Indian J Physiol Pharmacol 1999; 43 (1) : 5–24

REVIEW ARTICLE

HUMAN INDISPENSABLE AMINO ACID REQUIREMENTS: NEW PARADIGMS OF MEASUREMENT AND THE IMPLICATION FOR PROTEIN QUALITY

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(Received on June 24, 1998)

Abstract: Essential amino acids, now called indispensable amino acids (IAA) are not synthesized by the body, and have therefore to be supplied from an exogenous source. IAA requirements as set out in 1985 by the FAO/WHO/UNU have been challenged by the data derived from the application of obligatory nitrogen loss measurement technique and the stable isotope tracer amino acid technique. These measurements suggest that IAA requirement in adults may be between two to three times the requirements set out in the 1985 FAO/WHO/UNU recommendations.

Key words: recommended dietary allowances, essential amino acids, indispensable amino acids, proteins, amino acids, nutrient requirements

INTRODUCTION

Man and other mammals are dependant on at least 8 amino acids that have to be supplied from an exogenous source. These amino acids, which are not synthesised by the body, were initially called 'essential', as contrasted to those 'non–essential' amino acids which were synthesised in the body. More recently, the terms "indispensable' and "dispensable" have been used more often, instead of essential and non–essential respectively, and they are now in general acceptance (1). Estimates of the dietary intakes necessary to meet the requirements for these indispensable amino acids are of crucial significance in formulating sound nutrition and health policies, as well as in assessing and maintaining the health and well being in individuals.

The current international estimates of indispensable amino acid (IAA) requirements in human at various ages are set out in the 1985 report of the Joint FAO/WHO/UNU Expert Consultation on energy and protein requirements (2). These estimates, for pre-school and school–age children and adults, are summarised in Table I. Recently however, there has been

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a change in the recommended intake of essential amino acids for adults, as set out by the 1991 WHO/FAO Expert Consultation (3). The tentative new requirements have resulted from a *paradigm shift* in the approach to measuring the IAA requirements of adults. The need for this new paradigm arose from the inadequacies of the old method of measuring IAA requirement from measurements of nitrogen balance. In tha case of adult IAA requirments, they were so low that it would have been possible to achieve adequate intakes of IAA's on an exclusively cereal diet. Indeed, protein quality in this case would be of little practical consequence for adult human protein nutrition.

Two new methods of estimating IAA requirements have been proposed; the first from obligatory nitrogen loss measurements, and, the second, from the measurement of daily amino acid balance, by the use of a stable isotope tracer amino acid technique. These measurements have led to the suggestion that the IAA requirements in adults may be between two to three times the requirements set out in the 1985 WHO/FAO/UNU recommendations (2). The arguments for these measurements, presented by Young and co–workers in their publications, were sufficiently compelling to persuade an Expert consultation of the FAO/WHO in 1991 to recommend, *as an interim measure*, that the pattern of requirements in adults be set at that required by the pre-school child (3). This pattern was, in fact, similar to, and slightly higher than the pattern suggested by Young (4) and which is now known as the "MIT pattern of IAA requirement" (Table II).

2. The problem of accepting the 1985 WHO/FAO/UNU estimates in adults.

The 1985 FAO/WHO/UNU Expert Committee (2) recommendations on IAA requirements were based on the early studies of Rose and co-workers in men (5) and on separate but similar studies by various investigators in women (summarised in 6). These estimates (5) were based on nitrogen balance studies, which had several drawbacks. The drawbacks included (a) an overestimation of N balance and, hence, an underestimation of requirements due to *high*

TABLE I : 1985 WHO/FAO/UNU Recommendations for IAA Intake.

Amino acid	*Recommendations (mg/kg/day)*		
	Infants (3–4 month)	*Pre-school (2 years)*	*Adult*
Isoleucine	70	31	10
Leucine	161	73	14
Lysine	103	64	12
Methionine+cystine	58	27	13
Phenylalanine+tyrosine	125	69	14
Threonine	87	37	7
Tryptophan	17	12.5	3.5
Valine	93	38	3.5
Total	714	352	84

TABLE II : A Comparisón of the IAA requirements by different paradigms.

Amino acid	*Requirement (mg/kg/day)*			
	FAO/WHO/UNU	*OAAL*	*New "MIT"*	*FAP/WHO*
	1985 (1)	(2)	Pattern (3)	1991 (4)
Isoleucine	10	23	23	31
Leucine	14	40	39	73
Lysine	12	30	30	64
Methionine+cystine	13	13	15	27
Phenylalanine+tyrosine	14	39	39	69
Threonine	7	15	15	37
Tryptophan	3.5	6	6	12.5
Valine	3.5	20	20	38
Total	84	186	187	352

(1) From Ref. 2; (2) From Ref. 4; (3) From Ref. 20; (4) From Ref. 3.

dietary energy intakes, (b) incomplete measurement of N losses via routes other than faeces and urine, and (c) a confounded experimental design (7, 8, 9). The important feature of the Rose nitrogen balance studies was that the energy intakes given to the subjects were high, potentially leading to an enhanced degree of amino acid economy, and therefore, to apparently lower amino acid requirements. These problems with the interpretation of the nitrogen balance studies, have been reviewed extensively (10, 11, 12), and it is now clear that the results of N balance studies should not be used to establish the requirement for indispensable amino acids to maintain protein nutritional status in adults, in the long term.

There are other reasons for questioning the 1985 FAO/WHO/UNU adult IAA requirements. Table I, depicting these requirements, clearly shows that there is a sharp drop in the IAA requirement of adults, when compared to the pre school child. This difference is based on assumption of the relative importance of "growth" and "maintenance" requirements in the human body, i.e., that the requirement of the human pre-school child is skewed towards growth while the requirement in the adult is skewed towards maintenance.

The assumption of a difference between the partitioning of the requirements of a pre-school child and that of an adult may be flawed. The growth requirements for human is about 50% at 6 months of age, and this growth maintenance declines to about 20% in the 2 year old child, and 15% or less in the 4–5 year old child (2, 13, 14). This is in contrast to the pig, (which has been studied extensively, and has been used to extrapolate to humans (15)), where, 90% of the requirements in the young pig are for growth (recalculated in 13). A similar picture is seen in the weanling rat (16), and in both these mammals, differs profoundly from human growth, where a large proportion of the amino acid requirements in the young, are for maintenance. In the pig therefore, one would expect a large difference between adult pig and piglet

requirements, while the same reasoning would not necessarily be true for man. Further, while differences between the growth and maintenance patterns of IAA requirement, have been shown to be different in pigs (16), the comparison between the amino acid requirements, in adult animals (maintenance), show no great difference from the composition of mixed body proteins in both rats and pigs (17, 18). One measure of the maintenance requirement, is the measurement of the N required to sustain the existing fat free mass. This minimal N requirement can be derived from the basal N loss, which is a function of the body weight $^{0.75}$ (18). If the composition of this N (protein) loss could be determined, then the recommended intakes of IAA in adults could be predicted. While the maintenance pattern of IAA requirements has been studied in pigs (17–19) and shown to be high in dispensable amino acids, Young and El-Khoury have reviewed this issue and conclude that, in adult pigs and rats, there is not much difference between the maintenance pattern of IAA requirement and body protein composition (13). Hence, there is a case here for relating the basal N loss in humans to the amino acid composition of body protein.

These various observations support the development of a new paradigm of measuring amino acid requirements, based either on obligatory nitrogen loss and the body protein composition, or on the concept of body amino acid balance. These new paradigms for IAA requirements will be explored briefly in this review, which will finally concern itself with the estimates for the IAA requirements in adults, with specific reference to lysine.

3. New approaches to developing measures of indispensable amino acid requirements in adults.

3.1: The obligatory Amino acid loss (OAAL) method :

One of the paradigms for assessing IAA requirements has been put forth by Young et al (4) based on estimates of the intakes of amino acids necessary to balance the minimum obligatory losses of amino acids as *predicted* from the composition of mixed body proteins. This pattern was based on the daily obligatory nitrogen loss (ONL), with the assumptions that a) the ONL was 54 mg/kg/day in the adult, b) that the oxidation pattern of amino acids was in proportion to the pattern of amino acids in a reference body protein, and c) that the efficiency of absorption of the IAA (at requirement level), in humans, was about 70% (13). Clearly, this method, if anything, will underestimate the true requirement, since the OAAL is derived from the ONL, disregarding the small losses of amino acid that could occur in the urine or via the intestine.

Based on these estimates of OAAL, as well as ^{13}C-tracer studies (below), new revised values for the amino acid requirements of adults were proposed by Young, and these are presented in Table II, expressed in terms of mg amino acid required/kg/day. As can be seen from the data presented in this table, the proposed new requirement pattern is fundamentally quite different from the adult amino acid requirement pattern proposed by FAO/WHO/UNU (1985), and is similar to that for the pre-school child (see Table I).

3.2: The tracer balance technique :

The definition (20) of the requirement for an indispensable amino acid in healthy individuals, is as follows: "The requirement for an amino acid is that minimal intake level, which represents a single point on a dose–response curve, when applied to a well–characterised population group can, with some reliability, be predicted to achieve a criterion of adequacy (e.g. growth performance; target composition of body weight gain; *body amino acid balance*; measure of organ (liver, muscle) and/or system (immune/defence, nervous), or function."

With this definition in mind, Young and co–workers, have developed a second paradigm of measuring IAA requirement based on a stable isotope tracer technique (see below) to measure body amino acid balance as the criterion of adequacy. This technique is based on the measurement of the irreversible oxidation of tracer labelled test amino acids (21–27), and involves correlating the measured oxidation rate with controlled diet studies at different levels of intake of the test amino acid. The minimum intake at which the estimate of daily balance was obtained (intake–oxidation), was taken to be the requirement level. The procedure involves giving subjects, over a run–in period varying from 6 to 21 days, diets based on amino acid mixtures that supply limiting to generous levels of the amino acid being tested, and to measure the test amino acid balance at each level of test amino acid intake. This balance could be measured by the intravenous infusion (or oral administration) of a stable isotope (^{13}C) labelled amino acid, such that the flux and the irreversible oxidation of the test amino acid could be measured. The measurement of this irreversible oxidation, is based on the highly accurate quantification of the respiratory loss of oxidised tracer as carbon dioxide (by indirect calorimetry and isotope ratio mass spectrometry), as well as the measurement of the isotopic enrichment (by gas chromatography–mass spectrometry) of the precursor pool of the amino acid in the body. With the fulfilment of these conditions, the studies of Young and co–workers have provided a large body of data over the last decade, that consistently support and validate their original hypothesis (4) that the FAO/WHO/UNU (2) recommendations are inadequate. Measurements of oxidation performed over selected hours of feeding and fasting, appeared to confirm the general applicability of the proposed requirements (21–27) and a comparison of these estimates is presented in Table II. Except for the sulphur containing amino acid requirement, these ^{13}C–derived estimates are far higher than the 1985 WHO/FAO/UNU values that are shown for adults in Table I. For pragmatic reasons, in these studies, the body 'test' amino acid balance was used as the criterion of requirement, rather than measures of organ or system function, which have not been sufficiently explored to permit their use for the purpose of estimating requirements for specific indispensable amino acids.

There have been both technical and biological arguments against the immediate, world wide acceptance of the tentative new MIT pattern of amino acid requirement, based on the tracer technique, which thus far, has also validated the original OAAL

paradigm of measuring amino acid requirements. On the technical side, while there is little doubt that the Rose estimates are too low, it has been suggested that results from earlier tracer studies carried out over a few hours of fasting and feeding cannot be easily extrapolated to a 24 hour data base and, therefore, a daily requirement value (28). There are diurnal variations in the rate of amino acid oxidation. Studies at MIT on Western subjects (29–31), with the 24 hour tracer technique in adults, have clearly defined the temporal changes in the whole body rate of leucine oxidation, particularly with reference to meal intake and level of amino acid feeding. A similar pattern in the rate of leucine oxidation has also been observed in our Indian subjects (32, Fig. 1). An extrapolation of selected portion of this diurnal pattern, to a whole day, could therefore result in significant error.

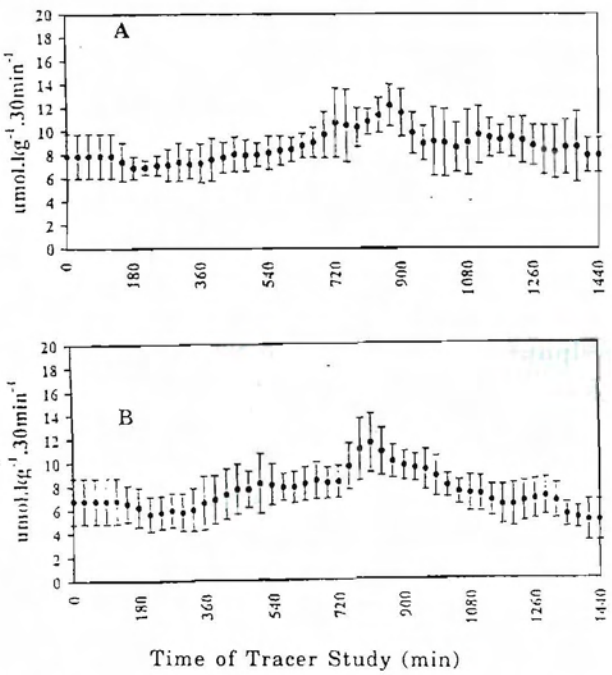

Fig. 1.: Leucine oxidation measured for each 30–min interval throughout a 24–h tracer infusion with [1–^{13}C] leucine. (A) Lysine intake of 12 mg/kg/d. (B) Lysine intake of 28 mg/kg/d. The time on the x–axis refers to time in minutes of the experiment. Feeding began at 0600 (720 min) as hourly meals and ended (last meal) at 1500 (1260 min), to provide 40 mg (approx.) dietary leucine/kg/d. Data are for seven subjects, each studied at both lysine intakes. Values plotted are mean ±1 SD.

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An analysis of the series of 24 hour tracer experiments with ^{13}C leucine has been carried out both at MIT as well as at Bangalore (29, 30, 32), and has confirmed that the daily leucine oxidation rate is approximately the same as that derived from shorter term studies. It is clear that the chosen hours of measurement, for extrapolation to a 24 hours pattern, were fortuitous for the short term experiments. However, subsequent studies with aromatic amino acids like ^{13}C–phenylalanine and ^{13}C–tyrosine (31) underscore the desirability of continuing use of the 24h isotope tracer protocol, despite its technically and experimentally demanding nature.

A second major argument, of a biological nature, against the acceptance of the tracer technique estimates being accepted world–wide, is that there may be metabolic adaptations, including sparing effects of dietary non essential nitrogen and urea nitrogen recycling through the gut and metabolic synthesis of indispensable amino acids for inclusion in host metabolism, particularly in populations with chronically low IAA intake (33).

There is little data that can be used to predict whether the indispensable amino acid needs are similar or different among

various population groups. One recent study by us in well nourished Indian adult males (32), using the tracer technique (indicator amino acid technique, see below), suggests that, the requirements for lysine are similar to those in Western subjects. Another approach to assessing the similarity (or dissimilarity) of IAA requirements among different populations, is to compare ONL losses. Studies of obligatory nitrogen losses in US (34, 35, 36), Chinese (37), Indian (38), Nigerian (39, 40) and Japanese men (41) reveal that they are remarkably uniform (42). By implication, the dietary requirements for indispensable amino acids, according to the OAAL model, would be similar (13), unless there is evidence that the efficiency of specific amino acid retention differed among apparently similar subjects in the population groups.

It is still possible that in *undernourished* populations with low intakes, there is an "adaptation" of requirements. Studies by Nicol & Philips (39, 43) suggesting that Nigerian men of low income are adapted to low-protein diets and utilise dietary protein more efficiently than, for example, US students (35) are not appropriate to answer this question. This is because the N balance results in the Nigerian subjects studies were indicative that they were depleted and that they were undergoing a body protein repletion response to the "good" diet given during the course of the experiments. Later studies in young Nigerian adult males (44) indicate that at maintenance nitrogen intakes, the efficiency of dietary protein utilisation is essentially the same as that for Caucasian and Oriental subjects. It is also important in these studies to ensure that there is no positive energy balance, as this could also 'spare' protein oxidation. Clearly, the need of the moment is for well-conducted 24 hour tracer balance studies, in well-nourished populations, from different ethnic backgrounds, at an adequate but not excess intake of energy, protein and indispensable amino acids.

There have also been suggestions that an economy of body nitrogen metabolism, and, hence IAA's may occur through urea recycling in the gut (45), wherein, the nitrogen in urea in the gut, is cycled into microbial amino acid N, and this N is subsequently absorbed into the body amino acid pool. There is no doubt that urea production exceeds urea excretion (46), and that urea recycling does occur (47). This degradation of urea, occurs in the gut, and can account for upto 40% of the urea production in subjects with a normal protein intake (46-48). In addition, there is evidence that dietary urea nitrogen can be used for amino nitrogen synthesis in breast-fed and formula-fed infants (49, 50). Further, ^{15}N urea administration to adults resulted in the detection of ^{15}N labelled *dispensable* amino acids in the plasma (51). In this study, ^{15}N was also detected in lysine, and this may have resulted from degradation of microbial protein. Given evidence that the urea utilisation process is variable, inversely dependent on the IAA intake, and growth of the animal (52), and that quantification of this process is difficult, it is possible that in the well nourished, adequately fed individual, this process may not be of major quantitative importance in the IAA economy of the whole body.

The leucine tracer balance approach has been extensively studied, and there is a

general acceptance of the technicalities of this method (33), as well as of the requirement for leucine determined by this paradigm. If it is assumed, as has been argued (1), that the maintenance requirement of IAA closely resembles that of the tissue composition of IAA's, and if the requirement for leucine is indeed higher as revealed by the tracer technique, then the requirement for the other indispensable amino acids would probably also follow this pattern (33).

3.3: The Indicator Amino acid Technique :

The methods detailed above examine irreversible oxidation of the test amino acid and correlate this with the intake of the same amino acid. This technique has been validated for leucine (29, 30) and investigations with phenylalanine, tyrosine (31) and lysine (53) that are underway at MIT further indicate that the technique can be applied to these amino acids. One of the objections of the "test" tracer approach, involving different levels of feeding of the test amino acid, which is also used as the labelled tracer, is that it would lead to changes in the *flux* of the test amino acid, and since the oxidation of the amino acid would be a percentage of the flux through the cell, that these changes in flux would inevitably change or complicate evaluation of the oxidation rate as determined by this model (54).

Recently, another technique has been used to assess the "inflection point' in the relationship between intake of one test amino acid and the oxidation of *another* indispensable amino acid (54). This method was proposed originally by Kim et al (55) and later by Ball et al (56) for studies of the amino acid requirements of pigs. This technique has been extended by Zello and others (57, 58) to studies of amino acid requirements in adult human subjects. In this technique, dietary intakes of the test amino acid are varied, but the irreversible oxidation and balance, of another indispensable amino acid (indicator amino acid) is measured. A relationship between the two is expected, because, at low intakes of the test (limiting) amino acid, protein synthesis will be reduced, and so, the oxidation of other (non limiting) amino acids will be increased. Therefore, as the dietary intake of the test amino acid increases and reaches the requirement level, the oxidation of the indicator amino acid decreases and reaches an inflection in the line of "protein or amino acid" oxidation vs. amino acid intake relationship (Fig. 2). Alternatively, the status of body balance of the indicator amino acid (achievement of 'zero' balance) can be used to identify the requirement level of feeding of the test amino acid. It has been suggested that the indicator amino acid technique may have an advantage over the

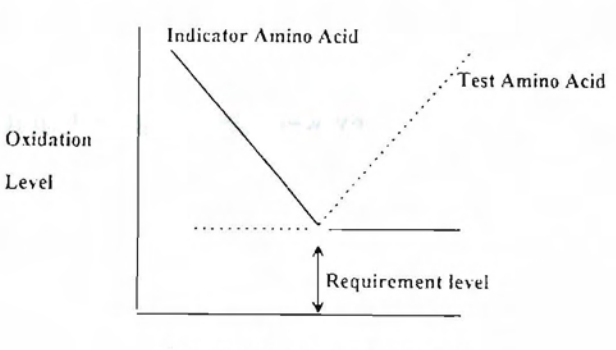

Fig. 2: Effect of dietary intake of test amino acid on its oxidation level and that of the indicator amino acid in relation to the requirement level of the test amino acid.

direct (test) amino acid oxidation method in that there may be changes in the free pool size of the test amino acid, as its level of intake is altered (54). This would be particularly a difficult problem if the intercellular enrichment of the tracer varied independently from the tracer enrichment in the plasma compartment. Since the indicator amino acid (leucine) intake is not varied, the major changes in the enrichment of the free pool of leucine or in its flux would not be expected, leading to reliable oxidation estimates of the indicator amino acid, and therefore, accurate breakpoint estimation.

We have recently carried out studies on the requirement of lysine in adult, well nourished Indian males, in Bangalore, using the indicator amino acid technique. In this study, a group of subjects was fed a diet containing one of two levels of lysine. Therefore each of the subjects was studied twice, with a feeding period of 7 days on each occasion. The levels of lysine in the diet were fixed at 12 mg/kg/day, and at 28 mg/kg/day. These levels corresponded to the 1985 FAO/WHO/UNU level (2) and the new MIT level (4) of requirement, respectively. The habitual intake of lysine in these subjects was between 40 and 60 mg/kg/day.

After completing each of the 7 day feeding periods, a 24 hour tracer balance study was performed, using leucine as the indicator amino acid. Twenty four hour leucine balances were estimated, and it was found that there was a significant negative leucine balance when the subjects were on the 12 mg/kg/day lysine intake diet. The balance, while on the 28 mg/kg/day diet was not significantly different from zero. It seems therefore, that the leucine oxidation measurement is serving as an "indicator" of the status of dietary lysine adequacy. This suggests that the requirements for lysine are close to the 28 mg/kg/day diet, and that the 1985 FAO/WHO/UNU requirements are far too low to maintain a neutral amino acid balance. Therefore, this is the first evidence that the IAA requirements in man may be relatively uniform in well–nourished populations across the world. Further studies are underway in Bangalore to confirm this finding, and to extend this paradigm to measure the requirements of other IAA's.

3.4 : Technical issues with the Tracer Technique :

The stable isotope tracer technique is a demanding method, and attention has to be paid to several issues, in order to avoid serious errors. The diet (particularly the carbohydrate part) eaten by the subjects can be a potential source of error, as the ^{13}C content of plants is variable. If the ^{13}C content of these plants is high, then it's oxidation during a tracer study with a ^{13}C labelled amino acid, will contribute variably to the breath $^{13}CO_2$ enrichment. In general, foods with a low ^{13}C content should be fed during the feeding period, and a correction made, for the contribution of this diet to the breath $^{13}CO_2$ enrichment. Such an error, if present, would over-estimate amino acid oxidation and requirement.

Another potential source of error is the sequestration of the CO_2 produced from oxidation in the body, such that it is not completely recovered in the breath. The reason for the incomplete recovery of $^{13}CO_2$ are well known, and include the

equilibration and entry of the label into slowly turning over pools (59). However, to this may be added potential methodological issues, such as the contribution of dietary ^{13}C to the enrichment of breath $^{13}CO_2$ and the preparation of the isotope solution. Correction factors have to be used in order to accurately predict the actual $^{13}CO_2$ production, as the measured value in the breath may be underestimated by upto 30%. This sequestration is corrected for in the form of a 'recovery factor', which is estimated by infusing ^{13}C labelled bicarbonate into the body, and measuring its recovery as $^{13}CO_2$ in the breath. We have done this, in exactly the same conditions as the tracer amino acid experiment, and have obtained a recovery of about 70% in the fasted state and 75% in the fed state. These $^{13}CO_2$ recovery studies gave values that were slightly lower than what has been reported previously in Westerns studies (60, 61); this difference was more marked in the "fed" pattern of recovery in the present study. The subjects of this Indian study had a smaller body size than the subjects in the previous Western studies (for example, 60), but the body size of the individual has not been shown to influence the recovery of $^{13}CO_2$ as similar recoveries have been recorded in obese and non obese individuals (62, 63), as well as in children and adults (64, 65). The duration of the infusion is another important variable that can influence recovery, as longer duration of infusion gives more time for label to cycle through the slowly turning over pools. However, in the Indian study, which followed a protocol (including duration of infusion) exactly similar to a previous Western study carried out in MIT (29, 30), the fed state recovery was still lower (32). The reason for this lower recovery could be a relatively larger, slowly turning over pool, such as bone (66). An under–or over-estimation of this factor would lead to an over–or under-estimation of IAA oxidation and requirement.

Another issue of importance in the tracer paradigm is the identification of, and the accurate measurement of, the precursor pool. This pool has been accurately identified in the case of leucine, in the muscle, and the enrichment of this pool is accurately represented by the enrichment of it's keto–analog in the plasma (67). The enrichment of alpha keto iso–caproic acid (KIC) is taken as indicative of the enrichment of the intracellular leucine pool, and studies by others (67–70) have supported the use of plasma ^{13}C–KIC enrichment to calculate whole body protein turnover and oxidation rates. In the case of those substances with dominant precursor pools in the liver, the measurement of the enrichment of the amino acid in rapidly turning over liver proteins in the plasma, such as APO B100, will give a reasonably accurate enrichment of the hepatic precursor pool (71). In general, these issues have been dealt with particularly for the leucine tracer model, and similar progress has been made in the case of lysine (24, 53), aromatic amino acids (31), threonine (23) and sulphur containing amino acids (27).

The composition of the amino acid mixture fed also deserves consideration. In general, the composition of the mix should be at the reference value, other than for the amino acid being tested. It has been suggested that the provision of an amino

acid mixture, which is "ideal" or generous, could induce the oxidative machinery of the cell, such that a higher oxidation of the test amino acid could occur (33). However, the intake level of the test amino acid is the *most* significant influence on the oxidation of the test amino acid (33, 72), particularly in the fed state, and an analysis of the influence of *other* amino acids, in the fed mixture, on the oxidation of leucine, in the MIT series of studies, showed no such relationship. Hence, this criticism may not be valid. However, in general, it is worthwhile to maintain the other amino acids in the dietary mixture at reference levels.

Other technical issues that could also lead to error include the neglect of the mass of the tracer in balance calculations, as stable isotope labelled tracers are not mass-less. In addition, the extrapolation of selected time point measurements, during the 24 hour day, to the entire period could also lead to error, as discussed in Section 3.2. Finally, the *energy* balance, during a nitrogen or tracer balance study, must be equal to, or near zero. It is important for activity patterns to be maintained at habitual levels, and for the energy intake to be estimated as a function of the BMR and the activity levels.

4. Lysine

4.1: Daily adult requirement:

With respect to lysine, the 1985 FAO/WHO/UNU (2) upper requirement value was set at 12 mg/kg/day for healthy adults and a ***mean*** requirement value of 30 mg/kg/day (20) has been proposed, based initially on results of short-term tracer studies. Together with the ^{13}C-tracer (indicator) studies from Toronto by Zello et al (57) and by Duncan et al (58), which suggest a mean lysine requirement in the region of 35–45 mg/kg/day, it seems reasonable to propose, for healthy, well nourished North American subjects, a mean lysine requirement value of 30 mg/kg/day.

Nitrogen balance studies give far lower estimates than this value of 30 mg/kg/day. However, the balance data are difficult to interpret and cannot be used readily to assess the minimum lysine requirement of adults. Although Bolourchi et al (73) gave college students a diet supplying about 12 g N daily, for 50 days, from which 90–95% of the N was derived from wheat flour, and found that N balance was positive, their findings are not sufficiently definitive for purposes of assessment of the minimum requirement level of lysine. While the lysine intake provided by the experimental diet approximated 18 mg/kg/day, the dietary energy intake required to prevent body weight loss was determined to be about 54 kcal/kg/day. Such a high energy intake limits the significance of the results obtained from this otherwise important study.

As detailed above, a recent study on Indian males did show that the requirement for lysine was close to 28 mg/kg/day. In that study, the subjects chosen were considerably smaller and much leaner than the MIT subjects studied in the USA. (Subjects from ref. 32 compared to ref. 53). Hence, it is reasonable to draw the tentative conclusion that if the US and Indian subjects have a similar lysine requirement when expressed

per unit of body weight, then the Indian subjects would appear to need somewhat less lysine per unit of existing lean body mass. This does raise the question as to whether healthy Indian subjects use lysine somewhat more effectively to maintain overall amino acid homeostasis than do healthy US subjects. Equally however, one may speculate that these Indians were using their lysine intake more effectively, if one assumes that they may have been in a 'repletion–type' pattern of protein synthesis, given a 'perfect' diet. In such a circumstance, it is possible that once these subjects reach their ideal pattern of lean tissue content, their lysine requirement may actually increase from its present level. More detailed studies, particularly linked to measures of body composition are required, over a longer term period of feeding, in order to resolve this question. The possibility exists that these populations might have adapted, through metabolic mechanisms, to more limiting intakes of lysine in comparison with the generous levels experienced by populations in technically advanced nations. An understanding of this issue is of fundamental importance in human nutrition, as was emphasised by Waterlow in 1985 (74) who stated," I believe that the nature and extent of metabolic adaptation to low intakes of energy and protein is one of the most important subjects in nutritional science at the present time".

It is important therefore, that the requirement for lysine in healthy adults be more firmly established, in order to provide a secure basis for developing food and nutrition policies and programs in regions where populations are at risk of dietary protein/amino acid inadequacies.

4.2: Implications of the new lysine requirement level :

The actual requirement value for lysine that is now established, has profound implications with respect to an assessment of the protein nutritional quality of diets, especially in developing regions, where cereal–based diets supply the major proportion of the indispensable amino acid intake (75, 76). Thus, it is evident that the populations at greatest risk of a dietary lysine inadequacy are those in developing regions of the world (76). There is already increasing evidence that the quality of protein influences linear growth in children (77, 78).

On the other hand, it is also evident that populations consuming diets containing largely evidently poor quality cereal protein, have survived. Assuming that the new IAA requirements are correct, the question that remain is, are these populations physically and functionally healthy? Alternatively, was the adaptation to a low quality protein intake "cost–less" or "costly"?

These questions could be answered by correlating some criterion of health with IAA intake. For instance, the physical (anthropometric) characteristics and activity (functional) patterns of an individual could be used to diagnose a state of Chronic *Energy* Deficiency (79). However, we do not, as yet, have anthropometric, or functional criterion, that can be used to define a minimum but safe level of intake of IAA's.

It is possible however, to evaluate the protein quality in the diets eaten in

developing regions. Protein nutritional quality can be measured by an amino acid score. This concept, first introduced by in Block & Mitchell in 1946 (80), is now defined as the concentration of the limiting amino acid in the food protein as a proportion of the concentration of the same amino acid in a reference amino acid pattern (81). The reference amino acid pattern (which should be ideal) is now taken to be the pattern of human amino acid requirements (82, 83), and is shown, for the different patterns that have been recommended, in Table III. The next step is to identify the limiting (of the least concentration in mg/g protein) amino acid in various proteins, from different sources, and to use these amino acids in the consideration above. The identification of the limiting amino acid is derived from the ratio of the amount of the amino acid in 1 gm of a dietary protein source to the amount of the same amino acid in 1 gm of an ideal standard protein, or, the reference pattern of IAA requirement (84). The amino acid score can be made more accurate by the correction for digestibility of the protein source. Thus, the digestibility of mixed vegetable protein diets by Indian children may approximate 65–85% (85). This method yields a new score, which is called the Protein Digestibility Corrected Amino Acid Score (PDCAAS, ref. 3). While the digestibility factors may vary, this still gives a more accurate scoring pattern for proteins than earlier patterns. The IAA lysine has been shown to be the most limiting in cereal protein, and in general, is at a much lower concentration in most plant foods (86, 87). In addition, the lysine content of legumes is high, and their sulphur containing amino acids are limiting, while animal foods have a high concentration of these amino acids, and are limiting in tryptophan (86, 87).

If an amino acid score ([amino acid content in the food protein/amino acid content in the reference amino acid requirement pattern] x 100) is calculated for wheat flour, it would be ≥ 100, when the 1985 FAO/WHO/UNU amino acid requirement pattern for the adult is used as the reference pattern. This says that the nutritional value of wheat would be *equal*

TABLE III : Amino Acid Scores for different Recommendations.

Amino acid	*Score (mg/gm protein)*			
	FAO/WHO/UNU 1985 (1)	*OAAL (2)*	*New "MIT" Pattern (3)*	*FAO/WHO 1991 (4)*
Isoleucine	13	35	38	28
Leucine	19	65	65	66
Lysine	16	50	50	58
Methionine+cystine	17	25	25	25
Phenylalanine+tyrosine	19	65	65	63
Threonine	9	25	25	34
Tryptophan	5	10	10	11
Valine	20	35	35	35
Total	111	310	313	320

(1) From Ref. 2; (2) From Ref. 4, 86; (3) From Ref. 20; (4) From Ref. 3.

TABLE IV : Amino acid scoring patterns for different food products.

Protein source	*Amino acid score* based on*			
	Lysine content (mg/g protein)	*FAO/WHO/UNU 1985*	*FAO/WHO 1991*	*MIT Pattern*
Wheat	27.2	>100	40	48
Rice	35.2	>100	60	70
Sorghum	24	>100	41	48
Millet	22.4	>100	38	44
Nuts/Seeds	34.5±12.3	>100	59	69
Vegetables	43.2±14.7	>100	74	86
Legumes	72.7±6.04	>100	>100	>100
Animal Protein	81.92±7.8	>100	>100	>100

* =Not corrected for digestibility
1. Lysine Scoring Pattern (mg/g protein)
FAO/WHO/UNU 1985 : 16; FAO/WHO :58; MIT Patter :58;
2. Lysine content from refs. 86,89.

to that of high quality animal protein foods, such as milk, egg or meats, and there would be no concern with the assessment of the quality of plant protein in adults (86). On the other hand, for scoring purposes, if the 1991 FAO/WHO/ pattern were used, a relative nutritional quality of 40% would be obtained. The MIT pattern would predict a slightly higher value of 48% (Table IV, ref. 20), and if digestibility was taken to be 85%, then the PDCAAS would be even lower. In each case, lysine was determined to be the most limiting amino acid (20). In either of these circumstances, a diet containing predominantly cereal as its protein source, would be a cause of concern, for risk of lysine inadequacy.

It is worth considering the impact of this in the context of an Indian diet, supplying 10% of the caloric intake as protein, which could come largely from cereal sources. For example, recent surveys (AV Kurpad, unpublished data) on small groups of urban and rural Indians in and around Bangalore show that a large proportion of the protein intake comes from cereals, which is also reflected in larger surveys (88). Assuming a protein intake of 62 gm (c.v. =20%), a cereal protein intake of 48 gm, a legume (assuming that all non cereal plant protein was legume) intake of 10 gm, and an animal protein (milk/eggs/meat) of 4 gm per day, the lysine intake per day would be about 2400 mg (assuming cereals to contain 30 mg lysine/gm protein, legumes to contain 64 mg lysine/g protein, and animal protein to contain 85 mg/gm protein; these lysine values taken from ref. 86). For a 60 kg individual, the lysine intake would be 40 mg/kg/day. Further, assuming that this would be utilised to an extent of 70%, this would amount to the physiological equivalent of 28 mg/kg/day, which is just about the estimated *minimum* requirement for lysine.

This crude analysis also points out the efficacy of legumes or animal protein, in dramatically increasing the lysine content

of the diet. For example, the ratio of cereal to legume in the above diet, was about 80:20. In order to improve this diet, to achieve a lysine intake of about 3000 mg/kg/day (or, a lysine score of 100 for the mixed dietary proteins), a change in the cereal: legume ratio, from it's previous value, to about 60:40, would suffice. Therefore, a judicious mix of different plant protein sources, would be adequate to meet a desirable lysine intake, even when the amount of animal protein was small or negligible.

Predictions about the desirable level of *population* intakes of lysine can also be made. If we now assume a reference weight of 60 kg in the population, then the *minimum* lysine requirement (based on the new MIT recommendation, ref. 20), in this population would be about 1800 mg/day. With an observed coefficient of variation of the above Bangalore diet of about (it may be more) 20%, and an assumed normal distribution of intake, we would obtain a safe population intake value of this distribution (assuming 1800 mg as falling at two standard deviations below the mean) of 3000 mg/day. This would be the target mean population intake, to ensure that there would be less than 5% of the population at risk of consuming less than their required intake of lysine. Once again, the importance of mixing different plant sources of protein is underscored by this analysis.

4.3: Implications for protein quality and vegetarianism :

The current dogma, as reflected by 1985 FAO/WHO/UNU recommendations (2), that "digestibility appears to be the most important factor determining the capacity of the protein in a usual mixed diet to meet the protein needs of adults", is now being challenged by the new MIT recommendations (20) for IAA intake. It now appears that protein quality is also an important issue for adult dietary intakes.

Throughout history, humans have used some 3000 plant species for food (86). Livestock production diverts the available energy and protein from plants into feed, rather than into direct human consumption, and it is a popular view to recommend the reduction in the amount of legumes and cereals to be used as animal feed (86). There are also many potential benefits of vegetarianism, and these benefits have been the subject of reviews and symposia (89, 90). There are many myths about plant proteins in human nutrition, and these are listed in reference 86. Some of these are that plant proteins are of "poor" quality, and are "incomplete". This is not true, as the analysis in section 3.1 showed, and while plant proteins in isolation may be variable in quality, in *combination*, they are "complete", and adequate to meet the balance requirements of an individual. Further, it is not necessary to have the "right" combination of plant proteins at one meal: the balance achieved over a day, is more important (86).

Apropos the dogma that has been in vogue, about the non–importance of protein quality; this is completely dispelled when accepting the new 1991 WHO/FAO interim recommendations, or the MIT recommendations for IAA intake. Clearly, protein quality is important, this quality in

the diet can be manipulated by food combinations to achieve balance, and excitingly, there are new possibilities of food fortification, with amino acids, in order to render a better quality protein in, for example, cereals.

5. Summary

In summary, the current yet limited evidence, suggests that it is unlikely that there would be any major differences in the minimal physiological requirements for lysine among groups of normal healthy adults of different genetic, nutritional and environmental backgrounds. It is hoped that there will be a broader appreciation of this fact by national and multi-national authorities concerned with improving the nutritional well-being of underprivileged populations world-wide.

Based therefore, on the discussed paradigms, it is our view that the new, tentative "MIT" derived requirements for indispensable amino acids, as summarised earlier in Table II, represent the best available approximations of the needs for these nutrients in adults. Hence, we recommend that they be used as a rational basis for the formulation of food policies world wide. In addition, these recommendations can be used in the formulation of amino acid mixtures, or of protein sources, intended for meeting the nutritional support of individuals in a clinical setting.

The new recommendations are for the minimum amounts of IAA required, and from the beginning of this century, there has been a concern that the requirements of nutrients be set at the most desirable level. As Atwater wrote: "A man may live and maintain body equilibrium on either a higher or lower nitrogen level. One essential question is: what level is the most advantageous? The answer to this question must be sought...in broader questions regarding bodily and mental efficiency, general health, strength and welfare" (91, quoted in ref. 14). The challenge of the future is to identify these functional indices of optimal health, which will guide future recommendations for IAA requirements in human.

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