A large proportion of extremely low-birth-weight infants requires parenteral nutrition for variable lengths of time. Amino acids are the key ingredients of parenteral nutrition. The goal of appropriate amino acid administration is to promote anabolism and normal cellular development in order to limit the incidence of postnatal growth restriction, which is associated with neurodevelopmental delays. The benefits of early amino acid commencement soon after birth are compelling, especially on nitrogen balance, while long-term outcome studies are lacking. Amino acid administration at 2.5 g/kg per day has been shown to be superior to lower intakes; however, the benefits of intakes above 2.5 g/kg per day remain controversial.

Introduction

Proteins are the major structural and functional components of body cells. They are made of chains of amino acids joined together by peptide bonds. Amino acids can be classified as essential, nonessential and semi-essential (table 1) [1, 2]. Essential amino acids are those that cannot be synthesized and must, therefore, be provided by diet or parenteral solutions. Nonessential amino acids can be synthesized by other amino acids or other precursors. Semi-essential amino acids are those that can be synthesized from other amino acids but not
in all circumstances: preterm infants have a developmental delay of activity of selected enzymes that can limit the synthesis of amino acids, for example of cysteine.

In more recent years, the notion of essentiality has changed and a more dynamic concept of essentiality has been put forward based on the concepts of supply and demand rather than growth and nitrogen balance [3]. Some amino acids that are classified as nonessential are utilized as precursors of other amino acids with important biological functions. With new knowledge emerging on the physiological and pharmacological properties of amino acids, the concept has changed further and nonnutritional functional outcomes of amino acids have also been considered [4, 5].

### Table 1. Essential, nonessential and semi-essential amino acids

<table>
<thead>
<tr>
<th>Essential</th>
<th>Nonessential</th>
<th>Semi-essential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>Alanine</td>
<td>Arginine</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Aspartic acid</td>
<td>Cysteine</td>
</tr>
<tr>
<td>Leucine</td>
<td>Asparagine</td>
<td>Glycine</td>
</tr>
<tr>
<td>Lysine</td>
<td>Glutamic acid</td>
<td>Proline</td>
</tr>
<tr>
<td>Methionine</td>
<td>Glutamine</td>
<td>Taurine</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Serine</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Threonine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Amino Acid Provision in Utero

Earlier studies on fetal amino acid metabolism are limited to animal research, mainly performed in sheep. More recently, human fetal amino acid metabolism has been studied in vivo using stable isotope techniques. A better understanding of how nutrients are transported through the placenta and metabolized by the fetus may help in the development of better nutritional therapies for preterm infants. It is known that intact proteins, with the exception of IgG, are transported only in negligible amounts through the placenta, while amino acids are actively transported in large quantities to the fetus. The most common substrates for active transport are ATP or sodium, but many amino acids are also transported inwardly in exchange for other amino acids that are transported outwardly [6]. It has also been demonstrated that there is a net output of ammonia [7] and urea [8] from the umbilical cord toward the placenta, indicating that amino acids are used also as an energy source. As far as protein synthesis is
concerned, studies performed on albumin synthesis have demonstrated that premature fetuses have surprisingly high albumin synthesis rates [9], although the meaning of this finding is not clear.

**Amino Acid Solutions for Parenteral Nutrition of Preterm Infants**

The composition of parenteral amino acid solutions has been based on amino acid blood concentrations of breastfed infants or cord blood levels, or on food protein composition (e.g. human milk proteins). Although their quality has slowly improved through the years, none of the currently available amino acid solutions has been specifically designed for the preterm infant. As a result, the composition of most of the currently available amino acid solutions may be of suboptimal quality for the need of preterm infants. For example, because of poor solubility, solutions contain very little tyrosine and cysteine. Since amino acids are incorporated in protein in a fixed ratio, if one of the semi-essential amino acids is lacking, all the others cannot be used and will be oxidized. It is also important to note that parenterally fed infants have lower amino acid requirements because the supply bypasses the intestine, which is supposed to use approximately 30–50% of the protein intake [10]. On the other hand, a number of amino acids are also metabolized and converted into other amino acids within the intestine and/or liver, so the intestinal bypass will lower systemic availability of these amino acids and, therefore, increase their parenteral requirements. It seems also that some amino acids have different metabolic rates depending on the route of administration: parenterally administered phenylalanine and methionine are converted to tyrosine and cysteine, respectively, but to a lesser extent than when administered enterally.

Single amino acid requirements for parenterally fed preterm infants are still not known for most of the amino acids. Trials of single amino acid supplementation [11, 12] and studies on single amino acid requirements using stable isotope techniques [13–15] have been performed, but still, how, when or how much they should be supplemented has not been fully established [16]. Furthermore, limited information is available on the bioavailability of the pharmacological preparation of the intravenous amino acids [17].

**Arginine**

Arginine is essential for ammonia detoxification through the urea cycle and it is a precursor of nitric oxide, which is important in endothelial cell vasodilation. Preterm infants receiving low arginine supplements demonstrate elevated
plasma ammonia [18] and impaired nitric oxide synthesis during hypoargininemia, and it has been found to be associated with an increased incidence of necroting enterocolitis [19, 20].

Cysteine
Cysteine is a precursor of taurine and glutathione and its synthesis might be reduced in preterm infants due to low activity of the enzyme cystathionase, although some studies suggest that cysteine may be synthesized de novo in very-low-birth-weight infants 48 h after birth [21, 22]. Providing some cysteine (25–50 mg/kg per day) improves short-term outcomes in preterm neonates, e.g. nitrogen balance. Most parenteral solutions contain little or no cysteine because of its low stability. N-acetyl-cysteine, which is stable in solutions and has been supplemented to parenteral formulations, has a low bioavailability [17].

Glycine
Glycine is a precursor of glutathione and serves as an inhibitory neurotransmitter. Preterm infants may have increased glycine requirements in case of oxidative injury such as during critical illness or when receiving oxygen therapy.

Proline
Proline is the most abundant amino acid in tissue protein. Since preterm infants are unable to synthesize proline from glutamate and because of their high protein turnover and tissue accretion, they may have a higher proline requirement.

Taurine
Taurine contributes to intestinal fat absorption, bile acid secretion, and retinal and hepatic functions. It is endogenously synthesized from cysteine, but at low rates in preterm infants; furthermore, cysteine is present at low concentrations in amino acid solutions. Some evidence indicates a preventive role of taurine in intestinal failure-associated liver disease. Despite the lack of evidence of a benefit from randomized clinical trials, taurine is considered a conditionally essential amino acid in preterm infants and it is contained in amino acid solutions for the newborn infant.

Tyrosine
Information on tyrosine metabolism and requirements in preterm infants is very limited. Tyrosine is present in small amounts in parenteral solutions due to its poor solubility, and its synthesis from phenylalanine hydroxylation is thought to be insufficient in preterm infants. Studies show that currently used parenteral solutions have tyrosine contents below minimum requirements for preterm infants that are about 18 mg/kg per day [23].
Glutamine has been proposed to be conditionally essential for premature infants, and the currently used parenteral nutrient mixtures do not contain glutamine. Several high-quality studies and related meta-analyses show that glutamine supplementation in infants up to 3 months of age does not result in any beneficial effects [24, 25].

Table 2 shows the amino acid profile and composition of the most common amino acid solutions.

### Amino Acids in Parenteral Nutrition of Preterm Infants

The goal of an appropriate amino acid administration is to promote anabolism and normal cellular development in order to limit the incidence of postnatal growth restriction and because of the possible adverse effects on long-term neurodevelopment. A minimum of 30–40 kcal per gram amino acid is usually

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### Table 2. Amino acid (AA; g/100 ml) content of AA solutions for parenteral nutrition

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Primene 10% Baxter</th>
<th>TrophAmine 6% Baxter</th>
<th>Aminopad 10% Fresenius Kabi</th>
<th>Aminoplasmal B. Braun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.670</td>
<td>0.490</td>
<td>0.510</td>
<td>0.500</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.000</td>
<td>0.840</td>
<td>0.760</td>
<td>0.890</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.100</td>
<td>0.490</td>
<td>0.880</td>
<td>0.574</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.240</td>
<td>0.200</td>
<td>0.200</td>
<td>0.440</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.420</td>
<td>0.290</td>
<td>0.310</td>
<td>0.470</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.370</td>
<td>0.250</td>
<td>0.510</td>
<td>0.420</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.200</td>
<td>0.120</td>
<td>0.400</td>
<td>0.160</td>
</tr>
<tr>
<td>Valine</td>
<td>0.760</td>
<td>0.470</td>
<td>0.610</td>
<td>0.620</td>
</tr>
<tr>
<td>Conditionally essential AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.189</td>
<td>0.020</td>
<td>0.050</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>0.380</td>
<td>0.290</td>
<td>0.460</td>
<td>0.300</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.060</td>
<td>0.015</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.045</td>
<td>0.140</td>
<td>0.110</td>
<td>0.400</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.840</td>
<td>0.730</td>
<td>0.910</td>
<td>0.115</td>
</tr>
<tr>
<td>Proline</td>
<td>0.300</td>
<td>0.410</td>
<td>0.610</td>
<td>0.550</td>
</tr>
<tr>
<td>Nonessential AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>0.800</td>
<td>0.320</td>
<td>1.590</td>
<td>0.105</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.600</td>
<td>0.190</td>
<td>0.660</td>
<td>0.560</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1.000</td>
<td>0.300</td>
<td>0.930</td>
<td>0.720</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.400</td>
<td>0.220</td>
<td>0.200</td>
<td>0.120</td>
</tr>
<tr>
<td>Serine</td>
<td>0.400</td>
<td>0.230</td>
<td>0.200</td>
<td>0.230</td>
</tr>
<tr>
<td>Ornithine</td>
<td>0.318</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
recommended to guarantee amino acid utilization. Optimal glucose and lipid intakes that maximize protein accretion and growth in preterm infants have not been determined at various amino acid intakes.

**Early Amino Acid Intake**

No doubt exists regarding the fact that amino acid intake should be started from the first day of life or, even better, as soon as possible after birth so to avoid the ‘metabolic shock’ caused by the interruption of continuous feeding that occurs in utero. Early amino acid administration in preterm infants results in increased protein synthesis without a decrease in proteolysis \[26\]. Several studies evaluating amino acid administration directly after birth have found a positive nitrogen balance, calculated as the difference between nitrogen intake and estimated urinary nitrogen loss \[27–30\]. Consistent with these findings were the ones of a positive correlation between an increased amount of amino acid intake and an improved nitrogen balance. Few studies have looked at the effect of early amino acid administration on short-term growth, and, in other ones, growth was recorded as a secondary outcome \[29, 30\]. Overall, early amino acid administration, when compared to glucose administration alone, is associated with improved short-term growth \[31\]. Much less is known about the effects on longer-term outcomes such as long-term growth and neurodevelopment. In a cohort study, Poindexter et al. \[32\] found significant improvement in growth parameters at 36 weeks postmenstrual age in favor of the infants who received early amino acids, but no differences were found either in growth or in neurodevelopment at 18 months corrected age. Stephens et al. \[33\] reported a retrospective analysis of 150 extremely low-birthweight infants and found a positive association between protein intake in the first week of life and scores on the Bayley Mental Development Index at 18 months corrected age. van den Akker et al. \[34\] found no difference in growth but a neurodevelopmental advantage at 2 years corrected age for boys that received amino acids from the first day of life compared to the ones that received glucose alone.

No detrimental metabolic effects of commencing amino acid administration from birth onwards have been noticed \[29, 35–37\]. Some researchers did not find higher urea concentrations in patients receiving high amino acid supplements \[28, 30, 38, 39\] while others did find a positive correlation between amino acid intake and increased blood urea levels \[29, 40–42\], indicating a greater proportion of amino acids being oxidized. This resembles the intrauterine situation in which amino acids are also used as an energy source, and higher blood urea levels should not be interpreted as a sign of intolerance but rather as a reflection of oxidation. Furthermore, the definition of what is a safe blood urea level in preterm infants still has to be determined and, indeed, the incidence of metabolic acidosis is not related to amino acid intakes \[41, 43\].
High versus Low Amino Acid Intakes
The most commonly used method to estimate amino acid requirements is the amount needed to achieve a positive nitrogen balance. Studies show that a mean intake of 0.9–2.65 g/kg per day can result in a positive nitrogen balance, with an energy intake as low as 30 kcal/kg per day. Performing nitrogen balance studies in small, often unstable preterm infants during the first days of life is very challenging. Most of these infants are not in a steady state and nitrogen balance studies often fail to correct for a rapidly expanding urea pool.

The 2005 ESPGHAN guidelines on pediatric parenteral nutrition [44] recommended a minimum amino acid intake of 1.5 g/kg per day to prevent a negative nitrogen balance and a maximum of 4 g/kg per day, according to the evidence that up to 3.3–3.9 g/kg per day seemed to be well tolerated. There is still limited evidence that increasing amino acid intake above 2.5 g/kg per day is associated with a more favorable outcome. The impact of different amino acid intakes on growth during parenteral nutrition has been studied in non-randomized controlled trials (RCTs) [36, 45, 46] or as secondary analyses of studies designed for other purposes [32, 47]. Other studies have evaluated different protein intake schemes with varying nonprotein energy or different timing of administration [35, 30, 48–50]. To date, only a few RCTs have been conducted to solely compare the effect of increasing amino acid intake in parenteral nutrition on growth and neurodevelopment in small preterm infants. In the study by Clark et al. [51], 122 patients were randomized to receive a maximum amino acid supplementation of 2.5 or 3.5 g/kg per day. Growth at 28 days was nearly identical between treated and control patients. In the study by Burattini et al. [41], 114 extremely low-birth-weight infants were randomized to receive standard (2.5 g/kg per day) versus high (4 g/kg per day) amino acid intake. Infants in the intervention group received an extra 8 g/kg of amino acids over the first 10 days of life without any significant difference in short- and long-term growth. Of interest, we recently conducted a blinded randomized study in which we gave an extra gram of protein (first as amino acid during parenteral nutrition and then as protein supplement during enteral nutrition) to small preterm infants from birth to 1,800 g of body weight. We found that this intervention did not improve short-term growth and body size at 2 years. There was also no difference in neurodevelopment at the 2-year follow-up [52]. Only a few other studies have looked at the relation between the dose of parenteral amino acids and neurodevelopment. In a randomized study, Blanco et al. [47, 53] found lower mental developmental scores at 18 months in infants who received the higher amino acid intake, but the difference was no longer significant at the 2-year follow-up. Their study was a secondary analysis of a study originally designed with the aim of reducing hyperkalemia and not powered for neurodevelopment [53].
It is worth mentioning that some studies reported better glucose control in infants who received amino acids/higher amino acid intakes [29, 30, 41]. These findings come from studies including a small number of patients and should be interpreted with caution.

High amino acid intake in small preterm infants has been reported to affect electrolytes and mineral metabolism [54, 55].

In summary, the present data provide strong evidence for a beneficial effect of early administration of amino acids in premature infants, while the optimal maximum amount still has to be determined. There is no evidence that increasing amino acid intake above 2.5 g/kg per day with a nonprotein energy <65 kcal/kg per day improves growth. Probably further strategies such as increasing nonprotein energy and improving the quality of amino acid solutions could partially help ameliorate the problem of the poor growth of small preterm infants [48].

RCTs including a large number of infants will be needed to assess the effect of early nutrition interventions on growth and, more importantly, on neurodevelopment.

Disclosure Statement

The authors declare no conflicts of interest.

References