is isotopically labeled (the fraction of the pool that is labeled), and ! represents the factorial function.

Since the intracellular pool is generally inaccessible for direct analysis, precursor enrichment p is determined by examining the relative distribution pattern of enriched isotopomers in the product (ratio of doubly to singly labeled product, triply to doubly labeled product, etc.), and the rate of synthesis of the product is derived from the rate at which enrichment increases in the product. The MIDA approach has been used by a number of investigators to measure hepatic fatty acid synthesis from ^{13}C-acetate[20] or ^2H_2O[21] and cholesterol synthesis from ^{13}C-acetate[22] or ^2H_2O,[21] and for numerous applications of gluconeogenesis.[23-25]

Multicompartmental Modeling Analysis

Compartmental modeling is an important tool for analyzing metabolic tracer kinetics with stable isotopically labeled tracers, as it is for radioactive tracers. The advantage of this type of analysis over a noncompartmental approach is that a compartmental model attempts to account for the kinetic heterogeneity inherent within most systems; in contrast, noncompartmental models generally assume that the metabolic pool being sampled is kinetically homogeneous.

Many or most compartmental models are based on the assumption that an infinitesimal quantity of tracer does not perturb the mass of the system, and therefore the tracer will follow linear first-order kinetics with respect to tracer concentration in various compartments.[4] This assumption is valid for radioactive tracers, since the quantity of tracer material administered is generally insignificant with respect to the system. However, the

validity of this assumption may break down when stable isotopically labeled tracers are used for some metabolic kinetics studies.[4] Because stable isotopes exist at natural abundance and the precision of measuring isotopic enrichment is limited for some MSs (GC/MS), it is often necessary to perturb the pool to a significant degree so that enrichments are sufficiently elevated to enable reliable quantification. Tracer to tracee ratios greater than 10% may be necessary for some applications, particularly if the tracer is administered as a bolus and is measured in metabolic products in successive compartments.

It is generally necessary to use a more complicated experimental design to resolve the structure and tracer kinetics of a multicompartmental system, in contrast to the noncompartmental approaches already outlined. To develop a comprehensive multicompartmental model of a metabolic system, it may be necessary to increase the number of data points, use multiple tracers that can simultaneously monitor different pathways within the system, or measure enrichments in additional compartments (different physiologic spaces and/or different metabolic products that incorporate the tracer(s) used). This increased complexity in experimental design places an additional burden on the tracer administration protocol, on the cost of the study (especially if additional tracers are needed), and on laboratory resources for sample processing and mass spectrometric analyses. Furthermore, compartmental modeling analysis is inherently more difficult than noncompartmental analysis. For these reasons, application of compartmental modeling analysis to stable isotopically labeled tracers has been hindered. Nevertheless, several examples can be illustrated to exemplify these efforts.

A compartmental model of apoB-100 in very–low-, intermediate-, and low-density lipoproteins has been described[26] and used in subjects with familial hypobetalipoproteinemia resulting from truncations in apoB-100.[27,28] The clinical protocol used for these studies was an 8-hour primed continuous infusion of either ^{13}C- or $^{2}H_3$-leucine; after 8 hours, the infusion was stopped, and plasma was sampled over the next several days. This experimental design offers the advantage of combining a long-term infusion to project the plateau enrichment of VLDL–apoB-100 with a transient tracer kinetic curve after the infusion is halted.

Matthews and Downey[29] presented a two-compartment model of urea kinetics following a bolus injection of ^{13}C- or ^{18}O-urea with measurement of plasma enrichment over 6 hours. The model showed that plasma urea exchanges with a slower nonplasma pool. Because the total-body urea pool is demonstrably heterogeneous and has a slow rate of turnover, it could be argued that the compartmental modeling approach provides a more accurate measure of plasma Ra than does a shorter-term primed constant infusion protocol with a noncompartmental approach.

One of the most frequent applications of compartmental modeling analysis to stable isotopically labeled tracers has been in the field of amino acid kinetics. For example, Irving et al[30] presented a five-compartment model for plasma lysine coupled with a four-compartment model for bicarbonate to describe the distribution and oxidation kinetics of ^{13}C- and $^{15}N_2$-lysine given as an intravenous or oral bolus, respectively. Cobelli et al[31] have presented a complex multicompartmental model to describe the distributions of and transaminations between leucine and α-ketoisocaproate following a bolus injection of either tracer. Biolo et al[32] recently developed a three-compartment model of leg muscle amino acid transport that resolves transmembrane amino acid transport kinetics in conjunction with measurements of muscle protein synthesis and breakdown. This represents a novel application for compartmental modeling in that the model is not used to fit transient tracer kinetics. Rather, the model is used to develop a set of steady-state equations based on the laws of mass action that define relationships between isotopic enrichments in arterial plasma, venous plasma, and the intracellular pool that are dependent on the concentrations of amino acids in those compartments and blood flow rates. The three-compartment representation of leg amino acid metabolism was demonstrated to provide a more accurate description of muscle protein synthesis and breakdown rates than a more conventional two-compartment arteriovenous difference model. This model has been used to study leg muscle transmembrane amino acid transport under various metabolic conditions, including burn injury,[33] hyperinsulinemia,[34] and resistance exercise.[35]

CONCLUSION

Stable isotopically labeled tracers offer a versatile approach to studies in diverse areas of protein, amino acid, lipid, and carbohydrate metabolism. An extensive number of metabolic processes can be studied through the wide variety of tracers, clinical protocols, mass spectrometry analytical tools, and mathematical analyses of tracer kinetics that are available. Growth in this field is likely to continue as instruments become easier to operate, more reliable, and less expensive to purchase, and as more investigators become trained in the use of stable isotope tracer methodologies. Further developments in mass spectrometry analytical procedures and mathematical treatments of metabolic kinetics will be needed to meet the needs of future metabolic studies.

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