

3. Amino Acids

METHODS

Literature Search

Time frame: Publication search is 1992–2004 plus relevant publications before 1992.

Type of publications: Case control and cohort studies, randomised controlled studies and systematic reviews.

Language: English.

Key Words: Adolescents, children, neonates, preterm infants, parenteral nutrition, amino acids, requirements, toxicity, deficiency.

Patients

All children with age range from preterm to adolescent were considered in these guidelines.

Outcome

Recommendations were developed from a standpoint of nutrient adequacy. Depending on age groups, nutrient adequacy was based on intrauterine accretion rate, organ development, factorial estimates of requirements and amino acid interactions. Individual amino acids are discussed.

Minimal intakes of specific amino acids are those that meet the specific requirement of children in that age group. Maximal intakes are recommended to prevent excessive and potentially harmful intakes of amino acids.

AMINO ACIDS

Introduction

Proteins are the major structural and functional components of all cells in the body. They consist of chains of amino acid subunits joined together by peptide bonds. The chain length ranges from two amino acids to thousands, with molecular weights subsequently ranging from hundreds to hundreds of thousands of Daltons. From a nutritional perspective, an important aspect of a protein is its amino acid composition.

Some amino acids are classified as essential (indispensable). Those are amino acids that cannot be synthesized by humans and hence must be provided in the diet or parenteral solution. Non-essential amino acids can be synthesized from other amino acids or from other precursors. Some amino acids are categorized as semi-essential. These amino acids can be synthesized from other amino acids but their synthesis is limited under certain circumstances (1–6). These amino acids may be of particular importance for the preterm infant in whom

a developmental delay in specific enzymes involved in amino acid synthesis have been demonstrated (7–11).

The essential, non-essential and conditionally essential amino acids are listed in table 1. Although everybody agrees on the concept that some non-essential amino acids are essential under certain circumstances, some debate exists about the conditional essentiality of arginine and proline.

METHODS FOR ESTIMATING TOTAL AND INDIVIDUAL AMINO ACID NEEDS

Amino acid requirements are mainly determined by the rate of net protein synthesis, which depends on the availability of rate limiting amino acids. There are several physiological and biochemical ways to determine whether the amino acid intake is sufficient or in excess of the needs of children. Different measurements in assessing adequacy of amino acid intake include anthropometry (weight and length), nitrogen balance, metabolic indices (e.g. amino acid concentrations, albumin, pre-albumin, total protein concentrations, blood urea nitrogen, metabolic acidosis), whole-body nitrogen kinetics, specific amino acid kinetics and the indicator amino acid method. The intake of each essential amino acid required to maintain nitrogen equilibrium in children and infants has been defined as the amount necessary to obtain growth and nitrogen balance.

In veterinary medicine the free amino acid concentrations were used to detect deficiencies and excesses of dietary amino acids. This principle works more rapidly than weight gain or growth studies (12–15). Most current amino acid solutions are developed in a similar fashion (16–18).

The amino acid indicator method seems to be an accurate and fast way to determine specific amino acid requirements. It has recently been developed to measure specific amino acid requirements (19–21) and has been validated in animal models of infancy (22–24). The technique is based on the partitioning of essential amino acid outflow under steady state conditions between oxidation and protein synthesis. When a single essential amino acid is deficient in the diet, the amount of protein that can be synthesized is limited. Since the limiting amino acid also limits the use of all other dietary amino acids for protein synthesis, the body must oxidize excess amounts of these amino acids. If one increases the dietary amount of the limiting amino acid, protein synthesis will increase and so will the utilization of the other dietary amino acids which in turn reduces their oxidation. Once the requirement for the limiting amino acid is reached, further increases in its dietary intake will cause no further increase in protein synthesis, nor decrease in the oxidation of the other essential amino acids.

The indicator amino acid oxidation method uses this relationship (19). Subjects are given a series of diets containing varying amounts of the amino acid for which the requirement is to be determined. The amounts vary below and above requirement. All other amino acids are furnished at constant

TABLE 3.1. *Essential, non-essential and conditionally essential amino acids*

Essential	Non-essential	Conditionally essential
Histidine	Alanine	Arginine
Isoleucine	Aspartic acid	Cysteine
Leucine	Asparagine	Glycine
Lysine	Glutamic acid	Proline
Methionine	Glutamine	Tyrosine
Phenylalanine	Serine	
Threonine		
Tryptophan		
Valine		

amounts *above* requirement. At the end of each diet period, a dose of another essential amino acid with a ^{13}C or ^{14}C label (the indicator amino acid) is given, and its oxidation is measured. The oxidation of the labelled indicator amino acid will decrease as the amount of test amino acid increases, until requirement is reached, and then the oxidation will plateau. Plotting the oxidation of the labelled indicator amino acid against test amino acid intake should show a breakpoint at the requirement level for the test amino acid. A slightly different approach is the use of the oxidation rate of the investigated amino acid or its direct metabolite. Such an approach has recently been used in the determination of the requirement of tyrosine in parenterally fed infants (25).

Most currently used parenteral amino acid mixtures contain amino acid amounts that result in a plasma amino acid pattern resembling the plasma amino acid patterns of normally growing, breast fed infants and children, or cord blood. These paediatric parenteral amino acid mixtures provide more essential and less non-essential amino acids than normally deposited by the infant or child.

The utilisation of the amino acid supply depends on a sufficient energy intake, and often an energy supply of 30 to 40 kcal per 1 g amino acids is recommended.

TOTAL AMINO ACID NEEDS DURING PARENTERAL NUTRITION

Differences Between Enterally Fed and Parenterally Fed Children

The amino acid requirement is lower in parenterally fed infants and children than in enterally fed infants because the supply bypasses the intestine. No human data are available but animal studies suggest that the equivalent of approximately 30–50% of the protein intake is used by the intestine in neonates (26–28). However, there is a wide variation in the intestinal uptake and utilization of specific amino acids that changes with age. First pass (intestinal and liver) leucine utilisation in older children is 24% (29), while it accounts for approximately 50% of the dietary intake in preterm infants (30). Intestinal utilisation of lysine accounts for approximately 20% of the intake (31) whereas 50% of glutamine is used (30) in preterm infants. Thus, the total needs of amino acids in parenterally fed children are lower than in enterally fed children, but there are huge differences in intestinal utilization of specific amino acids. Besides utilization by

the intestine, a number of amino acids are also metabolized and converted into other amino acids within the intestine and/or liver upon first pass. Bypassing the intestine will lower systemic availability of these amino acids and thus increase the parenteral requirements. In addition, while ingested phenylalanine and methionine appear to be converted to tyrosine and cysteine, respectively. It seems that parenterally administered phenylalanine and methionine are converted to a lower extent. Systemically active peptides are produced within the intestine (e.g. sIgA) and animal studies show that the intestine uses predominantly dietary amino acids (more than amino acids that are offered to the intestine from the systemic circulation) for specific protein synthesis (32).

These metabolic considerations could not yet be taken into account during the development of currently available parenteral amino acid solutions for infants and children, which have been based on considerations of food protein composition (e.g. human milk proteins) and amino acid blood concentrations. However severe deficiencies will be detected by measuring plasma amino acid levels during 24 hours infusions of amino acids.

Preterm Infants

The most widely used method to estimate total amino acid requirements is the amount needed to achieve a positive nitrogen balance. Energy intake and substrate composition affect protein balance in parenterally fed neonates (33–37).

Optimal glucose and lipid intakes that maximize protein accretion and growth have not yet been determined at all amino acid intakes in neonates, particularly in those who are ill or extremely preterm. Preterm infants without amino acid supplementation excrete between 0.6 and 1.1 g protein/kg per day. (2,38–40). Supplementation of 0.85, 1.0 or 1.2 g amino acids /kg per day does not result in a positive nitrogen balance (40–42). Multiple regression analysis in the study of Thureen et al revealed that a mean intake of 0.9 g amino acids/kg per day is necessary to prevent significant protein loss (34). Van Toledo-Eppinga found a minimal catabolic state in preterm infants receiving 1.8 g/kg per day (43), whereas Rivera et al found a significant positive nitrogen balance at an intake of 1.5 g/kg per day (39). Positive nitrogen balances were also achieved with an intake of 2.3 g/kg per day and 2.65 g/kg per day (2,41). Parenteral intake of 3.2 g/kg per day results in a positive mean protein balance of 2 g/kg per day at a non-protein energy intake of 90 kcal/kg per day. No detrimental effects on plasma amino acid profiles were noticed (44). Very recently, Ibrahim et al showed that preterm infants are able to tolerate 3.5 g/kg per day from birth onwards (45). This amount resulted in a positive nitrogen balance already on the first day of life. Also high intakes such as 3.3 g/kg per day and 3.9 g/kg per day seem to be well tolerated (46).

No higher parenteral intakes were reported in the time frame of the literature search.

It seems safe to administer amino acids from birth onwards (40,45).

Recommendations

- Amino acid supply should start on the first postnatal day. **GOR B**
- A minimum amino acid intake of 1.5 g/kg per day is necessary to prevent a negative nitrogen balance. Higher intakes are needed to achieve physiological protein deposition. **GOR A**
- A maximum amino acid intake of 4 g/kg per day is recommended. **GOR B**

Term Neonates During the First Month of Life

At a parenteral supply of 2.4 g amino acids/kg per day, urinary nitrogen excretion ranges 0.10–0.12 g N/kg per day in stable, post surgical term infants (47) corresponding to 0.6–0.8 g protein/kg per day. This results in a positive nitrogen balance of approximately 1.8 g/kg per day.

Term neonates with a parenteral amino acid intake of 2.5 g/kg per day achieve a moderate but positive protein balance (0.27 g/kg per day) (48).

In a similar age group Zlotkin et al recommended a protein intake of 2.3–2.7 g/kg per day to achieve a similar weight gain rate as in full term infants who were fed human milk (49). No data are available on the minimum lower limit in this age group but presumably the lower limit is not different from that in preterm infants.

Recommendation

- A minimum amino acid intake of 1.5 g/kg per day is recommended to avoid a negative nitrogen balance while a maximum amino acid intake should usually not exceed 3 g/kg per day. **GOR D**

From 1st Month to 3rd Year of Life

The administration of 2.4 ± 0.3 g amino acids/kg per day to infants and children up to an age of 43 months ($n = 40$, median age 2.7 months) resulted in a mean positive nitrogen balance of 242 ± 70 mg/kg per day, with plasma amino acid levels within the reference range except for a low level of tyrosine (16). A positive nitrogen balance of 242 mg/kg per day corresponds to a positive protein balance of 1.5 g/kg per day. Infants (age 2–12 months) on the first day after cardiac surgery excrete 244 ± 86 mg N/kg per day corresponding to a negative protein balance of 1.5 ± 0.5 g protein/kg per day, whereas the supplementation of 0.8 g amino acids/kg per day resulted in a negative protein balance of -114 ± 81 approx. 0.7 ± 0.5 g protein/kg per day (50).

No more recent data, less than 20 years old, is available. Thus, based upon the knowledge that the amino acid needs gradually decline and that the above mentioned studies are all dealing with ill children, our minimum estimate of amino acid intake in this age group is 1 g amino acids/kg per day.

Recommendations

- A minimum amino acid intake of 1.0 g/kg per day is recommended to avoid a negative nitrogen balance. **GOR D**
- No firm recommendation can be made on the maximal intake although based on weight gain rates an intake higher than 2.5 g/kg per day seems not advisable. **GOR C**

3rd–5th Year of Life

A study by Coss-Bu shows that critical ill children at a mean age of 5 yrs have a negative nitrogen balance at a protein intake of 2.1 g/kg per day (51). The subjects with a positive nitrogen balance had a higher protein intake (2.8 ± 0.9 g/kg per day) than subjects with a negative nitrogen balance (1.7 ± 0.7 g/kg per day). No data is available on healthy children.

Recommendations

- There is a paucity of data in the age group 3rd–5th year of age, insufficient to draw any firm conclusions on as much the lower and upper limit of protein intake should be. Daily intakes of 1.0 to 2.0 g/kg per day are considered reasonable for stable patients. **GOR D**
- For critically ill patients the advisable amino acid intake may be higher (up to 3 g/kg per day). **GOR D**

6th–12th Year of Life

Critically ill children at a mean age of 8 years show a negative protein balance at an intake of 1.7 g protein/kg per day. Regression analysis showed a protein requirement of 2.8 g/kg per day in this study group (52).

Recommendations

- There is a paucity of data in the age group 6th–12th year of age, insufficient to draw any firm conclusions on defining the lower and upper limits. Daily intakes of 1.0 to 2.0 g/kg per day are considered reasonable for stable patients. **GOR D**
- For critically ill patients the advisable amino acid intake may be higher (up to 3 g/kg per day). **GOR D**

Adolescents

Young men, receiving an essentially protein free diet, excrete approximately 24–38 mg N/kg per day which corresponds to 0.15–0.24 g protein/kg per day (53,54).

Goulet et al administered different amino acid intakes to patients with a compromised gut function (55). The response of protein turnover to graded levels of amino acid intakes was assessed by using stable isotopes technology (leucine kinetics) in approximately 13 year old children in a stable nutritional status receiving home parenteral nutrition. Since the fat content of the body of adolescents changes very rapidly during this period, the estimates are based on lean body mass rather than body weight alone. Intakes ranged from 0.7 to 2.5 g amino acids/kg lean body mass per day. Positive nitrogen balance was achieved in these children at an intake of 1.5 g amino acids/kg lean body mass per day, whereas this was not the case at an intake 0.7 g amino acids/kg lean body mass per day. There was a significant positive difference in protein balance when the intake increased from 1.5 to 2.5 g/kg lean body mass per day.

Recommendations

- A minimum amino acid intake of 1.0 g/kg lean body mass per day is recommended to avoid a negative nitrogen balance. **GOR B**
- There is a paucity of data in the adolescent age group, insufficient to draw any firm conclusions on as much the upper limit of protein intake should be. An upper limit of 2.0 g/kg lean body mass per day is considered reasonable. **GOR D**

The recommendations are summarised in Table 3.2.

TABLE 3.2. Parenteral amino acid supply considered adequate for most patients (g/kg body weight and day)

Preterm infants	1.5–4.0
Term neonates	1.5–3.0
2 nd month to 3 rd year	1.0–2.5
3 rd to 18 th year	1.0–2.0

SPECIFIC AMINO ACID REQUIREMENTS DURING TOTAL PARENTERAL NUTRITION

Cysteine

Cysteine is considered a semi-essential amino acid in the newborn period, indicating that cysteine might need to be administered to circumvent low cysteine synthesis with subsequently low plasma levels and impaired protein synthesis. It is normally synthesized from methionine (S-donor) and serine (C-donor). Stability of cysteine is low in solution, making it hard to supply enough to the infant. However, it is possible to add

cysteine-HCL to the amino acid solution just before the administration to the infant. Cystine (the oxidation product of two cysteine molecules combined) is stable but has a low solubility making it unsuitable as alternative to cysteine.

Cysteine is a major substrate for glutathione, a tripeptide (glutamic acid/cysteine/glycine) with important antioxidant properties, but also important in maintaining redox potential and calcium homeostasis. Appropriate levels of cysteine are therefore warranted. An intake of 170 $\mu\text{mol/kg}$ per day (approx. 27 mg Cysteine-HCl/kg per day) resulted in plasma cysteine levels below the reference range whereas an intake of 345 $\mu\text{mol/kg}$ per day (≈ 54 mg Cysteine-HCl/kg per day) was enough to reach adequate plasma levels (56). The addition of 462 $\mu\text{mol/kg}$ per day (72 mg/kg per day) resulted in normal plasma amino acid levels (57). Acetylation of cysteine prevents the instability but the bioavailability is low, approximately 50% (56).

In older children (age range 2–8 years) receiving an amino acid solution with varying doses of cysteine-HCl (0–40 mg/g AA, approx. 0–255 $\mu\text{mol/g}$ AA), no changes were noted in free cysteine/cystine or methionine plasma levels were noted. Only plasma taurine levels varied with cysteine supplementation. (58)

Recommendations

- The minimum advisable intake lies between 200 and 350 $\mu\text{mol/kg}$ per day (approx. 30–55 mg Cysteine-HCl/kg per day) in infants and young children. **GOR B**
- There is insufficient data in preterm infants to allow any firm conclusions to be made on the upper limit of cysteine intake. **GOR D**

Tyrosine

Like cysteine, tyrosine is considered a semi-essential amino acid in the neonatal period (59). The hydroxylation of phenylalanine to tyrosine is argued to be limited although Denne showed significant hydroxylation in even very preterm infants (60). However, many studies show low plasma concentrations of tyrosine in unsupplemented infants.

Supplementation of 55–90 μmol tyrosine/kg per day (≈ 10 –16 mg/kg per day) resulted in plasma levels below reference range in preterm infants (56). Acetylation of tyrosine increases the solubility, but the bioavailability is low. In two studies only 60% of N-acetyl-Tyrosine is retained (17,56). An intake of approximately 700 $\mu\text{mol/kg}$ per day which corresponds to a net intake of 126 mg tyrosine as NAT/kg per day resulted in adequate tyrosine levels. An intake of less than 200 $\mu\text{mol/kg}$ per day (corresponds to a net intake of 36 mg tyrosine as NAT/kg

per day) did not. However, plasma levels of N-acetyl-tyrosine exceeded the plasma levels of tyrosine.

Due to the immaturities in the neonatal tyrosine catabolic enzyme pathway, tolerance of tyrosine intakes at levels greatly over requirement is limited (61). In addition, due to the known neurologic impairment caused by hypertyrosinemia to the developing brain as assessed by lower IQ and psychologic tests, excess intakes must be avoided (62,63).

Recently, a dipeptide, glycyl-L-tyrosine was used to determine the tyrosine requirement in parenterally fed term neonates (25). Using the elegant technique of the indicator amino acid method, the tyrosine mean requirement and safe level of intake (at which 95% of the infants will have sufficient intake) were found to be 74 mg/kg per day and 94 mg/kg per day respectively.

Recommendations

- There is a paucity of data in preterm infants, insufficient to draw any firm conclusions on as much the upper and lower limits of tyrosine intake should be. The lower limit should be more than 100 $\mu\text{mol/kg}$ per day (≈ 18 mg/kg per day). **GOR C**
- The advisable intake in term infants is 520 $\mu\text{mol/kg}$ per day (≈ 94 mg tyrosine/kg per day). **GOR C**
- There is insufficient data in term infants to allow any firm recommendations to be made on the upper limit of tyrosine intake. **GOR D**

Glutamine

In critically ill adult patients, glutamine supplementation may reduce sepsis and mortality (64). In 2005, a systematic review stated that there is no evidence from randomised trials to support the routine use of glutamine supplementation in preterm babies (65). In 4 day old preterm infants, additional glutamine did not have an effect on leucine balance (66). Ten days of glutamine supplementation in very-low-birth weight infants resulted in higher plasma glutamine levels but ammonia levels were not increased (67). No effect of glutamine supplementation on sepsis incidence or mortality was observed. Neither had glutamine an effect on tolerance of enteral feeds, necrotizing enterocolitis, or growth. (68). Thus, there is no new evidence that glutamine should be added to parenteral mixtures for preterm infants. No data are available in older children.

Recommendation

- There is no conclusive evidence for the need to provide glutamine supplementation to the preterm infant. **GOR A**

Taurine

Taurine is not a typical amino acid because, although it contains an amino group, it does not have the requisite carboxyl group. Despite this, it is being discussed here. Taurine deficiency may increase glyco-conjugates of bile acids and result in cholestasis. Although the cause of neonatal cholestasis probably is multifactorial, there are data indicating that adequate taurine may prevent cholestasis in neonates. In addition, taurine deficiency may result in retina dysfunction (69). Taurine is synthesized from methionine and cysteine and studies show that prolonged parenteral nutrition in children with a cysteine and taurine free parenteral solution resulted in reduced plasma taurine levels (70,71). Taurine supplementation (3 mg/g AA) maintained plasma taurine concentrations within the reference range in term infants but not in very low birth weight infants (18). Cysteine supplementation (50–100 mg/kg per day) normalizes taurine concentrations in 7 year old children with short bowel syndrome (58).

Taurine supplementation results in a slightly higher nitrogen retention in newborn babies. The amino acid solution used was based on human milk.

Recommendations

- There is no conclusive evidence to support the supplementation of the preterm infant with taurine. However it is advised that taurine is supplemented in the same amount as it is present in human milk, approx. 22 $\mu\text{mol/gram}$ amino acids or 2.8 mg/g amino acids. **GOR D**
- No firm recommendation can be made upon advisable lower or upper limits. **GOR D**

No other amino acids are discussed as there is insufficient data available to recommend any intake ranges.

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