Amino Acid Pharmacokinetics and Safety Assessment

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ABSTRACT  Tracer kinetic studies of amino acid metabolism during periods of high amino acid intake should allow insights into adaptive or maladaptive regulatory mechanisms controlling amino acid catabolic or disposal events before clinically evident effects. The principles of amino acid tracer kinetics have been well defined, but their application to establishing upper safe intake levels has been essentially nonexistent. Similarly, the pharmacology field has well-established disciplines of toxicokinetics (the relationship of toxicant dose and delivery to its site of action) and toxicodynamics (the relationship of toxicant at its site of action and downstream functional consequences), but these principles have not been transferred to the field of amino acid metabolism. In this context, a theoretical framework is presented for tracer kinetic experiments to help establish upper tolerable levels of amino acid infusion and/or ingestion. In addition, experiments to couple specific amino acid intake levels with their consequent physiological dynamic effects are suggested to lead to the construction of benefit-risk curves that may permit definition of safe amino acid intake ranges for the population. J. Nutr. 133: 2034S–2039S, 2003.

KEY WORDS: • pharmacodynamics • toxicokinetics • toxicodynamics • physiologically based pharmacokinetic modeling • precursor-product relationships

The amounts of protein and, therefore, of amino acids consumed by humans vary over a wide range. When dietary nitrogen and essential amino acid intakes are above the requirement levels, healthy individuals appear to adapt well to highly variable dietary protein intakes, because frank signs or symptoms of amino acid excess are observed rarely, if at all, under usual dietary conditions. Thus, definition of tolerable ranges of amino acid intake in healthy people will require approaches that identify deviations from normal physiological and biochemical adaptive processes at the subclinical level. Further, the studies necessary to do so must conform to the strictest safety standards because of the ethical concerns of studying normal people. Dose-response kinetic studies employing stable isotopically labeled amino acid tracer provide a readily accessible approach to satisfying these conditions and probing the metabolic dynamics of adaptation, or lack thereof, to high levels of amino acid intake. Sick individuals who require therapeutic delivery of amino acids by vein or who cannot tolerate specific amino acids because of inborn errors of amino acid metabolism are at greater risk for the detrimental consequences of infusion or ingestion of amino acids at higher levels. Nonetheless, the same tracer kinetic approaches employed in healthy subjects are equally valuable in these high-risk groups.

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3 Abbreviations used: dx/dt, rate of change in tracee, x, as a function of time, t; k, rate constant of fractional loss of a tracee from a sampled compartment, n, per unit time; LOEL, lowest observed adverse effect level; NOEL, no observed adverse effect level; LOAEL, lowest observed adverse effect level (LOAEL) and lowest observed adverse effect level (NOAEL) is the extension of these pharmacological principles to so-called toxicokinetics and toxicodynamics (3). As defined by Renwick (3), toxicokinetics is “the application of pharmacokinetic principles to animal toxicity studies and to human toxicity data to provide information on

Kinetics, dynamics and models

The term kinetics defines the time-dependent movement of material (amino acids in the present case) within a system; the related expression, dynamics, refers to the relationship of these kinetic events to consequent effects. The fundamentals of tracer kinetic analysis are well established (1). In the context of assessing the safety of amino acid intake ranges, it appears most appropriate to transfer to the amino acid metabolism domain the correspondingly well-established principles learned in the pharmacological testing of therapeutic drugs called, not surprisingly, pharmacokinetics and pharmacodynamics (2). These approaches appear particularly relevant because establishing the safe range of amino acid intakes is similar to defining the safe therapeutic range for drug treatment using kinetic information about the drug’s bioavailability, distribution and elimination coupled with corresponding dynamic information about its action(s).

Especially relevant to issues of potential adverse effects of amino acids at the upper intake boundary between the no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) is the extension of these pharmacological principles to so-called toxicokinetics and toxicodynamics (3). As defined by Renwick (3), toxicokinetics is “the application of pharmacokinetic principles to animal toxicity studies and to human toxicity data to provide information on
exposure to the parent compound and its metabolites, and other aspects such as accumulation during chronic exposure.” It is “concerned with the relationship between the external dose...and a measure of the internal dose of active compound delivered to the target of toxicity...such as the concentration in the general circulation or at the target for toxicity. (3)" In contrast, toxicodynamics describes the “the mechanism of action and potency of the chemical at the site of action (3), i.e., the sequence of biochemical and physiological effects that the molecule elicits at the cellular or organ levels. These distinctions are shown schematically in Figure 1.

Transferring these concepts to the field of amino acid metabolism provides corresponding approaches to estimating upper intake levels, one of which focuses on alterations in the movement of amino acids throughout the system (kinetics) and the other focuses on consequent downstream effects at the cellular or metabolic levels (dynamics). To do so requires a model of the system in question. Figure 1 is an obviously oversimplified generic model. Most biological systems, including the overall integrated system of protein and amino acid metabolism, as well as the subsystem models of each of the individual amino acids, are far more complicated (4). In fact, comprehensive kinetic models have been presented for only two amino acids, leucine (5) and lysine (6). Nonetheless, approaches to modeling the amino acid systems are necessary in human studies because we generally require information on the fine structure of a system's operation in areas of the body inaccessible to sampling and this can only be achieved through the use of tracers (1).

The study of upper levels of amino acid intakes using tracers, however, requires consideration of a variety of theoretical or practical issues (4). First, one must consider both acute ingestion (e.g., meals) versus chronic ingestion (e.g., diet or long term parenteral nutrition), the form in which the amino acids enter the system (e.g., free amino acids, peptides or proteins) and, in the case of parenteral nutrition, the site of entry (e.g., central or peripheral vein or peritoneum). Secondly, the nature of the protein amino acid system itself complicates tracer approaches. The primarily accessible pool, the circulation, contains only a tiny fraction of the amino acids present in the entire system. Further, these are in very rapid flux, especially compared to the turnover of protein amino acids. In addition, the circulation is principally the route of amino acid movement among organs, not the location of amino acid metabolic events. Conversely, the inaccessible compartments contain the vast bulk of amino acids and proteins, and are the site of the vast majority of the metabolic transformations and regulatory actions. Moreover, the flux rates of individual amino acids in the inaccessible intracellular pools range over orders of magnitude depending on whether these are in the free or protein forms. Third, one must carefully consider the location of the tracer label because the movement of the tracer must reflect the movement of the tracee, and this principle is compromised by selected aspects of amino acid metabolism such as transamination (7,8). Fourth, in long-term studies, there is the potential for serious confounding due to tracer recycling from labeled amino acids released by proteolysis of slowly turning over body proteins. Finally, however, perhaps the most significant barrier to tracer studies are the issues of steady versus nonsteady state. Conventional tracer kinetic approaches are most readily applied to the steady-state condition when tracee concentrations remain unchanged due to equal and constant rates of production and utilization. Nevertheless, much of the physiologically relevant activity in amino acid homeostasis takes place in the postprandial condition, a nonsteady state when amino acid inflow and disposal are not equal. Solutions to this problem have remained especially elusive in the amino acid tracer field.

Irrespective of the final approach(es), the resulting kinetic parameters themselves do not describe particular anatomical areas, physiological functions or biochemical reactions within the inaccessible parts of the system. To make these physical and functional constructs requires mathematical modeling which, in turn, requires other information about the working nature of the system derived from conventional anatomical, physiological and biochemical measurements (1). It is also important to remember that the model is a hypothesis, subject to continued testing and refinement based on new data. Kinetic models are top-down models; that is, experimentally obtained tracer kinetic data lead to estimation of system parameters which, in turn, lead to structure-function modeling based on biological information available from other scientific sources. An alternative approach, called physiologically based pharmacokinetic modeling (PBPK) (9), is a bottom-up approach that begins with definition of the conceptual and functional nature of the system, then uses available or newly collected anatomical, physiological and biochemical data to develop the parameters of the model, then develops predictive algorithms about the model's behavior under various conditions and finally advances to test the predictions by obtaining experimental data and fitting the model to these data (9). The PBPK approach has not been directly applied to questions of upper-limit amino acid intakes. However, several PBPK models of responses to toxicants have had implications, admittedly quite distant, for the metabolism of select amino acids including alanine (10–11), glutamine (12) and arginine (13).

**Toxicokinetics**

**Intravenous amino acid infusion: steady-state approach.** In kinetic studies in the steady state, tracer and tracee relationships are linear; that is, event velocity is proportional to concentration and can be described by first-order kinetics with a linear differential equation, for example

\[
dx/dt = kx
\]

where the rate of change in substance \(x\) is related to the concentration of \(x\) according to a proportionality rate constant \(k\). In contrast, when the capacity of regulatory systems are exceeded because of high concentrations of a substance, movement of the substance (tracee) through the system is no longer proportional to its concentration, resulting in nonlinear, or zero-order, tracee saturation kinetics (3). However, even under these circumstances, the very small perturbation of the system introduced by the tracer behaves linearly and the tracer model of the system can be described by a linear compartmental model.

![Figure 1](https://academic.oup.com/jn/article-abstract/133/6/2034S/4688207) Simplified model of the relationships between the dose of an administered toxicant, its delivery to the site of action (toxicokinetics) and the consequent toxic response (toxicodynamics). Reprinted from Reference 3 with permission.
This principle might be applied to estimating upper limits of amino acid transport through plasma in the fashion shown in Figure 2. Subjects would receive a stepwise series of constant intravenous infusions of specific amino acids (tracee) at increasing infusion rates. At each resulting steady-state amino acid concentration level, a pulse injection of the appropriately labeled amino acid tracer would allow calculation of the corresponding rate constant \( k_0 \). Because the rate constant represents the rate of tracee removal or disappearance \( (R_d) \) from the system as a function of tracee concentration \( (x) \), one can calculate the rate of tracee removal at each amino acid concentration level. Idealized results of such studies are shown in Figure 3. Thus, within a certain amino acid infusion range, tracee kinetics would be linear as shown in the left panel of Figure 3.

Presumably, at some higher rate of amino acid infusion, regulatory mechanisms affecting tracee removal or disposition will be altered (e.g., by exceeding the capacity of cellular membrane transporters, saturation of the enzymatic metabolic processes along major catabolic disposal routes, diversion of metabolic intermediates into minor metabolic routes, or changes in renal excretion) resulting in nonlinear tracee kinetics. Drawing from the large body of data available in the pharmacologic field, one plausible result would be the saturation kinetics shown in the right panel of Figure 3. Thus, this kinetic information might help define the rates of interorgan amino acid transport that can be accommodated without difficulty and identify the rates at which adaptive mechanisms are either altered significantly or deteriorate entirely. Similarly, if a model of the system is available or is developed from the data available in the linear tracee kinetic range, one will be able to determine which parameters within the inaccessible parts of the system were affected primarily. Although I am not aware of an application of this approach in the amino acid field, the principle has been applied successfully to define kinetic system adaptations in the field of carbohydrate metabolism (14–16).

**Oral amino acid ingestion: quasisteady-state approach.**

With minor modifications, the above principle might be extended to dietary studies as well. Single meals acutely perturb the steady state and, as mentioned, validated nonsteady-state models of amino acid kinetics have not been forthcoming and are sorely needed in the field. However, many (if not most) of the questions related to safe upper levels of amino acid ingestion deal more appropriately with intakes over the long term. In this context, the consequences of intermittent, nonsteady-state meal perturbations are likely to be small and blunted in the context of the total system adaptation to an overall new steady state reached through chronic ingestion. Thus, a series of stepwise adaptations to increasing intakes of test amino acids might be carried out in a fashion analogous to the intravenous infusion studies discussed above.

**Figure 4** depicts schematically two such studies. The design is similar to the studies shown in Figure 2 with two apparent differences. First, although the time frame of each steady-state infusion experiment in Figure 2 is likely hours, the time frame for chronic ingestion studies may conceivably be longer (possibly days or weeks). Akin to the delayed system adaptation seen when dietary protein intake is changed, time may be required to achieve new stable amino acid concentrations throughout the entire system when dietary amino acid intakes are changed. Although circulating amino acid levels will reach a quasisteady state within hours after individual meals, a new set point circulating amino acid level may take much longer to reach. Thus, for example, doubling the dietary intake of a particular amino acid might change its circulating level by only \( x\% \) on the first day, but by \( 2x\% \) or \( 0.5x\% \) after the amino acid is consumed for 7 d. Further, in the practical context of the potential safety consequences of long-term consumption, it will be important to define the kinetic similarities or differences between acute and chronic ingestion in any case. Naturally, these considerations hold too for the intravenous infusions discussed previously. I contend, however, that system adaptation period will be shorter and the dynamic linear tracee range will be narrower for amino acids infused directly into the vascular compartment.

Secondly, during intravenous infusion studies, circulating amino acids levels will remain essentially constant. However, in
Amino Acid Kinetics

Precursor-product relationships: biochemical consequences. Individuals with inborn errors of amino acid metabolism have significantly constrained safe ranges of specific amino acid intakes. Clinical and experimental data obtained from these subjects provide insights into potential approaches for studying the limits of amino acid intakes in normal individuals. Perhaps the most consistent underlying principle identified is the diversion of proximal metabolic intermediates into generally otherwise minor metabolic pathways when distal metabolic disposal mechanisms are impaired. Thus, for example, we find both dramatic increases and distinct patterns of urinary and/or plasma organic acid intermediates in persons with various enzymatic defects in the leucine or lysine catalytic pathways (17–18). In the upper NOEL or lower LOEL amino acid–intake levels, saturation kinetics at specific catabolic enzyme steps may lead to the diversion of metabolic intermediates into alternative, generally minor routes of disposal. These diversions are likely to be considerably less dramatic than those found in persons with significant, inherited single gene defects. Nonetheless, isotopically labeled amino acids should not only be able to trace these diversions, but also allow such diversions to be identified before frank and potentially harmful elevations of the metabolite concentrations become apparent.

The underlying tracer principle has been applied previously in a variety of inherited disorders of amino acid metabolism and studies in phenylketonuria provide one example (19). Normal individuals convert phenylalanine to tyrosine at a rapid rate. Little phenylalanine is converted to alternative products such as phenylpyruvate, phenylactate, phenylacetate, o-hydroxyphenylacetate, phenylthylamine, or phenylacetylglutamine. If phenylalanine tracer is administered to normal individuals, labelled tyrosine (19–21). Although label also appears in the alternative, generally minor routes of disposal. These diversions are likely to be considerably less dramatic than those found in persons with significant, inherited single gene defects. Nonetheless, isotopically labeled amino acids should not only be able to trace these diversions, but also allow such diversions to be identified before frank and potentially harmful elevations of the metabolite concentrations become apparent.

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production is significantly reduced to a degree reflecting to some extent the magnitude of residual phenylalanine hydroxylase activity (19–21,24). If the label represents part of a phenylalanine loading dose (i.e., analogous to amino acids administered at upper limit amounts) the quantity of phenylalanine alternative metabolic products is increased, is as the fractional amount of label incorporated into these products. Conversely, no tracer is found in the para-hydroxylated compounds of tyrosine metabolism (22).

**Precursor-product relationships: functional consequences.** An alternative pharmacodynamic approach to estimating NOEL-LOEL amino acid intake ranges would couple amino acid kinetic data with the physiological effects consequent to its metabolism. Thus, for example, histidine kinetics might be coupled with histamine effects on bronchiolar or gastric smooth muscle functions, heart rate and blood pressure, sensory nerve endings, urticarial responses, or gastric secretion of acid, pepsin or intrinsic factor. For example, in a related fashion, Tashiro et al. recently used PET scanning to examine arousal and cognition in humans after oral administration of antihistamines (25). Tryptophan kinetics might be coupled to similar serotonin effects on cardiovascular, bronchiolar and gastric smooth muscle functions, as well as serotonergic central nervous system effects on mood and sleep (26–27). In this respect, using PET scanning, Hagberg et al. have recently published a comprehensive kinetic model of tryptophan-serotonin relationships in human brain (28). Tyrosine kinetic parameters may similarly be linked to catecholamine effects at the peripheral or central nervous system levels (26) in a fashion akin to recent primate PET studies of modulators of central nervous system dopaminergic activity (29).

Finally, given the direct precursor-product relationship between arginine and nitric oxide and given the latter’s direct effects on endothelial function, angiogenesis, neurotransmitter signaling, and leukocyte, platelet and macrophage functions (30), it is important to consider experiments coupling arginine kinetics and physiological measurements of nitric oxide actions. Labeled arginine tracer methods for measuring nitric oxide synthesis have already been developed (31) and numerous noninvasive approaches to measuring endothelial function are clinically available. Similarly, additional techniques exist to approach correlations with the other dynamic, physiologically relevant consequences of nitric oxide production as, for example, a recent demonstration of the relationship between nitric oxide production and the circulating levels of the soluble vascular cell adhesion molecule-1 (sVCAM-1) (32).

The physiologically relevant, dynamic actions measured as a consequence of systematic stepwise variations in specific amino acid intakes can then be used to construct dose-effect relationship plots of the kind commonly employed in the field of pharmacodynamics (32). One can then take the composite dose-effect relationships defined for a single amino acid to construct an optimal effect range for amino acid in question. Thus, Figure 6 shows a theoretical, but eventually plausible, result of such efforts in which the beneficial and detrimental consequences of an amino acid are plotted against the amino acid’s circulating concentration (or ingested amount, etc.). From these data, a representative range of maximum benefit and least harm may be delineated (hatched area) and form the basis for the recommendation of safe intake ranges.

**LITERATURE CITED**


