THE RECENT WHO/FAO/UNU REQUIREMENT PATTERN FOR INDISPENSABLE AMINO ACIDS AND THEIR IMPLICATION FOR PROTEIN QUALITY
Kurpad A V
St John’s Research Institute, Bangalore, India

Introduction

The previous international estimates of indispensable amino acid (IAA) requirements in humans at various ages were set out in the 1985 report of the Joint FAO/WHO/UNU Expert Consultation on energy and protein requirements 1. In the last two decades, an expanding body of evidence has emerged to suggest that the requirements of IAA are probably higher than previously thought. A review of these data led a recently constituted WHO/FAO/UNU Expert Committee to recommend, in the case of adult humans, the use of revised indispensable amino acid requirement values 2, which are about two to three times higher (Table 1) than the earlier international recommendations 1.

The earlier estimates 1 of IAA requirements for adults were so low that it would have been possible to achieve adequate intakes of IAA’s from any diet (Table 2). Indeed, dietary protein quality in this case would be of little practical consequence for adult human protein nutrition.

Methodology leading to the new requirement

The need for a re-evaluation of the IAA requirement was based
on the inadequacies of the method of measuring IAA requirement from estimations of nitrogen balance\textsuperscript{3}, and
from initial thoughts and experiments based on short-term tracer studies\textsuperscript{4,5} and predicted obligatory amino acid losses\textsuperscript{6}, driven by the work of Vernon Young.

In the first case, the nitrogen balance method, as used in the classical studies of Rose, was flawed in terms of the excessive energy intake that the subjects received and because the miscellaneous nitrogen losses were not measured. Re-evaluations of the original N balance data by other investigators\textsuperscript{7}, for example for the evaluation of lysine intake in which corrections for the miscellaneous N loss were made using either 5 or 8 mg N/kg/d, by Hegsted\textsuperscript{8}, Millward\textsuperscript{9} and Rand and Young\textsuperscript{10}, have suggested higher lysine requirements, although the degree of difference in the amino acid requirement estimate was dependent on whether an allowance of 5 or 8 mg N/kg/d was made. The same applied for other amino acids as well. The N balance technique is also prone to severe error from the assumed value of the miscellaneous loss, because the slope of the N balance – amino acid intake curve is so shallow near the zero balance point that small differences in the values assumed for miscellaneous loss will have a large impact on the intake of amino acid required for equilibrium. Therefore the remaining uncertainty was sufficient to preclude the use of these data as a primary source for deriving IAA requirements.

In the predicted obligatory amino acid losses model, the basal rate of N excretion in persons receiving a zero or very low protein diet was used to estimate the requirement for specific amino acids by relating it to the amino acid composition of body protein. The obligatory loss of nitrogen will be determined by the individual amino acid with the highest rate of obligatory loss relative to its concentration in protein. The other amino acids would be released based on their concentrations in the protein that is broken down, and oxidized based on the amount that is in excess of their requirement. This is a derivative method, which relies on relating the mean value of the obligatory N loss to the amino acid composition of mixed body proteins that are broken down, along with an assumption of the efficiency of utilization of dietary amino acids, to provide an estimate of indispensable amino acid requirement\textsuperscript{6}. Because of its theoretical nature, this method could not be considered for providing primary data; however, these calculations and reports led to a sustained effort to accurately measure IAA requirements, which in turn resulted in a paradigm shift in the approach to measuring the IAA requirements of adults.

The current method of choice in estimating the IAA requirement is by the measurement of daily test amino acid balance (24h intake – 24h irreversible oxidation) using a stable isotope tracer amino acid technique, with different levels of the test amino acid being fed to adult human volunteers. The minimum level of intake of the test amino acid that results in a zero balance is considered to be the daily requirement of that amino acid. An important requirement of this tracer-based technique is the precise determination of the rate of test amino acid oxidation. This in turn requires precise measurements
of the isotopic enrichment of the pool directly supplying substrate for oxidation, and this is difficult to determine for most amino acids, except leucine\textsuperscript{11} and probably methionine \textsuperscript{12}.

However, the kinetics of leucine are well established\textsuperscript{13}, and allow for a direct determination of leucine balances over 24 hours. The 24h balance method has been validated\textsuperscript{14}. Earlier short-term tracer balance studies had shown that the daily requirement for leucine was greater than the 1985 FAO/WHO/UNU value of 14 mg/kg/day and was probably ~40 mg/kg/d\textsuperscript{3}, \textsuperscript{4}. One of the arguments against the short-term amino acid oxidation studies was that they were done over a few hours, with extrapolations to a 24h day; it is possible that variations in the rate of leucine oxidation at different periods of the day would not allow these extrapolations to be easily made. A significant development in this field was made by Young’s group, using a demanding 24h tracer balance protocol after adapting subjects to their experimental diets (or levels of test amino acid intake) for one week\textsuperscript{14}, \textsuperscript{15}. These 24h direct amino acid balance studies confirmed that the leucine requirement was almost 3 times as high as the earlier estimate\textsuperscript{1}.

These findings of a higher leucine requirement in young, well-nourished American men had not been validated in populations from different areas of the world. Specifically, these findings needed to be applied to populations from developing countries, in whom there may be adaptations to lower-than-normal protein or leucine intakes, thereby reducing the daily leucine

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**Figure 1: Different tracer methods used in measuring indispensable amino acid requirements**

<table>
<thead>
<tr>
<th>Indicator / Tracer Oxidation or Balance</th>
<th>Test Amino Acid Intake</th>
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<tbody>
<tr>
<td>DAAO - Increasing oxidation of ‘test’ amino acid at supra-maintenance test amino acid intake levels</td>
<td></td>
</tr>
<tr>
<td>IAAO - Decreasing oxidation of ‘indicator’ amino acid at supra-maintenance test amino acid intake levels</td>
<td></td>
</tr>
<tr>
<td>IAAB. DAAB - Increasing balance towards zero balance of either ‘test’ or ‘indicator’ amino acid balance with increasing test amino acid intake levels. Note that at supra-maintenance intake levels, the balance stays at zero.</td>
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Legend:
- Inflection or breakpoint on all lines indicates the measured requirement level. **Solid line:** DAAO - Increasing oxidation of ‘test’ amino acid at supra-maintenance test amino acid intake levels
- **Large broken line:** IAAO - Decreasing oxidation of ‘indicator’ amino acid at supra-maintenance test amino acid intake levels
- **Small broken line:** DAAB or IAAB - Increasing balance towards zero balance of either ‘test’ or ‘indicator’ amino acid balance with increasing test amino acid intake levels.
requirement. We studied the leucine requirements of healthy, well nourished Indians using the 24h daily amino acid balance (DAAB) technique with L-amino acid mixtures (that supply graded levels of leucine) given to the subjects for an adaptation period of one week\textsuperscript{16}; the requirement for leucine was set as the level at which there was a zero leucine balance (Figure 1).

It is worth remembering that $\sim 50\%$ of the adult population in India was chronically undernourished according to a body mass index (BMI) standard of 18.5kg/m\textsuperscript{2}\textsuperscript{17} as a defining cut-off. Therefore, an additional study was undertaken to assess the leucine requirement of chronically undernourished young Indian men, using of the same 24h DAAB technique\textsuperscript{18}. Both these studies confirmed the finding of earlier short-term studies that had indicated a higher leucine requirement.

These developments in tracer methods really meant that one could measure the requirements of leucine with some certainty. However, could the requirement of the other IAAs simply be taken to be similarly higher, or, could the requirements of the other IAAs be independently and experimentally confirmed? The key problem was in the measurement of the precursor pool of the tracer amino acid, since this was critical to the measurement of the amino acid oxidation. A more recent technique (that had its roots in earlier animal experiments) that really broke the impasse in experimentally measuring the requirements for all the other IAAs was the indicator amino acid oxidation technique (IAAO). In this method, the test amino acid and the tracer amino acids are different, the nature of this difference being such that the behaviour of the tracer oxidation and balance under different test amino acid intakes determines (or indicates) the test amino acid requirement. In this approach, the oxidation and balance data of the indicator amino acid (whose intake is held constant) at different test amino acid intakes can be analysed to yield a breakpoint of a plateau in indicator amino acid oxidation (IAAO), or a zero balance (IAAB), which indicates the requirement of the test amino acid (Figure 1). This is because, at suboptimal (limiting) intakes of test amino acid, protein synthesis is ‘frustrated’ leading to increased oxidation of the other IAAs. At intakes of test amino acid that are optimal or above, the indicator amino acid oxidation would plateau off at its lowest level, and its balance would fall to zero (Figure 1). In general, the technical problems of precursor pool identification that are related to the DAAO/DAAB method are not present in this method, since the indicator chosen is one in which the precursor pool is known and/or validated. This method is either applied as a short-term IAAO method\textsuperscript{19}, or a longer-term 24h IAAO and IAAB method\textsuperscript{20}. The requirement is set as the intake level that provides for an inflection (or breakpoint) in the pattern of the indicator amino acid oxidation or balance response to different test amino acid intakes.

The short-term IAAO method has been used with lysine or phenylalanine as the indicator amino acid\textsuperscript{21,22} wherein the appearance of the $^{13}$C label (from the labelled amino acid) in the breath is measured after food intake, over the course of a few hours. Importantly, these studies are conducted after a short (48h) dietary adaptation period. The relative ease of the method, the short duration of dietary adaptation and the relatively non-invasive nature of
experimentation, are advantages that allows for many experiments at different levels of test amino acid intake to be carried out on the same subject. This, in turn, allows for the measurement of the variances of the requirement estimates for each amino acid. On the other hand, the lack of a sufficient dietary adaptation period, the lack of measurement under fasting conditions, and the short-term nature of the after-feeding evaluation may or may not influence the result of the amino acid requirement; there is insufficient evidence available at present to make a judgement about this.

The longer-term, 24h IAAO and IAAB method\textsuperscript{20} were developed using the kinetics of leucine as the indicator amino acid. In the case of leucine, there is no technical uncertainty about the adequacy of the method of measuring leucine oxidation and balance over a 24h period using a 12h fasted and 12h fed protocol. The output measures used for assessing the adequacy of a test amino acid intake include the 24h oxidation or balance, or a 12h post-feeding oxidation measurement. These measurements are performed after an adequate dietary adaptation period of one week, thereby removing the uncertainty about the measurement of the amino acid estimate in un-adapted or inadequately adapted individuals. The long-term dietary adaptation and 24h protocol render these experiments demanding, thereby restricting the numbers of studies that can be performed in a single subject. Nevertheless, these studies using the 24h IAAO or IAAB measurements with an adequate dietary adaptation period are considered to be the best available measurement for the determination of amino acid requirements. Based on the 24h IAAO model as the primary source of data\textsuperscript{20, 23-30}, the recently constituted WHO/FAO/UNU Expert Committee on protein and amino acid requirements\textsuperscript{2} recommended a generally increased pattern of IAA for adults (Table 1).

**Implications**

These new recommendations have an implication for protein quality requirements. 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, in which cereal-based diets supply the major proportion of the indispensable amino acid intake\textsuperscript{31, 32}. Thus, it is evident that the populations at greatest risk of a dietary lysine inadequacy are those in developing regions of the world\textsuperscript{32}. There is already increasing evidence that the quality of protein influences linear growth in children\textsuperscript{33, 35}. On the other hand, it is also evident that populations that consume diets consisting largely of evidently poor quality cereal protein have survived quite effectively. Assuming that the new IAA requirements are correct, the question that remains is, are these populations physically and functionally healthy? Alternatively, was their possible adaptation to a low-quality protein intake “cost-less” or “costly”? Do they have potential contributions from gut microbes, of nutritionally significant amounts of IAA input, to supplement that coming from the diet?

The first two questions may 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. However, we do not, as yet, have an anthropometric, or functional criterion, that can be used to define a minimum but safe level of intake of IAA’s. Populations consuming low lysine diets have been shown to have a lower level of selected immune markers as well as responses to stress\textsuperscript{36, 37}, and in some cases, a relatively higher lysine intake over 3 weeks resulted in a slightly improved muscle function\textsuperscript{29}. Clearly, more functional studies are required. Of concern, however, is the possibility that the environmental and parasitic burden imposed on poorer, undernourished communities may actually result in a higher lysine requirement\textsuperscript{38, 39}. Indeed, the splanchnic uptake of leucine is better in subjects with a higher BMI (Figure 2)\textsuperscript{40}, indicating that gut function may well be an area of research in relation to the adaptive price paid by undernourished humans.

This would imply adaptation with costs, since it is likely that a large proportion of such populations, also subsisting on mainly cereal-based diets, would face the risk of deficiency. To answer the third question posed above, it is also possible that the gut microbes contribute significantly to intestinal lysine (and other IAA) intake. For example, there is evidence of significant absorption of essential amino acids synthesized by the gut microbes in simple-stomached animals and humans\textsuperscript{41, 42}. From labelling experiments using \textsuperscript{15}NH\textsubscript{4}Cl incorporation into microbial lysine in normal human subjects and ileostomates it has been estimated that microbial lysine absorption was 29–68 mg/kg/d, which is of the same order as the estimated average requirement of lysine (30 mg/kg/d) in human adults\textsuperscript{43}. However, while this suggests that nutritionally significant amounts of microbially derived lysine are absorbed, the quantification of the amount absorbed is technically complicated by the need to estimate the \textsuperscript{15}N enrichment of the lysine that is being absorbed, which seems to occur mostly in the small intestine\textsuperscript{44, 45}. Using the indicator amino acid technique specified above, we have determined, through the reduction in leucine input into the body after antimicrobial treatment, to reduce the...
intestinal microbes. Using this method, we were able to determine that the potential leucine (and by extension, other IAA) input from the gut microbiota was of the order of ~ 20%, and in the potential range of nutritional relevance\textsuperscript{46}.

**Protein quality**

Regardless of functional evaluations, or of alternative sources of IAA in the diet, it is possible to evaluate the protein quality in the diets eaten in developing regions. Protein nutritional quality can be measured in terms of an amino acid score. This concept, first introduced in 1946\textsuperscript{47}, 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\textsuperscript{48}. Table 2 shows the reference amino acid pattern for the different reference patterns that have been recommended\textsuperscript{1, 2}. The next step is to identify the limiting amino acid (having the least concentration in mg/g protein) 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 g of a dietary protein source to the amount of the same amino acid in 1 g of an ideal standard protein, or the reference pattern of IAA requirement\textsuperscript{2}. The amino acid score can be made more accurate by correcting for digestibility of the protein source. Thus, the digestibility of mixed vegetable protein diets by Indian children may approximate 65-85\%\textsuperscript{49}. This method yields a new score, which is called the Protein Digestibility Corrected Amino Acid Score\textsuperscript{2} (PDCAAS). While the digestibility factors may vary, this still gives a more accurate scoring pattern for proteins than earlier patterns. 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\textsuperscript{50, 51}. In addition, the lysine content of legumes is high, and their sulphur-containing amino acids are limiting, while animal foods have high concentrations of these amino acids, and are limiting in tryptophan. If the amino acid score 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 to that of high quality animal protein foods, such as milk, egg or meat, and there would be no concern with the assessment of the quality of plant protein in adults. On the other hand, for scoring purposes, if the recent 2007 WHO/FAO/UNU pattern were used, a relative nutritional quality of <50 would be obtained (Table 2). Therefore, a diet containing predominantly cereal as its protein source would be a cause for concern, as regards 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, if a large proportion of the protein intake comes from cereals, and assuming a protein intake of 62 g (with a coefficient of variation of 20%), a cereal protein intake of 48 g, a legume (assuming that all non cereal plant protein was legume) intake of 10 g, and an animal protein (milk/eggs/meat) of 4 g per day, the lysine intake per day would be ~ 2400 g (assuming cereal, legume and animal protein to contain 30, 64 and 85 mg
lysine/g protein respectively). For a 60 kg individual, the lysine intake would be 40 mg/kg/d. Further, assuming that this would be utilised to an extent of 70%, this would amount to the physiological equivalent of 28 mg/kg/d, which is just about the estimated minimum requirement for lysine. This also underscores the efficacy of legumes or animal protein in increasing the lysine content of the whole diet. For example, the ratio of cereal to legume in the above diet was about 80:20. In order to improve this diet and achieve a lysine intake of ~ 3000 mg/kg/d, a change in the cereal:legume ratio to ~ 60:40, would suffice. Therefore, a mix of different plant protein sources would be adequate to meet the desired lysine intake, even when the amount of animal protein in the diet is small or negligible.

One should not however, lose sight of the absolute need for dispensable amino acids and a utilizable source of non-specific nitrogen for the synthesis of the dispensable amino acids and other physiologically important nitrogen-containing compounds, such as purines and pyrimidines, glutathione, and creatine. The rate of formation of dispensable amino acids in the body appears to be determined by the total intake of nitrogen, and their requirement can increase when stresses such as infection exist. At lower levels of total nitrogen consumption the formation of adequate amounts of dispensable amino acids is impaired, and the critical limiting ability may be the ability to provide adequate amounts of glycine and glutamine52.

Summary

In conclusion, protein quality is likely to be something that concerns nutritionists in India. For example, in a calculation of the risk of dietary protein deficiency based on a protein: energy (PE) ratio adjusted for protein quality or PDCAAS, it was estimated that, based on their habitual intakes, 23% of the sedentary adult male population of India could be at risk of IAA or quality protein deficiency53. Based on more recent data from tribal, rural and slum populations54-56, and recalculating the PDCAAS-adjusted PE ratio from these diets (excluding tribals whose diets are worse and constitute a special case), the risk of protein deficiency in elderly sedentary men and women was 34 and 45% respectively, and in younger adult sedentary men and women it was 8 and 14% respectively. Further, many of the reported diets (taken from NNMB surveys) were also suboptimal in micronutrients. For instance, in the tribal intake data, no less than 10% of the men and 30% of the women showed some clinical sign of micronutrient deficiency, and the NNMB report stated that their fruit and vegetable intake was “woefully inadequate”. It seems impossible to quantitatively judge the optimal utilization of protein in these situations, and this is made more difficult since not all micronutrient intakes are energy-dependent. If it were possible to relate the PDCAAS-adjusted PE ratio to some functional or pathophysiological outcome, a much better definition of risk would be available, but that is very difficult to do. In addition, there are so many environmental hazards that it is difficult to pin down protein as the cause of suboptimal function or an adverse outcome; however, studies on lysine requirements in undernourished Indians before and after eradication of intestinal parasites38, 39 would suggest that the environmental stresses are real.
Since the PE ratio has energy in the denominator, increasing this variable would inevitably reduce the requirement for a high PE ratio. The example of the sedentary individual is a case in point here: to satisfy this individual's reference PE ratio, one would have to drive protein intake upward, which would also result in an increased energy intake. This would inevitably result in higher energy intakes and fat accretion. It is tempting to speculate that this is one reason that there is a burgeoning obese population in India, but that argument would have to assume the existence of some protein-stat mechanism that drives appetite. However, from a public health viewpoint, it is worth inverting that reasoning to say that it is very important for the sedentary individual to lead an active life and exercise thereby increasing his/her energy requirement and lowering the required PE ratio to a more attainable level. Complementing cereal intake with high-quality protein sources is also a desirable and effective way to meet IAA requirements.

References


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