# AMINO ACID KINETICS IN HUMANS: METABOLIC ASPECTS AND NUTRITIONAL SIGNIFICANCE

V.R. YOUNG

## I. INTRODUCTION

Tissue and organ proteins and the free or peptide-bound forms of amino acids in cellular and extracellular pools serve multiple functions (table 1) and there continues to be an exciting explosion of knowledge concerning the genetic transcription, translation and intra-cellular translocation and orientation of gene products and of the molecular events and regulatory mechanisms that underlie these various functions. Of equal interest, to the nutritional biochemist, is that in the course of meeting their physiologic roles, the proteins and amino acids are synthesized and degraded at a collective rate that considerably exceeds a "usual" or sufficient level of dietary protein (nitrogen) and amino acid intake.

Table 1 Some Functions of Proteins and Amino Acids

| Function | Example |
|---|---|
| **Proteins** | |
| Enzymatic catalysis | BCKADH |
| Transport | $B_{12}$ binding proteins; ceruloplasmin; apolipoproteins |
| Messengers/signals | Insulin; Growth hormone |
| Movement | Kinesin; Actin |
| Structure | Collagens; Elastin |
| Storage/Sequestration | Ferritin; Metallothionein |
| Immunity | Antibodies; TNF; Interleukins |
| Growth; Differentiation Gene Expression | EGF; IGFs; Transcription factors |
| **Amino Acids** | |
| Substrates for protein synthesis | Those for which there is a codon |
| Regulators of protein turnover | Leucine; Arginine |
| Regulators of enzyme activity (allosteric) | Arginine and NAG synthetase; Phe and PAH activation |
| Precursor of signal transducer | Arginine and nitric oxide |
| Methylation reactions | Methionine |
| Neurotransmitter | Tryptophan (serotonin); Glutamate |
| Ion fluxes | Taurine; Glutamate |
| Precursor of physiologic molecules | arg (creatine); glut ($NH_2$)-purines |
| Transport of nitrogen | Alanine; Glut ($NH_2$) |
| Oxidation-Reduction properties | Cystine; Glutathione |
| Precursor of conditionally indispensable amino acids | Methionine (cys); Phe (tyr) |
| Gluconeogenic substrate and fuel | Alanine; Serine; Glut ($NH_2$) |

Laboratory of Human Nutrition, School of Science and Clinical Research Center, Massachusetts Institute of Technology, Cambridge MA 02142; Shriners Burns Institute, Boston, MA USA 02114

Thus, a major feature of the amino acid economy of the organism is a quantitatively significant reutilization of amino acids for the continuing synthesis and maintenance of cellular protein content and of other physiologically important N-containing compounds. However, because this process of amino acid recycling is not completely efficient there is, even under the most favorable of conditions, a continuous loss from the body of amino acid carbon and nitrogen, via oxidative metabolism. The maintenance of an adequate protein nutritional status and, and in parallel, of body function requires a replenishment of these losses via a dietary supply of utilizable nitrogen and of specific, nutritionally indispensable amino acids, in addition to the intake required to support a net gain of body protein in the growing organism or where there is a regeneration of tissue protein following catabolic illness.

The overall losses of nitrogen (largely in the form of urea, ammonia, uric acid, creatinine and other nitrogenous products in urine, as well as the N in feces, sweat and losses via other miscellaneous routes or in products (such as milk)) can be measured and with a simultaneous determination of nitrogen intake, an estimate of the status of body N balance can be obtained. This technique of N balance has provided the major basis for current estimations of the dietary requirements for nitrogen and for the indispensable amino acids in human subjects. Although it has been a useful method to explore and define, in qualitative terms, the nutritional needs of man under different physiological and pathological conditions, it has major technical and conceptual limitations. These are now well-appreciated (e.g. Hegsted 1976; Young 1986), and some of these had been pointed out more than fifty years ago by Schoenheimer and Rittenberg (1938), whose classical studies, involving stable isotope tracer techniques, have been an important stimulus for our investigations of human amino acid kinetics.

To improve upon an understanding of the metabolic and physiologic basis changes in body nitrogen balance that occur when nitrogen or amino acid intakes are altered, or following differing hormonal and disease states, as well as to sharpen our definition and methods for determination of nutritional requirements for N and specific amino acids, we concluded some time ago that alternative approaches to those involving the N balance procedure should be developed and exploited. Furthermore, since the intact organism, being the most complex system of biological organization, is the level of greatest interest to us we have attempted to investigate how the protein and amino acid pool sizes, their turnover and the activity of relevant metabolic pathways change in relation to specific nitrogen and amino acid intakes. The discussion that follows will review, in brief, a number of selected examples of studies concerned with the study of human amino acid kinetics and their nutritional significance.

## (a) Stable isotope tracer approaches

While different experimental techniques might be used to explore quantitative aspects of body amino acid metabolism in vivo, all derive from an appreciation that the free amino acids and their precursors are the currency of tissue and organ protein metabolism. Further, the major in vivo pathways of amino acid flow, with reference to whole body amino acid homeostasis, can be schematically presented as shown in Figure 1 (Munro 1983). Again, for the nutritional biochemist, it is particularly important to understand the consequence of dietary factors on the overall flows of amino acids, and of nitrogen, and how they influence the nutritional requirements for specific indispensable amino acids and for total dietary nitrogen.

Measurement, in vivo, of the interorgan and whole body transport, and fate, of nitrogen and of the individual amino acids, may be based on tracer approaches. These have been reviewed and discussed in detail by various investigators (e.g. Waterlow et al. 1978) and by us (Young et al. 1991). For the present purposes I will not review, or critique, the different tracer approaches used to study the kinetics of body proteins and of their constituent amino acids in vivo, except to say that our research has exploited, largely, the stochastic measurement of plasma or interorgan amino acid and nitrogen transport. We have utilized stable isotope probes in these investigations because of their major advantages for studying in vivo kinetics of amino acid metabolism in the human subjects. Specifically, I would like to discuss a number of applications of stable isotope tracer protocols, in order to emphasize how they might enhance an understanding of the metabolic basis for, and determination of, the nitrogen and amino acid requirements in healthy adult man.

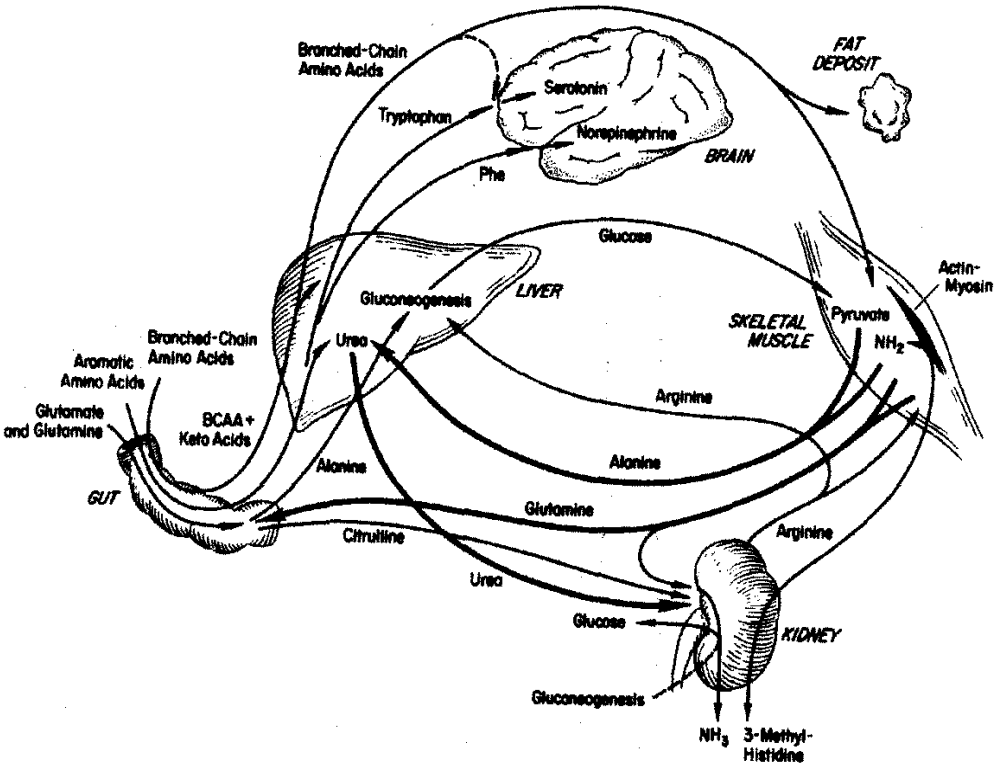


Figure 1. Inter-organ flows and associations of amino acid metabolism. Modified from Munro (1983).

## (b) Plasma amino acid fluxes

During the past about ten-to-fifteen years there has been an increased interest in the study of in vivo kinetics and interorgan transport of the nutritionally dispensable and indispensable amino acids in human subjects. Thus, Bier (1989) has made a survey of published values for the fluxes of the individual indispensable and of dispensable amino acids in the post-absorptive adult human. If the flux estimates for the indispensable amino acids, under these metabolic conditions, are a reasonably good index of their whole body flux, there should be a close correlation between the value for the flux and the amino acid composition of mixed body proteins. Indeed, Bier (1989) has shown this to be the case, as depicted in Figure 2. From this it can be concluded that, in the post-absorptive state, the flux of an indispensable amino acid serves as an index of the dynamic status of body protein breakdown. These findings also are supported by Nair et al. (1988) who found a significant correlation between whole body muscle protein synthesis and whole body protein synthesis.

Furthermore, the summary presented in Figure 2 reveals that the fluxes of three dispensable amino acids (alanine, glutamine, glutamic acid) greatly exceed the rates that would be predicted from their relative concentrations in muscle proteins. Clearly, this indicates that their appearance in plasma is due, in large part, to de novo synthesis of the amino acids.

Thus, it might be apparent that not only do the fluxes differ according to the specific amino acid but they are likely to be under different controls. Indeed, as Christensen (1986) has, thoughtfully, pointed out the fluxes of amino acids are not simply a consequence of an indiscriminate and intermittent inflow of amino acids arising from protein-containing meals but they are regulated at specific organ sites via various mechanisms. An identification and description of these in vivo mechanisms and of the points of control of amino acid fluxes in healthy subjects is necessary if we are to define the metabolic basis, and importance, of various pathophysiological factors on, the requirements for nitrogen and amino acids in the human subject. This has been a premise behind our investigations, a selection of which I will summarize in the sections to follow.

To establish a metabolic framework for the specific studies of "metabolism in real time", which is the focus of this portion of the annual meeting program, to be surveyed it might be worth distributing the amino acids into one of four different classes, according to the general structure of their major pathways of metabolism (see Figure 3). Thus, for class 1, amino acids are catabolized via a series of consecutive reactions where one or more of the early steps are irreversible, as for the case of threonine and lysine. The branched-chain amino acids (e.g. leucine), follow a similar general metabolic sequence, except in this case the initial step involves a reversible transamination reaction. With respect to class 2, for which methionine is used here as an example, the amino acid is converted to another amino acid (homocysteine) which may either undergo an irreversible conversion to a metabolite (cystathionine) or it may be reconverted to the parent amino acid. Hence, it is possible that the flux, and fate, of this amino acid (methionine) is controlled at the locus where this reconversion, or recycling, occurs. For class 3, these amino acids are derived, in part, from other amino acids; examples are cysteine (from methionine) and tyrosine (from phenylalanine) and they are now regarded from a nutritional standpoint as being conditionally indispensable. Metabolically more complex examples are proline and arginine, to which I will refer again below. Finally, in the case of class 4, these amino acids can arise from non-amino acid carbon sources, with alanine, glutamine and glycine being examples. These amino acids were earlier labeled as being nutritionally dispensable (Rose et al. 1954) although there is now increasing evidence that many of these are more appropriately to be regarded as being conditionally indispensable (e.g. Laidlaw and Kopple 1987).

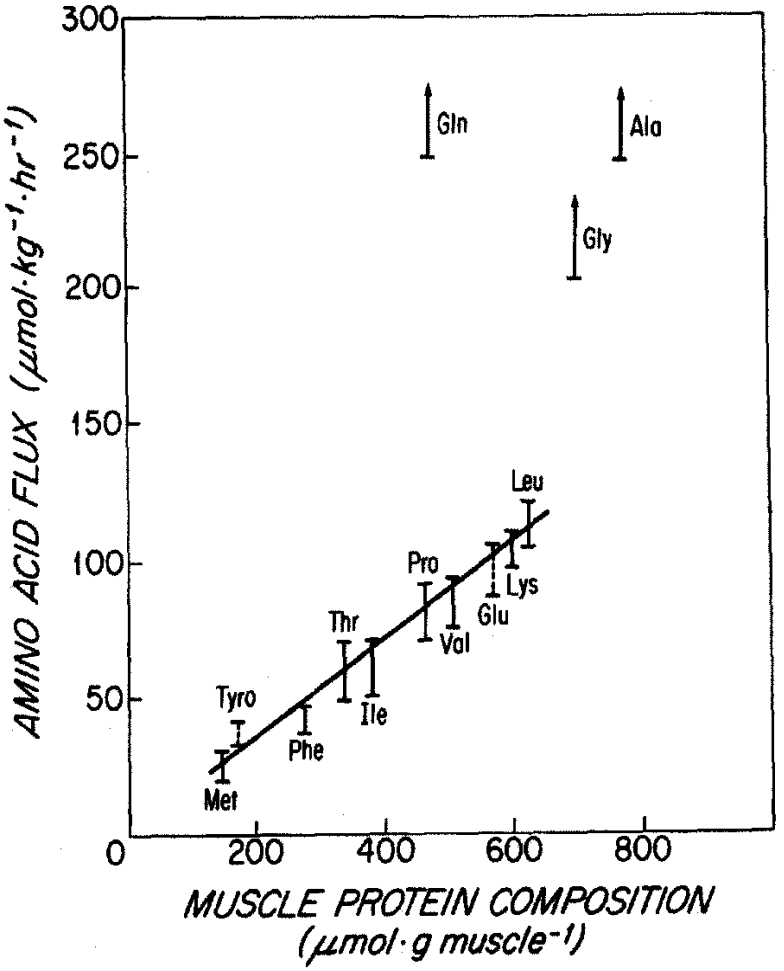


Figure 2. Relationship between plasma amino acid fluxes, in post-absorptive human adults and the amino acid composition of muscle mixed proteins. From Bier (1989).

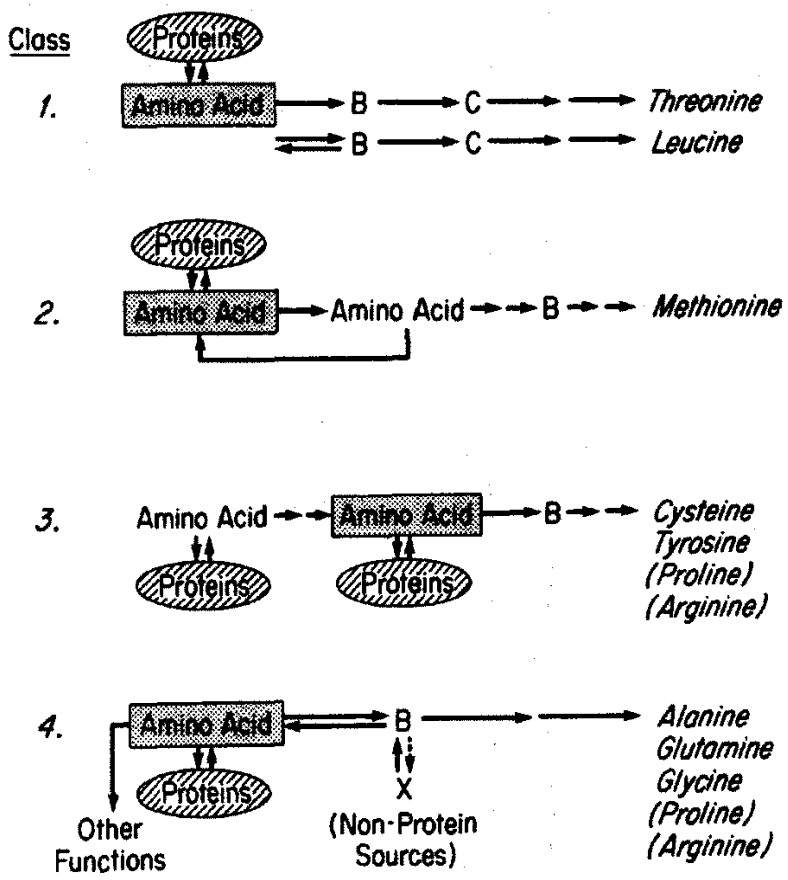


Figure 3. A categorization of the amino acids according to the general structure of their metabolic pathways. From Young and Marchini (1990).

(c) Studies of proline and arginine kinetics - are they dispensable amino acids?

With the foregoing metabolic picture (Figure 3) in mind, we now turn to a brief account of our initial explorations of the regulation of proline metabolism in human subjects (Jaksic et al. 1987). These investigations were initiated because it had been shown that there is feed-back regulation of proline synthesis cultured cells in vitro. Hence, we considered it important to establish whether this applied to the intact human under various conditions. Using a dual stable-isotope amino acid tracer model (e.g. Robert et al. 1982) to estimate the in vivo rate of proline synthesis, we found when proline was given intravenously to healthy subjects, at rates approximating a "usual" daily level of proline ingestion, there was a significant reduction in the rate of de novo proline synthesis (Figure 4). It was apparent that the synthesis of proline in man is also subject to feed-back regulation and is affected by nutritional factors. We were also interested in knowing whether this regulation remained intact, for example, under conditions of major stress as in the case of the severely burned patient. In this situation rates of whole body protein turnover and of collagen synthesis and degradation are very high, and it seemed likely that there would be an altered status of proline metabolism and possibly
of proline synthesis.

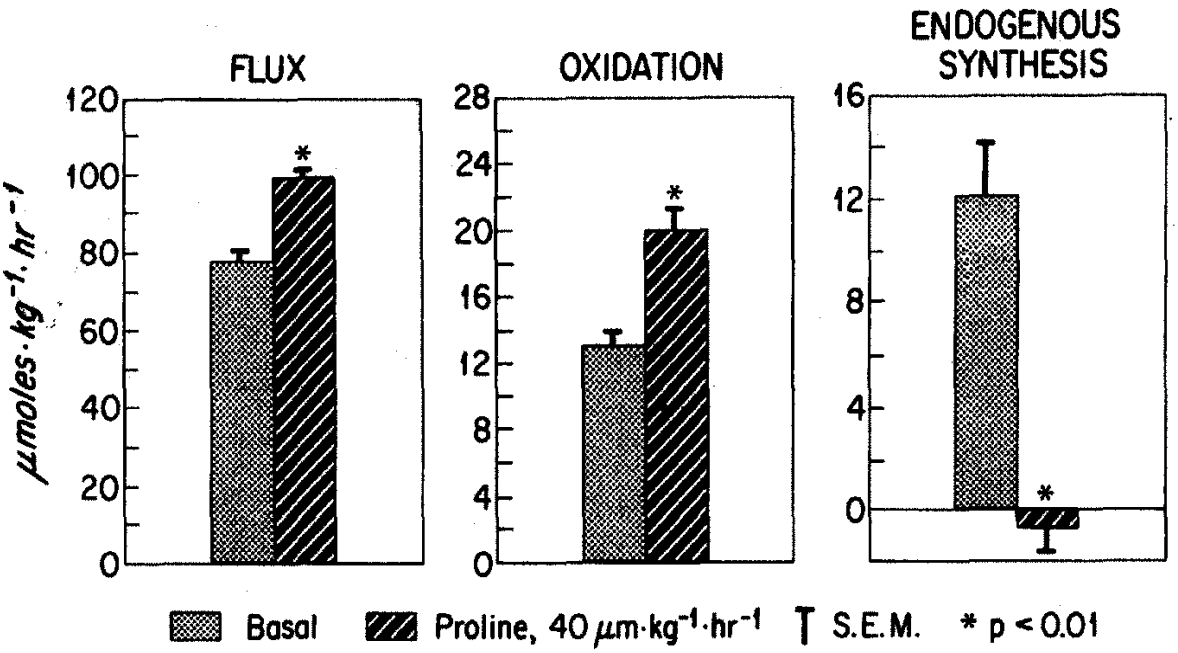


Figure 4. Plasma proline flux, oxidation and endogenous synthesis in young adult men in basal state and during an intravenous infusion of 40mol proline.kg$^{-1}$.h$^{-1}$. Drawn from Jaksic et al. (1987).

As shown in Figure 5, we (Jaksic et al. 1991) found a significant, about three-fold increase in proline oxidation in burn patients compared to that for healthy controls. Furthermore, the burned patients were in distinct negative proline balance and this was associated with the relatively low rate of de novo proline synthesis (Figure 5).

The suggestion emerging from these findings is that burned patients might require an exogenous source of proline to effectively maintain an overall daily proline balance and protein nutritional status. Obviously, additional studies will be needed to test this possibility but it is of possible interest to point out that the plasma proline flux in the healthy adult is considerably lower than that for the other, so-called, dispensable amino acids (alanine, for example) as shown in Figure 2. Indeed the flux of proline in relation to its content in body protein follows the pattern shown for the indispensable amino acids. This implies a relatively low rate of endogenous proline synthesis which, might arise from the catabolism of ornithine. Thus, it could be speculated that the amino acid may be conditionally indispensable in human nutrition. This hypothesis is strengthened by tracer studies indicating that proline is required in the diet for growth in the young pig (Ball et al. 1986) and by the novel investigation of Berthold et al. (1991) who found that uniformly $^{13}$C-labeled proline, when given as part of a diet containing U-$^{13}$C-labeled *Spirulina platensis* to a laying hen, was incorporated without change into egg and body proteins. Again, this finding in the bird suggests that endogenous proline synthesis was limited and that the metabolic behavior of dietary proline was like that of the other conventional indispensable amino acids. In any event, we believe that these observations of proline metabolism in human subjects point out the potential value of tracer studies in helping to probe more deeply the dietary significance of specific amino acids in human nutrition.

A second example of such studies of amino acid kinetics, with reference to developing a more refined definition of the role of dietary amino acids in the maintenance of protein nutritional status and tissue and organ function, concerns arginine; again Rose and coworkers (1954) classified this as a "non-essential" amino acid in human adult nutrition, as was also found to be the case by Snyderman et al. (1959), with reference to the young infant. Our interest in arginine was heightened by reports that arginine supplementation following trauma in animals had a beneficial effect on nitrogen balance and survival and improved the rate of wound healing (Barbul 1986). Additionally, the fact that arginine is the precursor for synthesis of nitric oxide, which is an endogenous vasodilator and cellular communication signal (Moncada et al. 1991) made it of even greater importance to learn how plasma arginine flux fitted into the relationship shown in Figure 3.

Hence, we have recently used L-[di-$^{15}$N-guanido,5,5$^{2}$H]-arginine and L-[$^{13}$C-guanido]arginine as tracers, in combination with $^{2}H_3$-leucine, to determine, in healthy young adult men receiving an adequate diet, plasma arginine and leucine fluxes and the rate of endogenous arginine synthesis. Our results are still quite preliminary, as well as incomplete, but they are summarized here because they raise a number of intriguing and important points about the physiology of arginine metabolism and its nutritional implications. Again, they also point out the desirability of conducting in vivo studies at the whole body level, to measure "metabolism in real-time."

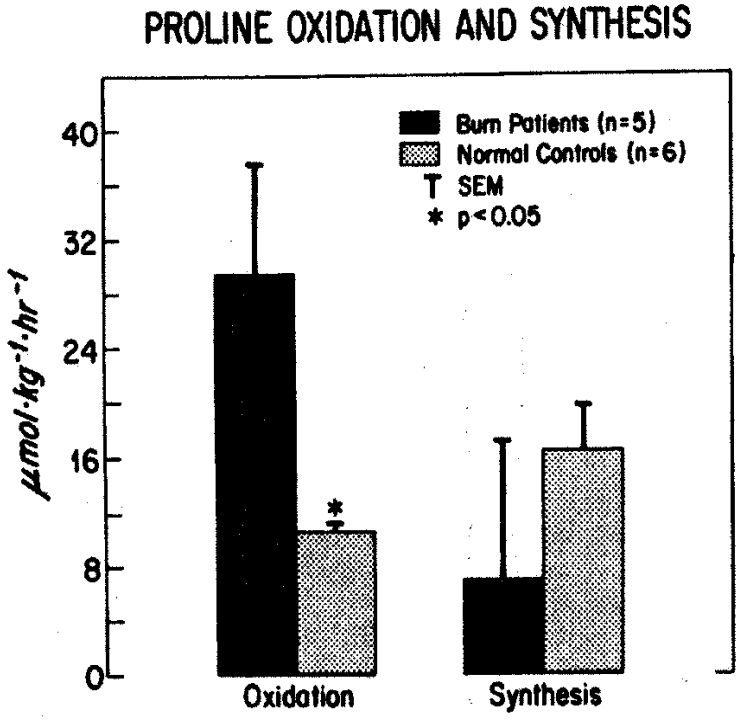


Figure 5. Proline oxidation and proline endogenous synthesis in five adult burn patients and six healthy adult volunteers. From Jaksic et al. (1991).

Thus, in summary, table 2 presents our initial findings for plasma arginine and leucine fluxes, determined simultaneously, in healthy young adult men. From these values we also estimate the extent to which the plasma arginine flux exceeds that which would be predicted to arise only from protein breakdown, as judged from the leucine flux. Thus, as indicated in this table the "excess" arginine flux amounts to 15 and 50 mol $kg^{-1}h^{-1}$ in subjects receiving an adequate dietary protein intake, for the fasted and fed states, respectively. In the fasted, or post-absorptive state, this excess presumably is an index of endogenous arginine synthesis, whereas it is due to net arginine synthesis and also the entry of dietary arginine into plasma compartment for the fed state. Therefore, making a number of simplifying assumptions, it is possible to derive an approximate rate of endogenous arginine synthesis and this estimate is also shown in table 2. It should be emphasized that these represent gross approximations of in vivo, arginine synthesis rates, for a number of reasons; first, in the case of the fed state, we have assumed in our calculations that all of the dietary arginine intake enters the plasma compartment. However, it is likely that a significant

amount of absorbed arginine disappears during a first-pass in the splanchnic region. Thus, in this case, the values shown in table 2 will be an underestimate of the true rate of arginine synthesis.

Second, and importantly, the measured plasma flux (table 2) is much lower than we would expect for the whole body flux of the guanido moiety of arginine. Indeed, the sum of the whole body (plasma?) arginine flux contributed by protein breakdown together with that due to the turnover of the guanido moiety during formation of arginine and its hydrolysis to urea, via the urea cycle, might be anticipated to amount to about 350+ mol.kg$^{-1}$h$^{-1}$, for subjects consuming an adequate protein diet. This value is far higher than the measured flux. The probable explanation for this discrepancy, between expected and measured fluxes, is that the intravenously administered tracer failed to label significantly, or exchange with, the arginine pool that is being formed and replenished via the cooperative activity of the five urea cycle enzymes within the liver.

Table 2. Comparison of Plasma Leucine and Arginine Kinetics in Young Men Receiving an Adequate Diet, during Fed and Fasting States

| | Fast | Fed |
|---|---|---|
| Leucine Flux* | 79±3[1] | 85±2 |
| Arginine Flux | 66±4 | 71±5 |
| Predicted Arg Flux[2] | 51±2 | 20±1 |
| "Excess" Arg Flux[3] | 15±6 | 50±8 |
| "Synthesis" | 15±6 | 13±7 |

* Based on KIC x 0.8.

1. μmol.kg$^{-1}$h$^{-1}$; mean ± SEM (n=5).
2. From an estimate of protein breakdown based on leu flux, corrected for leu intake.
3. "Excess" = arg intake + de novo synthesis(intake = 36 μmol.kg$^{-1}$h$^{-1}$).
4. Synthesis = "Excess" - intake.

Biochemical evidence now reveals that there is a tight channeling of intermediates between the interacting enzymes of the urea cycle. This includes "external" or cystolic ornithine which is tightly channeled to mitochondrial ornithine transcarbamylase (Cohen et al. 1987) and a channeling of citrulline, argininosuccinate and arginine between the three "soluble" cytoplasmic, urea cycle enzymes (Cheung et al. 1989), due to these being grouped around and spatially linked to the mitochondria. Furthermore, from immunocytochemical and biochemical evidence, it appears that carbamoylphosphate synthase I, which is the most abundant protein in the mitochondrial matrix, is not randomly arranged but associated with the inner mitochondrial matrix (Powers-Lee et al. 1987). Hence, the arginine formed via the urea cycle does not readily equilibrate with the liver free arginine pool and so, in turn, it is not readily exchangeable with the plasma free arginine pool. We estimate, from the $^{15}$N-glycine infusion studies of Yudkoff et al. (1984) when considered together with our arginine flux data, that only a small portion (possibly less than 5%) of the plasma arginine flux derives from arginine that "leaks" from the urea cycle. A major research challenge, therefore, is to devise a satisfactory tracer approach and model of arginine kinetics which allows this metabolically active, but tracer-inaccessible, "urea-cycle arginine" pool to be probed.

The intracellular compartmentation of arginine metabolism within the liver, as revealed by these various biochemical studies and further exposed by the present tracer studies in humans, is of considerable, functional significance; this spatial organization evidently contributes to the maintenance of arginine homeostasis while permitting the formation of significant quantities of urea, and thus, of arginine hydrolysis, without leading to a drain on the cystolic-free arginine compartment, required for protein synthesis and degradation in the liver, or without depleting the plasma pool of arginine which is used to meet the needs for the amino acid by extrahepatic tissues and organs. Without this metabolic and structural compartmentation the daily requirement for

arginine, either furnished by way of the diet or by way of endogenous synthesis, presumably would be exceedingly high. Parenthetically, there is now extensive evidence that cellular metabolism is largely spatially organized (as for arginine, as discussed above) and this is on a scale much smaller than that of the well known organelles (Welch et al. 1988), with the benefits being efficient substrate transfer, protection of labile intermediates and improved coordination. Whether this compartmentation remains effectively intact under various pathophysiological conditions, particularly where liver function might be compromised, is not known. Possibly it is not, so altering arginine homeostasis and perhaps this might be a basis for the favorable effects of arginine supplementation in stressed animals as referred to above.

Hence, an important point that needs to be made, at this stage, and emerging from these preliminary arginine flux data, is that it is obviously no longer sufficient to be content with "plasma amino acid kinetic data" per se, if we are to enhance significantly our view of the quantitative and regulatory status of amino acid metabolism at the integrated whole body level. The metabolic channelling and inter-organ trafficking of substrates and their metabolites, while complicating interpretation of tracer data, at least as we have generated them, are critical features of the physiology of amino acid metabolism in vivo. I believe that this issue represents an area of nutritional biochemistry that will receive increasing investigation, in part through application of more complex tracer models (e.g. Cobelli and Saccomani 1991), in the next few years. Certainly, for us the distinct inter-organ, intra-organ and intracellular compartmentation to arginine metabolism makes in vivo studies of arginine metabolism in the human subjects a conceptual and technical challenge, as well as a fascinating problem of amino acid regulation and control.

### (d) The indispensable amino acids, with reference to their oxidation

The two examples of studies of amino acid kinetic that I selected above for brief discussion dealt with those earlier classified as being non-essential (or a better term is dispensable (Harper 1974)). I now turn to the kinetics of indispensable amino acids metabolism, with a particular focus on their rates of oxidation. This will pave the way for the final section of this paper and concerning amino acid requirements and how kinetic studies have, in our opinion, improved upon previous estimates of the quantitative needs for these nutrients in humans throughout various stages of life. Again, returning to the metabolic framework in Figure 3, I have attempted to convey in this picture the well-known fact that protein turnover is a significant contributor to the amino acid flux. Thus, changes in protein turnover would be expected to have both an important influence on flux and possibly on the metabolic fate of the amino acid. In turn, this well might have a significant impact on the nutritional requirements in an individual. Indeed, this point is supported by the parallelism of the changes in the rates of whole body protein turnover and of the dietary needs for total protein that occur throughout growth and development in healthy individuals (Young et al. 1985). It follows then that an increased rate of turnover would be expected to lead to a greater dietary protein need. However, the nutritional requirement for indispensable amino acids might not be driven directly by the rate of protein synthesis (or breakdown) per se, but it may be more a direct function of the mechanisms and status of amino acid oxidation.

The importance of the regulation of oxidative catabolism in the maintenance of body amino acid homeostasis is , of course, well appreciated and the results obtained in our various tracer kinetic studies underscore this fact. For example, we have become interested recently in the metabolism of and nutritional requirements for the aromatic amino acids, phenylalanine and tyrosine. Now, the rate-limiting step in mammalian phenylalanine catabolism is hydroxylation, with production of tyrosine, catalyzed by phenylalanine hydroxylase [PAH]. Additionally, tyrosine aminotransferase (TAT) has been identified as rate-limiting for the degradation of tyrosine (Goldsmith and Laberge 1989) and both PAH and TAT activities are modulated by a range of nutritional and hormonal stimuli. However, these two enzymes, differ from each other in their sensitivity, time course and possibly direction of change, following a given stimulus and blood concentrations of phenylalanine and tyrosine do not change quantitatively in any simple predictable way as a result of enzyme changes (Pogson et al. 1989). Consistent with this view, we (Ozalp et al. 1971) reported earlier that free phenylalanine and tyrosine concentrations in plasma from post-

absorptive subjects showed little change when they had consumed an aromatic amino acid-free diet for as long as 12 days, but their concentrations in plasma during the prandial period were lower than for a control diet period. From control analysis of hepatic aromatic amino acid metabolism it has been concluded that transport across the plasma membrane plays a crucial role in determining the oxidation of these amino acids (Pogson et al. 1989). Thus, phenylalanine oxidation would probably be retarded during the prandial period when phenylalanine intakes are reduced substantially but its oxidation rate during the post-absorptive state might be little affected by the immediate past daily level of phenylalanine intake. We wished to explore this hypothesis and also to determine how phenylalanine and tyrosine fluxes respond to altered dietary protein and amino acid intakes. For this purpose we applied a tracer approach, essentially according to Thompson et al. (1989), to explore the dynamic status of whole body phenylalanine metabolism in healthy young men following (a) adjustment to an adequate "protein" diet, (b) a diet supplying adequate total nitrogen but with reduced phenylalanine and tyrosine and (c) a diet low in both nitrogen and phenylalanine/tyrosine.

In brief, we found, as summarized in table 3, that the phenylalanine oxidation or hydroxylation, rate was the components of whole body phenylalanine kinetics most affected by an acute reduction in total "protein" or restricted phenylalanine and tyrosine intake, especially during the fed state. Phenylalanine oxidation was stimulated by the ingestion of the adequate diet but with a restricted intake of phenylalanine and tyrosine, there was no meal-induced increase in phenylalanine hydroxylation and oxidation rates.

Table 3. The rate of phenylalanine oxidation, studied with the aid of $^{13}$C-phenylalanine, in five young men receiving different intakes of dietary nitrogen (N) and of phenylalanine and tyrosine, for 1 week

| | Phenylalanine oxidation | |
|---|---|---|
| Diet | Fasted state | Fed state |
| Adequate N | 5.9±0.7[1] | 13.6±2.4* |
| Adequate N: Low Phe/Tyro | 6.2±1.6 | 7.3±1.1 |
| Low N: Low Phe/Tyro | 5.1±0.3 | 6.9±1.5 |

1. $\mu mol.kg^{-1}h^{-1}$: Mean +SEM.
* Different ($P<0.01$) from fast.

This study reveals the importance of amino acid availability on the status of phenylalanine oxidation, particularly during the absorptive phase of metabolism, and both extends and confirms our earlier studies with leucine and lysine, that showed the rate of oxidation of these indispensable amino acids was highly sensitive to changes in the amino acid supply/availability within the plasma and presumably tissue pools. For example, as shown in Figure 6, leucine oxidation in healthy adult men changes with the level of dietary leucine intake, and particularly when increases in intake occur above an intake level of about 28 mg/kg/day. We (Young et al. 1987; 1989) consider this level to be close to the physiological requirement for leucine in this population-age group. This type of intake-oxidation response relationship appears to apply to the other indispensable amino acids, namely that the dietary supply and absorptive phase of amino acid metabolism are major determinants of the rate of amino acid oxidation in healthy subjects. At levels of intake substantially below requirements changes in oxidation with altered intakes are less marked than for changes occurring at maintenance or supramaintenance levels of intake.

A purpose in drawing attention to these two illustrations of amino acid kinetic - oxidation studies has been to introduce the topic of oxidative amino acid metabolism and how estimates of amino acid oxidation might help to strengthen our values for the requirements for specific indispensable amino acids under various conditions.

(e) Oxidation in relation to requirements

Initially, therefore, it might be instructive to compare estimates of minimum rates of amino acid oxidation (i.e. under conditions of prolonged low amino acid intakes) with current values for adult amino acid requirements. Hence, this comparison is given in Table 4 (Young and Marchini 1990). These minimum rates of amino acid oxidation were derived from tracer studies that we have conducted in healthy adults receiving graded reductions in the amount of a specific indispensable amino acid over a period of ~4-5 weeks; each graded level was given during separate, consecutive 5-7 day periods. As can be seen, the rates of oxidation for most of the amino acids examined are greater than the upper range of the amino acid requirement estimates proposed by FAO/WHO/UNU (1985). Thus, it can be suggested from this comparison that the requirement values shown in Table 4 are probably underestimates of the actual physiological requirement.

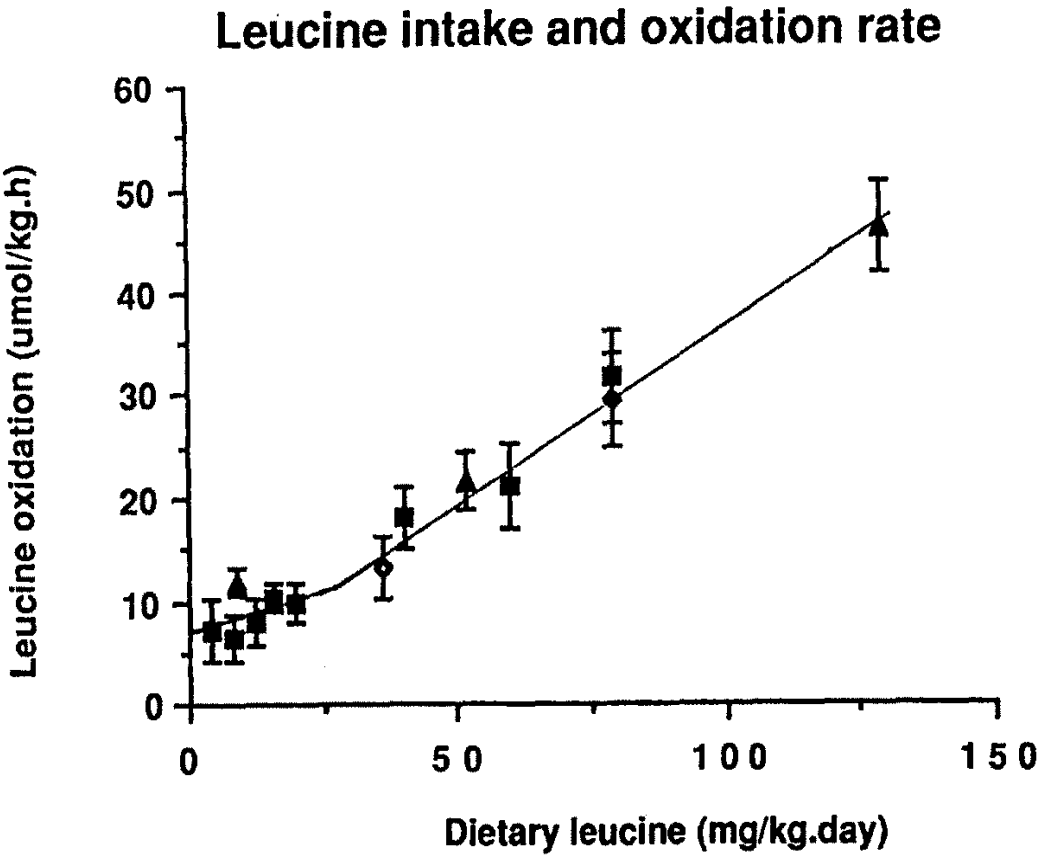


Figure 6. Relationship between leucine oxidation and leucine intake in adult men studied in the fed state. Based on a series of investigations carried out in the author's laboratories.

Is there an explanation for what appears to be a major discrepancy between requirement estimates and oxidative losses, assuming the rates given in Table 4 are acceptable approximations of minimum oxidative losses? We believe there is and that it concerns, in part, the inappropriate design and interpretation of many of the earlier nitrogen-balance studies conducted to establish the minimum physiological needs for specific amino acids. I will not go into these details, for lack of space, but they have been discussed elsewhere (Young et al. 1989; Young and Marchini 1990), leading us to the further conclusion that the internationally accepted figures for the amino acid requirements in adults are probably invalid and without practical nutritional significance.

Thus, in the context of the overall topic of this paper, we have explored two approaches for reassessing the requirements for the branched chain amino acids, and other indispensable amino acids, in adults; the first is predictive in character and based on estimated obligatory rates of amino acid oxidation. The second is a more direct approach, involving the conduct of $^{13}C$-tracer protocols, as carried out by our group at MIT.

Table 4. Estimates of minimum losses of amino acids derived from $^{13}C$ tracer studies relative to requirements[1]

| Amino acid | Minimum daily loss | 1985 FAO/WHO/UNU upper level of requirement |
|---|---|---|
| | $mg.kg^{-1}.d^{-1}$ | |
| Methionine + cysteine | 11 | 13 [85]* |
| Lysine | 18 | 12 [150] |
| Valine | 11 | 10 [110] |
| Threonine | 11 | 7 [157] |
| Leucine | 22-37 | 14 [157-264] |

1. From Young and Marchini (1990).
* Given in brackets: percent efficiency with which intake would need to be utilized to maintain balance at stated requirement intakes in relation to the minimum daily loss.

### (f) Obligatory oxidative losses and minimum amino acid requirements

A suggestion was made by Millward and Rivers (1988) that minimum rates of oxidative losses of the indispensable amino acids might be estimated from obligatory nitrogen losses. We were attracted by this idea and then extended it as a basis for predicting the minimum physiological intakes (requirement) necessary to maintain body amino acid balance in the healthy, well-nourished subject. Our approach has been described (Young et al. 1989) and involves three major assumptions: (i) the total obligatory N losses, are taken to be approximately 54 mg N $kg^{-1}day^{-1}$ in the adult (FAO/WHO/UNU 1989), (ii) the average amino acid composition of body proteins can be used to estimate the contribution made by each amino acid to this obligatory N output (defined as the obligatory oxidative losses), and (iii) at requirement intake levels absorbed amino acids are used to balance the obligatory oxidative losses of each indispensable amino acid with an efficiency of 70%. This value is assumed from N balance data in adults obtained from studies carried out in our laboratories and by others (e.g. FAO/WHO/UNU 1985).

An additional impetus to explore this approach comes from an appreciation for the importance of the oxidative catabolism of amino acids in determining the quantitative needs for specific amino acids. For example, from the recent amino acid kinetic studies by Thompson et al. (1990a,b) in patients with maple syrup urine disease (MSUD) it was observed that, despite significant elevations of plasma leucine in MSUD subjects, mean rates of whole body proteins synthesis and catabolism were similar to control values as also were their growth rates. However,

leucine oxidation rates in the MSUD subjects were about 5 to 10 times lower than the rates expected in healthy subjects who receive adequate, but not excessive, intakes of leucine. These findings appear, to us, to be a convincing demonstration that the status of oxidative catabolism is a primary determinant of the requirement value for the indispensable amino acid since the leucine need by MSUD patients is low, compared to that for the healthy. Further, Ruch and Kerr (1982) state "...obligatory catabolism constitutes a significant fraction of the normal infant requirement for essential amino acids and that loss of specific catabolic pathways due to enzymatic deficiency results in decreased requirements."

Therefore, we have estimated obligatory oxidative losses (OOL) and minimum dietary intakes to balance these in adult subjects (Table 5). As can be seen, these predicted requirement intake levels are two-to-three times those accepted by FAO/WHO/UNU (1985) which, and not incidentally, represent the upper range of the minimum requirement for this age group.

Table 5. Obligatory oxidative losses (OOL) of amino acids and intakes required to balance those in adult subjects

| Amino Acid | OOL | Intakes for balance (A) | FAO/WHO/UNU (1985) (B) $mg.kg^{-1}day^{-1}$ | Ratio A/B |
|---|---|---|---|---|
| Isoleucine | 16 | 23 | 10 | 2.3 |
| Leucine | 27 | 39 | 14 | 2.8 |
| Lysine | 30 | 42 | 12 | 3.5 |
| SAA | 13 | 16 | 13 | 1.2 |
| AAA | 27 | 39 | 14 | 2.8 |
| Threonine | 15 | 21 | 7 | 3.0 |
| Tryptophan | 4 | 6 | 3.5 | 1.7 |
| Valine | 17 | 24 | 10 | 2.4 |

Millward et al. (1990), have criticized our approach and in doing so stated "...it can be in no way justifiable to take values for the OOL as the basis for a new requirement pattern as suggested recently by Young et al. (1989)" However, we believe that there is reasonable justification for our approach and, further, that the evidence used to make the criticism developed by Millward et al. (1990) has its own significant limitations. These have been discussed by us in a recent review article (Young et al. 1992) and so I will not repeat the detailed arguments here, except to say that (i) the amino acid requirements predicted from OOL agree well with those determined from metabolic studies in 2-year old children, giving some further reason to beleive that the predicted requirements for the adult are probably reasonably sound values. (ii) the pattern of the amino acid requirements derived from metabolic studies in young children is similar to the average amino acid composition of the mixed proteins in the body. Furthermore, as in the case of the adult, the requirement is due largely to maintenance and only minimally to a growth component. (iii) the arguments made by Millward et al. (1990) against our use of OOL for, at least, an initial prediction of amino acid requirements are based on their consideration of the amino acid requirements for maintenance and growth in young pigs. The validity of Millward's approach may be questioned due to major differences in the quantitative characteristics of nitrogen and amino acid nutrition in the growing pig as compared with humans beyond very early infancy (see Young et al. 1992). We conclude, based on these various lines of evidence and argument, that the prediction of adult amino acid requirements from OOL has some merit. It would be desirable, therefore, to seek further support for the conclusion drawn, from these predictions (Table 5), that the adult human amino acid requirements are two-to-three times higher, except for the S-amino acids, than those based on the

earlier N-balance studies of Rose (1957) and many subsequent investigators using a similar approach, as well as those requirement figures presented in the report of FAO/WHO/UNU (1985).

### (g) $^{13}$C-tracer studies for estimation of amino acid requirements

Investigations with laboratory and farm animal species have shown that it is possible to estimate amino acid requirements from use of radioactively labeled tracers studied under various experimental dietary conditions. Thus, also, we have undertaken a series of studies, involving use of stable nuclide labeled amino acids, to determine in vivo rates of amino acid oxidation in healthy adult subjects given defined dietary treatments. Our experiments were conducted on the premise that a direct measure of the oxidation of a specific indispensable amino acid, coupled with a determination of the minimal intake required to balance this oxidation, in well-nourished, normal individuals would yield a more satisfactory estimate of the minimum physiological requirement for the amino acid than is possible from classical N-balance measurements.

In considering the design of these studies we thought that relatively short-term experimental dietary periods would provide the best initial means of arriving at a minimal, physiological requirement value. Our reasoning has already been presented and detailed aspects of design and methodological approach have been described (e.g. see Young et al. 1989). Briefly, our studies have been carried out in healthy adult men, who received isonitrogenous, L-amino acid diets, containing graded levels of a test amino acid, each for 5-7 day experimental periods. At the end of each diet period a $^{13}$C-tracer study was conducted with a subsequent estimate being made, from the isotopic data, of the whole body for the test amino acid balance.

Table 6 summarizes the estimates of the amino acid requirements that have been generated from our $^{13}$C-tracer experiments and compares these values with those predicted from 00L. In view of the difficulties encountered in the design, conduct and interpretation of amino acid kinetic studies, and the reasonable, but not necessarily, precisely accurate assumptions concerning amino acid efficiency and retention, there is a remarkably good agreement between the estimated requirement levels for these five amino acids as judged from these two new approaches. Furthermore, given the close agreement between the requirement estimations based on the 00L and $^{13}$C-kinetic methods, we feel entirely justified in stating that the much lower requirement figures previously accepted by FAO/WHO/UNU (1985), and Williams et al. (1974) must be viewed with considerable caution. Indeed, from this analysis, the case for the inadequacy of current national and international amino acid requirement figures appears, to us, to be convincing and that our two new methods based on amino acid kinetic data, described above, provide a more accurate estimate of the probable minimum, physiologic amino acid needs in healthy adults.

Table 6. Comparison of $^{13}$C-amino acid derived estimates of requirements in adults

| Amino acid | 13C-tracer[1] | Predicted (OOL)[1] | FAO/WHO/UNU 1985* |
|---|---|---|---|
| | mg.kg$^{-1}$day$^{-1}$ | | |
| Leucine | 40 | 39 | 14 |
| Valine | 20 | 24 | 10 |
| Lysine | 30 | 42 | 12 |
| Threonine | 15 | 21 | 7 |
| Met (-Cys) | 13 | 16 | 13 |

1. Approximate mean requirement.
* Upper range of requirement (from FAO/WHO/UNU, 1985).

There are a number of issues concerned with experimental design and interpretation of our tracer data that need to be considered in relation to these $^{13}C$-derived estimates of amino acid requirements. Several of these issues and problems had been raised by Millward and Rivers (1988) and we have evaluated them in some detail elsewhere (e.g. Young 1989; Young et al. 1992; Young and Marchini, 1990). In summary, however, we do not accept that the major concerns raised by Millward and Rivers (1988), including the question of the sparing effects of dietary non-essential nitrogen and of the experimental creation of amino acid imbalances (Harper et al. 1970) are problems that seriously confound the interpretation of our studies. For example, two major experiments have now been carried out in our laboratories (Pelletier et al. 1990a,b) to address the question of a dietary amino acid imbalance, Our findings lead us to the conclusion that the pattern of BCAA intake, within the physiological range tested, does not affect the isotopically derived estimates of requirements for either leucine or valine. Thus, "amino acid imbalances" created by the dietary protocol that we have used to determine the requirements for the BCAAs do not appear to have any important, or confounding, effects on our estimations. We recognize, however, that there are metabolic interactions between the BCAAs and they, of course, should not be ignored, including interactions between the BCAAs in relation to their influx and efflux in the brain and other tissues such as muscle. Indeed a possible index of the adequacy of leucine intake is the minimum level of intake that maintains a normal plasma amino acid profile and, under the conditions of our tracer studies, this occurs at leucine intake of ~30-40 mg $kg^{-1}d^{-1}$ and above (Meguid et al. 1986; Young et al. 1987).

Second, we (Hiramatsu T, Young VR, unpublished results) have recently completed an extensive study designed to assess whether the level of dietary non-specific nitrogen, within a reasonably defined, or physiologic, range of intake, affects the $^{13}C$-derived estimates of the leucine requirements in healthy adult men. We conclude from this investigation that the differences between our estimates and those of Rose (1957) cannot be explained, as has been proposed by Millward and Rivers (1988), on the basis of the NSN component of the experimental diets.

### (h) Short-term studies and criterion of adequacy

Another possible limitation of our $^{13}C$-kinetic investigations is that they involved relatively short-term metabolic studies. Nevertheless, we believe that we have provided a persuasive argument for the proposition that the minimum physiological requirements for the indispensable amino acids are probably close to the values predicted from 00L and $^{13}C$-tracer amino acid oxidation studies. The endpoint, or criterion of adequacy, used was the minimum intake required to achieve a balance of the test amino acid. We have not attempted, in our studies, to identify a so-called "breakpoint" on the intake-amino-acid-oxidation response curve as a criterion, in a manner analogous to that used in studies of the amino acid requirements in pigs (e.g. Kim and Bayley 1983; Ball and Bayley 1984), for example.

Recent human studies by Zello et al. (1990) are relevant, however. Using $^{13}C$-phenylalanine and an experimental diet that provided an excess of tyrosine together with graded levels of phenylalanine, they observed a "breakpoint" type of relationship, as shown in Figure 7. From this, Zello et al. (1990) attempted to estimate the requirement for total aromatic amino acids (phenylalanine and tyrosine), assuming that at an excess intake of tyrosine there is a 70% sparing in the requirement for phenylalanine. Hence, these investigators suggest that the aromatic amino acid requirement is about 30 mg $kg^{-1}day^{-1}$. This requirement level is in reasonably close agreement with that which we have proposed (Young et al. 1989) but considerably higher than the FAO/WHO/UNU (1985) figures.

Hence, the investigation by Zello et al. (1990) supports the nutritional interpretation that we have given to our $^{13}C$-tracer studies, as discussed above. Unfortunately, there are few other investigations to which we can refer in the published literature for purposes of evaluating the validity, or otherwise, of our newer estimates of the amino acid needs (Table 7) in the healthy adult.

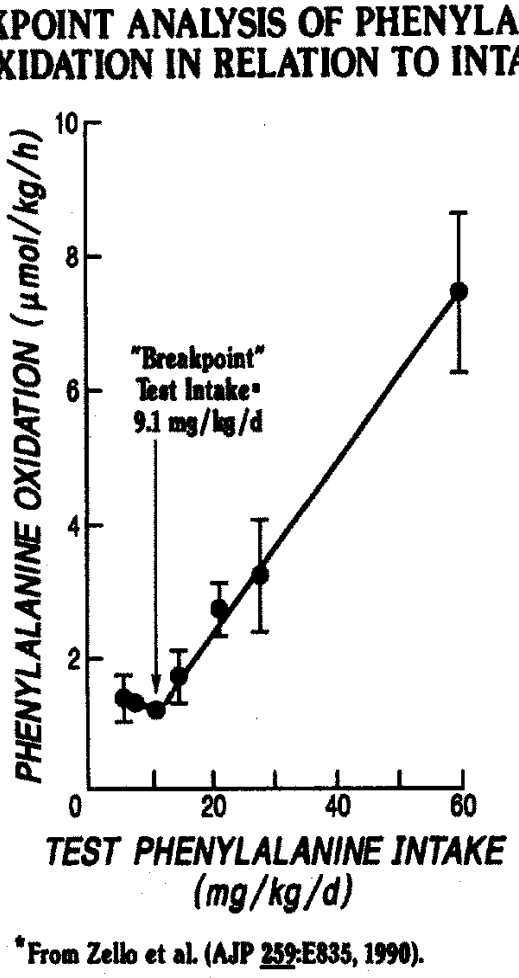


Figure 7. Relationship between L-[1-$^{13}$C]phenylalanine oxidation and seven test meals providing different phenylalanine intakes.
From Figure 1 in Zello et al. (1990), where the "breakpoint" was determined using a two-phase linear regression cross-over mode.

Basically, our $^{13}$C-tracer studies have attempted to avoid problems of the insensitivity and imprecision of the N balance data method and the conceptual problems surrounding the interpretation of N balance data. Nevertheless, our more direct studies of acute alterations in amino acid oxidation and balance with altered intakes still face difficult problems, particularly the unrealistically high balances at amino acid intakes that are substantially above the estimated minimum physiological requirement intake levels.

Finally, we wished to obtain additional evidence that our new estimates of the amino acid requirements, based on short-term experimental dietary periods, are valid and, therefore, represent intakes capable of supporting the longer-term maintenance of body protein homeostasis. Conversely, we were interested in determining that the FAO/WHO/UNU (1985) requirement values would neither permit body amino acid balance nor sustain adequate rates of whole body protein turnover. Hence, a major study has been undertaken in our laboratory (J.S. Marchini, J.C. Cortiella, T. Hiramatsu and V.R. Young (in preparation)) to test these hypotheses and we are still in the process of completing the analyses and evaluation of these data.

Table 7. Tentative, amino acid requirement estimates for the adult and corresponding requirement pattern for the pre-school child

| Amino Acid | Adult Tentative Requirement[1] (mg/kg/day) | Adult Amino Acid Pattern[2] (mg/kg/day) | 1985 FAO/WHO/UNU Pre-School Child Pattern[3] (mg/g protein) |
|---|---|---|---|
| Isoleucine | 23 | 35 | 28 |
| Leucine | 40 | 65 | 66 |
| Lysine | 30 | 50 | 58 |
| Total SAA[4] | 13 | 25 | 25 |
| Total AAA[5] | 39 | 65 | 63 |
| Threonine | 15 | 25 | 34 |
| Tryptophan | 6 | 10 | 11 |
| Valine | 20 | 35 | 35 |

1. From Young et al (1989).
2. Values rounded to nearest 5.
3. FAO/WHO/UNU (1985)
4. Sulfur amino acids.
5. Aromatic amino acids.

However, our preliminary findings lead to the following tentative conclusions: (i) the FAO/WHO/UNU (1985) amino acid pattern and intake level is not adequate to maintain protein homeostasis in healthy adults and (ii) the MIT amino acid requirement pattern (Table 6) maintains various parameters of leucine kinetics (balance, turnover) during a three-week experimental diet period. Thus, these results provide additional, and substantial, support for our newer requirement figures based on considerations of amino acid kinetics. Furthermore, they strengthen our argument that the "traditional" estimates of adult amino acid requirements should now be abandoned, particularly with respect to judging the adequacy and/or considering the design of therapeutic diets.

## II. SUMMARY AND CONCLUSIONS

It has been my purpose to provide some examples of investigations that we have carried out and involving use of stable isotope tracer techniques in an attempt to provide a more complete, quantitative description of the physiology of amino acid in the human subject. Our studies were initiated after we became aware of the pioneering stable-isotope tracer studies by Waterlow and collaborators (e.g. see Waterlow et al. 1978 for review), who were working in Jamaica at that time, and coupled with our dissatisfaction, based on a series of ongoing N-balance investigations, of the nutritional dogma that there was a quantitatively, profound decline in the proportion of indispensable amino acids required per unit of the total protein need in humans as they progress from age about 2 years to metabolic maturity in adult life.

It is hoped that the choice of the foregoing examples of studies of kinetics of amino acid metabolism have given the reader a useful, and possibly informative, view of the importance of studying in vivo aspects of metabolism with the aid of these techniques, particularly in terms of how they can contribute to basic knowledge of human physiology as well as for assisting in the resolution of practical nutritional problems.

Acknowledgements

I am deeply grateful to Sunrice, Australia and the Nutrition Society of Australia (NSA) for providing me with the financial resources so that I could deliver the talk, on which this article is based, at the 1991 annual meeting of the NSA. The author's unpublished results were obtained with the support of NIH grants DK 15856 and DK 42101.

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