Can amino acid requirements for nutritional maintenance in adult humans be approximated from the amino acid composition of body mixed proteins?

(obligatory amino acid losses/amo acid patterns)

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ABSTRACT The quantitative needs for the dietary indispensable amino acids in adult human protein nutrition are still poorly established. Tracer studies with 13C-labeled amino acids have been undertaken previously in our laboratories to reevaluate and further determine the minimum physiological needs for selected indispensable amino acids in healthy adult volunteers. For those amino acids that have not yet been studied by this approach we have proposed a tentative set of requirement figures based on considerations of the amino acid composition of body mixed proteins and the rate of obligatory amino acid losses (i.e., losses when the diet contains no proteins or amino acids). Here we provide an argument for, and a justification of, this approach as an interim measure until more comprehensive data become available on the quantitative aspects of amino acid metabolism in healthy humans.

We have carried out a series of stable nuclide-enriched, amino acid tracer studies to reevaluate the minimum physiological dietary requirement for the indispensable (essential) amino acids (IAA) in healthy adult humans (1–3). Further, we have concluded that the current international minimum amino acid requirement values are far too low for this population age group. For those amino acids that we have not yet examined in tracer studies we estimated the requirements for them based on an estimation of the obligatory oxidative losses (OOL; oxidative losses when the diet contains no proteins or amino acids) and minimum dietary intakes required to balance these (2). The pattern of total daily obligatory amino acid loss (OAAL) reflects that of the relative concentrations of amino acids in whole-body mixed proteins and so we used this pattern to approximate the required dietary intakes of some of the specific IAA (2, 3). However, our assumption that the amino acid requirement pattern for maintenance of protein nutritional status in adults is similar to that for mixed proteins in the whole body has been criticized by a number of investigators (4–7). Furthermore, Millward et al. (8) state that we (9) do not justify, or explain, our use of the OOL as the basis for our pattern, although we have attempted to do this, to a limited extent, in a number of our more recent papers (3, 10). These criticisms must be taken seriously since our tentative amino acid requirement pattern (11) has potentially important implications for the design of sound food and nutrition policies and programs, including agricultural research directed toward human nutrition. In this paper, we will further explain, and justify, the basis for this amino acid requirement pattern. In doing so, we will evaluate the basis for, and strength of, the arguments that have been used to disagree with our proposed pattern (4, 5, 7).

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Initial Proposal

In adults, when intakes of energy and other nutrients are adequate but the diet is essentially protein-free, the rate of body nitrogen (N) loss, principally via urine and feces, reaches a new, relatively steady-state level within about 1 week (12, 13). This new level is called the obligatory N loss (ONL) (14) and amounts to 54 mg of N·kg⁻¹·day⁻¹ in healthy adults (15). It can be assumed reasonably that the amounts of the different IAA contributing to these N losses occur in proportion to their concentrations in body mixed proteins, providing that those proteins contributing to the major proportion of the total N loss do not have amino acid patterns (concentrations) that differ markedly from the average of the body mixed proteins.

Initially, we assumed that the OOL were due entirely to oxidative catabolism and so we referred to these as obligatory oxidative losses of amino acids. Although the major route of loss of these amino acids is, indeed, via oxidative catabolism, the amino acids are also lost in urine in small quantities or via the intestine (particularly for threonine) in the ileal digesta (16). However, this is not a crucial issue for our proposal whether all of the amino acids are terminally oxidized or whether some are lost intact; body amino acid losses have been predicted from total ONL. Nevertheless, it would have been more appropriate to have referred to these as OAAL rather than to call them OOL; we will use the term OAAL in our further considerations below.

Having estimated the OAAL, we then assumed, from whole-body N balance studies in humans (14, 15), that at about a requirement level of intake, individual IAA would be retained with an efficiency of about 70%. Further research is needed, however, to determine whether it is valid to use a single figure for all of the amino acids for this purpose and so we need to know the actual value(s) with which the various absorbed IAA coming from the diet are retained (and before they are lost from the body). For present reference, however, our proposed amino acid requirement pattern is given in Table 1 and it is compared here with the adult amino acid requirement pattern prepared by the 1981 Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU) Expert Consultation on Energy and Protein (15). It can be seen that the international recommendation is about one-half to one-third of the level that we have proposed as a necessary minimum intake to maintain protein nutritional status in healthy adult humans.

Pattern of Amino Acid Requirements

Amino Acid Requirements in Preschool Children. To assess the validity of the approach described above it is instructive to

Abbreviations: OOL, obligatory oxidative loss(es); IAA, indispensable amino acids; OAAL, obligatory amino acid loss(es); ONL, obligatory N loss(es); SAA, sulfur amino acids; FAO/WHO/UNU, Food and Agriculture Organization/World Health Organization/United Nations University.
apply it to the preschool age group, for which amino acid requirements have been established using multiple criteria, including N balance and plasma amino acid levels, by workers at the Institute for Nutrition for Central America and Panama in Guatemala (17). These data were used by FAO/WHO/UNU (15) to establish the recommended amino acid requirement for preschool and young school-age children. Hence, we felt that it would be of interest to learn whether it is possible to predict, with reasonable accuracy, these requirements from OAAL, also taking into account the amounts of amino acids that are deposited as a net body protein gain. We have made these calculations (10) and, as summarized in Table 2, it is evident that our predicted requirement values are close to those proposed by the international group (Table 2, columns 1 and 2). It appears, therefore, that the requirements for preschool children can be approximated, initially, from OAAL. Further, the experimentally determined pattern of amino acid requirements is similar to that of body mixed proteins (Table 2, columns 6 and 7). Although there is a daily net deposition of body protein in this age group, it should be appreciated that “maintenance” accounts for about 90% of the total daily protein (nitrogen or IAA) requirement in the 5-year-old child (15). On this basis, the amino acid requirement pattern (essentially for maintenance) in the young child appears to be similar to that for mixed proteins in the whole body. Perhaps it might be pointed out that, in spite of the similarity in the requirement pattern as discussed above, the absolute concentration of each of the IAA per unit of dietary protein (or N) required is lower than for the concentration of amino acid in mixed proteins of the body (Table 2, compare columns C and D). This does not weaken our argument, however, because the efficient utilization of IAA requires an adequate level of dietary, nonspecific nitrogen, supplied in the form of the nutritionally dispensable amino acids or other, nontoxic, utilizable nitrogen sources, including urea (20, 21). Our (22) recent experiments reveal that the quantitative relationships between the indispensable and dispensable amino acid nitrogen components of the total protein requirement deserve further investigation in human subjects.

### Amino Acid Metabolism and Requirements in the Pig: A Model for the Human?

Millward et al. (7) have based their criticisms of our approach taken to arrive at a tentative set of requirement figures for adults largely on the basis of estimates of amino acid requirements for maintenance and growth in the growing 40- to 45-kg (body weight) pig [as reported by Fuller et al. (23)]. Hence, Millward et al. (7) have said that the pattern of the OOL is more like the dietary pattern of amino acids required for growth than for the amino acid pattern that they consider to be required for maintenance in this animal model. From such a comparison these investigators (7) have accepted that “the pattern of the OOL must (present authors’ emphasis) be quite different from the minimum requirement pattern.” However, we consider that this conclusion is based on a flawed, quantitative extrapolation from observations made with a pig model to the human nutritional context. Our reasoning is as follows. First, the proportionate contributions made to the total amino acid (and nitrogen) requirement by that for maintenance or for growth are profoundly different for the pig and the human.

### Table 2. Comparison of amino acid requirements in 2-year-old children: Estimations from OAALs with those of FAO/WHO/UNU/1985

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>1985 FAO/WHO/UNU</th>
<th>Body protein, g/16 g of N&lt;sup&gt;†&lt;/sup&gt;</th>
<th>C/D&lt;sup&gt;‡&lt;/sup&gt; (% of Lys)</th>
<th>C/D&lt;sup&gt;‡&lt;/sup&gt; (% of SAA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predictions from OAALs, mgkg&lt;sup&gt;-1&lt;/sup&gt;·day&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>1985 FAO/WHO/UNU</td>
<td>mgkg&lt;sup&gt;-1&lt;/sup&gt;·day&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>g/100 g of protein&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leucine</td>
<td>53</td>
<td>73</td>
<td>6.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>31</td>
<td>31</td>
<td>2.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Valine</td>
<td>38</td>
<td>38</td>
<td>3.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Lysine</td>
<td>60</td>
<td>64</td>
<td>5.8</td>
<td>0.94</td>
</tr>
<tr>
<td>Threonine</td>
<td>32</td>
<td>37</td>
<td>3.4</td>
<td>0.86</td>
</tr>
<tr>
<td>Aromatic (Phe + Tyr)</td>
<td>58</td>
<td>69</td>
<td>6.3</td>
<td>0.84</td>
</tr>
<tr>
<td>Sulfur (Met + Cys)</td>
<td>26</td>
<td>27</td>
<td>2.5</td>
<td>0.96</td>
</tr>
</tbody>
</table>

*See ref. 15 for FAO/WHO/UNU data. SAA, sulfur amino acids.

1. Details for predictions given in ref. 10.
2. Values are g/100 g of protein required, based on the FAO/WHO/UNU (15) estimate of 0.99 g of protein per kg of body weight per day.
3. From Reeds (18).
4. Figures adjusted with the ratio for lysine taken as 100.
5. Figures adjusted with the ratio for SAA (Met + Cys) taken as 100.
6. Value in parentheses for an assumed body mixed protein leucine concentration of 8.2% (19).
growing pig as compared to both the young child and human adult. From the data of Carr et al. (24), we can calculate that the growth component accounts for about 90% of the total requirement in the 40-kg pig and this component continues to remain high as the animal progresses toward a mature weight (>140 kg) (10). In the growing rat the maintenance need might be as low as about 5% of the total requirement (25). In the human subject, in contrast, the growth component of the total protein requirement is considerably lower than that for pigs and rats, being about 50% at 6 months of age and then declining to about 20% in the 2-year-old child and 15% or less in the 4–5 year old (15). Thus, on this basis alone, the quantitative importance of the metabolic processes responsible for protein acid requirement in the growing pig (and rat) differs profoundly from that for the preschool child. In addition, the efficiencies of dietary nitrogen (protein) utilization, and presumably that of dietary amino acid retention, at maintenance intakes in the 40-kg pig (i.e., Millward’s model for man) and the 2- to 5-year-old child are substantially different. According to Fuller et al. (23), the young pig uses dietary nitrogen with an approximate 100% efficiency for maintenance. Here we define efficiency as the dietary intake of N required to balance the losses of N that would occur with a N-free diet. In the young child, on the other hand, it is substantially less than the estimate for the pig, being about 70% according to FAO/WHO/UNU (15). Furthermore, this value also applies to that for healthy adults (15). Therefore, the data of Fuller et al. (23) cannot be used to resolve problems concerning quantitative aspects of human amino acid requirements.

If we compare the amino acid requirement pattern for adult pigs (boars) (26) with that for body proteins (18, 27) we also find that, when related to the lysine requirement, the pattern of dietary requirement is not too dissimilar from that for body mixed proteins. However, we have been informed by D. H. Baker of the University of Illinois (personal communication) that the National Research Council requirement pattern (26) is based on very weak data and so we do not emphasize unduly these numbers in our account of the issue at hand. Nevertheless, when we also compare the profile of amino acids required for near maximum growth in the rat (28) with the amino acid composition of the body of the rat (27) the relative values agree no better with each other than those based on our comparisons above for the mature pig. The point we are making here is that the pattern of amino acids required for rat growth does not necessarily resemble any more precisely the amino acid composition of mixed body proteins (or pattern of OAAL) as compared with that for maintenance in the preschool child (Table 2) (and presumably the adult). Again, this is in contrast with the opinion expressed by others (7).

Finally, the question can be raised as to the physiological, as well as nutritional, significance of maintenance amino acid needs for the growing organism; at these intake levels the organism would effectively be receiving a deficient intake in relation to its full metabolic potential. It will be of interest to learn whether the maintenance amino acid requirement pattern proposed by Fuller et al. (23), based on studies in the growing pig, is capable of efficiently maintaining protein homeostasis in the mature, nongrowing pig that is given an adequate but not excessive intake of total nitrogen. We raise this issue also because direct estimates of the maintenance requirements for threonine, SAA, isoleucine, and lysine in 145-kg gilts (29, 30) do not agree with those proposed by Fuller et al. (23). Indeed, the differences between these various published estimates may be as much as 2-fold, further weakening the arguments and conclusions drawn by Millward et al. (7). Also, while the University of Illinois pattern (UIP) of amino acids required by adult swine (29, 30) does not resemble that for mixed proteins in the body, the UIP is based on limited data, and the estimates of requirements show large differences depending upon whether a nitrogen balance equilibrium or a relatively small positive body nitrogen balance of 1 g is used as the criterion for evaluation. Finally, the rate of whole-body protein synthesis (WBPS) in growing pigs approximates 8–15 g kg⁻¹·day⁻¹ (31–33), in comparison with that in adult humans of about 4 g kg⁻¹·day⁻¹; when protein and energy intakes are at about maintenance levels in young pigs the rates of WBPS are substantially below those achieved when the ration supplies twice and three times the maintenance requirement (34) or adequate levels of protein (35). In contrast, according to Pacy et al. (36), in adult human subjects there is relatively little effect on whole-body protein turnover with changes in protein intake, during 2-week periods, over a wide range covering submaintenance and supramaintenance levels. Hence, again differences in the quantitative aspects of protein metabolism in the growing pig and adult human call for considerable caution when assessing amino acid metabolism and dietary requirements in adult human subjects.

Other Considerations and Counterarguments

The So-Called “Anabolic Drive.” Millward et al. (8) have proposed a descriptive model of whole-body protein and amino acid metabolism where a minimally adequate intake of protein (and IAA) is one that is sufficient to exert an acceptable anabolic drive, or the regulatory influence of amino acids in protein metabolism. In this context, they further propose that for a single, rate-limiting IAA the magnitude of the obligatory requirement may be similar to its OOL. We accept that this could be so, providing that there is a high effective mechanism for the conservation and retention of the amino acid when ingested at a physiologically limiting intake but not when intakes of this or other IAA are just adequate. These investigators (8) then use lysine, as an example, and compare the current international human adult requirement value of lysine of 12 mg·kg⁻¹·day⁻¹ with an estimated OAA L of 30 mg·kg⁻¹·day⁻¹ (2), which they say indicates that there is a high content of lysine in body proteins but a relatively low metabolic need. The effective conservation of lysine at limiting intakes is now well recognized (37, 38) but this does not mean that protein nutriment can be adequately sustained at extremely low amino acid intakes; indeed, improved conservation of lysine in growing animals is associated with diminished growth performance (37–39) and reduced protein turnover. However, we might further consider the lysine requirement value of 12 mg·kg⁻¹·day⁻¹, which is recommended by Millward et al. (8), in relation to the necessary replention of tissue protein that should occur during the fed period of the day so that a balance is achieved following the loss during the fasting period of the total 24-hr period. We can do this in relation to a recent study from Millward’s laboratories (40) in which it was found that the loss of body N during the 12-hr fasting phase of the 24-hr day was about 60 mg of N·kg⁻¹·12 hr⁻¹ for subjects receiving an adequate, but not excessive, 0.8 g of protein·kg⁻¹·day⁻¹ and who were in an overall daily, negative body N balance of about 19 ± 4.5 mg of N·kg⁻¹·day⁻¹. Taking, then, the value of 12 mg of lysine·kg⁻¹·day⁻¹ above and also assuming a quantitative net retention of this amino acid (i.e., no oxidative loss, which would be unlikely in reality) during the fed 12 hr, this replention would be equivalent of about 24 mg of protein N (N × 6.25)·kg⁻¹ during this anabolic phase. Clearly, this would be insufficient to balance the fasting loss of 60 mg of N·kg⁻¹ and so the subjects would remain presumably in a marked daily negative N balance. This further assumes that the N loss is equivalent essentially to the rate of net urea production (or urea production from nonrecycled N), which we believe to be the case (41), but if the latter rate is higher, as some have suggested (42), then the subjects would be in even greater negative N balance. Thus, from these N excretion data, it can be concluded that a minimum intake of about 29 mg of lysine·kg⁻¹ during the day would be required, assuming the
lysin content of retained protein to be 7.8% and that no oxidation of lysine occurred during the 12-hr fed phase, which would again be unlikely at this lysine intake level (43). Furthermore, it may also mean that an anabolic drive would not occur at 12 mg·kg\(^{-1}\)·day\(^{-1}\) intake because plasma lysine levels, and presumably also tissue free amino acid pool concentrations (44, 45), would decline (43, 44) with feeding at this relatively low intake level. In sum, the various arguments by Millward et al (8) against the amino acid requirement pattern being similar to that of body proteins do not stand up to close scrutiny.

Lysine may not be the rate-limiting amino acid, or the one that "drives" the ONL. Hence, there is some evidence that this driver may be methionine and/or possibly threonine (46) in the growing rat. The currently stated requirements for the SAA (methionine plus cystine) and threonine in healthy adult humans are 13 and 7 mg·kg\(^{-1}\)·day\(^{-1}\), respectively (17) (Table 1). In comparison, our proposed mean requirement values are also 13 mg for SAA but they are higher, or 15 mg·kg\(^{-1}\)·day\(^{-1}\), for threonine (2, 11). Using the same line of reasoning that we use in the foregoing paragraph concerning lysine retention and requirements, we predict that the 13-mg value for the SAA requirement (methionine plus cystine) would permit an achievement of body N equilibrium; on the other hand, with a 7-mg threonine intake, the N balance would be distinctly negative. However, a 15-mg threonine·kg\(^{-1}\)·day\(^{-1}\) level would permit a condition of close to body N equilibrium. Again, however, it seems unlikely that the oxidation rate of these two amino acids would decline to essentially a zero value during the entire feeding period, although these rates may fall below those for the fasting state when the intakes are limiting (47).

Actually, our OAAL procedure predicts a somewhat higher threonine requirement level of about 21 mg·kg\(^{-1}\)·day\(^{-1}\). This figure may somewhat exceed the minimum requirement if threonine oxidation adaptively, and without untoward functional consequences, declines to a relatively low level during a brief period of the absorptive/postprandial phase of amino acid metabolism.

**Amino Acid Use in Non-Protein Metabolic Pathways.**

Amino acids are used to meet a plurality of metabolic functions, in addition to their well-established role as substrates for protein synthesis (48). By essentially ignoring these additional functions and metabolic pathways in our calculations and predictions, this raises a potential criticism concerning the present approximation of adult human amino acid requirements that we have based quite simply on the composition of body mixed proteins. Further, these functions probably bear little relationship to the pattern of amino acids involved in polyribosome-directed protein synthesis and, thus, of the amino acid composition and turnover of tissue proteins. Although there is limited information on the quantities of amino acids involved in these other metabolic pathways, we make the tentative conclusion that they do not have any major impact on the quantitative values for irreversible amino acid losses and daily amino acid requirements, at least when these are assessed from body amino acid balance criteria. To give just two examples: (i) although the approximate turnover of glutathione in adults is about 24 μmol·kg\(^{-1}\)·hr\(^{-1}\) (49, 50), there is presumably an effective reutilization of the cysteine that is liberated, via the membrane-bound enzyme, γ-glutamyltranspeptidase, either into the plasma or following its intracellular release, with resynthesis of glutathione; (ii) the methionine used in creatine synthesis can be regenerated effectively via methylation of homocysteine. From a physiologic standpoint these other metabolic pathways and/or additional functions are of vital importance but from the data available they would not appear to represent quantitatively important irreversible routes of loss of the IAA. Therefore, considerations of protein synthesis, balance and body protein amino acid pattern would seem to be of paramount importance in predicting approximate dietary requirements for IAA in adult humans. Nevertheless, we accept that an analysis of the impact of altered levels of amino acid intake on the quantitative activity of these other metabolic pathways, and their functional significance, should help to improve upon current approaches taken to estimate the minimum dietary intakes of specific amino acids necessary for maintenance of physiologic function and performance.

**Relationships Between Amino Acid Oxidation of the Various Amino Acids.** In his evaluation of our previous \(^{13}\)C tracer studies (2, 3, 10) on the amino acid requirements of adult human subjects, Waterlow (5) suggested that the \(^{13}\)C tracer data implied that when the intakes of all the IAA, other than the limiting one under test, are high then the activity of various amino acid catabolizing enzymes is maintained at a high level and the oxidation of the test amino acid would be incompletely suppressed. His view suggests the requirements for the individual amino acids that have been determined in preschool children (15, 17) and in adults (15) by altering the level of a single, test amino acid while maintaining intakes of other IAA at adequate or generous levels, potentially would have led to overestimates of actual needs. Further, Waterlow's (5) point also implies that the pattern of amino acid requirements is determined by the pattern of amino acids in an experimental diet, which, in turn, could be very different from the amino acid composition of body mixed proteins. However, the suggestion made by Waterlow (5) is not supported by extensive unpublished data (V.R.Y.) in which the relationship between the rate of leucine oxidation in the fed state and the amino acid composition of various diets was examined. It can be concluded that the dietary level of leucine is the most important, quantitative determinant of its whole-body oxidation rate, with the amino acid composition (pattern) and level of total nitrogen in the diet having relatively little or no effect. This point is supported by findings, even under conditions of marked dietary disproportions of the branched-chain amino acids in human subjects (51) and of amino acid imbalances in rats (52), that the oxidation of the limiting amino acid is not necessarily increased and may be reduced to quite low levels (52, 53).

**Conclusions**

From the foregoing we conclude that (i) the amino acid requirements predicted from OAAL agree well with those determined from metabolic studies in young, preschool-age children; (ii) the pattern of the amino acid requirement as derived from metabolic studies in this young age group is similar to the composition of the mixed proteins in the body, despite the large maintenance and small growth components to the overall requirement for total nitrogen and amino acids; (iii) the arguments made by others against use of OAAL for, at least, a first approximation of the adult human amino acid requirements are not supported by the available data; (iv) the prediction of adult amino acid requirements from OAAL has merit as a tentative basis for establishing the adult human requirements for those IAA that have not yet been reevaluated and tested directly. Although the pattern of amino acids required by the adult subject that is ultimately established, and later agreed upon by authoritative national and international groups, may differ from that of whole-body mixed proteins it appears that the proposed approach we have taken to arrive at potentially more realistic amino acid requirement figures can be justified, in the interim. Thus, the answer to the question posed in the title to this paper is a qualified "yes;" indeed, there seems to be little alternative, at present.

We appreciate greatly the advice and direction given to us by Profs. D. H. Baker (University of Illinois, Urbana) and N. J. Benevenga


