REVIEW ARTICLE

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Targeting optimal protein delivery in parenteral and enteral nutrition for preterm infants: a review of randomized, controlled trials

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Close attention to nutritional management is essential for optimizing growth and neurodevelopment of the preterm infant. Protein intake and the protein to energy ratio are the main determinants of growth and body composition. Yet large, multi-center, randomized controlled trials are lacking to guide protein delivery for the preterm infant. Until these studies are pursued, smaller trials must be used to inform clinical practice. This review summarizes the randomized controlled trials that have been performed to test the impact of higher vs. lower protein delivery to the preterm infant. We consider the trials that varied protein delivery rates during parenteral and enteral phases of nutrition. Considerable heterogeneity exists across study designs. Still, cumulative evidence from these trials provides a framework for current recommendations for protein intake in the preterm infant.

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INTRODUCTION

The preterm infant requires enough protein to promote the growth and remodeling of cells, tissues, and organs to accrue lean body mass. Protein intake is the limiting nutrient regarding growth [1], so providing an adequate supply of protein to the preterm infant is critical. Several observational studies have shown that protein intakes are positively correlated with improved growth, brain size, and neurodevelopmental outcome, highlighting the benefit of enhanced protein delivery to the preterm infant [2–7]. However, associations identified in cohort studies limit the ability to fully measure the strength and influence of severity of illness as a confounding factor. Preterm infants in those cohorts who were sicker may have received less protein or utilized protein less effectively. Thus, randomized controlled trials (RCTs) to define the optimal dose and delivery of protein are essential, especially those that are adequately powered to measure differences in neurodevelopmental outcomes.

The current goals of nutrition for the preterm infant are to supply enough amino acids to avoid a catabolic state and achieve positive nitrogen balance. Recommendations for protein delivery generally range from 2.5 to 4.5 g/kg per day, depending on the route of delivery (enteral vs. parenteral), gestational and postnatal age of the infant, adequacy of growth, and degree of cumulative protein deficit [8–12]. Several studies have demonstrated that parenteral amino acids initiated on the first day of life are tolerated from a metabolic standpoint, increase protein synthesis rates, and promote positive nitrogen balance [13–16]. It has been recommended to provide enough protein to the preterm infant to support continued high rates of protein accretion by mimicking placental delivery of amino acids, which is 3.5–4 g/kg per day in the late second trimester and early third trimester [17, 18]. While such reference values may represent the protein requirement for a fetus, those delivery rates may not translate into optimal intakes for the preterm infant born at the equivalent gestational age (GA) [19]. Commercial amino acid solutions for parenteral nutrition do not replicate the same balance of amino acids transferred by the placenta to the fetus [20], and the placenta assists in the clearance of protein degradation products from the fetus. The low birth weight preterm infant is often critically ill during the immediate postnatal period, resulting in alterations in metabolic demand, cytokines, and hormone profiles, all of which impact the utilization of nutrients [21]. Several studies and commentaries have called into question the safety of using doses of parenteral amino acids that exceed 3.5 g/kg per day, especially in the first 1–2 postnatal days or when preterm infants are critically ill [11, 19, 22–24]. Furthermore, there are differences in safety profiles and efficacy for promoting growth and neurodevelopment when protein is delivered parenterally vs. enterally, requiring route of protein delivery to be a critical consideration.

Unfortunately, there has been a dearth of large, multi-center, RCTs that also account for the wide range of metabolic stress and postnatal maturity in preterm infants to specifically guide protein delivery. Thus, care providers in the NICU are left with many unanswered questions about what dose of protein will safely support optimal growth. Therefore, the goal of this review is to summarize the smaller RCTs available that test the efficacy and safety of different doses of amino acids given parenterally, and protein given enterally, in the preterm infant.

Estimating protein requirements for preterm infants

Two methods have been used to estimate protein requirements in preterm infants. The first is the factorial approach which uses the chemical composition of the human fetus based on a constructed

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Table 1. Protein and energy requirements for preterm infants by the factorial approach.

Body weight (g)	Protein (g/kg per day)		Energy (kcal/kg per day)		Protein:Energy (g/100 kcal)	
	Parenteral	Enteral	Parenteral	Enteral	Parenteral	Enteral
500–700	3.5	4.0	89	105	3.9	3.8
700–900	3.5	4.0	92	118	3.8	3.7
900–1200	3.5	4.0	101	119	3.5	3.4
1200–1500	3.4	3.9	108	127	3.1	3.1
1500–1800	3.2	3.6	109	128	2.9	2.8
1800–2200	3.0	3.4	111	131	2.7	2.6

Adapted from Zeigler [1].

Table 2. Enteral protein requirements for preterm infants by the empirical approach.

	Birthweight < 1200 g (g/kg per day)	Birthweight > 1200 g (g/kg per day)
Zeigler [1]	4.0	3.7
Kashyap S et al. [26, 27]	-	3.0
Rigo J et al. [28]	3.8–4.2	3.4–3.6

"reference fetus" [25]. Macronutrient requirements are calculated based on the sum of nutrients required to replace losses and those needed for accretion of tissue to meet average fetal weight gain per day [1] (Table 1). An alternate way to estimate macronutrient requirements is the empirical approach, which tests feedings with varying energy and protein content and uses growth and/or nitrogen balance as an outcome [26–29] (Table 2). Estimation of protein requirements using factorial and empirical approaches as well as systematic reviews of the literature have been used to inform guidelines published by international societies regarding protein delivery to preterm infants. Current guidelines for enteral protein delivery are shown in Table 3. Guidelines for parenteral amino acid delivery advise more generally for all preterm infants without specification by either birthweight, GA at birth, or chronological age. The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends that parenteral amino acids should be administered to the preterm infant immediately after birth with at least 1.5 g/kg per day and increase to 2.5 g/kg per day the next day. From day 2 onwards, parenteral amino acid intakes should be between 2.5 and 3.5 g/kg per day with at least 65 kcal/kg per day of energy [11]. The American Academy of Pediatrics Committee on Nutrition and the American Society of Parenteral and Enteral Nutrition (ASPEN) similarly recommend a maximal parenteral dose of 3–3.5 g/kg per day of protein [8, 10]. There is consistent guidance that amino acids should not be advanced beyond a maximum of 3.5 g/kg per day and that higher doses be provided on a research basis only [11].

RANDOMIZED CONTROLLED TRIALS EVALUATING THE EFFECT OF PARENTERAL AMINO ACID INTAKE ON GROWTH AND NEURODEVELOPMENTAL OUTCOME

There have been several small RCTs aimed to test the effects of different doses of parenteral amino acids on preterm infant growth and neurodevelopment (Table 4). Most trials compared a "higher" vs. a "lower" (or standard) dose of amino acids, with amino acid delivery rates in the higher groups that ranged from 3 to 4.5 g/kg per day vs. the lower groups that ranged from 2.5 to 3 g/kg per day. Several trials also varied the rapidity of advancement of amino acids, with the higher group starting either at the maximal dose within the first 24 hours of life or using a more rapid advancement to a maximum dose over 1–2 days compared to the lower group which used a stepwise increase over the first week of life. The mean GA among trials was approximately 27 weeks, with a range of 23–33 weeks. Some trials varied both parenteral and enteral protein delivery as well as carbohydrate and lipid delivery and even additional nutrients. The primary outcomes of most trials were growth and/or neurodevelopmental outcome and included biochemical measurements to track tolerance of higher amino acid doses. Results from each trial are briefly summarized in Table 4 and reviewed in more depth below. We report targeted amino acid delivery from each RCT; however, actual protein delivery did not always match targeted delivery and sometimes differed only minimally between groups.

Growth outcomes

Reported growth outcomes are variable as a result of higher vs. lower parenteral amino acid intake (Table 4). Several studies did not find differences in short term growth outcomes from increasing amino acid delivery in the first week of life [30–36]. In four of the trials that demonstrated greater growth when infants were randomized to higher parenteral amino acids, dextrose and/or lipid doses were also increased. For example, Can et al. randomized infants <34 weeks GA to receive a faster (3 g/kg per day amino acids advanced by 1 g/kg per day to 4 g/kg per day with a similar lipid advancement) vs. standard advancement of amino acids and lipid (1.5 g/kg per day amino acids advanced by 1 g/kg per day to 4 g/kg per day and slower lipid advancement) [37]. Length and occipital frontal head circumference (OFC) at 40 weeks postmenstrual age (PMA) were higher in the faster advancement group. Vlaardingerbroek et al. randomized infants <1500 g to one of three groups that included differences in amino acid and lipid delivery: higher (3.6 g/kg per day amino acids and 3 g/kg per day lipid), standard (2.4 g/kg per day amino acids and 3 g/kg per day lipid), or control (2.4 g/kg per day amino acids and 2.8 g/kg per day lipid) [38]. Amino acid dose was adjusted downward when blood urea nitrogen concentrations (BUN) exceeded 39 mg/dL, which occurred more frequently in the higher nutrient groups. Weight and OFC were greater at 6 weeks and weight was greater at 2 years in the higher amino acid group [39]. Morgan et al. randomized infants <29 weeks GA and <1200 g to receive enhanced (3.8 g/kg per day amino acids and lipid, 15.6 g/kg per day glucose) vs. standard parenteral nutrition (2.8 g/kg per day amino acids and lipid and 13.5 g/kg per day glucose) [40]. Enhanced nutrition resulted in larger OFC at both 28 days and 36 weeks corrected GA. Infants were not randomized until 120 h of life, so differences in amino acid delivery likely did not occur until later in the first week. Moltu et al. randomized infants <1500 g to receive higher (3.5 g/kg per day with multi-component intravenous lipid containing fish oil and an enteral protein supplement with amino acids and vitamin A) vs.

Table 3. Recommended enteral protein and energy intakes for very low birth weight preterm infants (<1500 g).

Society	Protein (g/kg per day)	Energy (kcal/kg per day)	Protein:Energy (g/100 kcal)
European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)	3.5–4.5[a]	110–130	2.2–3.1
Life Sciences Research Office (LSRO)	3.4–4.3	110–135	2.5–3.6
Canadian Pediatric Society	3.0–4.0[b]	105–135	2.5–3.0
American Academy of Pediatrics Committee on Nutrition	3.5–4.0	105–130	2.9–3.3

[a]Protein intake of 4.0–4.5 g/kg per day for infants weighing < 1000 g.
[b]Protein intake of 3.5–4.0 g/kg per day for infants weighing < 1000 g.
Adapted from Zeigler [1].

standard amino acids (2 g/kg per day increased to 3.5 g/kg per day using only enteral nutrition and a two-oil intravenous lipid without fish oil) [41]. The higher amino acid group had a lower proportion of infants discharged with a weight below the 10th%, though the study was stopped early due to higher septicemia in the intervention group. Scattolin et al., however, randomized infants <1250 g to receive higher (2 g/kg per day advanced by 1 g/kg per day to 4 g/kg per day) vs. standard amino acids (1.5 g/kg per day advanced by g/kg per day to 3 g/kg per day) without differences in other macronutrients or energy delivery [42]. The higher amino acid group had faster growth rates and higher weight and length at 36 weeks PMA.

Worse growth outcomes also have been reported in infants randomized to higher parenteral amino acid delivery, though in all these studies, the rate of advancing amino acids was also faster. Blanco et al. randomized infants with a birth weight <1000 g to receive early and high (2 g/kg per day advanced by 1 g/kg per day to 4 g/kg per day) vs. standard amino acids (0.5 g/kg per day advanced by 0.5 g/kg per day to 3 g/kg per day). There was no difference between groups for the primary outcome of hyperkalemia [43]. Secondary analyses demonstrated no differences between groups in weight, length and OFC z-scores at NICU discharge but lower growth z-scores at 6-, 12-, and 18-months corrected GA in the early and high amino acid group [44]. Uthaya et al. randomized infants <31 weeks GA in a multicenter RCT to receive immediate (3.6 g/kg per day from day 1) vs. incremental amino acids (1.7 g/kg per day and increased to 2.7 g/kg per day by day 3) in a 2 × 2 multifactorial study design with different lipid sources [45]. There was no difference in non-adipose mass measured by MRI at term corrected GA; however, infants in the immediate and higher amino acid group had smaller OFC at 40 weeks PMA. Balakrishnan et al. randomized infants <1250 g to receive rapid (3–4 g/kg per day advancing to 4 g/kg per day by day 1) vs. standard advancement of amino acids (1–2 g/kg per day advancing by 0.5 g/kg per day to 4 g/kg per day) [46]. Both groups reached equivalent amino acid delivery by day 7. After controlling for small for GA status, the more rapidly advanced group had smaller OFC at 36 weeks PMA and at NICU discharge.

Neurodevelopmental outcomes

Some of the RCTs that evaluated growth also evaluated neurodevelopmental outcomes at 2 years corrected GA and found no differences in those randomized to receive higher vs. lower parenteral amino acids [38, 39, 44, 46] (Table 4). Additionally, Burattini et al. randomized infants 500–1249 g to receive higher (2.5 g/kg per day advanced by 0.5 g/kg per day to 4 g/kg per day) vs. standard amino acids (1.5 g/kg per day advanced by 0.5 g/kg per day to 2.5 g/kg per day) and found no difference in neurodevelopmental outcome at 2 years [32]. Bellagamba et al. randomized infants 500–1249 g to receive higher (1.5 g/kg per day advanced 0.5 g/kg per day to 3.5 g/kg per day) vs. standard amino acids (1.5 g/kg per day advanced 0.5 g/kg per day to 2.5 g/kg per day) and found no difference in neurodevelopmental outcome [30]. Bloomfield et al. randomized infants <1000 g to receive a supplement of amino acids via an umbilical arterial catheter in addition to standard parenteral nutrition (total amino acid dose of 3.4 g/kg per day) vs. saline (total amino acid dose of 2.6 g/kg per day) beginning on the first day of life [47]. There were no differences in either growth or survival without disability at 2 years corrected GA between groups. Concerningly, however, moderate-to-severe neurodisability was more common in infants randomized to the higher amino acid group.

Biochemical differences and adverse outcomes

Plasma biochemical markers have been monitored as a measure of tolerance to parenteral amino acid dose. Amino acids in the circulation either enter energy-dependent protein synthetic pathways or are directly oxidized as an energy source through entry into the tricarboxylic acid cycle and ultimately the urea cycle for clearance of nitrogen [48, 49] (Fig. 1). Therefore, BUN is often used as a marker of amino acid utilization. While it was evaluated at different times during the intervention in each trial's protocol, BUN was always higher in the higher vs. standard amino acid groups (Table 4). To monitor metabolic tolerance of higher amino acids, some studies monitored blood gas measurements and serum electrolytes, calcium, and phosphorus concentrations. Moltu et al. found a higher incidence of hypophosphatemia and hypokalemia in the higher amino acid and nutrient delivery group [50], and Bloomfield et al. found that more infants randomized to receive additional amino acids via an umbilical arterial catheter developed refeeding syndrome, including hypophosphatemia, hypercalcemia, and hypokalemia [47]. Some studies also monitored serum amino acid concentrations as a measure of protein sufficiency and/or toxicity and found either minimal differences [31] or elevated concentrations in the higher amino acid groups [33, 51, 52]. Clinical implications of higher amino acid concentrations could not be determined by these studies.

RANDOMIZED CONTROLLED TRIALS EVALUATING THE EFFECT OF ENTERAL PROTEIN INTAKE ON GROWTH AND NEURODEVELOPMENTAL OUTCOME

Several RCTs have compared higher vs. standard amounts of enteral protein on preterm infant growth and neurodevelopmental outcome (Table 5). Enteral protein delivery in the higher groups ranged from 3.2 to 4.8 g/kg per day vs. standard groups, which ranged from 2.9 to 4.1 g/kg per day. The average GA of subjects enrolled among studies was 29 weeks, ranging from 25 to 32 weeks. Different combinations of mother's own milk, donor human milk, and preterm formulas were used in each of the studies. This is an important variable given the inter-individual variation in protein content in mother's own milk and that protein is almost always lower in donor human milk because it is often donated by mothers of term infants later in lactation [53–55]. Some studies directly measured protein content using human milk analyzers [56–58], but most studies estimated milk protein content (estimates ranging between 1.1 and 1.7 g/100 mL). However, the

Table 4. Summary of randomized controlled trials evaluating parenteral amino acid (AA) intake on growth and neurodevelopment.

Lead author and references[a]	GA (w, range) N subjects	Target maximum AA (g/kg per day) and rate of advance[b]	Results relative to lower protein group	Metabolic effects of higher AA
Tan [35, 36]	26w (24–27) $N = 142$	4.0 (slow) vs 3.0 (slow) plus dextrose, lipid	No difference in growth at 36 w PMA or ND outcome at 9 m corrected GA	
Clark [33]	27w (26–28) $N = 122$	3.5 (fast) vs 2.5 (slow)	No difference in growth at 28 d	8 AA out of range, higher BUN
Blanco [43, 44, 52]	26w [24–28] $N = 61$	4.0 (fast) vs 3.0 (slow)	No difference in growth at 28 d; reduced growth and no difference in ND outcome at 2 y chronological age	Higher BUN, NH_3, AA; no hyperkalemia
Bulbul [31]	29w (28–31) $N = 44$	3.0 (fast) vs 3.0 (slow)	No difference in growth at NICU discharge	No difference in AA (except Arg, Asp) and all within normal range
Can [37]	31w (27–33) $N = 50$	4 (fast) vs 4 (slow) plus lipid	Greater length and OFC at 40 w PMA	
Burattini [32]	28w (26–30) $N = 114$	4.0 (slow) vs 2.5 (slow)	No difference in growth at 36 w and 2 y PMA; no difference in ND outcome at 2 y PMA	Higher BUN
Scattolin [42]	27w (23–32) $N = 115$	4.0 (fast) vs 3.0 (slow)	Faster weight gain and greater weight and length at 36 w PMA	Higher urea
Moltu [41, 50]; Strommen [51]	28w (24–33) $N = 48$	3.5 (fast) vs 2.0 (fast) plus lipid type, enteral protein, vitamin A	Greater weight gain at 36 w PMA	Lower serum PO_4 and K; higher AA; higher sepsis rate
Vlaardingerbroek [38]; Roelants [39]	27w (25–29) $N = 144$	3.6 (fast) vs 2.4 (fast) plus lipid	No difference in growth at NICU discharge; greater weight and no difference in ND outcome at 2 y corrected GA	Higher urea (40 mg/dL) AA normal range
Morgan [40]	26w (25–28) $N = 152$	3.8 (slow) vs 2.8 (slow) plus lipid, dextrose	Larger OFC at 28 days and 36 w PMA	None reported
Bellagamba [30]	27w (26–29) $N = 164$	3.5 (slow) vs 2.5 (slow) plus enteral protein	No difference in weight gain; no difference in growth or ND outcome at 2 y corrected GA	Higher urea, more HCO_3 given
Uthaya [45]	27w (25–30) N = 168	3.6 (fast) vs 2.7 (slow) plus lipid	Smaller OFC, no difference in fat free mass or brain volume at term corrected GA	Higher BUN
Balakrishnan [46]	27w (24–30) $N = 168$	4.0 (fast) vs 4.0 (slow)	Reduced growth at 36 w PMA; no difference in ND outcome at 18–24 m corrected GA	Higher BUN
Li [34]	33w (30–35) $N = 191$	4–4.5 (fast) vs 3.5 (slow)	Shorter time to regain birthweight, no difference in weight or length in first 2 weeks	Higher rate of hypokalemia
Bloomfield [47]	26w (23–31) $N = 434$	Additional 1 g AA through UAC vs no additional AA	No difference in ND or growth outcomes at 2 y corrected GA	Higher incidence of refeeding syndrome

AA amino acids, *PMA* postmenstrual age, *TPN* total parenteral nutrition, *GA* gestational age, *ND* neurodevelopmental, *BUN* blood urea nitrogen, *Arg* arginine, *Asp* aspartate, *UAC* umbilical arterial catheter, *OFC* occipitofrontal circumference.

[a]Multiple references indicate follow-up studies that stem from the original study.

[b]"Fast" advancement of AA represents immediate initiation of maximum AA goal or stepwise advancement ≥ 1 g/kg per day; "Slow" advancement of AA represents stepwise advancement ≤ 0.5 g/kg per day.

proportions of milk type were comparable between groups within each individual study and included a majority of mother's own milk. Likewise, type of human milk fortifier (HMF) varied across studies; some directly compared the same fortifier and varied the amount of protein, some compared two different formulations of fortifier (liquid vs. powder, intact vs. hydrolyzed protein, whey vs. casein), and others adjusted the protein within a fortifier based on BUN concentrations. All HMF used in the reported studies was bovine derived. Some studies varied other components of enteral nutrition such as lipid content. Results from each trial are briefly summarized in Table 5. Formulation of fortification that was used and actual protein delivered to each group are described in more depth below.

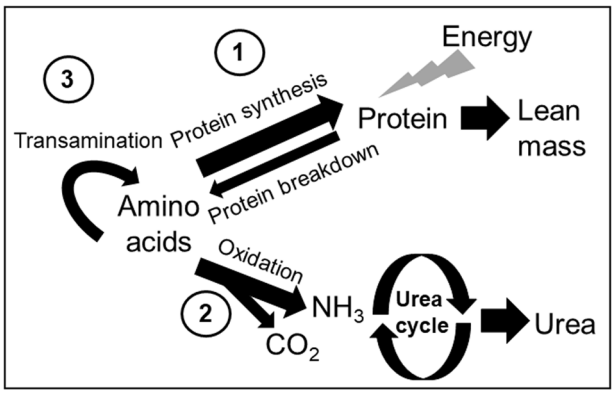

Fig. 1 Amino acid utilization by the preterm infant. Amino acids have three fates. They are used for (1) protein turnover, or continuous cycling of protein synthesis and breakdown, (2) synthesis of tricarboxylic acid cycle intermediates (anapleurosis) and catabolism to form ATP and CO_2, or (3) transaminated to form other amino acids and metabolites.

Growth outcomes

Two studies showed no difference in growth outcomes from higher enteral protein delivery [58, 59], no trials found a reduction in growth, and most found an improvement in growth (Table 5). Porcelli et al. randomized infants <1500 g to receive higher (1 g/100 mL whey HMF; 4.3 g/kg per day) vs. standard protein content (0.7 g/100 mL 60% whey and 40% casein HMF; 4.1 g/kg per day) for 21 days [60]. Greater weight gain velocity and greater achieved weight and OFC were observed in the higher protein group. Arslanoglu et al. randomized infants between 24 and 34 weeks GA to receive an adjustable regimen using supplemental protein based on BUN (0.8 g/100 ml hydrolyzed whey HMF and 0.3 g/100 mL whey supplemental protein; 3.2 g/kg per day) vs. standard protein (2.9 g/kg per day) until 2000 g [56]. Greater weight and OFC gains were observed in the adjustable group. Miller et al. randomized infants <31 weeks GA to receive higher (1.4 g/100 mL extensively hydrolyzed whey HMF; 4.2 g/kg per day) vs. standard protein (1 g/100 mL; 3.6 g/kg per day) for 4 weeks [57]. Achieved weight was greater and fewer infants were discharged <10th% for length in the higher protein group, though there was no difference in the rate of weight gain. Moya et al. randomized infants <1250 g to

Table 5. Summary of randomized controlled trials evaluating enteral protein intake on growth and neurodevelopment.

Lead author and reference	GA (w, range) N subjects	Protein (g/kg per day), type, and duration	Results relative to lower protein group	Side effects of higher protein
Porcelli [60]	29w (25–32) $N = 64$	4.3 (whey) vs 4.1 (whey/casein) HMF for 21d	Greater weight gain and higher achieved weight and OFC	Higher BUN
Arslanoglu [56]	31w (29–33) $N = 34$	3.2 vs 2.9 HMF; adjusted based on BUN until 2000g	Greater weight and OFC gain	
Miller [57]	28w (25–30) $N = 92$	4.2 vs 3.6 ext. hydrol. whey HMF	Higher achieved weight, fewer with length <10% at NICU discharge	Higher serum urea nitrogen
Moya [61]	28w (26–30) $N = 150$	4.5 (liquid) vs 3.6 (powder) HMF for 28 d	Greater length gain, higher achieved weight, length and OFC	Higher prealbumin and albumin, BUN
Kim [62]	28w (27–29) $N = 129$	3.9 (ext. hydrol. liquid) vs 3.3 (intact powder) HMF for 28 d	Greater weight and length gain	Higher prealbumin, BUN
Biasini [65]	23–32w $N = 61$	4.8 (powder) vs 3.5 HMF adjusted based on BUN for 28 d	Higher hearing/language score at 12 and 18 m corrected GA; greater length gain at 9 m	Higher BUN
Dogra [66]	30 w (28–32) $N = 120$	4.2 vs 3.6 HMF	Greater OFC, weight gain at 40 w PMA; no difference in ND outcome at 18 m corrected GA	Higher BUN
Rigo [63]	28w (26–30) $N = 153$	4.5 (part. hydrol.) vs 3.8 (ext. hydrol.) HMF; +/- lipid, CHO for 21d	Greater weight gain, higher achieved weight and head Z-score	Higher prealbumin, Alk Phos, Ca, BUN
Maas [59]	30 w (25–32) $N = 60$	4.1 vs 3.5 bovine protein to HMF for 6w	No difference in weight, length, OFC, lower leg length gain	Higher serum urea
Reid [58]	30 w (28–32) $N = 60$	4.2 (casein powder) vs 3.5 HMF until 40 w PMA	No difference in weight, length, OFC or FFM gain	Higher BUN, Phe and Tyr; more feed interruptions
Salas [64]	27 w (26–28) $N = 56$	3.9 (ext. hydrol. liquid) vs 3.3 HMF for 23d	Greater FM, FFM at 36 w PMA; greater weight and length change in z-score at 3 m PMA	No difference in BUN

All human milk fortifiers were bovine derived.
HMF human milk fortifier, *ext. hydrol.* extensively hydrolyzed, *part. hydrol.* partially hydrolyzed, *PMA* postmenstrual age, *GA* gestational age, *ND* neurodevelopmental, *BUN* blood urea nitrogen, *OFC* occipitofrontal circumference, *Alk Phos* alkaline phosphatase, *Phe* phenylalanine, *Tyr* tyrosine, *FFM* fat free mass, *FM* fat mass.

Table 6. Indications to increase enteral protein delivery > 4 g/kg per day.

Weight <1200 grams; GA < 28 wks
Promotion of wound healing
Prolonged infusion of parenteral nutrition through peripheral venous catheter
Inadequate parenteral nutrition due to fluid restriction
History of feeding intolerance and interrupted feeds
History of multiple procedures/surgeries requiring NPO status
Inadequate growth in length and/or head circumference
Partial unfortified human milk feeds (direct breastfeeding)
Prolonged use of donor human milk

receive higher (1.8 g/100 mL hydrolyzed whey liquid HMF; 4.5 g/kg per day) vs. lower protein (1 g/100 mL hydrolyzed whey powdered HMF; 3.6 g/kg per day) for 28 days [61]. While there was no difference in weight gain, linear growth rate and achieved weight and length at the end of the study were higher in the higher protein group. Kim et al. randomized infants <33 weeks GA to receive higher (1.67 g/100 mL extensively hydrolyzed casein liquid HMF; 3.9 g/kg per day) vs. lower protein (1 g/100 mL intact whey powdered HMF; 3.3 g/kg per day) for 28 days [62]. There was no difference in rate of weight gain; but in a subgroup analysis of pre-defined strict protocol followers, those fed the higher protein fortifier achieved greater weight and linear growth. Rigo et al. randomized infants <32 weeks GA to receive higher (1.4 g/100 mL partially hydrolyzed whey HMF; 4.5 g/kg per day) vs. standard protein (1 g/100 mL extensively hydrolyzed whey; 3.8 g/kg per day) for 21 days [63]. Rate of weight gain and weight and OFC for age z-score were greater in the higher protein group, without a difference in linear growth. Finally, Salas et al. randomized infants 25-28 weeks GA to receive higher (1.67 g/100 mL extensively hydrolyzed casein liquid HMF and 0.75 g/100 mL extensively hydrolyzed casein liquid protein supplement; 3.9 g/kg per day) vs. standard protein (3.3 g/kg per day) until 32 weeks PMA [64]. Fat free mass and fat mass z-scores were higher at 36 weeks PMA and weight and length change in z-scores from birth to 3 months were higher in the high protein group.

Neurodevelopmental outcomes

We identified two RCTs that evaluated neurodevelopmental outcome from varying enteral protein delivery. Biastini et al. randomized infants <1250 g to receive higher (0.6 g/100 ml hydrolyzed powder HMF and 0.8 g/100 mL supplemental protein based on BUN; 4.8 g/kg per day) vs. standard protein (3.5 g/kg per day) for 28 days [65]. Infants receiving higher protein had higher hearing and language scores at 12 and 18 months and greater length gain at 9 months corrected GA. Dogra et al. randomized infants <1500 g to receive higher (1 g/100 mL extensively hydrolyzed whey HMF; 4.2 g/kg per day) vs. lower protein (0.4 g/100 mL whey HMF; 3.6 g/kg per day) [66]. There were no differences in growth or neurodevelopmental outcome at 12- and 18-months corrected GA, though OFC and weight gain were greater in higher protein group at 40 weeks PMA.

Biochemical differences and adverse outcomes

Minimal differences in biochemical markers were reported from higher enteral protein delivery, except for higher BUN and improved prealbumin concentrations (Table 5). No adverse events were reported.

SUMMARY AND INTERPRETATION OF RESULTS

Parenteral amino acid intake

Outcomes related to growth and neurodevelopment from trials that have compared higher vs. lower parenteral amino acid intake in preterm infants are mixed. Some trials showed a benefit in growth outcomes from higher amino acid delivery, yet those studies either advanced to higher amino acid intake outside of the first few days of life or concurrently increased non-protein energy delivery. Some trials showed no difference between higher vs. lower amino acid dose on short-term growth outcomes or neurodevelopment. These conclusions are consistent with a Cochrane Review assessing trials that compared higher vs. lower intake of parenteral amino acids [67]. The conclusions were that higher amino acid intake in parenteral nutrition reduced the incidence of postnatal growth failure but did not affect mortality, with insufficient evidence to show an effect on neurodevelopmental outcome. Of particular concern, however, are the reports of smaller head circumference and worse neurodevelopmental outcomes when amino acid dose is at or above 3.5 g/kg per day beginning in the first 1–2 days of life. Given the current state of evidence from the literature, initiation of parenteral amino acids in the first day of life, with an increase in delivery rates that span the first 3–4 days of life to a maximum dose of 3.5 g/kg per day may be considered the safest and most efficacious approach for the preterm infant <1500 g and <32 weeks GA. Per ESPGHAN and ASPEN recommendations, parenteral amino acid intakes above 3.5 g/kg per day should only be administered as part of clinical trials [11, 24]. Close attention to amino acid delivery is warranted when administering starter TPN in the first day of life, or individually prepared TPN formulations thereafter, that contain a fixed percentage of amino acids. If higher fluid intakes are required, non-protein containing fluids may be administered to not exceed recommended amino acid delivery.

Enteral protein intake

Most trials demonstrated that in preterm infants, higher enteral protein intake ranging from 3.9 to 4.5 g/kg per day improved short-term growth. The lack of any adverse events or biochemical changes other than higher BUN reflect the gastrointestinal and metabolic tolerance of higher enteral protein delivery. Furthermore, several studies found an increase in prealbumin as a result of higher protein delivery, which is considered an indicator of growth in the neonate [68]. While improvements in weight gain were the predominant growth outcome, there were also beneficial effects on linear and head growth. Linear growth represents lean body mass and reflects organ growth and development, including the brain, and thus should be monitored, in addition to weight gain [69]. The current paucity of trials limits the ability to make conclusions about the effect of increased enteral protein delivery on neurodevelopmental outcome. Current recommendations for enteral protein delivery in preterm infants by major international societies range from 3.5 to 4.5 g/kg per day (Table 3). Based on our own experience, recent evidence [70–72], and ESPGHAN guidelines [9], we recommend enteral protein delivery at the higher end of that range (>4 g/kg per day, provided concomitant energy intakes are optimal) when there is evidence for cumulative protein deficit, administration of donor milk or unfortified mother's own milk, and/or poor growth, especially poor linear growth (Table 6).

Considerations for why delivering high doses of amino acids parenterally is not as impactful on growth as delivering similar doses of enteral protein for the preterm infant

Review of the current RCTs highlights the potential risks of excessive administration of parenteral amino acids and reinforces the safety and benefit of using higher enteral protein intakes on growth. There are several reasons for why this may be the case. First, there is no storage site for free amino acids, which may raise the risk for toxicity from elevated concentrations of specific amino acids and/or a nitrogen load that cannot be adequately cleared by the urea cycle. However, the level at which higher amino acid, ammonia, or urea concentrations become harmful or toxic in the preterm infant is unknown. Second, there have been persistent concerns about suboptimal composition of amino acids in commercially available solutions for the preterm infant [19]. An imbalance in amino acid concentrations, with some amino acids in excess and others limiting, in the context of suboptimal ratios with nonprotein calories, may result in higher amino acid oxidation rather than enhanced protein synthesis. Third, delivering the ideal protein to energy ratio is less likely in the infant receiving parenteral nutrition; if hyperglycemia or hyperlipidemia develops, clinicians commonly restrict dextrose and lipid infusions yet do not alter amino acids accordingly [19, 20]. It is essential to maintain a total energy delivery of 30–40 kcal/g of protein to optimize the protein to energy ratio [19]. Finally, preterm infants randomized in parenteral amino acid trials were gestationally and postnatally younger when receiving higher doses compared to those infants in enteral protein trials. Nutritional provision in excess of metabolic capacity in the critically ill, more immature infant may contribute to their inability to use amino acids for protein accretion.

Limitations of currently available RCTs that test protein dose

When reviewing trials that have tested parenteral and enteral protein delivery, there are several limitations to consider. Most trials are small and enrolled patients from only one or two centers. Several different types of commercially available parenteral amino acid solutions were used among the studies. What defines an optimal parenteral amino acid formulation for preterm infants remains unknown, particularly for infants born at the lowest GA or when considering infants in the acute phase of illness. Similarly, trials that compared enteral protein delivery varied the type of protein (whey vs. casein), formulation of HMF (powder vs. liquid), degree of protein hydrolyzation (intact vs. partial vs. extensive), generation of the fortifier (older vs. newer HMF formulations), amount other macronutrients (lipid content, dextrose), whether diets were isocaloric, and the relative amounts of milk type (mother's own milk vs. donor human milk vs. preterm formula). Only a few studies used human milk analysis to report the actual content of protein [56–58] and most studies estimated human milk protein content prior to addition of HMF, thereby limiting accuracy of determining actual protein delivery. These variables make it difficult to independently and strictly compare the amount of protein that was received and evaluate studies in a systematic way. Furthermore, the difference in amino acids or enteral protein that was received by the infants often was less than the difference that was intended by the study design. Very few preterm infants born <24 weeks of gestation were included in RCTs, yet these infants are at highest risk for postnatal growth failure and adverse neurodevelopment. It is important to note that neurodevelopmental outcomes were seldom the primary outcome of clinical trials and thus results are underpowered.

CONCLUSION

Close attention to nutritional delivery and protein intake is of utmost importance for the health of preterm infants to promote growth and neurodevelopment. Avoiding prolonged interruption of amino acids for very low birth weight infants prevents a catabolic state, reduces risk of hyperkalemia and hyperglycemia, and promotes positive nitrogen balance [20]. At the same time, caution is warranted when rapidly advancing amino acids to doses of >3.5 g/kg per day in the first 1–2 days of life. Given the preponderance of evidence for the safe and effective use of higher enteral protein doses, it may be prudent to increase protein delivery during the convalescent growth phase of a preterm infant when they are receiving full enteral feeds and are in a period of relative medical stability. However, it is still not known whether restoring a protein deficit is as effective for neurodevelopment as preventing it from happening in the first place. Expanded outcomes as a result of protein delivery to include body composition and lean mass growth [2], adequately powered school age neurodevelopmental outcome assessments, and later life incidence of metabolic disease are critically needed, as are more sophisticated and predictive biomarkers to monitor protein tolerance and effectiveness. Finally, many questions remain about the ceiling for safe and effective amino acid administration for the more complex preterm infant who is born prior to 24 weeks of gestation, or affected by intrauterine growth restriction, or who develops critical illness so that the approach to protein delivery may be personalized to the individual patient.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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