Proteins, Peptides and Amino Acids in Enteral Nutrition: Overview and Some Research Challenges

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The importance of adequate nutritional support as a component in the comprehensive clinical management of patients is now widely appreciated, and over the past two decades advances have been made in enteral feeding techniques. However, as outlined by Klein \textit{et al.} [1], the most clinically effective and cost-effective form of nutritional treatment for patients with gastrointestinal diseases, wasting conditions, and in the critically ill remains to be determined from research-based evidence rather than from expert opinion as at present.

The respective contributions of parenteral and enteral nutritional support [2, 3] and the merits of preoperative, perioperative, and postoperative nutritional support also have been reviewed [4–7]. Clearly, there are still large gaps in our knowledge about the qualitative and quantitative aspects of the form of enteral nutrition that would be best for individuals or groups of hospital inpatients and for others who might benefit from a dietary supplement.

\textit{A sine qua non} of the stress response, triggered either by infection, trauma, or even by clinical interventions, is an increased rate of loss of nitrogen from the body. This leads to negative nitrogen balance, and if prolonged or profound, it impairs the clinical outcome [7]. Thus an adequate, or possibly an enriched, dietary source of indispensable and conditionally indispensable amino acids, together with an intake of sufficient utilizable nitrogen is needed to counteract the losses, as well as to promote the repair and repletion of tissues and organs. In this context, amino acids have multiple functions (Table 1) in addition to their substrate role in protein synthesis. It is the requirement to support and maintain these various functions that determines the level and quality of
Proteins, Peptides and Amino Acids in Enteral Nutrition

Table 1. Some functions of amino acids

<table>
<thead>
<tr>
<th>Function</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrates for protein synthesis</td>
<td>Those for which there is a codon</td>
</tr>
<tr>
<td>Regulators of protein turnover</td>
<td>Leucine, glutamine</td>
</tr>
<tr>
<td>Regulators of enzyme activity</td>
<td>• Arginine and N-acetyl glutamate synthesis</td>
</tr>
<tr>
<td></td>
<td>• Phe and phenylalanine dehydroxylase activation</td>
</tr>
<tr>
<td>Precursor of signal transducer</td>
<td>Arginine and nitric oxide</td>
</tr>
<tr>
<td>Neurotransmitter</td>
<td>Tryptophan, glutamate</td>
</tr>
<tr>
<td>Ion fluxes</td>
<td>Taurine, glutamate, oxoproline</td>
</tr>
<tr>
<td>Precursor of N compounds</td>
<td>Nucleic acid, creatinine</td>
</tr>
<tr>
<td>Transporter of N</td>
<td>Glutamine, alanine</td>
</tr>
<tr>
<td>Translational regulator</td>
<td>Leucine [4E-BP1 and P70(s6k) via MTOR-dependent pathway]</td>
</tr>
<tr>
<td>Transcriptional regulator</td>
<td>Leucine limitation induces CHOP expression</td>
</tr>
</tbody>
</table>

the amino acid/nitrogen supply required by the individual consumer or patient. Recently, the roles of amino acids as regulators of the transcriptional [8, 9] and translational [10–14] phases of gene expression are becoming more clear. With a more complete understanding of this particular function of amino acids we might anticipate a less empirical approach to the design of enteral feeds and so an improvement in their efficacy.

In short, an assessment of the state of the art of one major aspect of enteral nutritional support is made in this volume – that is, the nitrogen component of enteral nutrition – and attention will be given to our understanding of the roles that intact proteins, peptides, and free amino acids play in nutritional physiology and their clinical corollaries. Our remit and challenge, therefore, is (i) to critically review and update knowledge on the utilization, metabolic fate, and function of ingested protein, peptides, and amino acids; (ii) to consider the consequences of disease states on their metabolism, utilization, and physiologic attributes; (iii) to integrate this information for the formulation and use of enteral nutrition in the support of hospital inpatients and other individuals who might benefit from this type of feeding, and (iv) to identify necessary areas of further research.

In this introductory chapter we will touch upon a few areas of research that we consider relevant to the stated goals of the workshop. We concentrate entirely on enteral nutrition, which offers support for mucosal function and integrity, improves body protein balance, and possibly reduces septic morbidity.

**Fate of Ingested Amino Acids during the Absorptive Phase of Metabolism**

The intestines and liver modify the profile and amount of amino acids that disappear from the intestinal lumen and enter the portal and peripheral blood
Proteins, Peptides and Amino Acids in Enteral Nutrition

Table 2. Dual isotope tracer model estimates of splanchnic uptake of amino acids: fed state in healthy adults

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Uptake % of intake</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine (adequate)</td>
<td>21 ± 6</td>
<td>Cortiella et al. [22]</td>
</tr>
<tr>
<td>Leucine (low)</td>
<td>37 ± 5</td>
<td>Cortiella et al. [22]</td>
</tr>
<tr>
<td>Leucine (adequate)</td>
<td>10 ± 6</td>
<td>Castillo et al. [23]</td>
</tr>
<tr>
<td>Phenylalanine (adequate)</td>
<td>25</td>
<td>Sánchez et al. [24]</td>
</tr>
<tr>
<td>Phenylalanine (low)</td>
<td>58 ± 4</td>
<td>Biolo et al. [25]</td>
</tr>
<tr>
<td>Tyrosine (adequate)</td>
<td>37</td>
<td>Sánchez et al. [24]</td>
</tr>
<tr>
<td>Arginine (adequate)</td>
<td>34 ± 8</td>
<td>Castillo et al. [23]</td>
</tr>
<tr>
<td>Methionine (adequate)</td>
<td>23 ± 2</td>
<td>Storch and Young, 19901</td>
</tr>
<tr>
<td>Cystine</td>
<td>&gt;50</td>
<td>Hiramatsu et al. [26]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Raguso and Young, 19951</td>
</tr>
</tbody>
</table>

1 Unpublished results.

supply [15]. Furthermore, as discussed in greater detail by Reeds elsewhere in this volume, following their absorption, amino acids enter pathways of protein anabolism and are used for synthesis of physiologically important compounds such as glutathione [16], or undergo oxidative catabolism, with some contributing significantly to the total energy economy of the intestinal tract [17–21]. It is important, therefore, that the splanchnic phase of amino acid metabolism be more fully characterized and its quantitative aspects understood, especially since the splanchnic tissues might limit the availability of enterally supplied amino acids to peripheral tissues.

Using dual isotope tracer models, estimates have been made in our laboratories and by others of the extent to which amino acids are removed by the splanchnic region during their uptake from the intestinal lumen. A summary of our estimates together with those of Biolo et al. [25] for a low phenylalanine diet is given in Table 2. These data illustrate that the extent of uptake by the splanchnic region differs among amino acids and this might also be dependent on the level of amino acid intake. It is noteworthy that the uptake of cystine is very high and this is consistent with data obtained in the pig [27]. This might explain why the concentration of cysteine in the circulation shows little postprandial change in response to a wide range of cystine intakes [28].

An obvious question emerging from the values given in Table 2 is the quantitative extent to which this uptake reflects the entry of an amino acid into anabolic pathways or those responsible for amino acid catabolism (oxidation). The available data for human subjects are insufficient to answer this question precisely, but an approximation might be derived from comparisons of estimates of amino acid oxidation when 13C-labeled amino acid tracers are given by the oral vs. the
Proteins, Peptides and Amino Acids in Enteral Nutrition

Table 3. Some estimates of amino acid oxidation according to route of tracer administration

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Diet/condition</th>
<th>Route</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>intravenous</td>
<td>oral</td>
</tr>
<tr>
<td>13C-leucine</td>
<td>NP/fed</td>
<td>53.21</td>
<td>58.4 Hoerr et al. [29]</td>
</tr>
<tr>
<td></td>
<td>LP/fed</td>
<td>12.0</td>
<td>14.3 Hoerr et al. [29]</td>
</tr>
<tr>
<td>13C-lysine</td>
<td>GL/fast</td>
<td>11.2 ± 1.1</td>
<td>14.8 ± 3.2 El-Khoury et al. [30]</td>
</tr>
<tr>
<td></td>
<td>GL/fed</td>
<td>23.5 ± 4.8</td>
<td>24.9 ± 4.9 El-Khoury et al. [30]</td>
</tr>
<tr>
<td>13C-lysine</td>
<td>LL/fast</td>
<td>9.8 ± 0.3</td>
<td>11.1 ± 3.6 Unpublished MIT data</td>
</tr>
<tr>
<td></td>
<td>LL/fed</td>
<td>25.7 ± 6.3</td>
<td>29.0 ± 7.1 Unpublished MIT data</td>
</tr>
<tr>
<td>13C-phenylalanine</td>
<td>GP/fast</td>
<td>5.8 ± 0.36</td>
<td>13.2 ± 1.76 Sánchez et al. [24]</td>
</tr>
<tr>
<td></td>
<td>GP/fed</td>
<td>14.6 ± 2.24</td>
<td>22.8 ± 3.74 Sánchez et al. [24]</td>
</tr>
</tbody>
</table>

NP = Normal protein; LP = low protein; GL = generous lysine; LL = low lysine; GP = generous phenylalanine intake.
1 Values are µmol/kg/h.

intravenous route. Therefore, in Table 3 we summarize estimates of the rates of whole body leucine, lysine, and phenylalanine oxidation when determined by giving tracers orally or intravenously. For leucine and lysine the rates of oxidation do not seem to be markedly different for the two routes. In contrast, it appears that the splanchnic uptake (disappearance) of phenylalanine following its absorption from the intestinal tract is associated with a relatively higher rate of catabolism, possibly implying that the efficiency of utilization of phenylalanine for anabolic purposes may be less when given orally than when supplied by the parenteral route. Although the differences between the intravenous and the oral tracer based estimations of leucine and lysine oxidation rates were apparently not large, when the difference is accumulated over an entire day it could be large enough to mean that the requirement for body maintenance is somewhat greater when the supply is by the oral than by the intravenous route.

Recently, Bertolo et al. [31] have concluded that the threonine requirement of neonatal piglets during parenteral nutrition is 45% of the mean oral requirement. This could be a result of a combination of factors, including a lower rate of threonine oxidation by intestinal tissues when threonine is given by vein, and also reduced losses of threonine by the gastrointestinal tract because of reduced mucin production, these glycoproteins being rich in threonine [32].

It is important, therefore, in developing optimal protein/peptide/amino acid feeding strategies that a more complete quantitative picture be developed of the immediate metabolic fate of absorbed nitrogen and amino acids under various dietary and host conditions. Furthermore, in this context, Boirie et al. [33] have reported that the splanchnic extraction of dietary leucine was twice as high in elderly men (50 ± 11%) as it was in young men (23 ± 3%), although whole
body leucine oxidation was similar for the two age groups. These investigators concluded that this difference in splanchnic uptake might limit the availability of leucine for peripheral tissue metabolism. Volpi et al. [34] also found that the splanchnic extraction of oral phenylalanine was significantly higher in the elderly (47 ± 3%) compared with the young (29 ± 5%). However, these investigators also measured the rates of muscle protein synthesis and breakdown and they observed that an oral amino acid mixture stimulated synthesis with increased net phenylalanine balance similarly in the young and the elderly. Again, we need to know more about the metabolic relations between amino acid uptake, oxidation, and utilization in the peripheral and splanchnic regions under different conditions to understand fully the nutritional implications of the above observations.

**Urea Nitrogen Metabolism and the Bowel**

It follows that if amino acids are oxidized during their passage through the splanchnic region – and for glutamate it is known that oxidation is extensive in rodents [15], the pig [20], and the human [18] – then there would be an expected increase in the rate of urea N production with the ingestion of protein. This response might also be predicted on the basis of considerations of hepatic energy transformation and metabolism [35]. Thus the metabolism and fate of urea within the intestine, and its relation to nitrogen metabolism in other organs, are another aspect of the overall topic being examined in this volume. Jackson examines urea N metabolism in somewhat greater depth, and in a review [36] he has emphasized the key role played by the hydrolysis of urea N within the intestinal lumen (assumed to be largely a function of the activity of microflora in the large bowel), and the significant contribution that this liberated nitrogen makes to the nitrogen homeostasis of the host. As disease and treatment can alter the functioning of the bowel, including the relation between the resident microflora and host metabolism, this area of nitrogen metabolism is relevant to a quantitative definition of the importance of protein or its derivatives in enteral nutrition.

In contrast to the conclusions drawn by Jackson [36], our experience on the relations among amino acid (leucine) oxidation, urea production, and hydrolysis has been summarized recently [37]: we find that urea production (Fig. 1) and excretion rates are linearly correlated over a wide range of N intake, as is also the extent of urea hydrolysis. In the latter case we have seen considerable variation in the rate among individual subjects, especially at a nitrogen intake of ~160 mg N/kg-day (1 g protein/kg-day). Thus our findings are consistent with the prevailing view that control of body N balance is achieved by regulation of urea production, and they do not support the concept [36] that urea hydrolysis is the more important site in the control of N loss through urea or N retention. Furthermore, our observations make it clear that the rates of urea N production and excretion reflect the rate of amino acid (leucine) oxidation and
they also question the generality of Jackson’s [36] view that ‘... it is unsafe to presume that the rate of urea excretion is a measure of the rate of urea production and hence of the rate of amino acid oxidation.’ Our analysis supports a conclusion that we drew earlier concerning the value of a measure of whole body leucine oxidation in predicting the rates of irreversible protein nitrogen loss (IPNL) or of whole body amino acid oxidation [38]. It should be noted that IPNL can only be determined precisely from leucine oxidation rates when the dietary leucine to nitrogen intake ratio approximates that of whole body mixed proteins.

**Nonspecific Nitrogen**

Perhaps more importantly than the foregoing comparison of our own and Jackson’s data is the specific question of whether healthy people, or for that matter sick patients, are capable of incorporating sufficient ammonia released through urea hydrolysis to make a significant net contribution to body N retention under conditions of a limiting N intake. Thus to make a net contribution, the ammonia would have to be incorporated into the α-amino N pool through the action of glutamate dehydrogenase, located in the mitochondrial matrix. However, it can be questioned whether this would make any real or significant net contribution to NH₄ assimilation in the animal system, because the Kₘ for NH₄⁺ is quite high (5–40 mM [39], and 1 mM [40]). Another pathway of NH₄⁺ assimilation could be through glycine synthase, and the glycine formed could then be converted to serine via serine hydroxymethyl transferase. However,
through the action of serine dehydratase, serine liberates its nitrogen as \( \text{NH}_4^+ \), so this pathway would not serve as a means for accumulating \( \alpha \)-amino N from non-amino nitrogen sources. For these biochemical reasons, it appears plausible that under the usual conditions, and possibly in catabolic states, the process of urea production would be the more important site for the regulation of body N loss. In this context, and as clearly outlined by Waterlow [41], there is still a great deal of uncertainty about the in vivo mechanisms responsible for both the short and the longer term regulation of urea production under different pathophysiological circumstances, and hence the mechanisms responsible for the maintenance of body protein balance.

An issue that emerges from our discussion of this particular aspect of N metabolism is the implication that there is a dietary requirement for a readily utilisable source of preformed \( \alpha \)-amino N. This could mean that an amino acid such as glutamate, which fulfills multiple functions and is a central ‘player’ in the nitrogen transactions of body [42], is a desirable dietary constituent even if not essential in the traditional context, although this remains to be completely clarified. The work of Reeds and coworkers, summarized elsewhere in this volume, strongly supports this notion. If correct, this introduces a new and important perspective on the ‘nonspecific’ nitrogen (NSN) [43, 44] component of the total protein requirement.

In 1965, the FAO/WHO [45] Expert Group stated: ‘The proportion of non-essential amino acid nitrogen, and hence the E/T [g total essential amino acids to total nitrogen] ratio of the diet, has an obvious influence on essential amino acid requirements ...To make the best use of the available food supplies there is an obvious need to determine the minimum E/T ratios for different physiological states ...Finally, the question arises whether there is any optimal pattern of non-essential amino acids.’ This statement can just as well be repeated today, but it is also encouraging that recent studies are beginning to provide deeper insight into the nature of the NSN needs of the human body. Not only is there an optimal E/T ratio [46], but it now seems likely that there is a desirable qualitative character to the dietary NSN supply, which raises the question of the optional sources and levels of \( \alpha \)-amino nitrogen compounds in enteral formulations. For example, this includes considerations of glutamate-proline-arginine interrelations [47] – not only in terms of the nitrogen economy of the host but also with respect to other functions such as the maintenance or stimulation of the immune system, wound and tissue repair, and the impact of NSN on polyamine and hormonal balance. For example, we have studied the impact of a relatively high arginine intake in healthy subjects on arginine/citrulline/ornithine kinetics [48] and, although we did not find changes in the activity of the L-arginine-NO pathway, the generous level of arginine supplementation reduced rates of urea production and excretion and increased circulating insulin concentrations. How this apparent protein anabolic effect of high arginine supplementation is brought about (perhaps enhanced by insulin action), what impact it has on the immune
system in the light of current interest in immune-enhancing diets, and finally what role this amino acid plays in aggravating or attenuating renal injury [49, 50] are just a few of the questions relevant to a better understanding of the role played by the NSN component in supporting protein metabolism and function in the host.

**Intestinal Amino Acid Synthesis**

With respect to the intestinal phase of amino acid metabolism, urea N metabolism, and enteral protein/nitrogen nutrition, it is also important to consider briefly the question of whether amino acids synthesized de novo by the gastrointestinal microflora are absorbed, and whether they contribute to the amino acid economy of the host in any significant way. Based on the interpretation of urinary 15N urea excretion following labeled urea administration, it has been suggested [36, 51] that urea nitrogen can be salvaged by urea hydrolysis in the colon and that the nitrogen can be incorporated by the intestinal microflora into amino acids which are subsequently absorbed by the host. Although it has been shown in the pig that colonic absorption of amino acids is possible [52, 53], much of the experimental evidence in non-ruminant animals does not suggest there is quantitatively important amino acid absorption from the colon [54–56]. However, the results in the pig have shown that amino acids synthesized by the microflora can be absorbed, supposedly in the small intestine, and used for tissue protein synthesis [57, 58]. Tracer studies in animals and man have shown a transfer of NSN (ammonia, urea N, glutamate, and so on) into dispensable and indispensable amino acids [59, 60]. For a majority of amino acids this input of 15N from urea may reflect nitrogen exchange or reversible transamination. However, lysine and threonine do not undergo transamination in mammalian tissues. Thus the appearance of 15N-labeled lysine or threonine in body proteins and plasma amino acids after administration of a 15N nitrogen source, such as ammonium or urea, must reflect the de novo synthesis of lysine and threonine by the intestinal microflora and their subsequent absorption from the gastrointestinal tract. Comparative experiments with germ-free and conventional rats [61] have confirmed that de novo synthesis of lysine is due to the activity of the indigenous microflora in the gastrointestinal tract.

While in uremic patients and human subjects consuming a low protein diet, microbial lysine can be shown to be made available to the human host [62, 63], there have been no extensive attempts to quantify the significance of this source of lysine and threonine (or other amino acids) in relation to host tissue metabolism. Recently, we [64] have investigated in healthy adults whether there is a net contribution to the lysine and threonine synthesized de novo by the gastrointestinal tract to lysine and threonine homeostasis. These experiments involved giving six healthy adults either 15N-urea or 15N-ammonium chloride for 6 days, during which we monitored the appearance of 15N in the plasma-free...
Table 4. The question of microbial amino acid synthesis and its contribution to amino acid economy of the host; studies in healthy adults

<table>
<thead>
<tr>
<th>15N-lysine enrichment, APE</th>
<th>Plasma-free lysine</th>
<th>Plasma/microbial lysine enrichment ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>With 15N-urea</td>
<td>0.0059 ± 0.0053</td>
<td>0.049 ± 0.043</td>
</tr>
<tr>
<td>With 15N-NH₄Cl</td>
<td>0.0118 ± 0.0034</td>
<td>0.090 ± 0.060</td>
</tr>
<tr>
<td>15N-threonine enrichment, APE</td>
<td>0.0073 ± 0.0072</td>
<td>0.08 ± 0.06</td>
</tr>
<tr>
<td>With 15N-urea</td>
<td>0.0331 ± 0.0113</td>
<td>0.14 ± 0.04</td>
</tr>
</tbody>
</table>

APE = Atom percent excess.

1 Summarized from data of Metges et al. [59, 64].

lysine and threonine pools and in the amino acids of bacterial protein extracted from feces. These results are discussed in detail elsewhere [59, 64], but in Table 4 we give a summary of the 15N labeling present in plasma lysine and threonine and the plasma to microbial amino acid 15N-enrichment ratio. This latter ratio is a crude index of the relative contribution made by the microbially derived amino acid to the total plasma amino acid turnover. These results confirm the presence of intestinal, microbially derived amino acids in host tissues, and the findings further suggest that a significant proportion of the circulating lysine and threonine in plasma could be derived from this intestinal source. However, insofar as microbial amino acid synthesis and absorption are accompanied by microbial breakdown of endogenous amino acids or the oxidation of ingested/endogenous amino acids by host intestinal tissues, these data may not reflect a significant net increase in the daily availability of lysine or threonine through absorption from this microbial source. As discussed more completely elsewhere [64], we cannot reliably estimate the quantitative contribution of microbial amino acid synthesis to host amino acid homeostasis using this tracer paradigm. Nevertheless, the results confirm the significant presence of lysine and threonine of microbial origin in the plasma-free amino acid pool and they raise the question as to how various clinical states and diseases and enteral nutrition affect the nutritional/metabolic interrelationships between the microbial flora of the intestine and the host.

Form and Pattern of the Protein and Amino Acid Supply

A second major aspect of proteins/peptides/amino acids concerns the physiochemical nature of the amino acid supply. Therefore, we will raise here some matters related to qualitative, quantitative, and temporal aspects of the problem,
which will also be discussed in greater detail by others elsewhere in this volume, especially Finot, Beauffrère, and Millward.

By way of introduction, it is worth reminding ourselves that, under the usual circumstances at least, the content of body protein is relatively constant from day to day throughout most of adult life, despite wide variations in the daily intake of dietary protein. There is, however, a slowly progressive decline in the content of total body protein over a relatively long time frame with progression of the adult years, and this occurs especially in the skeletal musculature; however, it is difficult to quantify by current methods of body composition measurement. Thus the essential daily maintenance of body protein content is achieved through a complex set of integrated changes in rates of whole body protein turnover, amino acid oxidation, urea production, and nitrogen excretion that occur at different rates during the postabsorptive, prandial, and postprandial periods of the 24-hour day [65]. Depending upon diet composition, smaller or larger gains and losses of body proteins occur during the diurnal cycle of feeding and fasting [66]. In this particular context, there has been a recent surge of research interest in the postprandial phase of protein and amino acid metabolism, especially in terms of trying to understand the physiological mechanisms responsible for protein gains and losses at different intakes of protein, or with different sources of protein. These investigations have been facilitated by the development of new paradigms, including the availability and use of food proteins intrinsically labeled with $^{15}$N or $^{13}$C [67, 68].

Millward and his group [65, 69] at the University of Surrey have investigated the postprandial deposition of protein in relation to an assessment of the efficiency of postprandial protein utilization (PPU). PPU is calculated – using labeled leucine as the tracer of amino acid retention and oxidation – from the ratio of the change in leucine balance to the change in leucine intake. We consider that this is a novel and potentially useful approach. The experimental value obtained is thought to be a comparable index to the protein quality estimate of net protein utilization, as would be derived from rat feeding experiments or human nitrogen balance studies [65].

Because there has been little investigation so far on the dietary or experimental design variables that might affect tracer-derived estimates of PPU, which will be discussed by Millward later in this workshop, we [Young VR, Raguso CA, El-Khoury AE, Regan MM, Forslund A, Hambraeus L; submitted, 1999] have examined some of our own data to explore the effects of (i) different levels of specific indispensable amino acid intake at constant nitrogen intake; (ii) different intake levels of total protein; (iii) use of discrete meals, and (iv) time of feeding on PPU, using $^{13}$C-leucine and $^{13}$C-lysine as tracers. Although our analysis will be published in detail elsewhere, the results for PPU at graded intakes of leucine, with constant intake of nitrogen and other amino acids, are summarized in Table 5, and for different intakes of good quality protein are shown in Table 6. Additionally, as the tracer experiments lasted for at least a feeding period of 10 h...
Proteins, Peptides and Amino Acids in Enteral Nutrition

Table 5. PPU\textsubscript{Leucine} at three leucine intakes (impact of intake level and time)

<table>
<thead>
<tr>
<th>Leucine intake mg/kg-day</th>
<th>PPU during feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>first 5 h</td>
</tr>
<tr>
<td>14 (FAO)</td>
<td>0.34 ± 0.71</td>
</tr>
<tr>
<td>38 (MIT)</td>
<td>0.68 ± 0.08</td>
</tr>
<tr>
<td>89 (generous)</td>
<td>0.77 ± 0.07</td>
</tr>
</tbody>
</table>

Diet were isonitrogenous. Data taken and calculated from El-Khoury et al. [38, 70]. Second 5 h: all pairs different (\(p \leq 0.005\)); first 5 h different from second 5 h (14 and 38: \(p < 0.0001\); \(p < 0.05\)).

Table 6. PPU\textsubscript{Leucine} at low and adequate protein intakes

<table>
<thead>
<tr>
<th>Protein intake mg/kg-day</th>
<th>PPU during feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>first 5 h</td>
</tr>
<tr>
<td>0.25</td>
<td>0.23 ± 0.56</td>
</tr>
<tr>
<td>1.0</td>
<td>0.77 ± 0.07</td>
</tr>
<tr>
<td>2.5</td>
<td>0.93 ± 0.10</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Different (\(p < 0.0001\)) from first 5 h.  
\textsuperscript{b} Different (\(p < 0.001\)) from 1.0 g intake.

[70], PPU results for the first and second 5-hour consecutive periods are also shown. From these findings it is apparent that PPU, as operationally defined, varies in a complex way with altered intakes of specific amino acids and total protein, and the value also depends on the time of observation, with PPU values exceeding 1 during the second 5-hour period for intakes of protein or specific indispensable amino acids that are grossly inadequate. We present these findings here because in principle the PPU approach would seem to offer a ‘patient friendly’ opportunity to assess the protein retention efficiency of the nitrogen component of different enteral formulations. However, if useful comparisons and assessments are to be made, then there will be a need to take into account these factors in the design and interpretation of sound clinical studies.

The physiological nature of the protein source(s) also will apparently affect the PPU as the speed of protein digestion and amino acid absorption from the gut has a major effect on whole body protein metabolism and amino acid oxidation after a single meal. The group at Clermont-Ferrand [71] reported that postprandial whole body leucine oxidation over 7 h was lower with casein than with whey protein, despite similar leucine intake (that is, the PPU for casein would be higher than for whey). Both of these protein sources are of high quality in adult human nutrition. Hence, a difference in PPU under some circumstances
might give a false indication of the comparative nutritional value of different formulations. Clearly, this new tracer-based/metabolic paradigm requires further definition and standardization.

In addition, the temporal nature of the amino acid supply or pattern of feeding has an influence on the efficiency of nitrogen and amino acid utilization. In our studies, involving 24-hour [1-13C]leucine tracer balance determinations, we have found that daily leucine oxidation is lower when three discrete meals versus 10 small hourly meals are given over a 12-hour fed period [72] (Table 7). This appears to be the case at both generous (Table 7) and limiting intakes of leucine, suggesting a better retention of oral amino acids with the less frequent meal intake. Whether this might be explained by the so-called ‘anabolic drive’ of amino acids [73] cannot be determined from these data, but it is evident that the pattern of protein/amino acid ingestion can determine the efficiency of utilization of an enteral formulation. Furthermore, Arnal et al. [74] have shown in studies with elderly subjects that protein retention was higher when 80% of the daily intake was consumed at midday as compared to giving the daily protein supply in four meals spread over 12 h (Table 8).
In summary, the efficiency of the postprandial utilization of ingested protein or free amino acids is determined by various dietary, physiological, and physical factors. Whether (and the extent to which) the utilization of peptide formulations is affected by the same set of factors remains to be determined. Nevertheless, we hope this brief consideration of PPU emphasizes the complexity of the host response to the nitrogen component of the diet, and that the formulation, application, and evaluation of protein/peptide/amino acid enteral products must take this complexity into full account.

**Sulfur Amino Acid Nutrition and Metabolism**

The last topic that be might covered within the space available relates to some aspects of sulfur amino acid (methionine and cyst(e)ine) metabolism and nutrition. We have chosen to raise a few issues and research challenges about the enteral supply of these amino acids in view of the following: (i) the postulated role of the redox state, as reflected by the plasma cysteine disulfide (cysteine)/cysteine redox couple, especially in the elderly [75], as a determinant of body cell mass maintenance/loss [76]; (ii) the fact that it is a substrate for glutathione (GSH) synthesis, the rate of which might be compromised in certain pathophysiological states [77]; (iii) because preformed cyst(e)ine seems to be a more effective source for this purpose than is the cyst(e)ine formed during the transsulfuration of methionine [78]; (iv) the conversion of methionine to cysteine seems to be impaired in catabolic states, such as burn injury [79], and, finally (v) because it brings back into focus the NSN component of the protein/amino acid requirement, as glycine is a substrate for GSH synthesis and it has been postulated that the endogenous supply of this amino acid might be limiting under conditions of rapid growth [80, 81], and perhaps also in catabolic states. In these cases a sufficient dietary content of glycine may be required.

First, with respect to dietary methionine-cystine relations we have found little or no evidence, using $^{13}$C-cysteine as tracers in young adults, for a major sparing effect of dietary cystine on the oxidation of methionine and therefore on efficiency of retention of methionine, except at an extremely low methionine intake [82, 83]. This means that the requirement for methionine is not substantially reduced by addition of cystine to the diet. Further, nitrogen balance studies by Tuttle et al. [84] led these investigators to suggest that methionine and lysine requirements in older individuals are much higher than those established, with similar procedures, in young adults. Hence, in comparison with the young, older individuals might also have greater requirements for the sulfur amino acids, as judged by results of $^{13}$C-methionine tracer balance studies, and the elderly might be more sensitive to a sparing effect of dietary cystine on the requirement for methionine. On the other hand, recent kinetic studies by Fereday et al. [85] imply that the protein requirements of healthy elderly people, at least as assessed from tracer leucine balance studies, may not be any higher than those of younger adults. Indeed, there is a great deal of uncertainty about the protein and amino
acid requirements of elderly subjects and the effects of aging [86–90]; therefore, further relevant studies in this older age group were considered desirable, so we [91] examined methionine kinetics, including oxidation, in healthy elderly subjects. As summarized in Table 9, elderly subjects were estimated to be in balance at the proposed FAO/WHO/UNU [92] requirement level for methionine, but they fell into a marked negative methionine balance when the methionine intake was reduced, despite a generous addition of cysteine. Again, there seems to be little sparing effect of cystine on the methionine requirement. From our perspective, much of the sulfur amino acid requirement for body amino acid maintenance should be provided as methionine but together, perhaps, with a sufficient although quantitatively uncertain level of intake of cystine.

As noted above, cysteine is a precursor of GSH and it is possible that the tissue status of GSH, a tripeptide which has evolved to serve diverse functions, might be sensitive to the dietary supply of cyst(e)ine. In rodents it has been shown that cystine is the limiting factor in GSH synthesis [93] and there is now a great deal of clinical interest in the modulation of GSH levels, especially during chemotherapy and radiotherapy. Clearly, a better understanding of the quantitative aspects of the physiology and metabolism and of the nutritional factors that regulate cellular GSH levels is the key to their effective manipulation during differing types of treatment. For this reason, we have been interested in exploring the use of tracers to assess GSH synthesis and turnover directly or indirectly in human subjects. Our initial investigations focused on 5-oxoproline (L-pyroglutamic acid; L-pyrrolidone carboxylic acid), an intermediate of the γ-glutamyl cycle, which is generated during GSH catabolism [94, 95]. Specifically, we [96] used L-[1-13C]5-oxoproline to examine the consequences of low sulfur amino acid and low glycine diets on oxoproline kinetics and excretion. As shown in Table 10, after 5 days of the dietary restrictions changes were observed in plasma oxoproline kinetics and (data not shown) in the urinary excretion of oxoproline. The key point here is that it seems that the activity of the γ-glutamyl cycle is affected acutely by the adequacy of intake of two of the precursors of GSH, even in healthy adults. Clearly, it will be important to expand these

### Table 9. Methionine and cystine intake and methionine balance in elderly subjects

<table>
<thead>
<tr>
<th>Diet</th>
<th>Intake</th>
<th>Daily $^{13}$C-methionine balance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>methionine</td>
<td>cystine</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>6.5</td>
<td>5.2</td>
</tr>
<tr>
<td>3</td>
<td>6.5</td>
<td>20.9</td>
</tr>
</tbody>
</table>

Extracted from Fukagawa et al. [91]. All values are mg/kg-day.

*Post hoc* comparisons between diets: $p < 0.001$ diet 1 vs. 2; $p < 0.005$ diet 1 vs. 3.
Table 10. Oxoproline kinetics in response to 6 days with a sulfur amino acid (SAA)-free or glycine-free diet (fed state) in healthy adults

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>SAA-free</th>
<th>Glycine-free</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flux (1), (\mu)mol/kg-h</td>
<td>38 ± 14</td>
<td>55 ± 16</td>
<td>52 ± 12</td>
</tr>
<tr>
<td>Oxidation (2), (\mu)mol/kg-h</td>
<td>31 ± 10</td>
<td>47 ± 12</td>
<td>43 ± 8</td>
</tr>
<tr>
<td>Plasma oxoproline, (\mu)M</td>
<td>54</td>
<td>62</td>
<td>54</td>
</tr>
</tbody>
</table>

Extracted from Metges et al. [96].

\(^1\) Control was significantly lower than glycine-free \((p = 0.0175)\) and tended to be lower than SAA-free \((p = 0.056)\).

\(^2\) Control was significantly lower than glycine-free \((p = 0.005)\) and SAA-free \((p = 0.03)\).

Table 11. Rate of whole blood glutathione synthesis in septic infants and children, based on continuous infusion of \(L\-[1-13C]\)-cysteine

<table>
<thead>
<tr>
<th>Group (n = 5)</th>
<th>Glutathione</th>
<th>FSR/day</th>
<th>ASR, (\mu)M/l-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>whole blood, (\mu)M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septic infants</td>
<td>756 ± 16</td>
<td>0.50 ± 0.20</td>
<td>395 ± 179</td>
</tr>
<tr>
<td>Septic children</td>
<td>574 ± 179</td>
<td>0.64 ± 0.23</td>
<td>340 ± 123</td>
</tr>
<tr>
<td>Combined septic</td>
<td>665 ± 194</td>
<td>0.57 ± 0.23</td>
<td>368 ± 156</td>
</tr>
<tr>
<td>Healthy infants</td>
<td>945 ± 91</td>
<td>1.01 ± 0.29</td>
<td>958 ± 321</td>
</tr>
<tr>
<td>Healthy children</td>
<td>1174 ± 435</td>
<td>0.77 ± 0.19</td>
<td>859 ± 200</td>
</tr>
<tr>
<td>Combined healthy</td>
<td>1059 ± 334</td>
<td>0.89 ± 0.27</td>
<td>909 ± 272</td>
</tr>
</tbody>
</table>


FSR = Fractional synthesis rate; ASR = absolute synthesis rate.

Studies in various clinical states in order to optimize the form and content of the sulfur containing amino acids in enteral products. We have recently determined, using a \([1-13C]\)-cysteine tracer, the rate of GSH synthesis in the whole blood of septic and healthy children. Our preliminary, unpublished [Castillo L, Lyons J, Young VR, et al., 1999] findings are summarized in Table 11 and they show a fall in the fractional synthesis rate with a marked reduction in the absolute synthesis rate of GSH. The extent to which this fall in synthesis and that accompanied by a lowered blood concentration (Table 11) might be attenuated by an appropriate modification of the protein/peptide/amino acid component of the nutritional support, or whether it is due to direct changes in the activities of one or more of the enzymes responsible for GSH production [97] remains to be established.
Nevertheless, the qualitative and quantitative nature of the amino acid adequacy of the diet for optimal maintenance of GSH synthesis need to be defined. Parenthetically, this raises the question as to whether a ‘cocktail’ or mixture of specific amino acids might be more useful than a supplement of any one alone. For example, it is possible that a balanced mixture of glycine, cystine, and glutamate might offer a particular protection of GSH homeostasis which any single amino acid given alone would not. This point might be useful to consider when designing clinical studies, which often cannot be as complete as those that might be possible to carry out in healthy subjects or such as those characteristic of more basic investigations in animal systems.

Summary and Conclusion

We have selectively reviewed various aspects of protein and amino acid metabolism and nutrition which we feel are relevant and important in any comprehensive consideration of proteins, peptides, and amino acids in enteral nutrition. These, together with various clinical aspects of the problem that we have not addressed, are covered in more specific detail elsewhere in this volume. Our hope is that by the time this workshop is at an end a reasonable picture of the state of the art will emerge in terms of an understanding of the physiology and biochemistry of protein, peptide, and amino acids in enteral nutrition. Simultaneously, we will need to know, through careful clinical investigation, how this knowledge can best be exploited for optimizing the design of the protein/peptide/amino acid component of enteral nutrition formulations. In doing so it should be possible to achieve a more effective enteral use of proteins, peptides, and amino acids in the comprehensive clinical management of individuals under various pathophysiological states.

Acknowledgement

The unpublished work referred to by the authors was supported by grants from the US National Institutes of Health (DK 15856, DK 42101, P-30-DK-40561, RR 88), the Shriners Hospitals for Children (Nos. 8370, 8470) and a grant from the Deutsche Forschungsgemeinschaft (Me 1420/1-1), Bonn, Germany. We thank Leticia Castillo, MD, and Cornelia Metges, PhD, for allowing us to refer to their unpublished data.

References

Proteins, Peptides and Amino Acids in Enteral Nutrition


Proteins, Peptides and Amino Acids in Enteral Nutrition


**Discussion**

*Dr. Millward:* How secure do you think we are in terms of the methodologies that we use to probe these different amino acids?
Dr. Young: For leucine I feel fairly secure, and I think you’d agree with that. I believe we have the necessary intracellular markers that can be identified in plasma in order to make assumptions about enrichment of the site of amino acid oxidation and so on. But you know very well that there are untested assumptions that we apply to virtually all the other amino acids. That’s a problem we’ve been thinking about recently. We’re going to approach this particular question by developing peptides or peptide analogs that will allow us to direct the entry of the tracer to the site of interest. Then we might actually be able to test some of these assumptions and strengthen the interpretation of the data from tracer studies.

Another approach to this particular problem relates to the desirability of developing bolus, non-compartmental system-type models of amino acid metabolism – not the complex models you’re familiar with and that I don’t understand, but minimal models that actually allow you to better understand what is going on in the so-called inaccessible compartments. I think we ought to take some lessons from pharmacokinetics in this context, and modify the approaches we have applied traditionally so that we can develop noninvasive approaches for application in people. But your question is well taken. Much of the tracer data that are generated in all our studies depend upon assumptions that have not been sufficiently validated. Some are reasonable, but nevertheless not validated. What would you do?

Dr. Millward: My own view at the moment is that – apart from leucine where, for the reasons that you said, we have a reasonably good probe of the intracellular precursor labeling – we are on extremely shaky ground with many of the other studies. I’m particularly concerned about much of the phenylalanine data, which I would argue are near worthless in terms of the magnitude of the errors involved in the transformation of the isotope data into actual values that relate to phenylalanine metabolism. I think this is a major problem and I would argue that it is never sufficiently addressed in publications using these methods. To some extent that may be the fault of many of the reviewers, who quite often have an inadequate understanding of the problems. I’m less certain about the methionine studies.

Dr. Jackson: Can I extend that? You showed data which implied important exchange of nitrogen with the bowel, and the quantitative relevance of that might be functionally significant. To what extent do you think that would affect the assumptions of the models we use for whole body amino acid kinetics, and whether or not they need to be revisited – in the light of the extent to which that component of flux may modify your interpretation?

Dr. Young: I don’t think that component of flux has an impact on the models we use. That particular source of entry of amino acids into the body certainly has a major impact on the interpretation we make of whole body amino acid balance data. You and I would probably disagree on the extent to which it affects the validity of the interpretation of the tracer-derived amino acid requirement data, but I don’t think it has a significant impact on the tracer models as we currently use them.

Dr. Reeds: I was very interested in your confidence in the validity of the methionine data, and Dr. Millward’s expression of confidence in the leucine data, and I wonder whether that is the result of the widespread metabolism of those two amino acids. It’s rare to find a tissue that doesn’t oxidize leucine, and I would argue on the basis of our data – which rarely if ever show a positive portal cysteine balance – that cysteine

21
synthesis is occurring throughout the body and much of it is being utilized at the site of synthesis. The result is that methionine is metabolized much more widely than a number of other amino acids.

Dr. Young: Well, that’s an interesting thought. I don’t know whether I can go further than that. Are there other indispensable amino acids that are broadly metabolized in the body, apart from leucine and the other branched chain amino acids and methionine? I suppose lysine is mainly limited to the liver, intestine and kidney; threonine similarly.

Dr. Reeds: Threonine is a fairly major problem, as I shall point out during my talk, because of its critical importance in the gut.

Dr. Beaufrère: Dr. Young, you focused your talk almost exclusively on the utilization of tracer techniques, and we are all convinced that these are very powerful tools, but in keeping with Dr. Millward’s comment don’t you think that there is still room for the more global techniques – and thinking of nitrogen balance, body composition, and so on? This is particularly true in the field of enteral nutrition. And don’t we need functional measurements such as determination of muscle strength?

Dr. Young: As you know, the focus of our research has been to gain an understanding of the quantitative nature of amino acid metabolism, and therefore we’ve focused our attention particularly on the use of tracer techniques. You’re right though – in the final analysis it is the maintenance of body protein or tissue protein and of tissue function that is of ultimate importance, and presumably we’ll hear from others about studies that relate to the use of those particular global measures.

Dr. Lundholm: I would like to comment on Dr. Millward’s question. I agree that the uncertainty about precursor pool activities is a major problem. On the other hand, we have compared flux data with balance data, and looking back 10 or 15 years, I think we must admit that a great deal of valid data has come out of these techniques. If we look at the information obtained from leucine kinetics, or phenylalanine, or tyrosine, they are quite similar, although the absolute values may not agree; but I think absolute values may not always be the most important thing. We should accept these techniques as semiquantitatively valid. In the field of tumor biology, people working with DNA synthesis, RNA synthesis and so on, never care about specific activities in compartments. They just inject the tracer and calculate incorporation. There have been some remarkable developments in that field, although they have never cared about specific activities in pools. So I think we need to moderate our hesitation about the validity of these things.

Dr. Young: Well, sometimes one can be satisfied with a kind of qualitative assessment, if you like, but I have been more interested in the quantitative aspects of the problem. Quantitative aspects in fact are very important in terms of my own interest, and this is where I think Dr. Millward’s concerns are appropriate.

Dr. Wernerman: You discussed the relation between cysteine and glutathione, and I’d like to raise the question of the chicken and the egg. Looking at data from muscle, you find that muscle cysteine is enormously stable, while muscle glutathione varies considerably, so the thought that comes to my mind is whether an important function of glutathione is to maintain cysteine homeostasis, not the reverse. Would you like to comment on that?

Dr. Young: That’s a very interesting thought. Glutathione is an important contributor to plasma cysteine flux, and presumably to plasma cysteine levels, and so in that context glutathione contributes to cysteine homeostasis. Half of the flux of plasma cysteine is actually contributed by the breakdown of glutathione, so this supports your contention. I don’t think I can go much beyond that. There is a theory [1] that cysteine plays an important role in determining the redox of cells and therefore ultimately in maintaining lean body mass. If that hypothesis is correct, then the maintenance of cysteine homeostasis becomes very important, and possibly glutathione may serve as a store in that particular context.
Proteins, Peptides and Amino Acids in Enteral Nutrition

Dr. Furst: The concentration of muscle cysteine is extremely low, about 50 µmol/l intracellular water, so at that level it is very difficult to speak about constancy of values because they are at the limits of detectability. I would like also to speculate that a major source of cysteine for glutathione synthesis is the protein-bound cysteine, which is hydrolyzed as required. I would like to discuss another point which might not come up in the general discussion. You spoke of giving nutrients in a ‘cocktail’, but when we are discussing glutamine nutrition, for example, I believe your approach is no longer a nutritional one, but a pharmacological one. When we give glutamine we are trying to influence several physiological functions. It doesn’t matter whether you include glutamine in the nutritional composition of the diet or if you add it as a supplement, you will still influence glutathione synthesis, immune responsiveness, fueling of the gut, and so on.

Dr. Young: We have some very preliminary data from burned rabbits that suggest that a cocktail approach is more effective than individual supplementation with cystine and glycine, for example. If the experiments had been ended after the cystine and glycine studies, neat though they might have been, one might have concluded that those particular amino acids are not limiting in terms of their cellular availability, whereas we now suspect that if you give them both together there’s a beneficial consequence. That is what I was trying to say.

Dr. Furst: My great concern in the practical setting is that by including several amino acids like cystine, glutamine and arginine you will have trouble fulfilling the requirement for essential amino acids in the correct proportions.

Dr. Young: I don’t know that I believe you.

Dr. Déchelotte: I would like to come back to the problem of tracer modeling, because a lot of studies dealing with the fate and metabolic effects of ingested proteins and amino acids have assumed that only the amount of bioavailable amino acid tracer should be taken into account for calculations, and that the amount extracted in the splanchnic bed should be subtracted from the calculations. I think that given the increasing evidence of splanchnic protein turnover, we should take into account the total amount of protein and tracer as affecting the whole body, including the splanchnic region.

Dr. Young: I agree.

Reference