What Are the Essential Elements Needed for the Determination of Amino Acid Requirements in Humans?1

Peter Fürst2,3 and Peter Stehle
Institute of Nutrition Science, University of Bonn, D-53115 Bonn, Germany

ABSTRACT The aims of this introductory article are to survey and critically evaluate the concepts and approaches that have been used to assess amino acid adequacy and to hypothesize about possible future directions of research. The issue in question is extensive, consequently this article will be limited to: 1) definitions of amino acid requirements; 2) available techniques to assess amino acid requirements; 3) actual recommendations for healthy adults; 4) factors influencing requirements; and 5) requirements in acute and chronic wasting diseases. Recommendations for amino acid intakes for healthy adults were proposed by the FAO/WHO expert committee in 1973 (2) and also considered again in its report in 1985 (3). These recommendations were based on the measurement of human nitrogen balance. The major factors affecting amino acid requirements are the stage of development, reproductive state, environmental factors, digestibility of dietary proteins, genotype of the individual, and pathological conditions. Remarkably, there are no conclusive data relative to changes in requirements induced by infection, injury, trauma, and renal or liver failure. Future research using modern methods to evaluate requirements must thus receive a high priority. Wasting diseases are associated with deficiencies and imbalances of particular amino acids causing specific changes in requirements. Consequently, a new approach has been used to categorize amino acids as conditionally indispensable according to their functional and physiological properties. Kinetic measurements of plasma amino acids might help to estimate qualitative requirements. Measurement of tissue intracellular free amino acid deficiencies or excesses is another method to estimate qualitative requirements. Based on these measurements tentative values for conditionally indispensable amino acids during disease are given in the article. J. Nutr. 134: 1558S–1565S, 2004.

KEY WORDS: • dispensable amino acids • indispensable amino acids • amino acid requirements • amino acid requirements in disease • factors influencing amino acid requirements

Introduction

According to Rose’s (1) classical definition, the adult human body can maintain nitrogen equilibrium on a mixture of eight amino acids as its sole source of nitrogen. The essential amino acid requirement as defined by Rose represented the highest estimate of individual amino acid requirement needed to achieve a positive nitrogen balance; the so-called “tentative minimum requirement.” Rose’s values were reproduced by FAO/WHO in 1973 (2) and also considered again in its report in 1985 (3). These recommendations were based on the assumption that the quality of protein available anywhere in the world was sufficiently adequate for adults to satisfy their essential amino acid requirement (4). These very low estimates were strongly criticized by many scientists and new methods were proposed resulting in a radically new approach based on the availability of amino acids labeled with stable isotopes (5). By using these novel techniques the requirements for many essential amino acids turned out to be considerably higher than those based on the early nitrogen balance method (6). Consequently, a provisional pattern of amino acid requirements similar to the values proposed by Young et al. (6) was recommended by an expert consultation of FAO/WHO (7). The intensive debate during the next 13 years included both controversial and constructive elements. Concern about the meaning of “requirement” to measure human needs in a real environment was one of the most important issues. Consideration of “metabolic needs” to ensure the flexible operation of protein turnover and also to allow for some reserve capacity is another matter of recent and current discussions.

This article will discuss amino acid intakes needed to meet physiological requirements, as well as evaluate dietary factors that affect these needs and the responses and variation in responses to levels of intake of amino acids among individuals. The major intentions are to survey and critically evaluate the concepts and approaches that have been used to assess amino acid adequacy and also to hypothesize about possible future directions of research. Indeed, the matter in question is
extremely complex and extensive, thus, this article by necessity will be limited to: 1) definitions of amino acid requirements, 2) available techniques to assess essential amino acid requirements, 3) actual recommendations of essential amino acid requirements in healthy adults, 4) factors influencing essential amino acid requirements, the concept of conditionally indispensable amino acids, and 5) the requirement of indispensable—conditionally indispensable amino acids in acute and chronic wasting disease

Amino acid requirements: definitions

At the meeting of the FAO/WHO in 1963 Dr. Waterlow maintained that “...there is only one level of requirement worth talking about—the minimal requirement...” (8). The minimal requirement was the object of innumerable measurements and is the lowest level of amino acid (protein) intake at which nitrogen equilibrium balance can be achieved and maintained. This minimal level could be variable within an individual (9). The conventional model is based on an intrinsic requirement that is a fixed function of body weight (5). An alternative model defines the requirement as a range of intake over which equilibrium can occur (10).

According to Millward (11) there are two additional levels of requirements to be considered: the optimal and the operational. The optimal requirement would be determined by functional criteria such as good health, growth, resistance to disease, etc. These criteria are very hard to define, yet a highly interesting future task would be to search for correlations between amino acid intake and functional criteria that can be expressed in quantitative terms (5).

The operational requirement is introduced on the basis of the net protein utilization (NPU₉⁴) (12). It considers the fact that nitrogen balance can be achieved over a wide range of protein intakes above minimal. According to this model the oxidative losses of amino acids can be divided into obligatory losses and regulatory losses. Importantly, regulatory losses are not simply wasted amino acids but they possess an operative-beneficial role, the “anabolic drive” of amino acids (10,11). Anabolic drive includes the stimulation of hormone (insulin) production and promotion of protein deposition (10,11). It is noteworthy that the minimum rate of the oxidative losses of the essential amino acids might be estimated from the obligatory nitrogen losses (10,11,13).

Independent of the level of requirement, three separate terms should be considered: 1) “metabolic need” may correspond to the magnitude of protein deposition that consumes dietary amino acids; 2) “dietary requirement” is defined as the quantity of protein that must be ingested to support the metabolic need; and 3) “recommended dietary intake (safe level)” corresponds to the dietary requirement plus 2SD; the intake that will satisfy the metabolic needs of 97.5% of the population (Reeds, P., personal communication). The biological basis of the amino acid requirements should be defined on the basis of experimental studies. Such investigations must be based on the relationship between the intake of total nitrogen (individual amino acids) and the nitrogen balance or amino acid carbon oxidation; the overall requirement being influenced by protein deposition and the metabolic needs to maintain body nitrogen or essential amino acid equilibrium.

The critical feature of human protein–amino acid nutrition is that the overall metabolic need (i.e., dietary requirement) is dominated by the maintenance needs (5). Indeed, it is conceivable that pathways of amino acid utilization that are not directly related to protein metabolism itself are of importance to maintenance needs. Pertinent processes are glutathione synthesis, mucin secretion, creatine, taurine, and neurotransmitter synthesis (14,15). Indeed, the amino acid pattern of the metabolic needs of maintenance remains obscure and the quantitative impact of the involved pathways unknown (Reeds, P., personal communication). There are some selected data available suggesting an active role of the turnover of the intestine, taurine, creatine, and glutathione on the maintenance needs for certain essential amino acids especially those of threonine, cysteine, and methionine (16–19). Finally, obligatory amino acid oxidation may also contribute to the maintenance needs because amino acids are potential energy sources. Consequently the quantification of pathways of amino acid utilization that are of importance at or near body nitrogen equilibrium remains a key research priority.

Methods to assess amino acid requirements

The estimate of the amino acid requirement is a highly controversial issue. About 10 years ago a critical review devoted to the evaluation of the human amino acid requirement raised the question: “Can the controversy be resolved?” (20). The major controversy relates to the discrepancy between the recent estimates of carbon oxidation and the previous measurements based on nitrogen balance. In a recent review the old controversy has now hopefully been resolved (21).

As repeatedly emphasized N-balance measurements are associated with many problems because they involve small differences between two large numbers, i.e., N-intake and N-output. The magnitude of miscellaneous (integumental and other) losses of N is still unresolved. According to current opinion, N-balance is poorly suited to assess amino acid requirements because it is not sufficiently sensitive, especially when N-intakes are kept constant.

Plasma amino acid response, defined as the occurrence of a break point in the amino acid response curve, has been previously used to estimate the requirement, yet, this method is probably not sufficiently reliable to yield more than supportive information. Obligatory amino acid losses (OAAL) were suggested to indicate the upper limit for the requirement of a specific amino acid. In this method the basal rate of nitrogen excretion at zero (very low) protein intake is related to the amino acid composition of body protein, therefore the obligatory nitrogen loss will be determined by the individual amino acid with the highest rate of obligatory loss relative to its concentration in body protein (6,22).

Young and co-workers at Massachusetts Institute of Technology pioneered the application of carbon dioxide tracer techniques that rely on the direct oxidation of a labeled test amino acid to produce labeled CO₂ that can be measured (23). The direct tracer balance is a direct estimation of the amount of the amino acid needed in the diet to replace its losses from the body (24,25). Technical problems associated with the direct tracer balance techniques are: 1) the substantial amount of tracer that is given; 2) the true rates of oxidation-isotopic enrichment of the free amino acid pool that serves as the site for oxidative losses cannot be directly probed; and 3) possible influx of essential amino acids from the gut microbiota may result in overestimation of the net loss of amino acids (26).

Another carbon oxidation model is the indicator amino acid oxidation approach. In this method oxidation of a ¹³C-labeled
"indicator" amino acid is assessed at different levels of the test amino acid. At the intake of the test amino acid at which the requirement is met, the oxidation of the indicator amino acid reaches its lowest rate (27,28). No prior adaptation to the level of the test amino acid is needed, yet 2 d of adaptation to the level of protein used before the indicator study is advisable (29,30).

Recent and current recommendations of indispensable amino acid requirements

As mentioned above, international recommendations for infants and preschool children proposed by the FAO/WHO/UNO expert consultation in 1985 (3) were derived from measurements of nitrogen balance. The proposed requirements for adults were considerably lower than those for children. In 1989 the expert consultation of FAO/WHO decided that the essential amino acid requirements for adults should be set equal to those of preschool children. The decision was based on new knowledge about the physiological function of amino acids; after 2 y of age the quantitative requirement for growth is negligible compared with that for body maintenance (7).

The most recent recommendations for requirements of indispensable amino acids have been proposed by the FAO/WHO expert committee in 2001 but have not yet been published. When data were available the recommendations were based on 24-h measurements of amino acid oxidation, either by direct or indicator approaches. For isoleucine and valine, requirements were estimated from an assumed proportionality with leucine, based on the amino acid composi-

tion of body protein, as these three amino acids share common enzymes in their oxidative pathways.

A comparison of the essential amino acid requirement as proposed by FAO/WHO/UNO for adults in 1985, 1990, and 2001 is illustrated in Figure 1.

The essential amino acid concentration of various food proteins in comparison with the current pattern of recommendation by FAO/WHO/UNO is illustrated in Figure 2. It is obvious that the availability of essential amino acids, especially that of lysine, might become critical in certain developing countries. Accordingly it was emphasized “that the poor nutritional value of the diets in some regions may be greater than our limited and somewhat crude analysis might suggest” (31).

Factors affecting amino acid requirements

The metabolic need will certainly vary with stage of development, and reproductive state and will be influenced by environmental factors as well as by diseased condition. The stage of development is an essential factor influencing amino acid requirements. This issue has been extensively discussed by Pencharz et al. (32) in another article in this issue. Past recommendations must be carefully reappraised because the existing data for amino acid needs for infants and children are based on N-balance, and plasma concentrations and urea/creatinine responses relative to amino acid intakes. These data are limited in terms of number of subjects, experimental design, handling of covariates, understanding variability, and adaptation to the diets, which make them of questionable quality. Pencharz and colleagues (32) are now applying the indicator tracer method to the study of amino acid requirement, thus more adequate data are to be expected.

The dietary amino acid requirement necessary to support metabolic need will be influenced by the digestibility and amino acid pattern of the dietary protein. As mentioned above, the overall metabolic need, and hence the dietary requirement, is dominated by the maintenance need. It was proposed that the dietary requirement will also be influenced by the genotype of the individual. This would be reflected in the variability in the efficacy by which absorbed amino acids are used to support the metabolic need.

Amino acid requirements in acute and chronic wasting diseases: a critical issue

The diseased condition considerably influences amino acid requirements. Remarkably, there are no conclusive data
available relative to changes of amino acid requirements induced by episodes of infection, injury, trauma, diabetes, and renal or liver failure. Consequently, future research evaluating amino acid requirements in these conditions by using modern methods must receive a high priority.

It is obvious that during episodes of catabolic stress, such as after an operation, burn or injury, or during infectious episodes or multiorgan failure, numerous factors could alter amino acid requirements. Hypercatabolism or malnutrition is associated with considerable changes in the release of hormones affecting protein turnover. Enhanced glucogenesis, glucose, or insulin intolerance are important factors influencing protein turnover and thus the metabolic needs of amino acids. Currently the essential importance of hyperglycemia on disease outcome was emphasized (33). Indeed, hyperglycemia affects protein turnover and thus amino acid requirements.

When dealing with the question of amino acid requirements in wasting diseases, one should also consider therapeutic approaches through which improvement, maintenance, and repletion can be achieved by changing the amounts and proportions of therapeutic amino acid preparations.

The concept of conditionally essential amino acids in disease. During episodes of various acute and chronic wasting diseases the classical definition of the essentiality of amino acids is seriously challenged. In 1962 Mitchell pointed out in the first volume of his treatise Comparative Nutrition of Man and Domestic Animals that an “amino acid may be a dietary essential even if an animal is capable of synthesizing it, provided that the demand for it exceeds the capacity for synthesis” (34). Thus, the strict nutritional classification of the common amino acids as formulated by Rose and later by Jackson, Chipponi, and others is not acceptable as we attempt to understand how dietary protein serves to meet our nutritional needs in disease (35,36).

Grimble proposes that, regardless of the definition used, a final judgment about the usefulness of an essential amino acid will be on the grounds of clinical and nutritional efficacy (37). A more general proposition is that “a possible and useful direction might put more emphasis on metabolic control and its regulation of tissue and organ function and nutritional status.” This definition offers suggestions as to how certain shared metabolic characteristics might be used to differentiate the various nutritionally important amino acids. It also implies that the dietary “essentiality” of a given amino acid is dependent on the ratio of supply to demand; the distinction between “essential” and “nonessential” largely disappears because it is dependent on conditions (35,36). In this context, chronic and acute wasting diseases are associated with particular amino acid deficiencies and imbalances causing specific changes in amino acid requirements. Thus, the new approach categorizes amino acids as indispensable, conditionally indispensable, or dispensable according to their functional and physiological properties as well as considering the ratio of supply to demand under various pathological conditions (35). Indeed, administration of the required conditionally indispensable amino acid might greatly facilitate an anabolic response to a life-threatening disease (38).

The major question is which amino acids are to be considered as conditionally essential (indispensable) during diseased conditions and in what amounts they should be administered. Lacking quantitative estimates of requirements, kinetic measurements of plasma amino acids after parenteral or enteral administration of amino acids might help to estimate qualitative requirements (39,40). The question raised is whether qualitative manipulations of amino acid intake could improve nutritional status especially in critically ill patients (41,42). Although, it is claimed that hyperaminoacidemia and hypoaminoacidemia indicate overload and deficit, respectively, no conclusive specific recommendations are suggested. Measurement of intracellular free amino acid deficiencies or excesses is another method to evaluate qualitative requirements. This method might be used as a guideline for composing suitable preparations for patients suffering from catabolic stress. Free amino acid patterns in muscle was measured in healthy men and in various catabolic conditions (43). One important finding is that the concentrations of free amino acids in muscle change in response to changes in diet and physiologic and pathologic states. Each catabolic condition appears to have its unique and reproducible pattern (44).

It was proposed that efficient protein metabolism requires the simultaneous presence of each proteic amino acid in appropriate proportions. The intracellular concentration of free amino acids would then represent the balance between the rate of production (entry of amino acids into the intracellular pool) and the rate of utilization of amino acids from this pool. Low intracellular amino acid concentrations may be due to decreased uptake from the extracellular space, a reduction in protein catabolism, or increased utilization. Intracellular depletion of amino acids is always a sign of deficiency, and a replenishment of the depleted amino acid pool with an adequate amount of supplementary amino acid may thus indicate the tentative requirement.

In the following section potentially indispensable amino acids will be discussed and their function scrutinized; their possible clinical application and tentative requirements will be suggested.

Histidine. In infants there is evidence for a dietary histidine requirement (45). Currently histidine was suggested to be indispensable (Pencharz, P., personal communication). This is supported by recent studies in normal men showing that a long-term histidine deficient diet (1–8 wk) leads to a significant decrease in plasma histidine concentrations (46).

In the early 1970s, Bergström and Fürst (47) provided the first evidence that histidine might be an indispensable amino acid in uremia. Fürst could demonstrate in 15N studies that the synthesis of indazolepyruvic acid or its transamination does not occur in severely uremic patients. This observation indicates that desamination of the histidine molecule is irreversible, similar to that of threonine and lysine (48). Since these first reports, several investigations confirmed the indispensability of histidine in severe renal disease (49,50). Accordingly, supplementation of tailored intravenous or oral diets with histidine resulted in improved nitrogen balance in uremic patients (51). On the basis of N-balance and muscle free intracellular amino acid results in uremia (47,52,53), a tentative requirement of 30 mg/kg body weight is suggested.

Serine. In healthy adults, serine can be readily synthesized from glycine and activated formaldehyde. However, in clinical situations with impaired kidney function, endogenous synthesis may not cover serine requirements, resulting in low extra- and intracellular serine concentrations (51,52,54). These findings suggest that serine may be indispensable for uremic patients who receive maintenance hemodialysis and that serine depletion may be another limiting factor for protein synthesis, thereby contributing to the increased protein requirements in these patients. It is also noteworthy that the kidney converts glycine to serine (55) and that this endogenous production of serine is extremely low in chronic renal failure (56,57). Supplementation with 35–40 mg serine per kg body weight normalizes low intracellular concentration (52,53). Thus, this amount is suggested for the tentative requirement in hemodialysis patients.
Taurine. Taurine (2-aminoethane sulfonic acid) is the most abundant free amine in the intracellular compartment (58). Taurine has functional roles in stabilizing the membrane potential, in bile salt formation, growth modulation, osmoregulation, antioxidation, promotion of calcium transport, and calcium binding to membranes. It exerts positive inotropic effects of the heart, as well as having antiarrhythmic and antihypertensive effects. It is involved in many metabolic responses in the central nervous system, has an anticonvulsant action, may have an insulinosgenic action, and is required for eye function (59). Taurine is capable of influencing programmed cell death in various cell types depending upon the initiating apoptotic stimulus (60) and of affecting Fas (CD95/APO-1)-mediated neutrophil apoptosis through the maintenance of calcium homeostasis (61).

There is some evidence that taurine might be indispensable during episodes of catabolic stress. We and others found low extracellular and intracellular taurine concentrations after trauma and infection (43). Low taurine concentrations in plasma, platelets, and urine were described in infants and children and also in adult trauma patients undergoing taurine-free long-term parenteral nutrition (62–64). Plasma taurine deficiency after intensive chemotherapy or radiotherapy is more severe in patients receiving taurine-free parenteral nutrition than in orally fed patients (65).

Low intracellular taurine concentrations in muscle are a typical feature in patients with chronic renal failure, probably because of impaired metabolic conversion of cysteine sulfonic acid to taurine (66,67). Intracellular taurine depletion may be associated with the well-known muscle fatigue and arrhythmic episodes that occur in uremia.

Taurine has been characterized as a conditionally essential amine in preterm infants and neonates and is currently incorporated in most neonatal dietary regimens (68).

On the basis of studies related to intracellular muscle free amino acids we propose the tentative requirements between 10 and 50 mg per kg body weight.

Free crystalline taurine is available for inclusion in intravenous or enteral preparations. However, we hypothesize that the extremely high intracellular to extracellular transmembrane gradient (250:1) might limit the cellular uptake of taurine. We proposed a novel binding of taurine to a suitable amino acid carrier in the form of a synthetic taurine conjugate (69,70). Experimental data strongly suggest improvement in transmembrane transport and intracellular utilization with this conjugate.

Tyrosine. The aromatic amino acid tyrosine has traditionally been considered a nonessential amino acid for adult humans. Tyrosine is synthesized exclusively from phenylalanine by hydroxylation; inclusion of tyrosine in the diet exerts a sparing effect on the dietary phenylalanine requirement (27,71). In premature infants, tyrosine is considered an indispensable amino acid; reduced endogenous tyrosine synthesis also may occur in full-term infants (72). The requirement is suggested to be 30–40 mg per kg body weight (53,54).

The suggested tentative glutamine requirement after uneventful recovery settings: a) instability, especially during heat sterilization and prolonged storage; and b) limited solubility (~3 g/100 mL at 20°C). The rate of breakdown of free glutamine depends on temperature, pH, and anion concentration. The decomposition of free glutamine is quantitative and yields the cyclic product pyroglutamic acid and ammonia. The drawback can be overcome by use of synthetic dipeptides (79,81). Clinical nutrition with glutamine supplementation has been associated with reduced hospital stay, morbidity, and mortality (82,83).

The suggested tentative glutamine requirement after uncomplicated major operations, major injury, gastrointestinal malfunctions, and during cachexy is ~0.15–0.20 g glutamine per kg body weight (corresponding to 20–25 g glutamine
TABLE 1
Suggested tentative requirements of indispensable amino acids in diseased state

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>mg (g) kg/BW</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infants</td>
<td>40</td>
<td>(45)</td>
</tr>
<tr>
<td>Adults</td>
<td>?</td>
<td>(46)</td>
</tr>
<tr>
<td>Severe CRF</td>
<td>30</td>
<td>(47, 52, 53)</td>
</tr>
<tr>
<td>Serine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemodialysis patients</td>
<td>35–40</td>
<td>(52, 53)</td>
</tr>
<tr>
<td>Taurine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trauma, infection</td>
<td>40</td>
<td>(43)</td>
</tr>
<tr>
<td>CRF/all conditions</td>
<td>40–50</td>
<td>(48–54)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infants</td>
<td>30</td>
<td>(72)</td>
</tr>
<tr>
<td>CRF</td>
<td>30–40</td>
<td>(48–54)</td>
</tr>
<tr>
<td>Cyst(e)ine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spare methionine</td>
<td>11</td>
<td>(75)</td>
</tr>
<tr>
<td>Infants</td>
<td>20</td>
<td>(77)</td>
</tr>
<tr>
<td>Liver failure</td>
<td>20</td>
<td>(75)</td>
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<tr>
<td>Glutamine</td>
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<tr>
<td>Moderate trauma Gl</td>
<td>0.15–0.20 g</td>
<td>(84, 85)</td>
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<tr>
<td>malfunction cachexy</td>
<td></td>
<td></td>
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<tr>
<td>Critical Illness Sepsis, MOF</td>
<td>0.3–0.5 g</td>
<td>(84, 85)</td>
</tr>
</tbody>
</table>

In Table 1 tentative requirements for conditionally indispensable amino acids are summarized in acute and chronic wasting diseases.

Final thoughts

Despite important achievements, many problems still remain to be solved concerning the requirements of specific amino acids before the nutritional treatment of wasting diseases can be optimized. Current results of metabolic studies indicate that the composition, amounts, and proportions of the presently available nutritional preparations are not suitable for the treatment of critically or chronically ill catabolic patients. In addition, glutamine, tyrosine, cystine, and taurine are normally not included in metabolizable form. For the treatment of such patients new preparations, including all conditionally indispensable amino acids, should be developed in appropriate amounts and proportions (Table 2).

Indeed, when Rose and his co-workers (86) discovered threonine as the last amino acid among 20 in 1935, they assumed that the job concerning amino acids and protein was done. Contrary to their belief, amino acid requirements are still a "tricky business" and today represent one of the most complex and controversial fields of research. Because amino acid imbalances and antagonisms remain a major puzzle in understanding pathogenesis of disease, a joint concerted attack unraveling these problems may shed light on the secrets of the regulation of protein and amino acid metabolism, in health and disease.

LITERATURE CITED


TABLE 2
Are current nutrition preparations unsuitable? Proposed measures to develop a suitable intravenous preparation for the treatment of the catabolic patient

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>mg (g) kg/BW</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Glycine</td>
<td></td>
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<tr>
<td>Valine</td>
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<td>Phenylalanine</td>
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<td>Serine</td>
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<td>Leucine</td>
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<td>Lysine</td>
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<tr>
<td>Threonine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 In association with I.V. arginine nutrition (87).
leucine oxidation, with particular reference to tracer estimates of leucine requirement
45. Först, P. (1987) Intracellular muscle free amino acids—their measure-
