Introduction

An improved definition of the requirements for nitrogen and indispensable amino acids in human protein nutrition depends upon a better understanding of the mechanisms responsible for, and nature of, the integration of protein and amino acid metabolism at the whole body level. Although the molecular and cellular events concerned with gene expression (e.g. 1, 2), the formation of tissue and organ proteins (3, 4) and their subsequent breakdown (5, 6) have been outlined, to some extent in great detail, in recent years, the relationships between these molecular, subcellular and organ-specific processes and the maintenance of nutritional status of the intact host remain to be vigorously explored. It is crucial that these relationships be defined if knowledge of protein and amino acid nutrition is to extend much beyond a stage that might be characterized, at present, as being rather empirical and, at times, almost naive. For this reason our research during the past few years has attempted to develop a more complete picture of the organization of whole body protein and amino acid metabolism and how it is affected by various nutritional and non-nutritional factors (e.g. 7–11).

In this invited paper, some recent work that we have undertaken at MIT, also in collaboration with others, will be reviewed in order to explore, in greater depth, the physiology of body protein and amino acid metabolism in adult human subjects. Our purpose has been to try to determine how the nitrogen economy of the host is maintained under differing nutritional and physiological conditions.

At the outset, it should be mentioned that although our work has been stimulated by many investigators a few of them deserve particular recognition; the pioneering work of Waterlow and his colleagues, conducted initially in Jamaica and...
then in London (e.g. 12, 13), of Munro (e.g. 14, 15), earlier in Scotland and later in
the U.S., and of Scrimshaw (e.g. 16, 17) in Guatemala and MIT created much of the
excitement that I have for the topics discussed below. Furthermore, the meticulous
balance studies by, and nutritionally significant findings of, Calloway and
Margen (e.g. 18) in Berkeley, California and Inoue and his co-workers (e.g. 19–21)
in Tokushima also greatly stimulated my desire to explore a further understanding
of human protein metabolism. Our hope has been that this would significantly
increase our capability of defining protein nutritional status, on the one hand, and
the dietary requirements for nitrogen and amino acids, on the other hand.

Desirability of using stable isotope probes

Much of the current knowledge of human protein and amino acid nutrition has
been gained from studies based on the use of the nitrogen (N) balance technique.
However, following Schoenheimer’s classic studies on the turnover of body
constituents (21, 22), it became widely accepted that a major proportion of total
body protein undergoes continuous synthesis and breakdown. For this reason, the
balance between the anabolic and catabolic phases of protein and amino acid
metabolism determines cell and organ protein content and, in turn, the nutritional
requirement for protein. Furthermore, protein synthesis and breakdown and the
metabolism of individual amino acids are affected by various factors and regulated
by specific control mechanisms. Also a complex biochemical organization provides
cells with a capacity to change the amount and type of protein made in order to best
meet a particular environmental condition. However, it follows that the body N
balance can be achieved within a wide range in the rates of organ protein synthesis
and breakdown. Thus, although N balance estimations can be useful in the study of
protein and amino acid nutrition (24) these measures do not provide a detailed
picture of the status of protein and amino acid metabolism within the body; they only
indicate the net balance between the rates of protein synthesis and breakdown.

The major pathways of amino acid metabolism involve protein synthesis and
breakdown, the formation of various N-containing compounds, such as nucleic
acids, the dispensable amino acids and creatine, and finally, those associated with
amino acid catabolism (Fig. 1). These three major pathways may either change in
parallel or in reciprocal ways (25), depending upon the particular tissue, dietary
factor(s) involved and/or nutritional health status of the host. Hence, to better
understand the ways by which whole body nitrogen and amino acid homeostasis is
achieved in healthy humans under various nutritional states and in disease, we have
used an isotopic tracer approach proposed and refined by Waterlow and his
colleagues (13). The long-term objective of this research is to develop more
appropriate diagnostic tests for determination of protein and amino acid require-
ments in healthy humans and in those who suffer illness.

It is necessary to resort to tracer methods, involving use of isotopically-labeled
compounds, in order to explore dynamic aspects of nitrogen amino acid metabolism
in the whole organism. The most extensively used labels have been the radioactive

isotopes $^3$H and $^{14}$C. However, with increased ethical considerations regarding studies in humans, and the stringent, though necessary, regulation of all human and clinical investigations, non-radioactive tracers, such as $^2$H, $^{13}$C, $^{15}$N and $^{18}$O offer a particularly attractive research tool for study of protein and amino acid metabolism in infants, children, women of child-bearing age and healthy adults.

There has been a rapidly growing use of stable isotopes in metabolic research, including human studies, during the past 10 years (26, 27) although, the discovery of the biologically significant stable isotopes goes back more than 50 years when Aston (28) described the stable isotopes of sulfur with a new mass spectrometer. Within the next 5 years the heavy isotopes of carbon, oxygen, nitrogen and hydrogen were discovered in the approximate order their abundance (26). Soon thereafter, studies were carried out by Schoenheimer and his collaborators (22, 23), utilizing $^{15}$N-labeled compounds, to explore whole body protein turnover and amino acid metabolism in the intact organism. These experiments set the stage for a re-awakening of interest in the study of dynamic aspects of protein and amino acid metabolism in humans. Increased production and availability of stable isotopes and compounds labeled with them (26, 29) coupled with advances, in techniques permitting convenient, practical determinations of isotope enrichment in biological samples by combined gas-chromatography, mass-spectrometry (e.g. 30, 31), now offer the reality for exploring many exciting and unresolved problems of the physiology of amino acid and protein metabolism in people.

Approaches taken with stable isotopes

Various approaches and labels might be followed in studies of human protein and amino acid metabolism (13). However, our work has concentrated on use of a method of continuous isotope infusion, that has been explored extensively by Waterlow and co-workers (13), in an attempt to avoid some of the difficulties inherent in the earlier, single isotope dose approaches. The continuous infusion of tracer may either be coupled with measurement of end products of N metabolism, as for the case of Picou and Taylor-Roberts model (32), or with determination of the enrichment of isotope ($^{15}$N or $^{13}$C) in plasma following administration of a labeled...
Fig. 2. General model of whole body amino acid metabolism as studied by the application of a continuous, stable isotope infusion approach. The author's studies have involved use of $^{15}$N-glycine, $[^{2}-{^{15}}$N]lysine and $[1-{^{13}}$C]leucine as well as other tracers, as indicated in the text.

Fig. 3. Model of whole body leucine metabolism studied using a continuous infusion of $[1-{^{13}}$C]leucine. An isotopic steady-state in the plasma leucine pool and in expired CO$_2$ can be rapidly achieved using priming doses of $^{13}$C-leucine and sodium bicarbonate-$^{13}$C, as described by Matthews et al. (34).

At plateau: 

$$d = \text{plateau enrichment of Leucine} \times \text{Flux}$$

$$\text{Flux} = \text{Leucine oxidized} + \text{Leucine for protein syn.}$$

$$= \text{Leucine intake} + \text{Leucine from protein breakdown}$$

Amino acid or precursor ($e.g.$ 31, 33, 34). When this latter approach is combined with measurements of $^{13}$C in expired CO$_2$ or $^{15}$N in urinary urea or other metabolic or excretory products the components of whole body amino acid flux can be determined. These are incorporation of amino acids into and release from proteins and conversion to intermediate- or end-products of their metabolism.

The constant isotope infusion model, involving measurement of stable isotope enrichment in plasma and in excretory products, views body N metabolism according to a metabolic pool from which amino acids leaves via pathways of protein anabolism or via oxidative catabolism (Fig. 2). The major inflow of nitrogen (or amino acids) into the metabolic pool is via the diet and/or via breakdown of cell
Fig. 4. Model used to estimate whole body flux and de novo synthesis of a dispensable amino acid, such as alanine or glycine.

and tissue proteins. These major routes of amino acid and N flow can be quantified by administration of specifically labeled, indispensable (essential) amino acids and measurement of the dilution of label in the free amino acid in blood plasma and its appearance in excretory products, such as expired air ($^{13}$CO$_2$) or urinary nitrogen constituents ($^{15}$N in urea, ammonia or total N) (34, 35). For example, the specific model, based on $[1-^{13}$C$]$leucine, but described earlier by Golden and Waterlow (36) for use with $^{14}$C-leucine, that we have used for measurement of plasma leucine flux and its major components (rates of leucine oxidation, incorporation into body protein and entry into the metabolic pool via protein breakdown and from dietary sources) is shown in Fig. 3. This represents a form of stochastic analysis in which the overall process is measured and not the intermediate pools and/or their exchanges. The model has also been applied in our studies of the metabolism of valine (37) lysine (11) and, more recently, in continuing studies with threonine. We have also used this approach for study of the metabolism of dispensable amino acids, such as glycine and alanine (e.g. 38). In this case, the extensive contribution made to the whole body flux of the amino acid due to de novo synthesis must also be determined. This can be achieved with a simultaneous infusion of the labeled dispensable amino acid and an indispensable amino acid, such as $^{13}$C-carboxyl-labeled leucine or lysine. In this way, as depicted in Fig. 4, it is possible to determine the inflow of the dispensable amino acid into plasma that arises from its liberation during protein breakdown. If the exogenous intake of the test amino acid is known, then the rate of synthesis of new molecules of the amino acid can be computed. Thus, the comparative aspects of metabolism of the various dispensable and indispensable amino acids can be explored under various nutritional status, as discussed below. Details of these models and the assumptions involved in their application have been discussed extensively by Waterlow et al. (13), and in recent reports from our group (e.g. 34, 35, 37).
Some applications of stable isotope labels

i) Responses of amino acid metabolism to dietary protein and energy intakes

The supply of amino acids and the availability of high energy intermediates (ATP and GTP) required for various stages in the synthesis of polypeptides and their subsequent breakdown affect the status of cellular protein and amino acid metabolism. It is not surprising, therefore, that body nitrogen metabolism is highly sensitive to altered dietary intakes of either protein and/or energy (e.g. 21, 39-41). Thus, N balance becomes less positive and then more negative as protein intake is reduced and falls below the requirement level. Furthermore, for a given intake of dietary protein, N balance is affected by the level and source of dietary energy (14, 39, 40). Among the more significant human N balance studies concerned with the responses to changes in protein and energy intakes in adult subjects are those of Inoue and his collaborators (20, 21), referred to earlier.

Stable isotope probes have been used to explore the whole body mechanisms that might account for changes in N balance and alterations in the efficiency of dietary N retention to these variable levels of protein and energy intake. Thus, we (42) have carried out a study to examine changes in whole body leucine and lysine metabolism in young men to alterations in dietary protein intake, using a primed constant infusion protocol, involving [1-13C]leucine and [α-15N]lysine given simultaneously. In this experiment, three protein intake levels were studied 1.5, 0.6, and 0.1 g egg protein kg⁻¹·day⁻¹. The 0.6 g protein kg⁻¹·day⁻¹ intake level was chosen to represent an average maintenance requirement level for healthy young men (42, 43) and to serve as a reference level of protein intake against which changes in leucine and lysine metabolism to increases or decreases in protein intake could then be evaluated. Whole body leucine and lysine fluxes showed a decline (Fig. 5) with reduced protein intake, although the actual fluxes were lower for lysine than for leucine, especially at the high protein intakes.

![Fig. 5. Comparison of whole body fluxes of leucine and lysine in young men, studied while in the fed and fasted (post-absorptive) states for three levels of protein intake. Drawn from data of Motil et al. (42).](image-url)
A summary of the changes in the components of leucine flux (oxidation, outflow of leucine from the metabolic pool into proteins and inflow via protein breakdown) with these alterations in protein intake is shown in Fig. 6. Rates of oxidation and incorporation of leucine into body proteins changed with altered protein intakes. For the post-absorptive state, an increase in dietary protein, from the requirement to

![Bar charts showing rates of leucine oxidation, release, incorporation, and percentage of flux under different protein intakes.](image)

Fig. 6. Rates of leucine oxidation, incorporation into body proteins and its release by protein breakdown for young men studied in the fed and fasted states after receiving an adequate or usual (1.5 g·kg⁻¹·day⁻¹), a marginal or requirement (0.6 g·kg⁻¹·day⁻¹), or a minimal or low (0.1 g·kg⁻¹·day⁻¹) level of protein intake. Drawn from data of Motil et al. (42).
generous intake level, was associated with an increased incorporation of leucine into body proteins, accounting for 100% of the change in flux. During the fed condition, leucine oxidation was greatly increased, together with an increased rate of leucine incorporation into tissue proteins. On the other hand, reducing the protein intake from the requirement to an inadequate level was associated with a small decline in the oxidation rate of leucine but a marked change in the rates of leucine incorporation into and release from body proteins.

These responses are consistent with observations showing higher rates of oxidation of indispensable amino acids when consumed in excess of needs of growth (44-46) or for maintenance in adult (47) rats. Our findings also suggest that more than one mechanism is responsible for the adaptive responses of whole body leucine metabolism and of body N balance to changes in dietary protein intakes. Furthermore, it appears that the mechanisms are related to the total nitrogen and amino acid needs of the host. Thus, at generous and excess levels of protein the major change observed in leucine metabolism is one of a marked increase in oxidation, seen especially in the fed state. On the other hand, within the sub-maintenance-to-maintenance range alterations in dietary protein intake bring about changes in leucine metabolism that are principally related to a reduced body protein turnover.

These dietary-induced changes in whole body leucine kinetics can be modulated by the energy status of the host and the level of energy intake. For example, we (48) have also examined the possible basis for changes in N balance when energy intakes exceed requirements (20, 21). For this purpose, we chose a dietary protein intake level of 0.6 g·kg\(^{-1}\)·day\(^{-1}\) to explore responses of whole body leucine and lysine metabolism to excess intakes of dietary energy from the different energy sources. Maintenance energy intakes averaged 44 kcal·kg\(^{-1}\)·day\(^{-1}\), comparable to an adequate energy intake for healthy adult men (49). The excess level supplied at 25% increment above the maintenance need.

As shown in Table 1, nitrogen balance improved with excess dietary energy intake and the response was somewhat greater for those diets containing an excess of carbohydrate (48). Furthermore, when the small changes in whole body leucine kinetics in response to excess energy are used to compute net leucine retention (leucine incorporation into proteins minus leucine liberated by protein breakdown) this parameter was found to be higher, for the fed state, when dietary energy intake exceeded maintenance needs (Table 1). In addition, diets containing a relatively higher carbohydrate content were associated with a greater change in net leucine retention than those based on fat as the source of additional energy (48). It appears, therefore, that the enhanced N balance at excess energy intake is a consequence of the summated, but individually small, changes in amino acid oxidation and rate of amino acid incorporation into and its release from body proteins.

These various findings reveal that the major pathway of leucine metabolism are affected by alterations in both the dietary protein and energy level. Furthermore, these pathways respond in a co-ordinated way to promote whole body leucine metabolism.
Table 1. Nitrogen balance and whole body leucine kinetics in young adult men receiving maintenance or excess level of dietary energy.a

<table>
<thead>
<tr>
<th>Measure</th>
<th>Maintenance</th>
<th>Excess</th>
</tr>
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<tbody>
<tr>
<td>N balance (mg·kg⁻¹·day⁻¹)</td>
<td>-20 ± 3</td>
<td>+2 ± 2</td>
</tr>
<tr>
<td>Leucine kineticsb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-absorptive state</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flux</td>
<td>94 ± 3</td>
<td>94 ± 3</td>
</tr>
<tr>
<td>Oxidation</td>
<td>16 ± 2</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>Retention</td>
<td>-12 ± 2</td>
<td>-11 ± 2</td>
</tr>
<tr>
<td>Fed state</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flux</td>
<td>102 ± 2</td>
<td>101 ± 3</td>
</tr>
<tr>
<td>Oxidation</td>
<td>18 ± 2</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>Retention</td>
<td>22 ± 3</td>
<td>27 ± 3</td>
</tr>
</tbody>
</table>

aSummarized from Motil et al. (48). bValues are expressed as μmol·kg⁻¹·hr⁻¹. Mean ± SEM for 12 subjects.

Fig. 7. Whole body glycine flux and rate of de novo glycine synthesis in young adult and elderly men adapted to diets providing generous (1.5 g protein·kg⁻¹·day⁻¹) or low protein (0.4 g protein·kg⁻¹·day⁻¹) intake. Drawn from data of Gersovitz et al. (38).

homeostasis under various nutritional conditions.

The responses of the metabolism of the dispensable amino acids are also important in considering the maintenance of body nitrogen economy under various dietary conditions. Therefore, using the model outlined above, we have also determined that glycine flux and de novo synthesis (Fig. 7) are reduced in healthy
Fig. 8. Rate of whole body de novo alanine synthesis in young men, studied in the post-absorptive states, after receiving for 7 days diets that supplied the various levels of protein indicated. Unpublished data of Yang et al.

Fig. 9. A schematic of possible associations between alanine, glucose and nitrogen metabolism in intact man, with an indication of the roles played by skeletal muscles and the liver in these interrelationships.

Young and elderly men when a diet supplying a protein intake level that is lower than the maintenance requirement is consumed (38). In contrast to these changes in glycine metabolism, the rate of formation of alanine is not reduced when protein intake is restricted within intake ranging from a generous to an essentially protein-free level. Indeed, as depicted in Fig. 8, alanine synthesis is apparently higher at lower protein intakes. Furthermore, ingestion of meals stimulates formation of alanine (Fig. 8), probably due to the intake of the major energy yielding nutrients (carbohydrate).

In the view of the significance of alanine metabolism in whole body glucose (50) and in nitrogen metabolism (51, 52) (e.g. Fig. 9) further studies on the metabolism of this amino acid under various dietary conditions would be desirable. Hence, we

Table 2. Alanine and glycine fluxes and de novo synthesis rates in post-absorptive young men.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Alanine</th>
<th>Glycine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flux</td>
<td>381 ± 26(^b)</td>
<td>240 ± 22</td>
</tr>
<tr>
<td>From protein breakdown</td>
<td>104 ± 4 (28 ± 3)</td>
<td>96 ± 12 (40 ± 3)</td>
</tr>
<tr>
<td>de novo Synthesis</td>
<td>277 ± 29 (72 ± 3)</td>
<td>144 ± 13 (60 ± 3)</td>
</tr>
</tbody>
</table>

\(^a\) From Robert (53). \(^b\) Mean ± SEM; \(\mu\)mol·kg\(^{-1}\)·hr\(^{-1}\). Numbers in parenthesis are percent of flux.

Fig. 10. Change in plasma glucose and in insulin concentrations in post-absorptive young men during an intravenous infusion of glucose at 4 mg·kg\(^{-1}\)·min\(^{-1}\). Unpublished MIT data of Robert et al.

have examined alanine and glycine metabolism in another study in young men while they were in the post-absorptive state and the response to an intravenous glucose infusion (53). This study was also performed with the aid of a continuous administration, by vein, of the amino acids labeled with \(^{15}\)N or \(^{2}\)H, with application of the model described above. Thus, by combining infusions of labeled alanine or glycine with \([1-13\text{C}]\)leucine or \([1-13\text{C}]\)lysine, respectively, to estimate the rate of body protein breakdown, we have estimated the de novo rates of synthesis of each of these dispensable amino acids. As summarized in Table 2, the synthesis of new alanine and glycine molecules during post-absorption accounts for about 60–70% of the whole body fluxes of the amino acids.

When glucose was infused by vein at a rate of 4 mg·kg\(^{-1}\)·min\(^{-1}\), which resulted in a significant hyperglycemia and increased plasma insulin levels (Fig. 10), the alanine flux was significantly increased, but glycine flux did not change; and this rise was entirely due to an enhanced rate of synthesis of new alanine molecules (Fig. 11). The mean increase in alanine synthesis was 101 \(\mu\)mol·kg\(^{-1}\)·hr\(^{-1}\) and if derived entirely from glucose, it would account for an additional conversion of 50 \(\mu\)mol of the infused glucose. This represents about 7% of the additional glucose disposal when glucose is administered at a rate of 4 mg·kg\(^{-1}\)·min\(^{-1}\) (53).
Fig. 11. Fluxes of alanine and glycine and their rates of de novo synthesis before and after an intravenous infusion of 4 mg·kg⁻¹·min⁻¹ of glucose in young men. Unpublished MIT data of Robert et al.

Fig. 12. A speculative outline of the metabolic relationships between alanine, glucose and urea metabolism in the normal state and during conditions of stress. It is hypothesized that this latter condition, associated with increased glucose turnover and urea N output, is consequent to an enhanced alanine synthesis.

These findings raise the speculation that a hyperglycemic state, coupled with a moderate increase in circulating insulin, might drive the synthesis of alanine in, and its subsequent release from, skeletal muscles and possibly other tissues, such as the intestine. If this occurs under conditions where the coordinated regulation of metabolic pathways may be altered, such as in stress or disease, the increased rate of alanine synthesis could lead to a depletion of N in muscles, and the body as a whole.
if the N from alanine is subsequently transferred to urea during its metabolism in the liver (51). This speculation is schematically depicted in Fig. 12.

Possibly, therefore, the hyperglycemia typical of injury or sepsis (54), coupled with an enhanced rate of glucose disappearance from plasma (55), might be of causal significance in the wasting of body N under stressful conditions (56) and the increased protein requirement. If this hypothesis is valid, therapy aimed at reducing alanine formation might improve the N balance status of patients exposed to these unfavorable situations.

These findings have been discussed in greater detail elsewhere (57) but an exploration of our hypothesis concerning the possible involvement of alanine metabolism in the causation of stress-induced body N losses, should be expected to provide a better understanding of the impact of disease conditions on the mechanisms responsible for maintenance of body N economy in the normally healthy individual.

ii) Specific protein metabolism

A plasma protein of particular interest in the biochemical evaluation of protein nutritional status is albumin. Whereas the catabolism of this protein has been measured relatively simply by injection of radioiodinated albumin, the rate of synthesis in man has been evaluated since 1963 by a procedure involving administration of a pulse dose of 14C-bicarbonate or 14C-guanidinoarginine (e.g. 58) which labels both the urea and the albumin synthesized in the liver. The labeling of urea was, therefore, considered to provide a measure of the precursor-free arginine pool in the liver, where albumin synthesis occurs. Therefore, we recently adapted these concepts for application of a stable isotope procedure to estimate albumin synthesis in humans. Our method involves labeling with 15N-glycine administered orally every 3 hr as a donor of 15N for liver free arginine (59). This method follows the nitrogen enrichment of the guanidine group of albumin bound arginine and monitors 15N-urea in the urine at isotopic steady state, as an index of the enrichment of the liver free arginine pool. As shown in Fig. 13, a progressive labeling of the arginine in serum albumin occurred with administration of 15N-glycine and this labeling could be related to the plateau level of enrichment of urinary urea to provide a measure of the albumin synthesis rate.

Applying this method in young adult and elderly male subjects, we have found that the fractional synthesis of the albumin pool is only slightly and insignificantly different in the two age groups (Fig. 14). However, albumin synthesis in the elderly was not affected by a reduced intake of dietary protein as compared with the response to lowered protein intake in the young. This implies that only the younger subjects are able to respond to increased protein N amino acid intake and that there is an upper rate of albumin synthesis limited by a set-point beyond which amino acid supply cannot stimulate it further. Based on this conclusion, it would appear that the concentration of serum albumin in elderly subjects is maintained at a lower level than in younger subjects because of a lower set-point. This would explain why elderly subjects fail to show an increment in serum albumin synthesis in response to
Fig. 13. Enrichment of arginine in serum albumin and in urinary urea in an adult subject receiving oral doses, at 3-hr intervals, of $^{15}$N-glycine. From Gersovitz et al. (59).

Fig. 14. Rate of albumin synthesis in young adult and elderly men receiving a generous or low intake of dietary protein. Drawn from data of Gersovitz et al. (59).

Increasing dietary protein intake, whereas albumin synthesis in young subjects is responsive to this dietary change. This hypothesis, based on observations made in a small population of elderly subjects, must be explored further in large groups of subjects before the metabolic and nutritional significance of our findings on albumin metabolism in young adult and older subjects can be more completely assessed.

Table 3. Rates of protein synthesis, expressed as amino acid incorporation, in young men receiving L-amino acid diets providing a generous or "requirement" intake of specific amino acids.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Intake level</th>
<th>Generous</th>
<th>&quot;Requirement&quot;</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>µmol amino acid incorporated kg⁻¹·hr⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>108 ± 1a (100)</td>
<td>49 ± 5 (12)</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>99 ± 6 (68)</td>
<td>46 ± 2 (12)</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>89 ± 8 (79)</td>
<td>59 ± 4 (12)</td>
<td></td>
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</table>

a Mean ± SEM for rate of incorporation into whole body protein of the amino acid indicated. Numbers in parentheses refer to the daily intake (mg·kg⁻¹·day⁻¹) of the specific amino acid. Based on unpublished MIT work of Meguid and Meredith, and obtained using a constant intravenous infusion of the L-[l-¹³C]amino acid.

iii) Relationship to nutritional requirements

The studies that we have conducted, as described briefly above, suggest that the dynamic responses of the whole body metabolism of specific amino acids and of protein, might be differentiated for those intakes of protein and/or specific amino acids that are generous as compared to dietary alterations that are within the inadequate range of protein intake. Hence, we have also attempted to examine whole body amino acid kinetics in a way that might have predictive value in the study of human protein and amino acid requirements.

Many of the estimates of human amino acid requirements are based on results obtained from metabolic N balance studies and their limitations are well recognized (e.g. 60). For these reasons, an evaluation of stable isotope tracer approaches for purposes of further evaluating current estimates of the requirements for indispensable amino acids in healthy young men has been undertaken in our laboratory (11). The initial findings are most encouraging. First, we have found that the rate of whole body protein synthesis is much lower in young adults receiving L-amino acid diet when a "requirement" level of a specific indispensable amino acid is consumed as compared with a more generous intake of the test amino acid (Table 3). Furthermore, the rates of amino acid incorporation into proteins at "requirement" levels of amino acid intake are less than those reported for subjects receiving adequate intakes of protein (13). Therefore, the observations summarized in Table 3 suggest that current requirement values for specific indispensable amino acids may not be sufficient to maintain a "normal" status of whole body protein turnover.

Second, we have determined amino acid oxidation rates and whole body protein turnover at intake levels of specific indispensable amino acids in the region both above and below the estimated requirement level for maintenance of protein nutritional status (i.e. N balance). Results are shown in Fig. 15 for lysine oxidation in young men receiving graded intakes of this amino acid and in Fig. 16 for the rate
Fig. 15. Lysine oxidation, determined with a primed constant intravenous infusion of L-[1-13C]lysine in young men receiving an amino acid diet providing graded levels of lysine. Preliminary MIT data of Meredith et al.

Fig. 16. Rate of incorporation of leucine into whole body proteins, as determined with a primed-constant intravenous infusion of L-13C-leucine in young men receiving graded intakes of leucine from an L-amino acid diet. Preliminary MIT data of Meguid et al.

of incorporation of leucine into whole body protein for subjects receiving graded intakes for leucine. From these preliminary data it is apparent that marked changes (i.e. "breakpoints") in these response curves occur at an intake of about 30 mg · kg⁻¹ · day⁻¹ for lysine and 23 mg · kg⁻¹ · day⁻¹ for leucine.

Whether these breakpoints reflect the requirements for these amino acids is uncertain. However, it is significant that the responses of whole body lysine and leucine metabolism that we have obtained with healthy adult humans are similar to those reported from studies in growing and adult rats (44–47). Furthermore, these dynamic responses of amino acid metabolism in rats have been used to estimate the dietary requirement for specific indispensable amino acids. Thus, the data we have obtained so far on whole body amino acid dynamics in healthy young men suggest that the current estimates of adult amino acid requirements might be too low and that an adequate level of amino acid intake for protein nutritional maintenance is best predicted from changes in whole body amino acid dynamics to altered amino acid intakes (11). Although we emphasize that this interpretation of our findings is highly tentative, our data support the reservations that we have expressed pre-
viously (11, 60) about the uncertainties of the nutritional significance of the published requirements for indispensable amino acids in healthy adults. We are extending these studies to cover other amino acids and to explore requirements under various dietary conditions so that the potential that this novel approach might offer for improving estimates of human amino acid requirements can be fully determined.

Summary and conclusions

In this paper, we have discussed some selected aspects of human N and amino acid metabolism. Particular emphasis has been given to recent studies conducted in our laboratories involving use of stable isotope probes and to explore the dynamic responses of body protein metabolism to changes in protein and amino acid intake. An exciting, but hardly unexpected, conclusion arising from these studies is the suggestion that the control mechanisms responsible for body N and amino acid homeostasis are, in some as yet unknown way, tied to the nutritional requirement of the host. This being so, a more vigorous application of these tracer techniques, using the safe, stable isotopes of carbon, nitrogen and also of hydrogen (2H) and oxygen (18O) will help to refine measurements of protein and amino acid requirements and of protein nutritional status in humans under various pathophysiologic conditions. Furthermore, I hope that the honor and opportunity of presenting this paper to the membership of the Japanese Society of Food and Nutrition may also further encourage a concerted effort by nutritional professionals to apply modern technology for purposes of advancing our understanding of the quantitative relationships between diet, essential nutrients and human health.

My research work is the fruit of close collaboration with many investigators and especially Dr. Dennis M. Bier and his colleague Dr. Dwight E. Matthews, Washington University School of Medicine, St. Louis, who have enlightened my thoughts and approaches in the study of human protein and amino acid metabolism. Many colleagues at MIT and elsewhere, as well as my past and present graduate students, have played an essential role in this work. They know who they are and I will always be appreciative of their significant help.

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