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Whole Body Protein and Amino Acid Metabolism: Relation to Protein Quality Evaluation in Human Nutrition

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Selected aspects of whole body protein and amino acid metabolism in human subjects are reviewed in relation to the assessment and significance of dietary protein quality. The limitations of N balance measurements for assessment of protein quality are emphasized. Examples of the use of amino acids labeled with the stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) to examine the responses of protein and amino acid metabolism to dietary change are given. These studies suggest that the mechanisms responsible for adaptations of body N are intimately linked to the requirements for these nutrients. Hence, it is proposed that this observation be exploited to develop new and "dynamic" approaches for assessment of amino acid requirements and evaluation of protein quality in human subjects.

It is a privilege for us to contribute this paper in honor of Elmer V. McCollum, who, through his vision and research, has had such a lasting and positive impact on the advancement of nutritional science and its application to the solution of problems of human health.

The purpose of this paper is to explore selected aspects of human protein and amino acid metabolism, with the hope that this may lead to a better understanding of the metabolic consequences of altered nitrogen and essential amino acid intakes. An improved knowledge in this area is essential if the practical significance of dietary protein quality is to be defined in precise, quantitative terms. Furthermore, it is necessary to know how dietary-induced responses in nitrogen and amino acid metabolism in humans, at various stages of development and ages, compare with those observed in experimental animals. This is important if results obtained in assays of protein quality involving use of nonprimate and subhuman primate species are to find maximum application in resolving issues of direct concern in human nutrition. Finally, improved knowledge of human protein and amino acid metabolism should lead to more sensitive measures of protein nutriture and, in turn, determination of the adequacy of the dietary protein and amino acid intake.

Because there are a number of relevant and extensive reviews [e.g., Munro (1964), Allison (1964), McLaughlan and Campbell (1969), NAS/NRC (1974), and Waterlow et al. (1978a)], only a selected coverage of the topic will be undertaken here, with emphasis given to recent investigations that we and our colleagues have been carrying out in our laboratories. We will be concerned with the metabolic evaluation of protein adequency in humans, and this could be approached by using various measurements that indicate the status of specific aspects or phases of body amino acid and protein metabolism (Figure 1). However, our major focus will be on dynamic aspects of body protein and amino acid metabolism, studied with the aid of amino acids labeled with the stable isotopes of carbon (^{13}C) and nitrogen (^{15}N). These isotopes can be safely applied in humans of all ages, and, therefore, they provide an opportunity to examine the physiology of human protein metabolism throughout the life-cycle and the response of protein and amino acid metabolism to dietary change in healthy normal subjects.

NITROGEN BALANCE

It is worth emphasizing that much of the current knowlege of protein quality in human nutrition has been derived from studies based on use of the nitrogen (N) balance technique.

Following Schoenheimer's classic studies, involving the use of stable isotopes to explore the turnover of body constituents (Schoenheimer and Rittenberg, 1938; Schoenheimer, 1942), it is accepted that a major proportion of total body protein undergoes continuous synthesis and breakdown. Thus, the balance between the anabolic and catabolic phases of protein and amino acid metabolism determines cell and organ protein content and, in turn, the efficiency with which dietary nitrogen is retained. Hence, as depicted in Figure 2, both protein synthesis and breakdown are affected by various factors and their rates regulated through specific control mechanisms [e.g., Schimke (1970), Munro (1970), Goldberg and Dice (1974),

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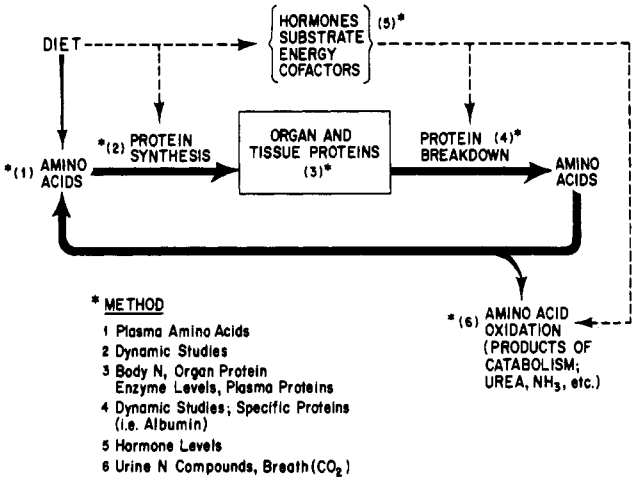

Figure 1. Illustration of the major flow of amino acids within the body together with an indication of the methods and measurements that may be used to assess the status of the various phases of body protein and amino acid metabolism. The dashed lines indicate that, in addition to the provision of amino acids and N, the diet influences the regulation of protein metabolism via its effects on hormones, energy metabolism, etc.

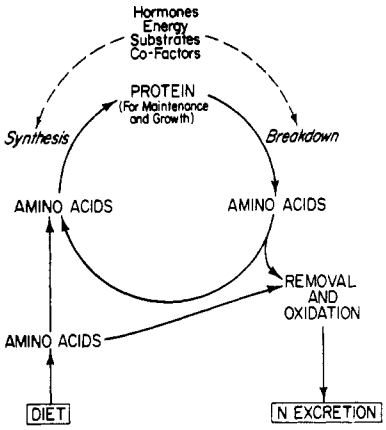

Figure 2. Body and organ protein content is determined by the balance between the rates of protein synthesis and breakdown, and each of these phases of protein metabolism is influenced by factors including hormones, substrate (amino acids; nitrogen), and energy supply.

Goldberg and St. John (1976), Ballard (1977), and Walker (1977)]. This biochemical organization provides the cell with a capacity to change its protein content and types of protein to suit a particular environmental condition. Hence, a given body N balance can, in theory, be achieved within a wide range in the rates of protein synthesis and breakdown; similarly, alterations in N balance can be brought about by various combinations of changes in these rates (Figure 3). Thus, although N balance estimations can be useful in the study of protein and amino acid nutrition (Waterlow, 1969), N balance measures do not provide a detailed picture of the status of protein and amino metabolism within the body; they only indicate the net balance between the rates of protein synthesis and breakdown.

In addition to this limitation in N balance measures, there are other problems associated with the N balance technique and interpretation of N balance data. For example, the high retentions of nitrogen in subjects receiving generous intakes of protein requires explanation. Current concepts dictate that body protein content is essentially constant in adults and declines only slowly during the advancing years of adult life (Forbes and Reina, 1970;

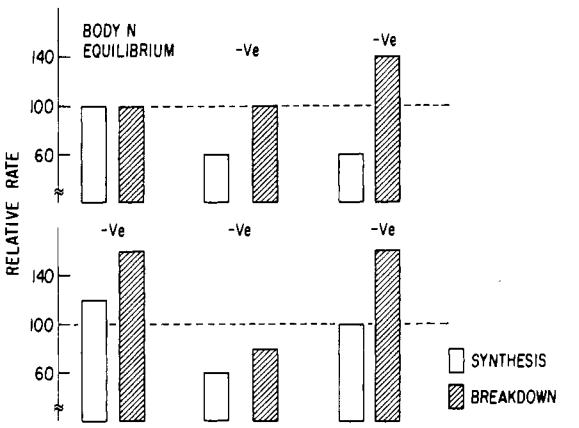

Figure 3. A reduced or negative nitrogen balance can arise through various combinations of changes in whole body protein synthesis and breakdown.

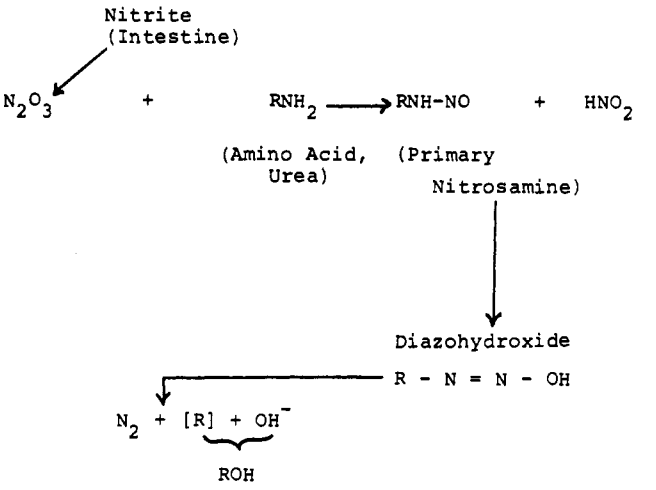

Figure 4. A possible pathway of formation of molecular nitrogen.

Young et al., 1976). However, as discussed by Hegsted (1976) and illustrated in a recent study by Oddoye and Margen (1979), adults retain considerably more nitrogen beyond that expected when receiving high N intakes and this is not explained on the basis of methodological errors. It has been suggested that there is a slow physiological adjustment to altered protein intake and this might take a considerably longer period of time than previously assumed (Forbes, 1973). However, our own findings (Rand et al., 1979) and those of Oddoye and Margen (1979) fail to support this suggestion.

Costa et al. (1968, 1974) have proposed that there is an evolution of gaseous nitrogen, arising during the course of amino acid and nitrogen metabolism. In mammals, it would be expected to be eliminated through the breath, but this is difficult to validate using conventional respiratory gas analysis methods. Furthermore, most reviews of this problem [e.g., Anonymous (1974)] have concluded that this does not occur and that there is no known pathway in mammals for the formation of N_2. We conclude that the necessary definitive studies required remain to be undertaken. On the other hand, indirect evidence supports the suggestion that formation of molecular nitrogen can occur, possibly through a pathway such as that shown in Figure 4. Thus, nitrite, known to occur in saliva (Tannenbaum et al., 1974), may arise via heterotrophic nitrification by the intestinal microflora (Tannenbaum and Young, 1980). Reaction of nitrite with primary amines can, through a series of *N*-nitroso reactions, eventually give rise to molecular nitrogen. Furthermore, in human metabolic

Table I. Urinary Nitrate in Subjects Consuming a Protein-Free and Essentially Nitrate-Free Diet[a]

subject no.	urinary nitrate, μmol of $NaNO_3$/day		
	day 1	day 5	day 8
1	985	1350	325
2	4050	2400	1390
3	1140	3040	918
4		1960	981
5	406	833	579
6	1130	1430	448

[a] Taken from Tannenbaum et al. (1978).

experiments we (Tannenbaum et al., 1978) have demonstrated a significant endogenous synthesis of nitrate that arises from nitrite (Table I). For this reason alone, it is unwise, without additional critical investigation, to perpetuate the notion that molecular N or gaseous nitrogen compounds, other than ammonia, cannot be formed during the course of whole body amino acid metabolism. However, the unanswered question is the quantitative significance of the pathway of molecular N formation or others leading to nitrogen loss. Until the amount of nitrite synthesized per day and its metabolic fate are known precisely, the potential importance of molecular N_2 formation as a route of body N loss will remain unclear.

Additional problems and issues relating to the use of N balance measures in protein quality evaluation studies in humans might be discussed, including the claim that there is a cyclic variation in N balance (Sukhatme and Margen, 1978) and that short-term N balances are unreliable indices of N retention. Although we have not been able to confirm this suggestion (Rand et al., 1979), this also might serve to emphasize the uncertainties that we face in a critical interpretation of N balance data. In addition, long-term metabolic studies in young men (Garza et al., 1977) lead us to the conclusion that it is not adequate to rely only on N balance as a measure of dietary protein adequacy. Hence, we have attempted to explore the status of human body protein and amino acid metabolism by applying methods that extend beyond application of N balance measures, and some of our observations are discussed in the following sections.

DYNAMIC ASPECTS OF BODY N AND AMINO ACID METABOLISM

We have applied a simple model of whole body N and amino acid metabolism, as developed by Waterlow and his colleagues [reviewed in Waterlow et al. (1978a)]. Studies of the quantitative aspects of whole body amino acid metabolism require application of tracer techniques, and previously major use has been made of radioisotopes, such as ^{14}C, and also ^{15}N, the stable isotope of nitrogen, for this purpose. However, our studies [e.g., Steffe et al. (1976), Young et al. (1975), Winterer et al. (1976), Pencharz et al. (1977), Uauy et al. (1978), Motil et al. (1979), and Conway et al. (1980)] have emphasized use of ^{15}N- and ^{13}C-labeled amino acids because these stable isotopes can be used safely and without any radiation hazard in humans of all ages and physiological states.

The simplified model of body amino acid and protein metabolism (Figure 5) views body N metabolism according to a metabolic pool from which amino acids leave via pathways of protein anabolism or via oxidative catabolism. The major inflow of nitrogen or amino acids into the metabolic pool is via the diet or breakdown of cell and tissue proteins. These major routes of amino acid and N flow can be quantified following administration of labeled amino acids and measurement of the label in the free

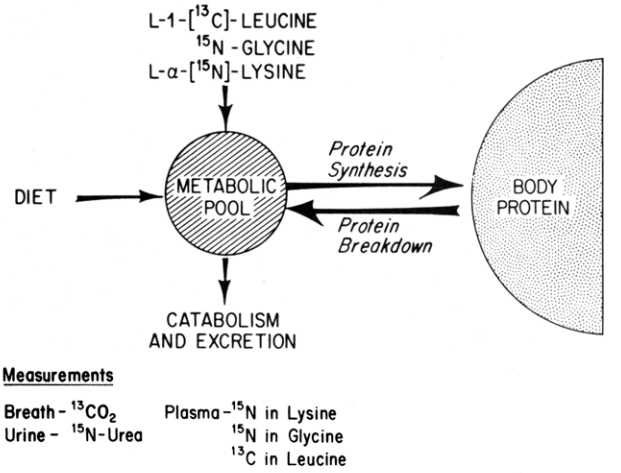

Figure 5. A general model of whole body protein and amino acid metabolsm in humans. The author's studies have applied [^{15}N]glycine, [α-^{15}N]lysine, and [1-^{13}C]leucine as the tracers.

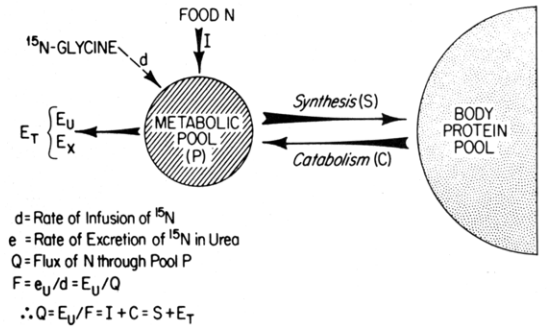

Figure 6. Picou and Taylor-Roberts (1969, p 283) model of whole body N metabolism, explored with use of a continuous infusion of [^{15}N]glycine. I, C, and S are intake, protein breakdown, and protein synthesis, respectively. E_U, E_X, and E_T are urinary urea, nonurea N, and urinary total N excretion, respectively. Q is the flux (milligrams of N per unit of t) of N in the metabolic pool (P). d is rate of ^{15}N administration; F is the fraction of administered dose excreted as [^{15}N]urea.

amino acid in blood plasma or its appearance in excretory products such as expired air ($^{13}CO_2$) or urinary nitrogen components (^{15}N in urea, ammonia, or total N) (Golden and Waterlow, 1977). Labeled amino acids may be administered as a single pulse dose (Waterlow et al., 1978b) or as a continuous infusion (Waterlow, 1967), and the advantages and disadvantages of these specific approaches have been discussed in detail by Waterlow et al. (1978a). We chose to use the continuous isotope-infusion approach in our earlier studies involving use of [^{15}N]glycine and the Picou and Taylor-Roberts (1969) model (Figure 6). More recently we have administered, by vein, essential amino acids labeled with ^{15}N (e.g., L-[α-^{15}N]lysine) or ^{13}C ([1-^{13}C]leucine and [1-^{13}C]valine). These investigations have been made possible with the aid of a combined gas chromatograph–mass spectrometer and selected ion monitoring (Bier and Christopherson, 1979; Matthews et al., 1980).

Thus, changes in rates of whole body protein synthesis and breakdown during growth and development and with passage of the adult years of life have been examined (Table II). Our findings reveal that these rates are high in the early stages of life, declining markedly during the first ~2 years so that the rates in young adults are about one-sixth of those in the newborn [compare Pencharz et al. (1977) and Steffe et al. (1976)]. Little further change occurs during passage of the adult years (Winterer et al., 1976; Uauy et al., 1978). However, two general observa-

Table II. Rates of Whole Body Protein Synthesis Based on [^{15}N]Glycine Administration in Humans during Growth and Development[a]

group	age	whole body protein synthesis, g kg^{-1} day^{-1}
premature infants	1–45 days	26
infants	10–20 months	6
children	9–16 years	4
young adults	20–25 years	~3
elderly adults	~72 years	~3

[a] Based on studies in the authors' laboratories except for data for infants obtained from Picou and Taylor-Roberts (1969).

Table III. Comparison of the Relationship between Net Protein Gain and Whole Body Protein Synthesis in Human Neonates and Young Rats[a]

	g of protein kg^{-1} day^{-1}		
	protein gain	whole body protein synthesis	gain, % of synthesis
human neonate	2.3	26	9
young rat	9.4	40	24

[a] Slightly modified from Young and Scrimshaw (1978). Based on the data of Pencharz et al. (1977) and, for rats, from Bernhart (1970) and Millward and Garlick (1972).

tions can be made from this series of studies, and these may have significance in relation to protein quality evaluation. First, the amount of protein synthesis associated with net body protein gain in the human neonate is only a small proportion of the total daily protein synthesis (Table III). Thus, protein turnover is *the* quantitatively important component of body protein metabolism rather than net protein synthesis per se, even at a very early stage in development when rapid growth is evident. Also, these estimates may be contrasted with those obtained in the growing rat where body protein gain appears to account for a much larger fraction of total body protein synthesis (Table III). On this basis, the rat may not be an adequate experimental animal model for the study of certain aspects of protein metabolism in humans. Second, associated with the change in the rates (grams of protein per unit of weight per day) of protein synthesis and breakdown during various phases of life, there is a redistribution in body protein metabolism. By combining studies of whole body protein synthesis and breakdown with measurements of urinary N^{τ}-methylhistidine (3-methylhistidine) as an index of the rate of muscle protein breakdown (Young and Munro, 1978), we have concluded that skeletal muscle makes an increasing contribution to whole body protein metabolism during growth and development, with this tissue accounting for ~25–30% of total body protein metabolism in young adult men (Uauy et al., 1978). Thereafter, during the advancing adult years, there is a progressive decline in the quantitative contribution made by skeletal muscle to whole body protein metabolism (Uauy et al., 1978). The significance of this shift in the distribution of body protein metabolism for dietary protein quality is, of course, uncertain. However, if the major organs exhibit different patterns of requirement for individual amino acids (Munro, 1969) and different efficiencies of reutilization or recycling of amino acids [e.g., Gan and Jeffay (1967)], it might be speculated that the efficiency of dietary protein utilization and, therefore, dietary protein quality would depend upon the quantitative contribution made by muscle vs. the visceral organs to whole body protein metabolism.

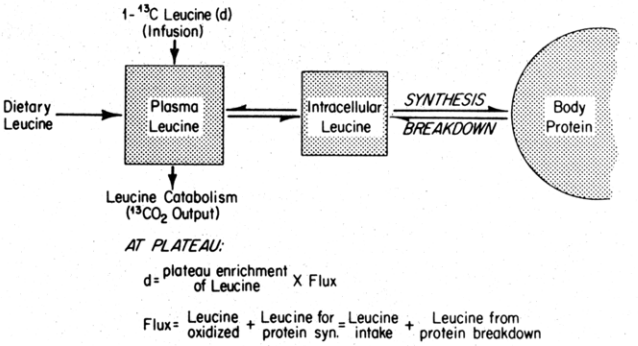

Figure 7. Model of whole body leucine metabolism used to explore responses of leucine and protein metabolism to alterations in dietary protein intake in healthy young men.

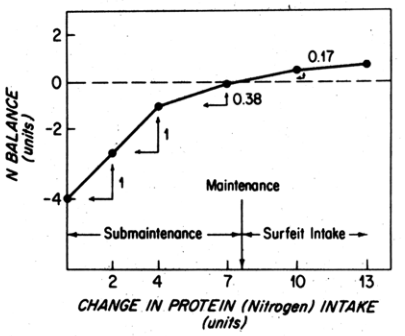

Figure 8. Diagrammatic representation of the change in efficiency of nitrogen retention with increments in N intake within the submaintenance to supramaintenance range of N (protein) intake.

RESPONSE OF PROTEIN METABOLISM TO DIETARY CHANGE

Recent studies in our laboratories concerned with an assessment of responses of whole body leucine metabolism to dietary protein intake may also be of interest in relation to protein quality. With [1-^{13}C]leucine as a primed-constant tracer infusion (Matthews et al., 1980), we have attempted to quantify various aspects of whole body leucine metabolism using the model shown in Figure 7. Because the carboxyl carbon of leucine is irreversibly lost during the decarboxylation of α-ketoisocaproic acid, measurement of the rate of appearance of ^{13}C in expired CO_2 following administration of [1-^{13}C]leucine allows a determination of leucine oxidation. Coupled with an estimate of leucine flux within the plasma pool, based on measurement of ^{13}C enrichment of leucine in plasma after achieving an isotopic steady state (Waterlow et al., 1978a), the rates of leucine incorporation into body proteins and of leucine entry into the metabolic pool from endogenous protein breakdown can be estimated. Thus, the quantitative status of the major components of whole body leucine metabolism in human subjects can be determined and examined in relation to how they change with alterations in dietary protein intake.

It would be useful to establish the metabolic basis for the change in efficiency of N retention that occurs with changes in protein intake within the submaintenance and supramaintenance range of protein intakes (Calloway and Margen, 1971; Young et al., 1973; Inoue et al., 1973; Kishi et al., 1978) since this might allow development of a more rigorous approach to the assessment of the capacity of different protein sources to meet the N and amino acid requirements of populations of human subjects. As depicted in Figure 8, the efficiency with which a change in N (protein) intake is retained declines as the total intake

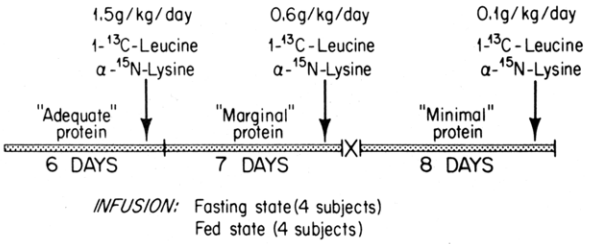

Figure 9. Design of the study used to examine response of whole body leucine and lysine metabolism to changes in protein intake in healthy young men. See the text for additional details (Motil et al., unpublished study).

of protein approaches that required for maintenance, and above this level of intake the retention of additional increments in N is low. Although there is debate as to whether the N balance response to changes in N intake remains linear throughout the entire submaintenance range (Kishi et al., 1978) or follows a curvilinear function (Young et al., 1973), as might be anticipated purely on biochemical grounds [e.g., Krebs (1972)], it is clear that the efficiency of N retention falls off markedly as changes in N intake approach or exceed requirement levels.

We have begun to examine this aspect of body protein metabolism by studying whole body leucine and lysine metabolism in healthy young adults receiving intakes that approximate "usual" (1.5 g of egg protein kg^{-1} day^{-1}), "requirement" (0.6 g of egg protein kg^{-1} day^{-1}), or "minimal" (0.1 g of egg protein kg^{-1} day^{-1}) intakes of protein. The design of the study, involving healthy young men who received [1-^{13}C]leucine and [^{15}N]lycine following an overnight fast (fasted state) or while receiving small meals (fed state) and after adaptation to these dietary protein intakes, is shown in Figure 9. Details of the ^{13}C–^{15}N-tracer infusion protocol applied at the end of each diet period have been described (Conway et al., 1980; Matthews et al., 1980). A schematic summary of the results obtained in this study is given in Figure 10, and our findings reveal that within the surfeit or supramaintenance range of protein intake, body leucine homeostasis is achieved largely by alterations in the rate of leucine oxidation. In contrast, only small changes in the rates of leucine oxidation occur with changes in protein intake below the maintenance needs, and the major responses occur in relation to altered rates of leucine incorporation into proteins (protein synthesis) and release from endogenous protein breakdown.

Changes in dynamic aspects of whole body lysine metabolism were also explored simultaneously with those of leucine metabolism in response to altered protein intake (see Figure 9). However, when [^{15}N]lysine is used as the tracer, it is not possible to estimate the major components of whole body lysine metabolism as might be achieved for leucine. This is because the ^{15}N from lysine enters the general amino acid N pool before appearing in urinary urea, thus complicating an estimation of lysine oxidation. However, the results summarized in Table IV indicate that whole body lysine flux responds in a qualitatively similar way to that observed for whole body leucine flux.

Because the 0.6 g of egg protein kg^{-1} day^{-1} intake level may be taken, for discussion purposes, to approximate a requirement level in healthy young adults [e.g., FAO/WHO (1973)], our results with [1-^{13}C]leucine indicate that the metabolic responses of whole body leucine metabolism to alterations in protein intake depend upon where, in relation to the requirement intake level of protein, the dietary change is made. Thus, for changes in protein intake below requirements, alterations in whole body protein amino acid metabolism occur mainly via changes in rates

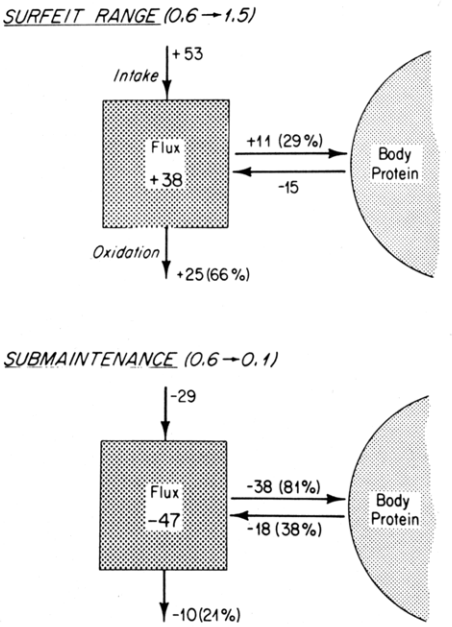

Figure 10. Schematic summary of responses of whole leucine metabolism to changes in protein intake in young men. Changes in leucine flux, leucine oxidation, and leucine incorporation into protein and release into the pool via protein breakdown (all values expressed as micromoles per kilogram per hour when protein intake is increased from 0.6 to 1.5 g of protein kg^{-1} day^{-1} (top panel) or decreased from 0.6 to 0.1 g of protein kg^{-1} day^{-1} (bottom panel). These data are mean values for four subjects receiving [1-^{13}C]-leucine in the fed state (see the text) and after adaptation to the different protein intakes (see Figure 9).

Table IV. Comparison of Mean Values for Whole Body Leucine and Lysine Flux, As Estimated by Simultaneous Infusion of [1-^{13}C]Leucine and [α-^{15}N]Lysine in Young Men after Adaptation to Different Intakes of Dietary Protein[a]

	protein intake, μmol kg^{-1} h^{-1}		
metabolic state	adequate	marginal	minimal
fast			
leucine	127	110	87
lysine	101	86	81
fed			
leucine	157	120	73
lysine	119	79	66

[a] Mean values for four subjects (Motil, Bier, Matthews, and Young, unpublished data).

of body protein synthesis and breakdown. In contrast, for changes in protein intakes that are above requirements, the homeostasis of body N and amino acid metabolism is achieved mainly via changes in amino acid oxidation. Of course this conclusion must be regarded as tentative until further labels, such as [^{13}C]methionine, [^{13}C]phenylalanine, and [^{13}C]threonine, are applied in a similar series of metabolic studies. Nevertheless, our initial results suggest that estimates of protein and/or amino acid requirements may be improved through a more detailed study of dynamic aspects of whole body N and amino acid metabolism throughout the submaintenance to supramaintenance range of protein and/or amino acid intake.

METABOLIC RESPONSES IN RELATION TO AMINO ACID REQUIREMENTS

From the foregoing, it was suggested that biochemical mechanisms responsible for maintaining protein and amino acid nutritional status are tied to the requirements for

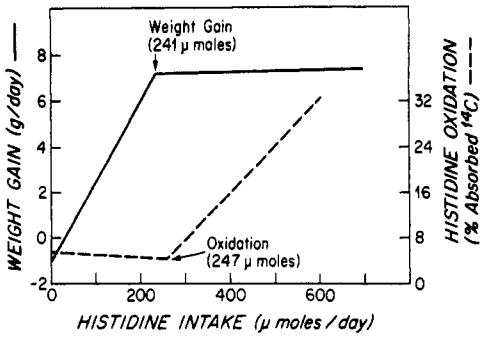

Figure 11. Schematic representation of the data of Kang-Lee and Harper (1977, p 1427) showing the relationships between histidine intake and oxidation and growth in young rats.

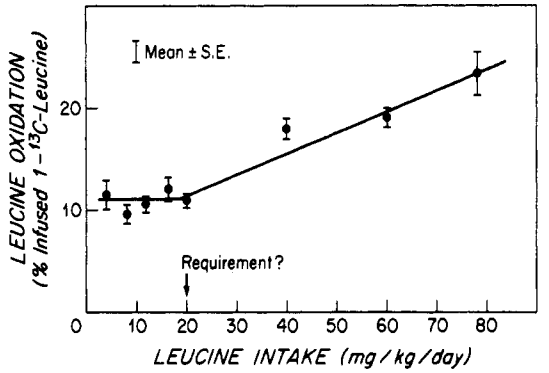

Figure 12. Preliminary results on the oxidation of leucine, studied with [1-^{13}C]leucine, in young men at various intakes of leucine supplied by an L-amino acid mixture. M.I.T. data of Meguid, Bier, and Young (unpublished data, 1979).

protein and amino acids. There is evidence to support this view. For example, we have observed that hepatic tryptophan oxygenase shows a cyclic change in activity in response to adequate protein-containing meals (Young and Munro, 1973), but this pattern is abolished when tryptophan intake is below requirements. This suggests that the liver monitors the adequacy of the tyrptophan intake in relation to the body's need. In addition, rates of oxidation of lysine (Brookes et al., 1972), threonine (Kang-Lee and Harper, 1978), and histidine (Kang-Lee and Harper, 1977) are low and constant when intakes of these amino acids are below requirements in rats, but the oxidation rates rise linearly with increased amino acid intakes exceeding a level required for maximum growth in young rats (Figure 11).

We have begun to explore, in healthy adult men, the nature of the relationships between whole body amino acid metabolism and requirements for individual essential amino acids. The preliminary results of a study designed to examine the rate of leucine oxidation at graded intakes of leucine, furnished by an otherwise constant diet based on an L-amino acid mixture, are shown in Figure 12. At leucine intakes ~20 mg kg^{-1} day^{-1} and below, the percentage of infused [1-^{13}C]leucine oxidized was constant, but above this intake level increases in leucine intake were accompanied by increases in leucine oxidation. Comparable data obtained in rats (Brookes et al., 1972; Kang-Lee and Harper, 1977, 1978) might lead to the conclusion that the break in the leucine intake–oxidation response curve that occurred at about a 20 mg kg^{-1} day^{-1} intake level indicates the mean leucine requirement in healthy young men. However, this estimate is higher than the requirement of 13 mg kg^{-1} day^{-1} based previously on short-term N balance studies (Williams et al., 1974; Harper, 1977), and longer term metabolic studies will be necessary to resolve this point. However, it is significant that the relatively

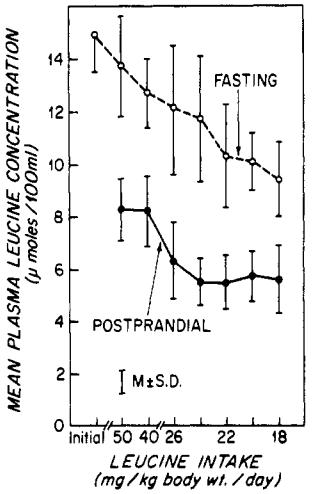

Figure 13. Plasma leucine concentration following an overnight fast or 3 h postbreakfast with changes in leucine intake in four young men (Perera et al., unpublished results).

Table V. Summary Statements on Implications of Studies of Whole Body Amino Acid Metabolism for Protein Quality Evaluation

(1) N balance is an inadequate sole criteria of protein status
(2) metabolic changes in amino acid and N metabolism show
(a) changes in relation to requirements and
(b) changes with development and age
(3) need to explore (2) in relation to
(a) dietary proteins and amino acid mixtures and
(b) development of "dynamic tests", i.e., breath tests with ^{13}C and urine tests with ^{15}N

constant rate of leucine oxidation at or below 20 mg kg^{-1} day^{-1} and the linear change in oxidation above this level of intake parallel the responses of plasma leucine concentration that we have observed in young men to altered leucine intake (Figure 13). Thus, these preliminary data suggest that not only the status of whole body amino acid metabolism but also the requirements for a number of the essential amino acids in young adults might be determined and more definitely explored by application of these modern approaches based on stable isotope tracers. Furthermore, it is important to know whether the current estimates of amino acid requirements (FAO/WHO, 1973; NAS/NRC, 1974), based largely on N balance measurements, can be confirmed by use of new alternative approaches. This is significant because it has been concluded that protein quality is of little importance in adult human nutrition due to the low estimates of the concentration of essential amino acids required per unit of protein intake [e.g., Arroyave (1974) and Harper (1977)]. We have questioned previously the strength of evidence to support this conclusion (Young and Scrimshaw, 1978), and there is a lack of sufficient critical evidence to establish reliably that dietary protein quality shows a marked age-dependent change between early childhood and adult life.

POSSIBLE DEVELOPMENTS

Studies of whole body amino acid metabolism in human subjects are still limited, particularly with reference to protein intake and issues of dietary protein quality. However, from the observations that we have made recently, it is evident that there exists a valuable and exciting field of study that should be pursued with the aid of stable isotope tracers, in order to better define the responses of protein and amino acid intake in healthy humans. Table V lists some of the points made in above sections, together

with the areas that deserve research emphasis. Information arising from these studies could be used not only to improve current understanding of the significance of protein quality in human nutrition but also to enhance our knowledge of the metabolic consequences for body protein metabolism due to changes in the quality of dietary proteins consumed. This will require a further exploration of the dynamic aspects of body amino acid metabolism in relation to various intake levels of proteins and amino acid mixtures and with different amino acid profiles. Furthermore, with the generation of a sufficient data base on the relationship between amino acid requirements and the oxidation and utilization of amino acids in humans, as studied with ^{13}C and ^{15}N, we believe it should be possible to develop dynamic tests of dietary protein quality and the status of body protein metabolism. These might be based on use of breath tests with ^{13}C, as has been proposed for assessment of gastrointestinal and liver function (Schoeller et al., 1977; Watkins et al., 1977; Solomons et al., 1977), or urinary tests with ^{15}N following oral administration of suitably labeled compounds together with meals of test proteins. Although considerable research must precede the attainment of practical tests, the latter represents a potential practical outcome of studies designed to examine the adaptations of protein and amino acid metabolism to altered nutritional conditions in healthy, normal subjects of all ages.

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 the limitations of the N balance method and also to recent studies conducted in our laboratories and involving use of ^{13}C and ^{15}N labels to explore the responses of body protein metabolism to changes in protein and amino acid intake. An exciting outcome of these studies is the suggestion that the control mechanisms concerned with body N and amino acid homeostatsis are, in some as yet unknown way, tied to the protein and amino acid requirements of the individual. If this is so, a more vigorous application of tracer techniques, using the safe, stable, or nonradioactive isotopes of carbon and nitrogen, in healthy human subjects will help define more precisely the practical significance of protein quality in human nutrition. This research should also lead to improved methods for the rapid and accurate assessment of the capacity of dietary proteins to meet human needs.

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