An Overview of Phenylalanine and Tyrosine Kinetics in Humans

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Abstract

The initial use of a tracer of phenylalanine was by Moss and Schoenheimer in rats in 1940 to determine that phenylalanine was hydroxylated to tyrosine, defining for the first time the primacy of this pathway. Phenylalanine and tyrosine kinetics were not measured in humans until the 1970–80s. The first application was to determine the degree of blockage of phenylalanine hydroxylation in patients with hyperphenylalanemia and phenylketonuria, but this approach was expanded to determination of phenylalanine hydroxylation in normal subjects. Far more uses have been demonstrated for measuring rates of phenylalanine disposal and tyrosine production in relatively normal subjects than in patients with in-born errors of metabolism. Key to use of tracers to determine phenylalanine and tyrosine metabolic rates has been the development of appropriate tracer models. Most applications have used relatively simple models ignoring the intracellular hydroxylation rate component. Because the liver is the primary site of hydroxylation in the body, the intracellular enrichment at the site of hydroxylation can be assessed from the tracer enrichments at isotopic steady state in rapid-turnover plasma proteins, such as Apo-B, made and secreted by the liver. Although there are potential problems with use of deuterated tracers of phenylalanine, suitable tracers are available and have been demonstrated for general measurement of phenylalanine and tyrosine kinetics in humans. J. Nutr. 137: 1549S–1555S, 2007.

The most obvious reason to measure rates of phenylalanine and tyrosine kinetics in humans is to understand the derangements of the metabolism of these amino acids that occurs in patients with the in-born error phenylketonuria (PKU) and in diseases that affect their metabolism, such as in liver or renal disease. However, phenylalanine and tyrosine also have specific characteristics as amino acids that make them useful as markers of protein metabolism. First, both phenylalanine and tyrosine are indispensable amino acids that are essential to our diet. In the postabsorptive state, there is no entry of amino acids from dietary sources and the flux of phenylalanine in the body is derived from entry of phenylalanine released from protein breakdown. That input is matched by phenylalanine removal via protein synthesis and via metabolic disposal by conversion to tyrosine. Therefore, the measurement of the whole body rate of appearance of phenylalanine in the postabsorptive state is a measure of the whole body rate of proteolysis (1). Additional reasons for determining phenylalanine and tyrosine kinetics are the determination of dietary requirements of phenylalanine and tyrosine and measurement of the production of tyrosine from dietary phenylalanine.

History of phenylalanine and tyrosine metabolic measurements

Prior to 1940, there was only circumstantial evidence that tyrosine was produced from phenylalanine. As reported by Moss and Schoenheimer (2), Embden and Blades had shown in 1913 that L-tyrosine was formed in livers perfused with DL-phenylalanine and assumed that phenylalanine metabolism proceeded by conversion to tyrosine. However, Shambaugh, Lewis, and Tourtellotte in 1931 suggested that phenylalanine was not converted to tyrosine. Others in the 1920–30s (2) had shown that hydroxylated metabolites of phenylalanine could be found in the urine of animals given a load of phenylalanine. Probably the most convincing evidence at that time came from the study of Womack and Rose (3), showing that phenylalanine was essential to the diet of rats but that tyrosine was not, arguing that tyrosine is formed from phenylalanine but not the reverse.

The 1940 study of Moss and Schoenheimer (2) provided the key evidence and ended the speculation about the metabolism of phenylalanine. They added deuterated D3-phenylalanine to a casein-containing diet given to both growing and adult rats. Samples of tyrosine were isolated from proteins of the animals and the 2H content determined. The tyrosine from protein in...
internal organs contained enough $^2$H to show that 20–30% of the tyrosine had come from the $^2$H-phenylalanine tracer, demonstrating that tyrosine was produced from phenylalanine and that phenylalanine was metabolized to tyrosine. Today, we take these isotopic study results for granted, but in 1940 the use of a deuterated tracer by Moss and Schoenheimer (3) was key in proving the metabolic link between phenylalanine and tyrosine and the ability of phenylalanine to substitute for tyrosine in the diet.

The 1940 Moss and Schoenheimer study was not really a kinetic study. Rittenberg, a colleague of Schoenheimer, later developed in the 1950s a kinetic model using $^{15}$N-labeled amino acids to determine rates of whole body protein synthesis in humans. Grümer et al. in 1962 (4) thought that the Rittenberg approach to measuring the rate of human protein synthesis had too many assumptions and limitations and gave a $^{14}$C-phenylalanine tracer to 4 PKU patients. Grümer et al. (4) reasoned that if phenylalanine conversion to tyrosine was effectively blocked in these PKU patients, then the only route of $^{14}$C-phenylalanine tracer disposal was through incorporation into newly synthesized protein. They therefore used these data to develop an alternative model to measure whole body protein synthesis (4). Surprisingly, these authors did not use the $^{14}$C-phenylalanine tracer to try to assess residual phenylalanine hydroxylase enzyme activity in these PKU patients.

What quickly became clear from studies in the 1960s was that measurement of whole body protein synthesis using tracers is actually very difficult. In contrast, whole body protein breakdown is easy to measure using an indispensable amino acid tracer. The dilution of the indispensable amino acid tracer in blood occurs due to release of unlabeled indispensable amino acid from protein breakdown and entry from the diet (1). Knowing the rate of indispensable amino acid intake in fed studies or neglecting this route in postabsorptive subjects, the rate of protein breakdown is readily calculated from the dilution of an intravenously infused indispensable amino acid tracer. The first demonstration in humans of measurement of whole body protein breakdown using an indispensable amino acid tracer was by James et al. in 1967 (7) using a $^{14}$C-lysine tracer. In 1974 O’Keefe et al. (5) used a $[\text{1-}^{13}\text{C}]$leucine tracer to measure whole body protein breakdown in humans and other indispensable amino acid tracers have been used since then. However, it was not until 1988 that Darmaun et al. (6) used a $[\text{phenyl-}^{2}\text{H}]$ phenylalanine tracer to measure phenylalanine kinetics and whole body protein breakdown. Waterlow and colleagues (7) tried a $^{14}$C-tyrosine tracer in 1976, but use of a tyrosine tracer to measure whole body protein breakdown via tracer dilution is inherently problematic because of the unknown amount of phenylalanine converted to tyrosine that also provides an input.

**Phenylalanine conversion to tyrosine**

The early principal use of a deuterated phenylalanine tracer was not to measure whole body protein breakdown but to assess the conversion of phenylalanine to tyrosine. Although considerable amounts of phenylalanine tracer are normally converted to tyrosine in humans, phenylalanine conversion to tyrosine is limited in PKU patients. Curtius et al. (8) used a $^{2}$H-phenylalanine tracer administered to 1 patient as a proof of concept of this method in 1972 and followed up this report with measurement of phenylalanine conversion to tyrosine in hyperphenylalanemic and PKU patients in 1977 (9). The approach used by Curtius et al. (8) is shown in the model in Figure 1. The key measurement gleaned is the enrichment of plasma $^{2}$H-tyrosine derived from conversation of the $[\text{phenyl-}^{2}\text{H}]$phenylalanine tracer normalized against the plasma $^{2}$H-phenylalanine enrichment. This tyrosine/phenylalanine enrichment ratio defines the fraction of free tyrosine that is produced from phenylalanine. In normal postabsorptive humans, $\approx$15% of indispensable amino acids are oxidized in the postabsorptive state (1) and thus phenylalanine disposal through conversion to tyrosine should produce a normal tyrosine/phenylalanine enrichment ratio of $\approx$15%. A PKU patient who has no phenylalanine hydroxylase activity would be expected to have undetectable amounts of $^{2}$H-tyrosine in plasma following administration of a $^{2}$H-phenylalanine tracer. Values between 0 and 15% have been used to classify the degree of impairment of phenylalanine metabolism in patients with derangements of phenylalanine metabolism (10). Because of the very limited conversion capacity of most PKU patients, this method is difficult to apply with good accuracy in PKU due to the low enrichment of $^{2}$H-tyrosine.

Rather than using phenylalanine tracer conversion to tyrosine to assess derangements of phenylalanine metabolism, Clarke and Bier (11) used the conversion to define normal metabolism. Although tyrosine is a dispensable amino acid, tyrosine synthesis in the body depends upon phenylalanine availability. When phenylalanine intake is limited, phenylalanine availability may be limited for production of tyrosine and tyrosine can become a conditionally indispensable amino acid (1). The model using a phenylalanine tracer and measurement of the tracer in free phenylalanine and tyrosine determines the fraction of tyrosine produced from phenylalanine, but a tyrosine tracer is also needed to determine tyrosine flux and define the absolute amount of tyrosine produced from phenylalanine. Clarke and Bier (11) infused a $[\text{1-}^{13}\text{C}]$tyrosine tracer in conjunction with the $[\text{phenyl-}^{2}\text{H}]$ phenylalanine tracer to measure simultaneously the turnover rates of both phenylalanine and tyrosine as well as the rate of phenylalanine conversion to tyrosine. The model for this approach is shown in Figure 2.
Tyrosine oxidation

The only rate left unsolved in Figure 2 as applied by Clarke and Bier (11) is the rate of tyrosine oxidation \( F_{\text{out}(b)} \). The normal approach to measure whole body oxidation of a tracer would be to use a \(^{13}\text{C}\) or \(^{14}\text{C}\) tracer that can be recovered in exhaled \( \text{CO}_2 \). The best tracers to measure oxidation are those that are quickly released as \( \text{CO}_2 \) during tyrosine degradation. The remainder of the molecule either ends up as fumarate or acetoacetate. Although both molecules may enter the tricarboxylic acid cycle and the label recovered as \( \text{CO}_2 \), there are also alternative nonoxidative fates. Therefore, a carbon label placed in the phenyl ring of tyrosine will have a lower recovery in \( \text{CO}_2 \) than a carbon isotope placed in the carboxyl position.

Although the choice for a tyrosine label for measurement of tyrosine oxidation should be one with a carboxyl-label, it has not been an easy tracer to obtain for carbon-14. Thus, the earliest measurement of human tyrosine oxidation used a \([\text{U-}^{14}\text{C}]\)tyrosine label (7) and this choice has continued for carbon-14. The situation is different for stable isotopes. A carboxyl-label is available as the nonradioactive \([1-^{13}\text{C}]\)tyrosine, making this label the preferred choice for measuring tyrosine oxidation. The first reported use of the \([1-^{13}\text{C}]\)tyrosine label for oxidation was the report of Cortiella et al. (12) in 1992 who also used a \([1-^{13}\text{C}]\)phenylalanine label to measure phenylalanine oxidation as well. They performed 2 series of experiments: one where 6 volunteers were infused with \([1-^{13}\text{C}]\)phenylalanine and \([2,2-^{2}\text{H}_2]\) tyrosine on 1 d and where they were infused with \([2,2-^{2}\text{H}_2]\)phenylalanine and \([1-^{13}\text{C}]\)tyrosine on another. In both cases, exhaled \(^{13}\text{CO}_2\) was determined to define the rate of \(^{13}\text{C}\)-tracer oxidation.

The key results of the Cortiella et al. (12) study are summarized in Figure 4. Although a variety of studies using dual tracers have been performed to study phenylalanine to tyrosine conversion and oxidation of these amino acids, this is the only study to our knowledge where the same subjects were infused with the different \(^{13}\text{C}\) labels on different days to obtain a complete picture of oxidation of a phenylalanine label as well as that of a tyrosine label. The results of this study highlight several
The [1-13C]tyrosine tracer appears to be an appropriate marker for tyrosine oxidation, but is the [1-13C]phenylalanine tracer an appropriate marker for phenylalanine oxidation? The 13C-phenylalanine tracer probably underestimates oxidation, because to oxidize the [1-13C]phenylalanine tracer, it must first be hydroxylated to form [1-13C]tyrosine. At that point, there are 2 possible fates for the tracer: oxidation (Fig. 2) or incorporation into newly synthesized protein. The 13C-phenylalanine tracer will understate phenylalanine oxidation by the amount that the tracer is trapped into newly synthesized protein. The appropriate measure of phenylalanine catabolism is using a phenylalanine-tyrosine tracer pair as described originally by Clarke and Bier (11) (Fig. 2).

Even if phenylalanine oxidation using a 13C-phenylalanine tracer underestimated phenylalanine oxidation, the oxidation of the 13C-phenylalanine tracer still has found important uses. The most extensive use of the [1-13C]phenylalanine tracer has been the indicator amino acid oxidation (IAAO) method of determining the requirements of individual indispensable amino acids. The IAAO method was developed by Ball et al. (13,14) in growing pigs using a 14C-lysine or 13C-phenylalanine tracer. The concept of the IAAO method is simple: when a single indispensable amino acid is deficient in the diet, the body is limited in how much protein can be synthesized. Under these conditions, there is an excess of the other indispensable amino acids and the body has no choice other than to oxidize them. The more deficient the diet is in a single indispensable amino acid, the greater the oxidation of the other indispensable amino acids. However, at requirement or just above, the oxidation of dispensable amino acids will be at a minimum. The result is a 2-line curve when the oxidation of the indicator amino acid is plotted vs. the intake of the indispensable amino acid being manipulated in the test animals or human subjects. An example of this type of plot is shown in the paper of Wilson et al. (15) where the recovery of [1-13C]phenylalanine as 13CO2 (the indicator amino acid) is plotted vs. dietary threonine intake in young adult men consuming different levels of dietary threonine. The intake of all other amino acids was held constant (including that of the indicator, phenylalanine). At threonine intakes above requirement, phenylalanine oxidation is constant, but phenylalanine oxidation progressively increases as threonine intake is decreased below the threonine requirement. A breakpoint between the 2 curves is realized indicating a threonine requirement of 19 mg kg\(^{-1}\) d\(^{-1}\). It is not important in the IAAO method that the measured indicator amino acid accurately determines oxidation; rather the measured oxidation value only needs to be responsive and produce a breakpoint as a function of amino acid intake. For this purpose the groups of Ball and Pencharz (13–17) have focused on the use of [1-13C]phenylalanine.

**Pitfalls of measuring phenylalanine and tyrosine kinetics**

Given the complexities of the methods described above for determining phenylalanine and tyrosine metabolism, what are the pitfalls and limitations of the existing methods? The paper of Cortiella et al. (12) was used as 1 example to highlight both the power of the measurements and problems where some of the measured phenylalanine and tyrosine kinetic parameters using the model (Fig. 4) were not congruent. There are several possible problems that can affect the kinetic results and produce disparate results. The problem is that most studies do not use a complete range of tracers with multiple infusions as per the Cortiella et al. study (12) and tracer problems will remain unnoticed.

**Problems with d-amino acid tracer impurities.** The first possible problem that can affect almost every amino acid tracer...
in the "l" stereoisomer, i.e., it is of the opposite "d" configuration. Most synthetic routes to produce either stable or radioisotope tracers of amino acids produce initially racemic mixtures of the d and l stereoisomers that must subsequently be resolved either by enzymatic degradation or purification via a physical chemical process such as crystallization or chromatography. The original studies of phenylalanine and tyrosine metabolism used racemic or mixtures of tracers (2), but studies since the 1960s have generally used tracers administered only as the l stereoisomer. However, it is not simple to determine whether small amounts of optical d stereoisomers are present in the tracer and even small amounts of d stereoisomer can produce untoward effects. Darling et al. (18) reported a study of the effect of some d-stereoisomer in the 1-[1-13C]phenylalanine administered. However, in general, the effect of d-stereoisomer contamination can be reduced to minimal levels with adequate assay of each lot of tracer before use to determine that it is correctly manufactured as the l-only form.

**Plasma sampling vs. intracellular metabolism.** A much more important consideration is that we infuse our tracers into plasma and sample their enrichments from plasma, but the metabolism and interconversion of the tracers is intracellular. We have discussed this problem previously with respect to leucine (1,19,20). The branched-chain amino acids have their first step of metabolism being the reversible transamination step to produce α-ketoacids. The only source of these ketoacids in the body is from these amino acids. As we have illustrated for leucine, the measurement of leucine’s ketoacid, α-ketoisocaproate (KIC), in plasma can be used as an intracellular index of the tracer enrichment of intracellular leucine. In general, the plasma KIC enrichment is ~75% that of the plasma leucine tracer, indicating that the rate of leucine transport into and out of cells is ~4 times faster than the rate of leucine entry into cells from protein breakdown. Therefore, there is a significant difference in the rate of leucine kinetics calculated when the KIC enrichment is used for the calculations compared with the leucine enrichment.

The intracellular compartments have been added in Figure 5 to the phenylalanine-tyrosine tracer model. Given that amino acid transport for phenylalanine is expected to be similar to that for leucine, we would expect that the intracellular enrichments of both phenylalanine and tyrosine to be ~75% that found in plasma. Arbitrary corrections could be applied to the model in Figure 5, but it would be better if we had a way to assess the intracellular enrichment directly. In some respects, we are fortunate, because, in contrast to leucine, the majority of phenylalanine metabolism occurs through hydroxylation in the liver. Therefore, the organ to focus on is the liver for phenylalanine and tyrosine kinetics.

Although the liver is very difficult to sample in humans, it is also fortuitous to study for amino acid metabolism because it makes a variety of secreted proteins that can be sampled from blood. One of the proteins that is made in abundance that has a relatively fast turnover rate in plasma is Apo-B packaged into VLDL. Several groups have measured labeled amino acid incorporation into Apo-B for the purpose of determining its synthetic rate. However, Reeds et al. (21) recognized another purpose for measuring tracer enrichment into Apo-B: the plasma Apo-B tracer enrichment will serve as an intracellular marker for liver intracellular amino acid tracer enrichments. Although the enrichment in tyrosine was not measured in this study, Reeds et al. (21) did infuse and measure the enrichment of a phenylalanine tracer in plasma phenylalanine and Apo-B. Based on the work of Clarke and Bier (11) and Reeds et al. (21), the intracellular compartments outlined in Figure 5 can be addressed by using a sufficiently long infusion of tracer (≥8 h) both to establish a steady-state equilibrium of phenylalanine tracer in tyrosine and to define a plateau enrichment in VLDL Apo B phenylalanine and tyrosine.

**Isotope effects.** The final concern in measuring phenylalanine and tyrosine kinetics is the possibility of an isotope effect influencing these measurements. Although it was not until 1988 that a [phenyl-2H5]phenylalanine tracer was first used in humans to measure phenylalanine kinetics (6), it has been a popular tracer since that time, because the multiple deuteriums increase the mass of the tracer well above natural abundance isotopomer levels when measured by GC-MS and it is cost effective. Alternative tracers do not increase mass significantly (e.g., [1-13C]- or [15N]phenylalanine) and must be measured against a significant natural isotopomer background, especially when a silylated derivative is used. Tracers that increase mass significantly, such as [phenyl-13C6]phenylalanine, are also considerably more expensive.

There is a potential, nonisotope effect problem with the [15N]phenylalanine tracer in that the same transaminase that begins the degradation of tyrosine may also affect phenylalanine. Branched-chain amino acids also have transamination as the first step in their metabolism and 15N tracers of these amino acids produce much higher fluxes than found for 13C- or 2H-labeled tracers, reflective of a rapid and reversible transamination process for branched-chain amino acids (22,23). Presumably a 15N label of a [1-14N]tyrosine tracer would be similarly affected by transamination in the first step of tyrosine degradation, as per the metabolic process shown in Figure 3. Because phenylpyruvate, the transamination product of phenylalanine, is found in small concentrations in blood and increases dramatically in hyperphenylalanemia or PKU, we assume that some transamination of phenylalanine occurs normally. The extent of the transamination has not been quantified in humans but would be expected to be dependent upon phenylalanine concentration as

![Figure 5](https://academic.oup.com/jn/article-abstract/137/6/1549S/4664866) Model of whole body phenylalanine and tyrosine metabolism including intracellular pools. The model is similar to that shown in Figure 2. The arrows are labeled with "B" for rate of phenylalanine or tyrosine entry into the intracellular phenylalanine and tyrosine pools from protein breakdown, "S" for rate of phenylalanine or tyrosine uptake from the intracellular pools for new protein synthesis, and "C" for rate of tyrosine oxidation. The model shows infusion inputs for a phenylalanine and a tyrosine tracer (wide arrows) and for sampling from plasma (ball and stick indicator) for phenylalanine tracer enrichment, tyrosine tracer enrichment, and phenylalanine tracer converted to tyrosine (Phe→Tyr).
it is for the branched-chain amino acids or alanine (22–24). Krempf et al. (25) infused 6 volunteers simultaneously with [1-13C] and [15N]phenylalanine both intravenously and via an intragastric route. The measured phenylalanine flux between these tracers did not differ. The power of the measurement was such that they should have been able to define a difference of 5% between the fluxes measured with the 2 tracers if it existed. These results (25) suggest that phenylalanine transamination plays a minor metabolic role and that the 15N-phenylalanine tracer provides an accurate measure of phenylalanine kinetics in normal subjects.

The concern of an actual isotope effect is primarily with the use of the [phenyl-2H5]phenylalanine tracer. As indicated in Figure 1, 1 of the deuteriums of this tracer must be removed to hydroxylate the phenylalanine. Depending upon the enzymatic mechanism of the hydroxylation reaction, an isotope effect slowing the rate of 2H-bond breaking could occur (a primary isotope effect) or a secondary isotope effect could arise via 1 of the adjacent phenyl-deuteriums in the molecule. A primary isotope effect could slow the reaction by one-half (26). Such an isotope effect for a [2H5]phenylalanine tracer would affect primarily the measurement of the rate of hydroxylation of phenylalanine to tyrosine, because this step involves removal of a deuterium. We would expect weak force interactions (e.g. van der Waals, hydrogen-bonding, or pi-bond interactions) to be limited upon the [1H2]phenylalanine in the free state in circulating plasma or inside cells and, therefore, not to be sufficient to alter the dilution of the [2H5]phenylalanine label in the body (measurement of flux). Two studies have been completed with the [2H5]phenylalanine tracer to define whether this tracer produces an isotope effect when used to measure phenylalanine kinetics in humans. Krempf et al. (24) also coinfused intravenously or via an intragastric route [2H5]phenylalanine along with [15N]phenylalanine and [1-13C]phenylalanine. The postabsorptive state in the flux of phenylalanine in 6 subjects did not differ when the tracers were infused intravenously (power of measurement being ~5% of the flux). However, the [1H2]phenylalanine tracer produced lower plasma enrichments (higher flux) in 5 subjects when given intragastrically compared with the other 2 tracers. Differences could also be seen within this limited group of subjects between the 15N and 13C tracers, albeit the difference was not as great as produced by the [2H5]phenylalanine tracer.

Marchini et al. (26) infused 4 subjects simultaneously with [1-13C]- and [2H5]phenylalanine and [2,2-2H2]tyrosine in the postabsorptive and fed states. Flux in the postabsorptive state did not differ between the 13C and 2H phenylalanine tracers. However, the [2H5]phenylalanine tracer produced a slightly higher enrichment (lower flux) in the fed state than the 13C-phenylalanine tracer did, implying an isotope effect. Because the effect observed in this study with a [2H5]phenylalanine tracer is opposite that observed by this same group in an earlier study (24), we cannot conclude from the 2 studies that the [2H5]phenylalanine tracer measures a different phenylalanine flux than the other tracers or whether the results from these studies are artifactual due to the small number of subjects studied.

The key parameter to focus on remains the measurement of the phenylalanine hydroxylation rate. The Marchini et al. study (27) also measured phenylalanine hydroxylation (via tyrosine enrichment) and found a substantial difference between the [1-13C]- and [2H5]phenylalanine tracers. In both the fasted and fed state, the hydroxylation rate measured with the [2H5]phenylalanine tracer was substantially lower (indicative of a much lower [2H4]tyrosine enrichment compared with [1-13C]tyrosine enrichment). These results depend on accurate measurement by GCMS of the 3 simultaneous tyrosine tracer species ([1-13C]-, [2H5]-, and [phenyl-2H4]tyrosine) that will have overlapping isotopomer patterns and will be difficult to measure simultaneously in the same sample (28). If there were no measurement problems of the tyrosine sample enrichments, then these results provide the clearest evidence for an isotope effect on the [phenyl-2H4] phenylalanine conversion to [phenyl-2H4]tyrosine in humans (27).

The most obvious application of determination of phenylalanine and tyrosine kinetics is in patients with impaired ability to metabolize phenylalanine or tyrosine (e.g. in PKU patients). However, the degree of impairment of phenylalanine hydroxylation is usually so large in PKU patients that the whole body tracer approach has not been very effective in defining hydroxylation rate differences due to the difficulty of measuring small enrichments of the phenylalanine tracer in tyrosine. The tracer method has been far more effective when applied to normal subjects. Obvious applications are in subjects whose phenylalanine or tyrosine kinetics are altered, as in the case when tyrosine is limited in the diet or in patients who have mild but considerable alterations in phenylalanine-tyrosine metabolism (e.g. in patients with liver disease).

A complete picture of phenylalanine-tyrosine metabolism can be accomplished with 2 tracers (a phenylalanine and a tyrosine tracer) and measurement of the phenylalanine tracer in tyrosine. Although a [1-13C]tyrosine tracer is ideal for determining tyrosine kinetics because we also get a direct measurement of tyrosine oxidation, it may not necessarily be used when combined with a phenylalanine tracer. Although [phenyl-2H4]phenylalanine is an obvious economical choice as a tracer, it may produce a significant isotope effect during conversion to [2H5]tyrosine, making this tracer less desirable for measuring phenylalanine hydroxylation. Therefore, a [1-13C]phenylalanine becomes the desired choice, forcing use of a [2,2-2H2]tyrosine tracer and forfeit of a tyrosine oxidation rate measurement. The [1-13C]phenylalanine tracer was also used in the IAAO method to determine indispensable amino acid requirements in humans.

A key to an accurate picture of whole body phenylalanine-tyrosine metabolism requires determination of intracellular enrichments at the site of phenylalanine hydroxylation. Determination of enrichments of faster turnover proteins secreted by liver is an obvious approach to get information about intracellular hepatic enrichments. Apo-B from VLDL has been demonstrated to be useful for phenylalanine in this regard and should be applicable to measurement of tyrosine as well. Because phenylalanine is an indispensable amino acid, its flux provides a good representation of whole body protein breakdown. One of the more consistent uses of phenylalanine tracers has been measurement or rates of protein breakdown in the whole body and in tissues that do not hydroxylate phenylalanine, e.g. muscle. There are a limited number of indispensable amino acids whose metabolism and availability of tracers coincide for use of determining protein kinetics in humans and phenylalanine and phenylalanine tracers are important in this regard.

**Literature Cited**


