Amino Acid Requirements of Infants and Children

Paul B. Pencharz a–d, Ronald O. Ball c,d

aResearch Institute, Hospital for Sick Children, Departments of bPaediatrics and cNutritional Sciences, University of Toronto, Toronto, Ont., dDepartment of Agriculture, Food and Nutritional Sciences, University of Alberta, Edmonton, Alta., Canada

Abstract

Nitrogen balances have been conducted in preterm infants, preschool children, and 6- to 10-year-old children to determine dietary indispensable amino acid. A recent review concluded that the data, being sufficiently uncertain, could not be used as the basis for defining amino acid requirements in infants and children. Therefore, it was decided to use a factorial approach (basal plus growth). This approach is based on the assumption that basal requirements are the same throughout the life cycle. Recently, using indicator oxidation, the requirements of the eight classical indispensable amino acids have been defined in adults. These values have been used as the basal component of requirement in childhood. The growth component was based on the changes in body protein with age. We have recently shown that the maintenance requirements for branched chain amino acids are similar in children and adults, thereby validating the factorial approach.

Introduction

This review is restricted to the 20 α-amino acids for which t-RNAs exist and hence are part of protein. These amino acids can be divided into the classical indispensable (essential) amino acids, the conditionally indispensable amino acids and the dispensable (nonessential) amino acids [1]. The 8 classically indispensable amino acids cannot be made by humans and hence must be obtained from the diet, hence dietary requirements have been defined for them. For the 9th classical indispensable amino acid in infants and children, histidine, it has proven impossible to determine its requirements in adults, since it takes >40 days to deplete body histidine stores [2]. Conversely, in infants [3] and in young pigs [4] it has proven straightforward to determine the histidine requirement,
since body histidine stores are much smaller. The term ‘conditionally indispensa-
ble amino acid’ implies that the infant or child is unable to make sufficient
amounts of that amino acid and hence all or a part of the daily needs for that
amino acid have to be provided by the diet. For infants the literature evidence is
that only 5 α-amino acids are truly dietary dispensable amino acids, namely, ala-
nine, aspartate, asparaginine, glutamate and serine [1].

Components of Requirements

Requirements for α-amino acids are made up of components, namely how
much is needed for net incorporation into protein, plus that which is needed
for other biological processes. While biologically important, some of these
processes such as neurotransmitter synthesis from tyrosine and tryptophan
are quantitatively small [5, 6]. Conversely others require significant amounts
of the amino acids, namely: (a) cysteine, glutamate and glycine for glutathione
synthesis; (b) arginine for urea cycle activity, and (c) arginine, glycine and
methionine for creatine synthesis [6].

Determination of Amino Acid Requirements

The determination of dietary requirements in infants and children has
proven to be a challenging task. Whatever method is used, graded levels of
the amino acid under study have to be fed to the subjects, ranging from below
to above the requirement level [7–9], and changes determined in a biological
response. The biological responses which have been used include: nitrogen
balance; plasma amino acid level; direct amino acid oxidation and balance,
and indicator amino acid oxidation and balance [5, 8]. Plasma amino acid lev-
els have not proven to be useful except possibly for tryptophan [8]. Nitrogen
balances have been conducted in preterm infants [3, 10, 11], preschool chil-
dren [12], and 6- to 10-year-old children [13–17] to determine dietary indis-
pensable amino acid. As recent extensive review of this work concluded that
the data were sufficiently uncertain so that they could not be used as the
basis for defining dietary indispensable amino acid requirements in infants
and children [5]. Direct oxidation and balance are limited to the few amino
acids whose carboxyl group is released as soon as the amino acid is committed
to degradation and, in addition, there are several potential biological limitations
with direct oxidation/balance which have been reviewed in detail [8]. Hence,
indicator oxidation and balance are regarded as the optimal methods to
determine dietary indispensable amino acids [5, 8, 18]. There is some dis-
agreement as to how much adaptation time is needed prior to conducting a
indicator study at a particular level of intake of the test amino acid [5, 8]. It
has long been known that if nitrogen balance is used as the method to determine
the requirement it takes 7 days to achieve equilibrium in the body urea pool and in urinary urea excretion. Hence if nitrogen balance is used then subjects must be adapted on the test intake for a minimum of 7 days. It is unacceptable to place an infant or young child on a markedly deficient level of test amino acid intake for 7–10 days (7 days of adaptation plus 3 days to conduct the nitrogen balance study). Conversely the indicator method is based on the partitioning of the indicator amino acid between net incorporation into protein or oxidation. In which case the adaptation needed does not relate in anyway to the urea pool but does relate to the turnover of acylated t-RNAs, which is a matter of a few hours [8]. Therefore we have developed a minimally invasive method to determine essential amino acid requirements in infants and children which uses fed state as an indicator of oxidation [7–9, 19, 20].

Since there are limited published data on dietary amino acid requirements in children using indicator amino acid oxidation (IAAO), and in light of the decision not to use the earlier nitrogen balance data [5], it was decided to use a factorial approach to define dietary indispensable amino acid requirements in infants and children [5, 18]. The factorial approach is based on the assumption that the basal requirements of a component are the same throughout the life cycle and that requirements in infants and children are higher than those in adults due to growth [5]. Using a combination of IAAO and indicator balance, the requirements of the 8 classical indispensable amino acids have recently been defined [5, 18] and these have been used as the basal component of requirement for infants and children. Recent data from the Children's Nutrition Research Center in Houston have provided much improved estimates of the growth component and this has been used to define the growth component for each indispensable amino acid [21, 22]. Because of all the assumptions involved in the factorial method, we are in the process of determining dietary indispensable amino acid requirements in children and comparing them with estimates obtained in adults using the same IAAO method. So far our studies support the use of the factorial method [8, 9, 23, 24]. Specifically for branched chain amino acids in 6- to 10-year-old children, their requirements approximate the maintenance (adult) requirement plus the small growth component.

**Requirement Estimates for Dietary Indispensable Amino Acids**

For infants <6 months of age estimates of dietary indispensable amino acids have been based on the average intake from mother's milk (table 1). These were calculated based on the average amino acid content of human milk protein [5, 18] multiplied by the average protein intake from mother's milk [5, 18].

For infants >6 months of age, children and adolescents amino acid requirement estimates were calculated using the factorial approach described above.
The protein deposition estimates used are shown in table 2. Since there were only small differences due to gender, it was decided to use an average of the results for the two genders [18]. Details are given in the footnote to table 3 as to how amino acid requirements were calculated. Briefly, the maintenance pattern derived from adult requirement estimates were multiplied by the maintenance protein requirement (0.686 g protein/kg/day) to calculate the maintenance need for each amino acid, to which was added the growth component. The growth component is calculated from tissue protein composition multiplied by the amino acid content of that tissue protein (table 3). Finally a correction is made for dietary protein utilization which has been estimated as being approximately 58% of the ingested protein. The efficiency factor is derived from the slope as regression analysis of nitrogen balance studies conducted in children with a variety of different protein sources [5, 18]. These new estimates of dietary indispensable amino acid requirements differ from the previous World Health Organization report [25] to a variable degree as shown in table 4. The variability is less when the estimates are compared as a requirement pattern, namely as milligrams of each amino acid per gram of protein intake. The biggest differences are for tryptophan and for the aromatic amino acid, the sum of phenylalanine and tyrosine. There is no doubt that the maintenance estimates for the dietary indispensable amino acids are based on more complete data than were available in 1985 [5, 8, 18]. Similarly the growth estimates are based on extensive new data [21, 22]. However, it is important to mention that the maintenance estimates for the aromatic amino acid are based on the averaging of two very different results and may be an underestimate. If that proves to be the case the requirement patterns will

Table 1. Indispensable amino acid intakes in exclusively breast-fed infants

<table>
<thead>
<tr>
<th>Age, months</th>
<th>AAA1</th>
<th>HIS</th>
<th>ILE</th>
<th>LEU</th>
<th>LYS</th>
<th>SAA1</th>
<th>THR</th>
<th>TRP</th>
<th>VAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>162</td>
<td>36</td>
<td>95</td>
<td>165</td>
<td>119</td>
<td>57</td>
<td>76</td>
<td>29</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>118</td>
<td>26</td>
<td>69</td>
<td>121</td>
<td>87</td>
<td>42</td>
<td>55</td>
<td>21</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>102</td>
<td>23</td>
<td>60</td>
<td>105</td>
<td>75</td>
<td>36</td>
<td>48</td>
<td>19</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>93</td>
<td>21</td>
<td>54</td>
<td>95</td>
<td>68</td>
<td>33</td>
<td>44</td>
<td>17</td>
<td>54</td>
</tr>
<tr>
<td>6</td>
<td>88</td>
<td>20</td>
<td>52</td>
<td>90</td>
<td>65</td>
<td>31</td>
<td>41</td>
<td>16</td>
<td>52</td>
</tr>
</tbody>
</table>

1AAA = Aromatic amino acids, the sum of phenylalanine and tyrosine; SAA = sulfur amino acids, the sum of methionine and cysteine.

2Values taken from the WHO/FAO/UNO Protein and amino acid report [18].

3Protein content in human for each age group (75% crude protein) multiplied by the average amino acid content as mg/g of protein shown above.

The protein deposition estimates used are shown in table 2. Since there were only small differences due to gender, it was decided to use an average of the results for the two genders [18]. Details are given in the footnote to table 3 as to how amino acid requirements were calculated. Briefly, the maintenance pattern derived from adult requirement estimates were multiplied by the maintenance protein requirement (0.686 g protein/kg/day) to calculate the maintenance need for each amino acid, to which was added the growth component. The growth component is calculated from tissue protein composition multiplied by the amino acid content of that tissue protein (table 3). Finally a correction is made for dietary protein utilization which has been estimated as being approximately 58% of the ingested protein. The efficiency factor is derived from the slope as regression analysis of nitrogen balance studies conducted in children with a variety of different protein sources [5, 18]. These new estimates of dietary indispensable amino acid requirements differ from the previous World Health Organization report [25] to a variable degree as shown in table 4. The variability is less when the estimates are compared as a requirement pattern, namely as milligrams of each amino acid per gram of protein intake. The biggest differences are for tryptophan and for the aromatic amino acid, the sum of phenylalanine and tyrosine. There is no doubt that the maintenance estimates for the dietary indispensable amino acids are based on more complete data than were available in 1985 [5, 8, 18]. Similarly the growth estimates are based on extensive new data [21, 22]. However, it is important to mention that the maintenance estimates for the aromatic amino acid are based on the averaging of two very different results and may be an underestimate. If that proves to be the case the requirement patterns will
only differ markedly for tryptophan. Clearly, there is a need to directly determine the tryptophan requirements of children.

### Requirement Estimates in Disease

The development of the minimally invasive indicator amino acid oxidation model for the first time opened the way to directly determine requirements for inborn errors of amino acid metabolism: phenylketonuria [19, 20] and maple syrup urine disease [26]. In addition we have been able to use the indicator oxidation technique to determine essential amino acid requirements during intravenous feeding and compare the results with estimates obtained during enteral feeding. The work was conducted initially in neonatal piglets as a model for human neonates [27, 28] and has been partially confirmed in human neonates [29]. Overall this work has shown that the intestinal mucosa is active in amino acid metabolism. The key findings are that: up to 60% of dietary threonine in the neonatal piglet is taken up and used for the synthesis of new proteins.

### Table 2. Protein deposition for infants and children

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Protein deposition, g/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>females</td>
</tr>
<tr>
<td>0.5</td>
<td>0.266</td>
</tr>
<tr>
<td>1.0</td>
<td>0.168</td>
</tr>
<tr>
<td>1.5</td>
<td>0.108</td>
</tr>
<tr>
<td>2.0</td>
<td>0.076</td>
</tr>
<tr>
<td>3.0</td>
<td>0.044</td>
</tr>
<tr>
<td>4.0</td>
<td>0.026</td>
</tr>
<tr>
<td>5.0</td>
<td>0.022</td>
</tr>
<tr>
<td>6.0</td>
<td>0.038</td>
</tr>
<tr>
<td>7.0</td>
<td>0.048</td>
</tr>
<tr>
<td>8.0</td>
<td>0.051</td>
</tr>
<tr>
<td>9.0</td>
<td>0.050</td>
</tr>
<tr>
<td>10.0</td>
<td>0.047</td>
</tr>
<tr>
<td>11.0</td>
<td>0.043</td>
</tr>
<tr>
<td>12.0</td>
<td>0.037</td>
</tr>
<tr>
<td>13.0</td>
<td>0.031</td>
</tr>
<tr>
<td>14.0</td>
<td>0.025</td>
</tr>
<tr>
<td>15.0</td>
<td>0.018</td>
</tr>
<tr>
<td>16.0</td>
<td>0.012</td>
</tr>
<tr>
<td>17.0</td>
<td>0.005</td>
</tr>
<tr>
<td>18.0</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Derived from Butte et al. [21] and Ellis et al. [22], data smoothed using non-linear regression. For details see [18].
### Table 3. Amino acid requirements of infants, children and adolescents

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Protein requirements for maintenance&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Amino acid requirements&lt;sup&gt;5&lt;/sup&gt;, mg/kg/day</th>
<th>Amino acid content (mg/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AAA HIS ILE LEU LYS SAA THR TRP VAL</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.686</td>
<td>0.46</td>
<td>59</td>
</tr>
<tr>
<td>1–2</td>
<td>0.686</td>
<td>0.19</td>
<td>40</td>
</tr>
<tr>
<td>2–10</td>
<td>0.686</td>
<td>0.06</td>
<td>30</td>
</tr>
<tr>
<td>10–14</td>
<td>0.686</td>
<td>0.07</td>
<td>30</td>
</tr>
<tr>
<td>14–18</td>
<td>0.686</td>
<td>0.04</td>
<td>28</td>
</tr>
<tr>
<td>&gt;18</td>
<td>0.66</td>
<td>nil</td>
<td>25</td>
</tr>
</tbody>
</table>

<sup>1</sup>Amino acid content of whole body protein [18].
<sup>2</sup>Maintenance amino acid requirements expressed as mg/g of protein per day.
<sup>3</sup>Maintenance protein requirements in childhood and for adults (>18 years) determined as described by WHO/FAO/UNO Protein and amino acid report [18].
<sup>4</sup>Calculated as an average value from the growth data in table 2 adjusted for a protein utilization of 58% [18].
<sup>5</sup>Sum of the maintenance protein × the adult maintenance amino acid pattern and growth (tissue deposition adjusted for a 58% dietary efficiency of utilization × tissue amino acid pattern).

### Table 4. Comparison of the factorial requirement estimates for preschool children (1–4 years old) with previous values

<table>
<thead>
<tr>
<th>Requirement pattern, mg/g protein</th>
<th>AAA</th>
<th>ILE</th>
<th>LEU</th>
<th>LYS</th>
<th>SAA</th>
<th>THR</th>
<th>TRP</th>
<th>VAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio New/1985 values</td>
<td>0.60</td>
<td>0.87</td>
<td>0.75</td>
<td>0.70</td>
<td>0.81</td>
<td>0.65</td>
<td>0.49</td>
<td>0.95</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Requirement pattern, mg/g protein</th>
<th>AAA</th>
<th>ILE</th>
<th>LEU</th>
<th>LYS</th>
<th>SAA</th>
<th>THR</th>
<th>TRP</th>
<th>VAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>New WHO/FAO Report [18]</td>
<td>47</td>
<td>32</td>
<td>64</td>
<td>53</td>
<td>27</td>
<td>28</td>
<td>7.5</td>
<td>42</td>
</tr>
<tr>
<td>Ratio New/1985 values</td>
<td>0.75</td>
<td>1.14</td>
<td>0.97</td>
<td>0.91</td>
<td>1.08</td>
<td>0.82</td>
<td>0.68</td>
<td>1.2</td>
</tr>
</tbody>
</table>
of intestinal mucins [27, 28]; about 40% of the branched chain amino acids are oxidized, and the gut is necessary for arginine synthesis [27, 28].

**Future Directions**

More work needs to be conducted to verify whether the factorial method of calculating dietary indispensable amino acid requirements is valid in infants as well as in children. Chronic diseases such as liver and kidney failure are known to alter amino acid metabolism and hence may also alter dietary indispensable amino acid requirements [8, 9]. The minimally invasive indicator model makes studies possible in these chronically ill children. Of much larger public health importance, many of the world's children live in poverty and have chronic diarrheal illnesses. We believe that this increases their amino acid requirements and there is a need to define by how much.

**References**


Discussion

Dr. van Goudoever: Thank you very much and congratulations on all the work you have done in improving all these requirements. The figures you showed on branched chain amino acid requirements in adults versus 6- to 10-year-old children saying that 6 mg/kg/day is the amount needed for growth, have you calculated how much g/kg/day that is and whether it would fit the normal growth pattern of infants of that age?

Dr. Pencharz: Those were actually based on the data of Butte et al. [1] and Ellis et al. [2] in children 6–10 years of age and then corrected for utilization; so that is the number we came up with. Now the actual direct isotope breakpoint we think really reflects expenditure. In one day the growth component cannot actually be measured anymore than with doubly labeled water; you are just measuring the expenditure. In
our case it is an 8-hour period; in Dr. Butte’s case with the doubly labeled water it is 5, 3 days, whatever, and then adding the growth on top of that.

Dr. van Goudoever: But if I understood correctly, the requirements were 1.43 for adults and 1.47 for 6- to 10-year-olds, and those were measured by your indicator of amino acids?

Dr. Pencharz: That is correct.

Dr. van Goudoever: And so the 6 mg/kg/day is calculated separately.

Dr. Pencharz: The 1.43 and 1.47 are the numbers we actually found. I will admit that we did not initially interpret our data completely correctly. We said there is a difference there of 4, and 6 and 4 are very close. So we come to realize that in piglets with the rapid growth we are measuring the growth component as well as maintenance. In our studies in the slow growing children we are measuring only maintenance, we are not measuring the growth component.

Dr. van Goudoever: Because the 6 mg would not represent let’s say 2 g/kg/day of growth.

Dr. Pencharz: I can’t remember but you have to go back and see what they are growing and what proportion of that is the three branched amino acids, etc.

Dr. Dewey: One of the issues that I think is interesting to explore is the assumption that in the first 6 months human milk does meet all the essential amino acid requirements. Does that fit with your estimates of requirements from 6 months onward? I was just comparing your tables showing intake from human milk and the requirements. It looks as though for most of them intakes are higher than requirements, except for histidine, lysine and the sulfur amino acids it is very close. Could you comment on that?

Dr. Pencharz: It is a really good question, and we don’t know because the data just don’t exist. The human milk data are based on analysis of the true protein amino acid composition plus adding free amino acids on top, and so it is just like being an accountant and adding everything together. We are in the process of doing measurements using indicator oxidation techniques in neonates and we hope to have estimates for both the enteral and parenteral requirements of branched chains of threonine and so on. But that hasn’t been done yet, so we don’t really have good data. Peter Reeds and I have tried to subject the Holt and Snyderman data [3] to nonlinear regression but the data are too weak, so we don’t have a good estimate. At the moment, we need more data to be able to answer your question.

Dr. Garlick: The major difference between your approach with the indicator and the approach that other people have used previously, either with isotopes or nitrogen balance, is the adaptation issue. Even when using an indicator method, people have still used the traditional adaptation period of a couple of weeks on each diet because they felt that this was required for the enzymes to readapt to the new level. You don’t; you use the time it takes for the subject to start with the meals with the new amino acid content, a few hours rather than 2 weeks. Could you elaborate on why it is that you feel that you can dispense with this adaptation period?

Dr. Pencharz: I did mention adaptation briefly in my written paper but I haven’t brought any slides. The adaptation business really relates to the urea pool. The urea pool in the body is distributed within body water, so 60% of our body composition as adults is water. Rand et al. [4] showed years ago that the adaptation when you go from one level of protein to another or one level of amino acid to another is 5–7 days; so that is what is behind Dr. Garlick’s question. The big question is does this apply to enzyme adaptation with regard to oxidation of an indicator amino acid and to formation of transfer RNA and protein synthesis. We thought not, and in fact in our piglet studies suggested that this was not the case and it was not necessary. So in our first studies in humans we actually looked at two very different levels of phenylalanine intake and
showed what prior adaptation did to our estimates, and we found no effect. So that is where the data are at the moment. We are actually conducting a study right now and I am pleased to be able to tell you that we are finding no effects on indicator oxidation with our adaptation of 6h versus 3 days versus 7 days. I will also say that for three amino acids there are data from Kurpad and Young [5] and our own work, and there is no systematic difference in our estimate of sulfur amino acids of lysine or threonine. So in fact although the adaptation question is an important one, it doesn’t affect the estimates we are obtaining in the breakpoint.

Dr. Thi Thanh Binh Nguyen: Is there any difference between the amino acid requirement for preterm babies and term babies. If we use a total parenteral nutrition and the enteral route, is there any difference between the quantity and quality requirement of amino acids for preterm infants?

Dr. Pencharz: The very small premature baby in utero is growing and accreting protein more rapidly than the term infant. So in terms of total protein there is a difference; a term baby needs less protein than the preterm baby. What we don’t really know, and at the moment it is only theory, is there may be differences in different amino acids. There clearly are differences if you feed parenterally than enterally and that is the work that Burrin et al. [6] and our own group [7] in Canada have done. The piglet work is lead by Ron Ball and the human work by myself, but we work together. We have defined a pattern of essential amino acids. The one we haven’t actually looked at is histidine. There are earlier data on histidine. So I do think that we are going to find some differences in very small premature infants, and we are embarking on studies looking at arginine and other amino acids in very small premature infants. So we don’t know the answer, but perhaps in the next 5 years or so we might have the answer.

Dr. Muhammad Heru Muryawan: You have shown that the essential amino acid requirements for the ages 2–10 and 10–14 years are the same for the 2 groups, except for threonine which is very different for these groups. Can you explain why?

Dr. Pencharz: I may have to go back and check and see if we have got the right numbers because in entering it I may have made a mistake. It should not be systematically different from all of the others, so I appreciate you pointing out that potential error. Dr. Garlick and I will look at that and make sure that in the book it is correct. This is not directly from the WHO document; this is my transcription and so I could well have made a mistake.

Dr. Yates: I can understand nitrogen balance, which is a fairly approximate evaluation methodology, when we talk about body weight based on kilograms. But when we are dealing with a nutrient that is so closely tied to lean body mass, how much of the decision to put these in terms of the amount/kg/day is due to convention and ease of determining body weight versus a real lack of concern about lean body mass as a better unit of measure?

Dr. Pencharz: That is a really good question and you have actually answered it yourself. The reason that we chose both the dietary reference intake and the WHO is that measuring body weight is so much easier. Scientifically there is no doubt that nutrients like this will relate to lean body mass. We have repeatedly tried to see if we could show differences when we measure body composition in our studies and we haven’t yet come up with anything, but perhaps in disease we may. Actually we did study this in liver patients and we still could not find any different interpretation using lean mass versus body weight.

Dr. Rigo: When you extrapolate the data on factorial approach to the first months of life and compare them to human milk composition, is there a good agreement for all the amino acids?

Dr. Pencharz: I haven’t done that for quite a while, so I can’t really answer that. It would be nice to go back and to see if this issue of maintenance applies in the first
Dr. Dewey: You mentioned that the maintenance requirement was determined to be similar between 6- and 10-year-old children and adults. How much evidence do we have that this is also true under 12 or 6 months of age?

Dr. Pencharz: We don't know the answer. I can only fall back on animal studies which we have done in pigs. It looks as though the maintenance in newborn pigs is the same as in adult pigs, so it is likely true in humans as well.

References
