Amino acid requirements of total parenteral nutrition (TPN) fed neonates: a narrative review of current knowledge and the basis for a new amino acid solution in neonatal nutrition

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Background and Objective: Globally, an estimated 11% of births, approximately 15 million babies are born preterm per year. Preterm infants have unique nutritional needs. However, gastrointestinal immaturity and congenital anomalies often preclude enteral feeding, necessitating intravenously administered total parenteral nutrition (TPN). Metabolic immaturity affects amino acid metabolism and requirements, decreases tolerance to excess intakes and ability to synthesize dispensable amino acids. Parenterally fed neonates therefore are at risk of toxicity and deficiency of amino acids which negatively affect clinical outcomes. The objectives of this narrative review were to summarize current knowledge on amino acid requirements and describe the importance of appropriate composition of amino acids in parenteral nutrition formulas for neonates.

Methods: Studies published on MEDLINE between 1950 and 2020 were included if they were conducted to determine amino acid requirements, evaluate parenteral amino acid solution, splanchnic amino acid metabolism in neonates or neonatal outcomes related to amino acid intake and protein quality.

Key Content and Findings: The gut is an important site of amino acid metabolism and when it is bypassed, the requirements for many amino acids are lower for TPN than for enteral feeding. Yet, the amino acid profiles in current commercial TPN formulas are based on extrapolations from plasma amino acid concentrations, human cord blood or human breast milk composition. The requirement for four amino acids in the TPN fed human neonate have been estimated and are up to 90% lower or higher than what is present in current commercial solutions. These data provide confirmation that current amino acid solutions for the neonate are inappropriate.

Conclusions: We conclude that Much work is left to be done to determine the requirements for the remaining indispensable and conditionally indispensable amino acids in the parenterally fed neonate. Only then, can an optimal pattern of amino acids be combined for formulating an appropriate parenteral amino acid solution for this vulnerable patient population.

Keywords: Total parenteral nutrition (TPN); neonate; amino acid requirement; indispensable amino acids; indicator amino acid oxidation method

Introduction

The global preterm birth rate is 11%, accounting for about 15 million live births (1) annually. Preterm neonates are metabolically immature, born during a period of rapid *in utero* growth and brain development, when nutrient requirements are high and nutritional imbalances can adversely affect outcome (2-5). Gut immaturity, congenital and acquired gastrointestinal disorders, which all occur frequently in preterm infants, can preclude enteral feeding necessitating the use of parenteral nutrition. Different centers have differing protocols regarding when to commence and how to advance parenteral nutrition; particularly relating to amino acid delivery (6-10). Some maintain that parenteral nutrition should be initiated within the first 24 h after birth with high amino acid intake (3.0–3.5 $g.kg^{-1}.d^{-1}$) while many argue for a slower, less aggressive introduction and progression (11). More recent data suggest that withholding parenteral nutrition for one week in term neonates was clinically superior to initiation within 24 h (12). However, regardless of the mode of introduction and advancement, many preterm infants require parenteral nutrition for days to months of life. The importance of amino acids for stimulation and maintenance of growth and bodily functions is well understood. Beyond their roles in protein synthesis and growth, amino acids perform functional and regulatory roles, and their intakes, physiological concentrations, as well as concentrations of their metabolites are associated with clinical outcomes in preterm infants (2,13-21). This demonstrates that knowledge of amino acid requirements is necessary in order to provide the ideal amino acid solution for parenterally fed neonates. However, current knowledge of amino acid requirements in total parenteral nutrition (TPN) fed neonates is inadequate and amino acid solutions used in TPN feeding are based on best judgements rather than scientifically derived estimates of requirements in this vulnerable population.

The objective of this review is to summarize current knowledge on amino acid requirements and describe the importance of appropriate composition of amino acids in parenteral nutrition formulas for neonates. The physiological basis for revising current amino acid solutions used in parenteral feeding of neonates is also provided. We present the following article in accordance with the Narrative Review reporting checklist (available at https://pm.amegroups.com/article/view/10.21037/pm-21-27/rc).

Table 1 Amino acid classification

Indispensable	Dispensable	Conditionally indispensable
Isoleucine	Alanine	Cysteine
Leucine	Aspartate	Tyrosine
Valine	Glutamate	Glycine
Phenylalanine	Serine	Arginine
Tryptophan		Proline
Methionine		Asparagine
Lysine		Glutamine
Threonine		
Histidine		

Methods

The authors conducted a literature review with a research database search using the following keywords:

"amino acid requirements and neonates";

"amino acid requirements and parenterally fed neonates";

"essential amino acid requirements and neonates".

In addition, personal conversation with the authors of the current manuscript was made to identify publications. Finally, we also review the references list of retrieved publications. A number of publications between 1950 to Aug 2020 were identified and subsequently evaluated to determine if they referred to amino acid requirement in human neonates and neonatal piglets, effect of parenteral nutrition on growth and body composition, splanchnic metabolism of amino acids, neonatal outcomes related to amino acid intake and impact of protein quality on neonatal growth. Papers cited at the back of the manuscript were those agreed upon by all authors to be included in this narrative review.

Discussion

Classification of amino acids and importance of amino acid composition

Classically the α-amino acids, that are the constituents of protein, were divided into essential (indispensable) and non-essential (dispensable) (*Table 1*). Indispensable amino acids are those which cannot be synthesized in the body and must be obtained exogenously from the diet. Dispensable amino acids on the other hand are those which can be synthesized endogenously. In infants, the classical

indispensable amino acids are: the branched chain amino acids (isoleucine, leucine and valine); histidine; lysine; phenylalanine; methionine, threonine; and tryptophan (22). We now recognize that quantitatively important functions of amino acids, which do not involve protein synthesis, must also be considered. These would include the role of cysteine and glycine as components of the major antioxidant, glutathione (23-28), as well as the role of arginine in nitric oxide (NO) synthesis and creatine synthesis (14,29). Hence, as knowledge has accumulated it has been recognized that most of the "dispensable" amino acids are conditionally indispensable (30) (*Table 1*). That is to say that in illness and in immaturity (both of which apply to premature infants) endogenous synthesis of these amino acids is inadequate. Thus of the 20 constituent amino acids, which make up body proteins, perhaps only alanine, aspartate, serine and glutamate are truly dispensable (22).

Protein quality is a term which refers to a mixture of amino acids in proportions which optimize growth, net protein synthesis (i.e., synthesis minus breakdown) and nitrogen accretion, without supplying any amino acid(s) in an excess, potentially toxic amount. Neonatologists (clinicians) often discuss protein quantity when determining nutritional intake. However, very little attention is paid to protein quality.

Current amino acid solutions used in TPN feeding of neonates

Preterm neonates are at increased risk of poor growth, as well as life-threatening conditions including respiratory distress syndrome, chronic lung disease, persistent pulmonary hypertension, necrotizing enterocolitis (NEC), and neurodevelopmental delay (31-35). While medical advances have increased survival of babies born prematurely, premature infants have poorer outcomes (which extends beyond infancy and childhood) than term infants (36). Optimal nutrition during the early post-natal period is a well-recognized strategy to decrease risk of adverse outcomes in this vulnerable population (16,37-40).

When less than optimal amino acid mixtures are fed parenterally to growing premature infants (41), growth and nitrogen accretion are sub-optimal (42,43). In addition, a variety of aberrations are seen in plasma amino acid profiles (2,20,44,45). However, when the quality of the amino acid mixture is improved, enhanced growth and protein accretion are achieved, largely by a reduction in endogenous protein degradation (46,47).

Current amino acid solutions used in parenteral feeding are patterned after three different approaches. The first approach, patterned the amino acid mixture based on the amino acid composition of egg protein (48), for use in adults. This approach was extrapolated to neonates but instead patterned the amino acids after human milk amino acid composition, the ideal protein source for enterally fed infants. This approach presupposes that the splanchnic bed (gut and liver) absorbs and metabolizes all enterally fed amino acids in the same way and that all enterally consumed amino acids enter the circulation—an assumption that isotope studies have disproved (42,49-55). The second approach was to use the amino acid composition of cord blood. This commercial solution is currently widely used in Neonatal Intensive Care Units (NICU) in North America and Europe (47,56). The third approach was to computer model the plasma amino acid response to parenteral amino acid administration (56). A commercial paediatric amino acid solution was developed based on this approach and is commonly used in NICUs in the USA. This approach cannot account for rates of entry and exit of amino acids from the plasma pool but presupposes that optimal plasma amino acid concentrations are known. *Table 2* presents the composition of the two most common commercial amino acid solutions for neonates.

Demonstrating the importance of protein quality, leucine turnover in preterm infants was assessed in response to two of the three amino acid solutions; the one patterned after cord blood (Primene®) and the other modeled after plasma amino acids (Trophamine®) (47). Despite a higher intake of leucine in the Trophamine group, leucine breakdown on day 7 was higher in the infants receiving Trophamine than those receiving Primene, indicating that protein quality was affecting leucine metabolism. Whilst this suggests that Primene may have a better protein quality/ amino acid profile compared to Trophamine, this does not provide evidence that the amino acid composition is optimal. Another important difference between Primene® and Trophamine® is the tyrosine source. While Primene® contains L-tyrosine, the tyrosine source in Trophamine® is N-acetyl-L-tyrosine which has a poor bioavailability in neonates (56). Indeed, as an initial step towards assessing parenteral amino acid requirements, others have evaluated available commercial amino acid solutions supplied to critically ill children (57). They found that the concentrations of amino acids in current amino acid solution used for parenteral feeding were either inadequate or excessive and concluded that "*amino acid composition*

Table 2 Amino acid composition of the most common used neonatal amino acid solutions (% amino acid by weight)

AA	Primene® (Baxter)	Trophamine® (McGaw)
Ile	6.7	8.2
Leu	9.9	14.0
Val	7.6	7.8
Lys	10.9	8.2
Met	2.4	3.4
Cys	1.9	0.1
Phe	4.2	4.8
Tyr	0.9[1]	2.3[2]
Thr	3.7	4.2
Trp	2.0	2.0
His	3.8	4.8
Arg	8.4	12.2
Gly	4.0	3.6
Ala	7.9	5.4
Asp	6.0	3.2
Glu	9.9	5.0
Pro	3.0	6.8
Ser	4.0	3.8
Tau	0.6	0.2
Orn	2.2	0
Total (mg)	105.4	101.8

[1], supplied as L-tyrosine and [2], N-acetyl-tyrosine.

of parenteral formulas is variable and lacks scientific support. Parenteral amino acid intakes should be based on measured requirements to maintain nutrition and functional balance and on a knowledge of toxicity" (57). In a more recent review on amino acid intake in parenterally fed newborn infants, the authors concluded that" adequately powered trials in very preterm infants are required to determine the optimal intake of amino acid" (58). Although they were referring to very preterm newborn infants, their observation underlines the lack of adequate information on amino requirements for all parenterally fed neonates.

Methods for determining amino acid requirements

Traditionally, amino acid requirements were studied using nitrogen balance in response to feeding graded intakes of the test amino acids (59). This approach has limitations which have been extensively reviewed (60). One such limitation is the long period required to adapt subjects to each amino acid intake (60). As far as neonates are concerned such studies would require exposing the infant to a deficient intake of the test amino acids for 7 to 10 days, which is unethical. Furthermore, nitrogen balance is not sensitive enough to detect differences in protein quality (61). Hence, alternate methods based on amino acid oxidation (62,63) have been developed. Recent reviews of this topic may be found in (64,65). Indicator oxidation/balance is now recognized as the optimal methods for determining amino acid requirements (66,67). Most applicable to neonates is the minimally invasive indicator amino acid oxidation method, since the infant is exposed to a deficient, or excess intake for only a period of 24 h and only breath and urine are collected (20,68-70).

The development of the indicator amino acid oxidation method provided a major breakthrough for the study of amino acid requirements (62,71). However, it was the development and validation of the neonatal piglet model (72-74) which allowed for a comprehensive assessment of the effect of parenteral nutrition on amino acid requirements and metabolism in neonates. Similarities in anatomy, physiology and metabolism has deemed the neonatal piglet the best model for the human neonate (75,76).

Amino acid requirements of parenterally fed neonates

Using the neonatal piglet model, the group led by Ball and Pencharz worked for almost 30 years to systematically determine parenteral requirements for dietary indispensable and conditionally indispensable amino acids and compared the estimates with enteral requirements (51-54,77-80). They began with an estimate of the phenylalanine requirement (79) using direct amino acid oxidation method and from then used phenylalanine as the indicator amino acid to estimate the requirement for lysine (78), tyrosine (77), threonine (52), methionine in the absence and presence of dietary cysteine (51,81), branched chain amino acids (leucine, isoleucine and valine) (53) and tryptophan (54). The most novel and significant findings are that the parenteral threonine, methionine and total branched chain amino acid requirements are 40%, 69%, and 56% respectively of enteral requirements, while tryptophan requirements are not altered by the route of feeding (51-54) (*Table 3*). These groundbreaking

Table 3 Amino acid requirements of the parenterally fed neonatal piglets and human neonate compared with enteral requirement

Model	Amino acid	Parenteral requirement	Enteral requirement	Present in commercial TPN solutions	References
Neonatal piglet	Phenylalanine (in the presence of excess tyrosine)	0.45 g/kg/d	ND		(79)
	Tyrosine	0.31 g/kg/d	ND		(77)
	Lysine	0.79 g/kg/d	ND		(78)
	Methionine (in the absence of cysteine)	0.29 g/kg/d	0.42 g/kg/d		(51)
	Methionine (in the presence of cysteine)	0.18 g/kg/d	0.25 g/kg/d		(81)
	Branch chain amino acids	1.53 g/kg/d	2.64 g/kg/d		(53)
	Threonine	0.19 g/kg/d	0.42 g/kg/d		(52)
	Tryptophan	0.145 g/kg/d	0.127 g/kg/d		(54)
Human neonates	Tyrosine	74 mg/kg/d	ND	<23 mg/kg/d	(82)
	Methionine (in the absence of cysteine)	49 mg/kg/d	ND	72–102 mg/kg/d	(20)
	Lysine	105 mg/kg/d	130 mg/kg/d	221–337 mg/kg/d	(69,83)
	Threonine	33 mg/kg/d	68 mg/kg/d	111–126 mg/kg/d	(68,84)

ND, not done; TPN, total parenteral nutrition.

results demonstrate that the gut is an important site of amino acid metabolism (42,49,85) and when it is bypassed the requirements for most amino acids during parenteral feeding are significantly lower. These findings were independently confirmed by others using different methodologies (73,74). Using the piglet model, Stoll *et al*. demonstrated that approximately 30% of dietary amino acid intake is utilized on first-pass metabolism. Interestingly approximately 60% of the amino acid taken up on first pass is catabolized by the intestine (73,74). The authors propose that this could be a significant source of energy for the small intestinal mucosa (73,74). Indeed whether used as a source of energy or for incorporation into mucin as in the case of threonine (86), small intestinal metabolism contributes to a significant portion of several amino acid requirements as demonstrated by the number of studies showing lower amino acid requirements of TPN fed compared to enterally fed neonates (51,53,81,86).

These differences in amino acid metabolism between routes of feeding need consideration when designing an amino acid solution for neonates. Additionally the neonate cannot be considered a small adult as immaturity of enzyme pathways and underdeveloped organs may be susceptible to the effects of early nutrition (3-5,87-89). Some have shown that excess protein and amino acid intakes have been linked to permanent damage to the brain (2,90), while more recent systematic

review has provided inconclusive results (58). In low-birth-weight infants (42), splanchnic extraction of leucine was observed to be twice as high as in adults (91-93) demonstrating significant differences in amino acid metabolism between the neonate and adult human. The piglet derived estimates also supported the conclusions made by others that current amino acid solutions patterned after cord blood, plasma amino acids or human milk are inadequate (47,57).

Building on knowledge and experience gained from the piglet studies, the minimally invasive indicator amino acid oxidation method (94) was applied in parenterally fed human neonates to determine the requirements for total sulphur amino acid (20) (as methionine only), threonine (68) lysine (69) and tyrosine (82). Since piglets grow at 5 times the rate of the human neonate, it was hypothesized that the requirement in the human neonate will be 1/5th the estimate obtained from the neonatal piglet model. While it was possible to accurately predict the requirement for methionine and threonine, it was not so for tyrosine and lysine. Thus, is it important that the requirement for each amino acid be derived separately in human neonates. More importantly however, the derived human neonate estimates were up to 90% lower for methionine, threonine and lysine but 70% higher for tyrosine than in current commercial amino acid solutions (*Table 4*).

In addition, using the indicator amino acid oxidation

Table 4 Amino acid intake of neonates on parenteral nutrition receiving protein intake of 3.0 g/kg/d compared to estimated requirements using the indicator amino acid oxidation (IAAO) method

Amino acid	Intake from Primene® (Baxter) (mg/kg/d)	Intake from Trophamine® (McGaw) (mg/kg/d)	Amino acid requirement derived using the IAAO method (mg/kg/d)	References
Lysine	327	246	104.9	(69)
Methionine	72.0	102	49.0	(20)
Threonine	111	126	33.0	(68)
Tyrosine	27.0	69.0	74.0	(82)

method, van Goudoever's group has estimated the requirement for several indispensable amino acids in enterally fed infants (83,84,95-98). When compared to the parenterally derived estimates (68,69), their estimates of threonine (84) and lysine (83) requirements in enterally fed neonates were 100% and 25% higher. This provides support for the data obtained in the piglet model that when the gut is bypassed, amino acid requirements for most amino acids are lower. Furthermore, this provides additional evidence to support the conclusion that current commercial amino acid solutions for parenterally fed neonates are inadequate (99).

Importance of optimal parenteral amino acid therapy for premature infants

Preterm neonates are vulnerable and complex patients and the long-term consequences of providing too little or too much amino acids are not known. Growth is a dynamic process, which from the view of protein metabolism is, a positive balance between whole body protein synthesis and breakdown (100). Accretion of body proteins during growth is dependent on optimal intakes of all indispensable amino acids as well as an adequate supply of dispensable amino acids (or nitrogen for their synthesis), plus non-protein energy (101). If the pattern of amino acids is not ideal, the rate of protein synthesis, and in turn growth, will be determined by the first limiting amino acid. The excess amounts of all other amino acids must be oxidized which could overload immature degradative pathways and increase the risk of toxicity (99). Provision of the correct balance of amino acids is also important because of the roles of specific amino acids in health outcomes, beyond protein synthesis and growth. Data from the piglet model suggest that the ideal amino acid composition (protein quality) of amino acid solutions used for parenteral fed preterm neonates will be different from enterally fed proteins (51-53).

Traditionally, the main focus of nutrition management

in preterm infants has been to duplicate *in utero* growth rates (102) with a "more is better" approach, and little understanding of the metabolic needs of individual nutrients, particularly protein and amino acids. However, there is evidence that premature babies are vulnerable to the neuro-cognitive programming effects of early nutrition (87). For example, the brain of preterm neonates is sensitive to the balance of amino acids in human milk *vs.* preterm formula (5,89) and older data demonstrate the vulnerability of the brain to excess protein and amino acid intakes affecting school performance (2,90). There is increasing evidence that the amino acid arginine plays a key role in the health of the premature neonate, in addition to its role in protein synthesis, and ammonia clearance, through the urea cycle (13). In the preterm neonate it exerts additional effects as a precursor of NO, which affects lung (14,15) and gut health (16,18). Further, the balance between the dietary sulphur amino acids methionine and cysteine affect plasma homocysteine concentration in preterm neonates (19,20) in whom hyperhomocysteinemia is a risk factor for ischemic and hemorrhagic stroke (21). This emerging body of evidence on the effects of early nutrition and the balance of nutrients on long-term outcomes provides a new lens with which current amino acid solutions for parenterally fed neonates should be evaluated.

The metabolic and physiological basis for a revised amino acid solution for the parenteral fed neonate

In addition to differences in amino acid requirements between enterally and parenterally fed neonates, data from neonatal piglets demonstrate that the amino acid composition (protein quality) of amino acid solutions used for TPN fed preterm neonates should be largely different from enterally fed proteins (51-53). Therefore, the requirements for all indispensable and conditionally indispensable amino acids should be determined as

precisely as possible in order to establish an appropriate proportion of amino acids in the amino acid mixtures, used for parenterally fed preterm neonates. In particular, the following amino acids need special consideration.

Arginine

Using the neonatal piglet model, it was demonstrated that arginine is an indispensable amino acid in preterm parenterally fed neonates (55,103). Neonatal piglets receiving arginine-free TPN develop severe hyperammonemia within hours (55). Many authors have reported on hyperammonemia in preterm TPN fed infants (44,104) which can be corrected by arginine supplementation (104). Our group demonstrated in piglets (55,105,106) as well as human neonates (107) that *de novo* arginine synthesis in neonates is dependent on small intestinal metabolism. Arginine is the only known biological precursor of NO and inadequate arginine has been associated with neonatal necrotizing enterocolits and persistent pulmonary hypertension (14,17,108,109). While arginine supplementation reduced all stage necrotizing enterocolits in a randomized control trial (17), the data was not as clear regarding persistent pulmonary hypertension. More recent data demonstrate that TPN fed preterm infants continue to have low plasma arginine despite adequate essential amino acid and protein intakes (110,111) and that the low plasma arginine was associated with poor glucose control and plasma arginine intake (112). Badurdeen *et al*. (113) proposed that arginine deficiency is the key factor which increases the susceptibility of neonates to infections. More recent data by Zheng *et al*. (114), showed that arginine supplementation to the diet of enterally fed low-birth-weight neonatal piglets improved intestinal barrier function and antioxidant capacity as well as weight gain. Arginine content of commercial TPN solution is the most variable of all essential amino acids ranging from 4.7% to 12.3% (99). Premakumar *et al*. (112) recently suggested that arginine concentration of amino acid solutions for preterm neonates should be 17–20%. In order to prevent deficiency or toxicity, the dietary requirement for arginine should be defined in the parenterally fed neonate.

Methionine and cysteine

Methionine and cysteine are the sulphur containing amino acids. Cysteine is generally considered dispensable because under normal conditions, it can be formed from methionine. Current TPN solutions contain little to no cysteine because cysteine is unstable in solution, oxidized to the insoluble form, cystine (115). Therefore, commercial TPN solutions contain relatively high methionine concentrations to

meet the total sulphur amino acid requirement. Despite this practice, many published studies report low plasma cysteine in preterm neonates receiving cysteine-free, high methionine TPN (116-119). Therefore, for many years cysteine was believed to be an essential amino acids in the preterm neonate (120). However, Zlotkin *et al*. were the first to demonstrate that preterm TPN fed neonates did not need pre- formed cysteine to maintain adequate growth and nitrogen balance (115). Although this was confirmed in neonatal piglet and human studies (20,51,121,122), when the total sulphur amino acids intake was provided as methionine only, hyperhomocysteinemia and high plasma methionine developed in both piglets and human neonates (19,20). This demonstrates that although neonates could synthesize adequate cysteine from methionine for growth, sulphur amino acids would be better provided as a balance between methionine and cysteine to avoid overload of the immature catabolic pathway (123). Methionine is considered one of the most toxic amino acids (124,125) and high plasma methionine has been identified as a contributing factor in the pathogenesis of TPN-associated cholestasis in neonates (126).

In addition to their role in protein synthesis, the sulphur amino acids have been of particular interest because cysteine is a key component of glutathione (127). Glutathione (GSH) is the most prevalent intracellular thiol (24), and the [GSH]:[GSSG] ratio is used as an indicator of cellular redox state. GSH is also the most important endogenous antioxidant (128). In TPN fed neonates, it was shown that when sufficient methionine is given to meet the needs for protein synthesis, additional cysteine did not increase GSH concentration or rates of synthesis (129). This along with data from van Goudoever's group (130) provide confirmation that cysteine is not a conditionally essential amino acid in neonates.

Although adequate cysteine for growth and GSH synthesis can be provided as methionine only, high plasma methionine and hyperhomocysteinemia observed when the total sulphur amino acids is provided as methionine only suggest that provisions of the sulphur amino acids as a balance between methionine and cysteine are important considerations when designing an amino acid solution for the TPN fed neonate. This is now possible due to the availability of a soluble form of cysteine; N-acetyl cysteine which is highly bioavailable precursor of cysteine (129,131) to meet the needs for nitrogen accretion and growth in the neonate. Currently, no commercial amino acid solution contains this cysteine precursor.

Phenylalanine and tyrosine

Phenylalanine and tyrosine are the aromatic amino acids. Phenylalanine is indispensable and is the precursor for tyrosine which in healthy individuals is dispensable. Tyrosine has low solubility; therefore, excess phenylalanine is added to TPN to meet the needs of the total aromatic acids. However, hyperphenylalaninemia and low plasma tyrosine have been observed in infants receiving high phenylalanine- low tyrosine containing TPN (132-134). This suggests that phenylalanine hydroxylation to tyrosine is limited making tyrosine a conditionally indispensable amino acid in TPN fed neonates. The requirement for tyrosine in TPN fed preterm neonates was estimated using commercial TPN solutions with graded amounts of the soluble form of tyrosine (glycyl-tyrosine) (82). The neonates had high urinary phenylalanine as well as metabolites of phenylalanine catabolism indicating an overload of the catabolic pathway involved in phenylalanine degradation (135). The derived tyrosine requirement was over 70% higher than available in current TPN solutions (*Table 3*). Data from the neonatal piglet model previously demonstrated that in TPN fed neonatal piglets, there is a limit to which excess phenylalanine can be oxidized and that free phenylalanine accumulates beyond this limit (79). This is very concerning since the long-term effects of high phenylalanine in TPN fed preterm neonates is not known. We do know however, that high phenylalanine in patients with phenylketonuria is associated with significant neurocognitive damage (136-138). It is now possible to provide tyrosine as glycyl-tyrosine, a soluble and highly bioavailable precursor of tyrosine (77,82), and discontinue the practice of providing excessive phenylalanine to meet the needs for tyrosine in parenteral neonatal amino acid solutions.

Leucine, isoleucine, valine

From the neonatal piglet model it was observed that parenteral requirement for the BCAA; leucine, isoleucine and valine were only 56% of the enteral requirement (53). In addition, the plasma amino acid pattern observed in the TPN and enterally fed piglets suggest that the optimum ratio of BCAA differ between routes of feeding (53). The pattern of BCAA in TPN solutions for preterm neonates is similar to human milk but the absolute amount of each BCAA is higher in TPN (99) than in human milk. This suggest, current BCAA composition and ratios of current TPN solutions are inappropriate for TPN fed neonates.

Tryptophan

Beyond its role in protein synthesis tryptophan is important for synthesis of the neurotransmitter serotonin and the hormone melatonin (139). Interestingly in neonatal piglet studies the TPN and enteral requirements for tryptophan were not different (54). Based on extrapolations from that data (54), the tryptophan content of current TPN solutions is likely double the requirement of the preterm TPN fed neonate. However, long-term effects of excessive tryptophan intake in TPN fed preterm neonates is unknown. Therefore, it is important to accurately define its requirement.

Histidine

Histidine is an essential amino acid in preterm infants (22). Beyond its role in protein synthesis it is involved in allergic reactions, and modulation of the immune response in skin. It is also involved in regulation of gut function (140). No data exist on histidine requirement in the TPN fed piglet or human neonate. Hence it is not possible to assess whether current commercial amino acids have an excess or deficiency of this amino acid.

Glutamine

Conclusive evidence on the essentiality of glutamine in neonates is lacking. Conflicting data exist on its efficacy in critically ill patients. While some data suggest that glutamine supplementation reduced mortality in critically ill adults (141), more recent data suggest no effect of glutamine supplementation (142). Glutamine is the most abundant amino acid in the muscle and plasma of humans (143). It is not included in commercial amino acid solutions because it is unstable in solution. Beyond its role in protein synthesis glutamine is involved in many metabolic reactions including the synthesis of glutathione (via glutamate). It is believed that during stress glutamine production may be insufficient to meet increased demands especially in low-birth-weight neonates who have limited reserve (143). Although glutamine supplementation did not result in apparent biochemical risk in TPN fed low-birth-weight infants (144), decreased tyrosine and phenylalanine concentrations were observed in the glutamine supplemented group. Furthermore, glutamine supplementation in parenterally fed neonatal piglets resulted in expansion of the plasma volume; a component of the extracellular fluid and indicated that glutamine is not conditionally indispensable in neonates (145).

Summary & conclusions

Despite the increased need for TPN in premature infants and the recognition that amino acid intake affects clinical outcomes, the scientifically derived requirement estimates for all indispensable and conditionally indispensable amino acids in TPN fed preterm infants have not been derived. Amino acid composition of current commercial solutions are estimates and neonatologists agree that "amino acid composition of parenteral formulas is variable and lacks scientific support". Preterm neonates are extremely complex and vulnerable patients and uncertainty exists concerning the long-term effects of excess or inadequate amino acid intakes. Randomized clinical trials demonstrate that long-term cognitive performance of preterm infants is adversely affected by suboptimal early nutrition (3).

Using a neonatal piglet model, it was demonstrated that the gut variably utilizes dietary amino acids (ranging from nil for tryptophan to 60% for threonine). It is now confirmed that both tyrosine and arginine are conditionally essential and that the sulphur amino acids are best provided in TPN as a balance between the two to prevent high plasma methionine and homocysteine in the preterm neonate. These observations have been made in human neonates, in whom the requirements for tyrosine, lysine, maximum methionine and threonine have been estimated. The quantitative significance of amino acids beyond their roles in protein synthesis and the impact of prematurity are important consideration when designing an optimal amino acid solution for the parenterally fed neonate. In addition, neonates are not small adults as differences in amino acid metabolism exist between neonates and adults. More research needs to be done so that an amino acid solution with a scientifically derived pattern of amino acid could be designed for neonates requiring parenteral nutrition.

Limitations of the review

A key limitation of this review is that all data on amino acid requirements in the parenterally fed neonates is based on data obtained from carbon oxidation studies using the indicator amino acid oxidation method. This is because data using other methods of which nitrogen balance is the classical method is not available. The adequacy of these data remains to be tested in long-term trials pending the formulation of an amino acid solution designed after all indispensable amino acid requirements have been estimated.

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