Criteria and Significance of Dietary Protein Sources in Humans

Dispensable and Indispensable Amino Acids for Humans\textsuperscript{1,2}

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ABSTRACT Here, we compared the traditional nutritional definition of the dispensable and indispensable amino acids for humans with categorizations based on amino acid metabolism and function. The three views lead to somewhat different interpretations. From a nutritional perspective, it is quite clear that some amino acids are absolute dietary necessities if normal growth is to be maintained. Even so, growth responses to deficiencies of dispensable amino acids can be found in the literature. From a strictly metabolic perspective, there are only three indispensable amino acids (lysine, threonine and tryptophan) and two dispensable amino acids (glutamate and serine). In addition, a consideration of in vivo amino acid metabolism leads to the definition of a third class of amino acids, termed conditionally essential, whose synthesis can be carried out by mammals but can be limited by a variety of factors. These factors include the dietary supply of the appropriate precursors and the maturity and health of the individual. From a functional perspective, all amino acids are essential, and an argument in favor of the idea of the critical importance of nonessential and conditionally essential amino acids to physiological function is developed. J. Nutr. 130: 1835S—1840S, 2000.

KEY WORDS: • amino acid nutrition • metabolism • dietary requirement • function

For at least 60 years, it has been the convention to divide amino acids into two categories: indispensable (or essential) and dispensable (or nonessential). This categorization provides a convenient, and generally useful, way of viewing amino acid nutrition. However, despite the longevity of the convention, as more information has become available, the distinctions between dispensable and indispensable amino acids, at least at the metabolic level, have become increasingly blurred. Indeed, W. C. Rose, who was responsible for the initial definition of the two terms, was not especially enamored with the way in which they were applied by others and wrote the following (Womack and Rose, 1947):

“We have emphasized on several occasions... that the classification of an amino acid like arginine or glutamic acid as dispensable or indispensable is purely a matter of definition.”

I wish to consider this “matter of definition” by examining the terms from a nutritional, metabolic and functional perspective.

Nutritional definitions of indispensable and dispensable amino acids

It is important to remember that the terms “indispensable” and “dispensable” were originally defined not only in dietary terms but also in relation to the role of amino acids in supporting protein deposition and growth. In fact, as far as I can ascertain, the original nutritional definition of an indispensable amino acid (Borman et al. 1946) was, “One which cannot be synthesized by the animal organism out of materials ordinarily available to the cells at a speed commensurate with the demands for normal growth.”

The key phrases in this definition, and phrases that were, in fact, italicized by the authors, are “ordinarily available,” “at a speed” and “normal growth.” Each is an important qualifier.

The phrase “ordinarily available” is important because a number of nutritionally essential amino acids, e.g., the branched-chain amino acids, phenylalanine and methionine, can be synthesized by transamination of their analogous \(\alpha\)-keto acids. However, these keto acids are not normally part of the diet and hence are not “ordinarily available to the cells.” The phrase “at a speed” is important because there are circumstances in which the rate of synthesis of an amino acid can be constrained, e.g., by the availability of appropriate quantities

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of metabolic nitrogen. Indeed, the rate of synthesis becomes of specific importance when we consider a group of amino acids, exemplified by arginine, cysteine, proline and perhaps glycine, that are frequently described as conditionally essential. For example, Womack and Rose (1947) made the important point that the degree to which arginine could be regarded as indispensable was very much a function of the quantities of its natural precursors, proline and glutamate, in the diet. Finally, the phrase “normal growth” is critical in two respects. First, it serves to emphasize that the definitions were originally constructed in the context of growth. For example, it is possible to show (Table 1) that the ingestion of diets completely devoid of glutamate, which in some ways can be regarded as the doyen of dispensable amino acids, leads to a small but statistically significant slower rate of growth. Second, constraining the definition of essentiality to growth does not encompass the importance of some amino acids to pathways of disposal other than protein deposition, a subject that I discuss later.

Amino acid biosynthesis

It is also possible to define amino acid essentiality and nonessentiality in chemical and metabolic terms. An examination of the amino acids that are generally considered to be nutritionally essential indicates that each has a specific structural feature, the synthesis of which cannot be catalyzed by mammalian enzymes (Table 2). In this regard, it is very important to note that the loss of the ability to carry out these biosyntheses appeared early in evolution and is a common feature of the metabolism of eukaryotic organisms in general, and not just of mammals. However, within this view, the important term is de novo synthesis. This is because some indispensable amino acids can be synthesized from precursors that are structurally very similar. For example, methionine can be synthesized both by transamination of its keto acid analogue and by remethylation of homocysteine. In this sense, then, the mammal is capable of synthesizing leucine, isoleucine, valine, phenylalanine and methionine. However, this is not new synthesis, because the branched-chain keto acids and homocysteine were originally derived from branched-chain amino acids and methionine, respectively. According to this restricted metabolic definition of essentiality, threonine and lysine (and perhaps tryptophan) are the only truly essential amino acids.

The reverse applies to dispensable amino acids. Strictly speaking, a truly nonessential amino acid is one that can be synthesized de novo from a non–amino acid source of nitrogen (e.g., ammonium ions) and an appropriate carbon source. According to this metabolic definition, the only truly metabolically nonessential amino acids are glutamic acid and serine. If this is so, then these two amino acids are the ultimate precursors of the other nonessential amino acids. This conclusion leads to the prediction that the contribution of endogenous synthesis to the systemic fluxes of glutamate and serine should be higher than its contribution to the fluxes of other nonessential amino acids. This appears to be so (Table 3). Interestingly, there is a reciprocal relationship between the contribution of endogenous synthesis to the plasma flux of a given nonessential amino acid and the degree to which the intestine metabolizes the dietary amino acids in first pass (see Reeds et al. 1996, Stoll et al. 1998).

Conditionally essential amino acids

Although the nitrogen of the amino acids that mammals can synthesize derives ultimately from either glutamate or serine, there are some amino acids that are synthesized by more complex pathways than the simple transamination of an appropriate keto acid. These amino acids are frequently termed “conditionally” essential, the term being used to imply that there are measurable limitations to the rate at which they can be synthesized. When this limit is attained, the amino acid in question becomes an essential component of the diet. The limitations can result from a number of factors.

First, the synthesis of these amino acids (Table 4) requires the provision of another amino acid, either as the carbon donor or as a donor of an accessory group, such as the sulfur group of cysteine. Thus, the ability of the organism to synthe-

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**Table 1**

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Complete diet</th>
<th>Glutamate devoid</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>21</td>
<td>55.0</td>
<td>36.8</td>
<td>Hepburn and Bradley 1964</td>
</tr>
<tr>
<td>49</td>
<td>11.1</td>
<td>8.8</td>
<td></td>
<td>Womack and Rose 1947</td>
</tr>
<tr>
<td>60</td>
<td>2.94</td>
<td>2.71</td>
<td></td>
<td>Horvath et al. 1996</td>
</tr>
<tr>
<td>Pig</td>
<td>24</td>
<td>37.1</td>
<td>32.8</td>
<td>Price, P. and Stoll, B. unpublished data</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Structural feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine, isoleucine, valine</td>
<td>Branched aliphatic side chain</td>
</tr>
<tr>
<td>Lysine</td>
<td>Primary amine</td>
</tr>
<tr>
<td>Threonine</td>
<td>Secondary amine</td>
</tr>
<tr>
<td>Methionine</td>
<td>Secondary thiol</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Indole ring</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Aromatic ring</td>
</tr>
<tr>
<td>Histidine</td>
<td>Imidazole ring</td>
</tr>
</tbody>
</table>

**Table 3**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Fed state</th>
<th>Fasted state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate¹</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>Serine¹</td>
<td>94</td>
<td>95</td>
</tr>
<tr>
<td>Aspartate¹</td>
<td>76</td>
<td>78</td>
</tr>
<tr>
<td>Alanine¹</td>
<td>45</td>
<td>46</td>
</tr>
<tr>
<td>Glycine¹</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Arginine²</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Proline²</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

as low-birth-weight infants, it is possible that conditionally (Jaksic et al. 1991). Moreover, in immature individuals, such case with regard to the proline nutrition of burned individuals biosynthetic capacity of the organism. Such appears to be the demands for the amino acids rise to values that are beyond the sized may be quite limited (Beaumier et al. 1995, Berthold et al. 1995). It follows from this that alterations in either intestinal metabolism or the route of nutrition can have a critical implications of conditionally essential amino acids that can be synthesized in only a limited number of tissues. For example, the synthesis of both proline and arginine is crucially dependent on intestinal metabolism (Wakabayashi et al. 1994, Wu et al. 1997). Moreover, in the case of these two amino acids, the available evidence suggests that dietary, as opposed to systemic, amino acid precursors are obligatory (Beaumier et al. 1995, Berthold et al. 1995, Brunton et al. 1999, Murphy et al. 1996, Stoll et al. 1999). The synthesis from parallel measurements of intake and body proteolysis do not agree with estimates based on isotopic incorporation from labeled precursors (cf Beaumier et al. 1995 and Berthold et al. 1995 for arginine; Jaksic et al. 1987 and Berthold et al. 1995 for proline). Furthermore, there is also evidence to suggest that newly synthesized conditionally essential amino acids may be used within their cells of origin and hence do not equilibrate with the plasma pool (Miller et al. 1996). Nevertheless, even with these uncertainties, it appears that the synthesis of these amino acids can become limiting for growth and other physiological functions and that an absolute, as opposed to a relative, dietary requirement can be defined.

### Amino acids and physiological function

As I emphasized earlier here, the original definitions of the terms "indispensable" and "dispensable" were focused on growth or, more correctly, on protein deposition. When the definitions are applied in this way, there is relatively little confusion, at least regarding the indispensable amino acids. Quantification of the minimum needs for indispensable amino acids to support growth is relatively easy because these are simply the product of the rate of protein deposition and the amino acid composition of the proteins that are deposited. In this regard, there is a good consensus that the relative needs of individual amino acids to support protein deposition are very similar among mammalian species (Table 5). In other words, the amino acid requirements for the support of protein deposition in the human infant differ from those of other mammals only to the degree to which their respective rates of protein deposition differ.

In humans, the obligatory amino acid needs for net protein deposition are a very minor portion of the total amino acid requirement (Dewey et al. 1996), and >90% of the total amino acid requirement, even of the young child, is associated with the maintenance of body protein stores (i.e., nitrogen equilibrium). Formulating the amino acids needs for "mainte-

### TABLE 5

<table>
<thead>
<tr>
<th>Species</th>
<th>Lysine</th>
<th>Phenylalanine</th>
<th>Methionine</th>
<th>Histidine</th>
<th>Valine</th>
<th>Isoleucine</th>
<th>Leucine</th>
<th>Threonine</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>77</td>
<td>43</td>
<td>20</td>
<td>30</td>
<td>52</td>
<td>39</td>
<td>85</td>
<td>43</td>
<td>Pellet and Kaba 1972, Davis et al. 1993</td>
</tr>
<tr>
<td>Human</td>
<td>72</td>
<td>41</td>
<td>20</td>
<td>26</td>
<td>47</td>
<td>35</td>
<td>75</td>
<td>41</td>
<td>Widdowson et al. 1979</td>
</tr>
<tr>
<td>Pig</td>
<td>75</td>
<td>42</td>
<td>20</td>
<td>28</td>
<td>52</td>
<td>38</td>
<td>72</td>
<td>37</td>
<td>Wu et al. 1999, Mahan and Shields 1998</td>
</tr>
<tr>
<td>Sheep</td>
<td>75</td>
<td>42</td>
<td>17</td>
<td>23</td>
<td>53</td>
<td>33</td>
<td>79</td>
<td>47</td>
<td>Meier et al. 1981</td>
</tr>
<tr>
<td>Calf</td>
<td>69</td>
<td>39</td>
<td>18</td>
<td>27</td>
<td>42</td>
<td>30</td>
<td>74</td>
<td>43</td>
<td>Williams 1978</td>
</tr>
</tbody>
</table>
nance” is difficult and is still the subject of controversy (see Young and Borgonha 2000).

At least as important as the technical and experimental difficulties associated with the measurements of maintenance amino acid needs (Fuller and Garlick, 1994) is the problem of identifying the processes that consume amino acids close to nitrogen equilibrium. A portion of these needs is, of course, directly associated with protein metabolism and reflects two related factors: that amino acids released from tissue protein degradation are unlikely to be recycled with complete efficiency, and that the presence of finite concentrations of free amino acids inevitably leads to some degree of catabolism. There also is increasing evidence that a significant portion of the needs for some essential amino acids may reflect the <100% efficient recycling of intestinal secretions (Fuller et al. 1994, Fuller and Reeds, 1998). This aspect of basal or maintenance amino acid needs is amenable to direct measurement, although some technical aspects of these measurements, notably those associated with intestinal protein metabolic function, pose difficulties (see Fuller and Reeds, 1998). However, as more information has accrued, it has become increasingly clear that amino acids are involved (and hence consumed) in a number of physiological functions that are not directly related to protein metabolism itself.

Before passing to a discussion of these pathways, it is critical to emphasize two additional points. First, at protein intakes that are just sufficient to maintain body protein equilibrium, metabolic nitrogen itself, rather than any single amino acid, may be the limiting nutrient. In other words, because nitrogen is in short supply, the ability of the organism to synthesize amino acids may become compromised to the extent that nonessential amino acid intake could become limiting. This might be particularly applicable to conditions associated with the consumption of low quantities of so-called high quality proteins (i.e., proteins that are well balanced for protein deposition and hence with a high indispensable amino acid/disposable amino acid ratio). Second, there is now evidence to show that the adult human is capable of lowering the catabolism of any single amino acid close to zero if that amino acid is strongly limiting (Raguso et al. 1999). However, the rate of catabolism of the amino acid observed under this circumstance is much lower than that found when protein as a whole is the limiting dietary nutrient. One explanation for this observation is that under protein-free feeding conditions, the free amino acid pool derives exclusively from tissue proteolysis so that all amino acids are equally limiting. The consequence is the utilization of any single amino acid in the support of a nonprotein process automatically limits the ability of the organism to recycle all others back into the protein stores of the body. The questions that arise are: What are these nonprotein pathways of consumption, and what is their quantitative impact on amino acid needs in general? The short answer to both questions is simple: There is not sufficient current information to provide accurate answers. Nevertheless, it is possible to hypothesize which pathways could be the most important at the level of overall physiological function.

To develop these hypotheses, it is useful to consider those functions that are necessary to maintain health. This is not a new approach, as its usefulness was clearly appreciated by some of the founders of nutritional science. For example, Voit (1902), as quoted by Lusk (1922), wrote the following:

“I therefore maintain my "older" point of view, that of pure metabolism... the more unifying development will be possible as one investigates what substances are destroyed under different circumstances... and how much of the different materials must be fed to maintain the body in condition.”

In my opinion, four systems are critical for the body to be “maintained in condition”: the intestine, to maintain absorptive and protective function; the immune system and other aspects of defense; the skeletal musculature system; and the central nervous system. Within each system, critical metabolic roles for some specific amino acids can be identified (Table 6).

Perhaps the most interesting point that emerges from a consideration of Table 6 is that with the exception of the involvement of phenylalanine and tryptophan in the maintenance of the adrenergic and serotonergic neurotransmitter systems and of methionine as a methyl group donor for the synthesis of creatine, the necessary precursors are nonessential or conditionally essential amino acids. That being so, it is tempting to argue that the ability to maintain the synthesis of these amino acids is of sufficiently high functional priority that under conditions in which protein (nitrogen) is limiting, essential amino acids are used to maintain these pathways. In Table 7 I attempted to compare estimates of the turnover or losses of some critical end products with the kinetics of their precursor amino acids. This comparison suggests that some pathways, e.g., taurine and nitric oxide production, have little quantitative impact on the nutrition of the precursors, whereas others are of much greater importance. Thus, creatine synthesis (as estimated from creatinine excretion) and the turnover of glutathione (estimated from measurements in the plasma and red blood cells) have a substantial impact on the utilization of some precursors, especially if the rate of product synthesis is set against the intake or net synthesis of the precursor.

The continuing synthesis of these two end products, one involved in energy transduction in both the musculature and central nervous system and the other a critical factor in detoxification mechanisms, appears to have a substantial potential impact on the nutritional status of the individual. Indeed,
data obtained in protein-depleted pigs (Jahoor et al. 1995) and in asymptomatic HIV-infected patients (Jahoor et al. 1999) demonstrate that the supply of protein and cysteine can measurably alter the ability of the organism to maintain glutathione synthesis.

In this necessarily brief account, I attempted to examine the terms "indispensable" and "dispensable" as they apply to amino acids, from three perspectives. The traditional nutritional view, which focuses specifically on growth, clearly differentiates amino acids that must be supplied in the diet from amino acids that do not necessarily have to be supplied by that source. The metabolic view leads to a somewhat more complex interpretation; this reveals that there may be significant limitations on the synthesis of some amino acids, thereby rendering them potential limitations to growth. Finally, the functional view not only serves to indicate the essentiality of all amino acids for specific physiological functions but also leads to the conclusion that under conditions in which protein is the primary dietary limitation, the ability to maintain the synthesis of some traditionally nonessential amino acids might be crucial for the continuing health and functional integrity of the individual. Clearly, the scene is now set for further, more detailed quantitative examination of these important issues. I look forward to an expansion in our knowledge of amino acid functions that extends beyond the traditional focus on protein.

**LITERATURE CITED**


**TABLE 7**

Potential contribution of functionally important end product synthesis to amino acid needs in adult humans

<table>
<thead>
<tr>
<th>Glutamate</th>
<th>Glycine</th>
<th>Cysteine</th>
<th>Arginine</th>
<th>Methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma flux</td>
<td>4200</td>
<td>3960</td>
<td>1320</td>
<td>1800</td>
</tr>
<tr>
<td>“Net” synthesis</td>
<td>358</td>
<td>2730</td>
<td>96</td>
<td>1807</td>
</tr>
<tr>
<td>End product production</td>
<td>170</td>
<td>170</td>
<td>170</td>
<td>7</td>
</tr>
<tr>
<td>Creatine9</td>
<td>1550</td>
<td>1550</td>
<td>1550</td>
<td>550</td>
</tr>
<tr>
<td>Taurine10</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione11</td>
<td>550</td>
<td>550</td>
<td>550</td>
<td>15</td>
</tr>
</tbody>
</table>

1 From Matthews and Campbell 1992.
2 From Yu et al. 1985.
3 From Fukagawa et al. 1996.
5 The difference between amino acid intake [at a protein intake of 1 g/(kg d)] adjusted for first-pass splanchnic utilization and entry from proteolysis (calculated from leucine kinetics).
6 Calculated from methionine transsulfuration (Fukagawa et al. 1996).
7 Calculated from the transfer of citrulline to arginine (Castillo et al. 1993).
8 Methionine intake, adjusted for first-pass splanchnic metabolism.
9 From daily creatine excretion.
10 From taurine excretion (Naismith et al. 1987.
12 Refers only to erythrocyte and plasma glutathione (Jahoor et al. 1999) and hence measures neither hepatic (Jahoor et al. 1995) nor intestinal (Reeds et al. 1997) glutathione synthesis.