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Clinical Investigation

Plasma Amino Acid Concentrations in 108 Children Receiving a Pediatric Amino Acid Formulation as Part of Parenteral Nutrition

Chasity M. Shelton, PharmD,[1,4] Amanda J. Clark, PharmD,[4] Michael C. Storm, PhD,[1,3] and Richard A. Helms, PharmD[1-4]

Departments of [1]Clinical Pharmacy and [2]Pediatrics, The University of Tennessee Health Science Center, [3]The Center for Pediatric Pharmacokinetics and Therapeutics, and [4]Le Bonheur Children's Medical Center, Memphis, Tennessee

BACKGROUND Plasma amino acid (PAA) levels can be largely normalized during parenteral nutrition (PN) in infants and children using a pediatric-specific amino acid (AA) formulation. However, these previous results were based on individual clinical studies of small populations of neonates and infants.
OBJECTIVE We have now examined AA levels in 108 children (0-7 years of age) receiving a pediatric-specific AA formulation in PN using a single analytical methodology.
METHODS Infants and children were enrolled in specific protocols and parents/caregivers gave informed consent. Patients were stable and receiving age-appropriate intakes of AA and non-protein calories. Samples were obtained between 8 and10 am, processed immediately, deproteinized, and AA concentrations (μmol/L) were determined on a Beckman 6300 analyzer. Means and SD were calculated for sub-populations stratified by age: 0-1 month (48 patients, n=139), 1-6 months (36 patients, n=124), 7-12 months (11 patients, n=41), and 1-7 years (13 patients, n=51). Z scores were calculated for each amino acid [(observed mean - normal control mean) / normal control SD].
RESULTS When compared to the neonatal reference range, nonessential AA had Z scores that ranged from -1.84 (asparagine) to +1.48 (threonine). Only plasma free cystine, free tyrosine, and phenylalanine had Z scores outside the −2.0 to +2.0 range (95% confidence limits). Plasma free cystine values were low in all groups except neonates. Free tyrosine levels were low in all groups despite the presence of N-acetyl-L-tyrosine in the pediatric AA formulation. Phenylalanine levels were elevated only in neonates. When children 1 to 7 years old were compared with an age-matched reference range, plasma free cystine values were low (Z score -2.47), as were plasma glutamine values (-3.11), but elevations were found in the dicarboxylic amino acids aspartic acid (+2.5) and glutamic acid (+4.27). Regardless of reference range used for comparison, all essential amino acids, except phenylalanine in neonates, were within range (-2 to +2 of the 95% confidence limits).
CONCLUSIONS While most AAs were within the normal range, formulation modifications are needed to normalize free cystine in infants and young children, free tyrosine in all children, and phenylalanine in neonates. The decrease in glutamine concentrations in older children has been noted by our group before, and may imply limited ability to convert glutamic acid to glutamine, or increased consumption of glutamine. In either case, increased concentrations of glutamine in older children, especially those receiving home parenteral nutrition, should be considered.

KEYWORDS amino acids, parenteral nutrition, pediatric

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INTRODUCTION

Neonates and infants receiving standard amino acid (AA) solutions as part of parenteral nutrition (PN) develop abnormal plasma amino acid (PAA) patterns.[1,2] These early observations caused practitioners to question the safety and efficacy of PN in this age group and led to the development of specialized AA products designed to meet the needs of neonates and infants in the mid 1980s.[3] TrophAmine (TA, B. Braun Medical Inc., Bethlehem, PA)) was formulated as a pediatric-specific

Address correspondence to: Richard A. Helms, PharmD, Professor and Chair, Department of Clinical Pharmacy, Professor of Pediatrics, The University of Tennessee Health Science Center, 847 Monroe, Suite 208, Memphis, TN 38163, email: rhelms@uthsc.edu

AA product to reproduce the 2-hour postprandial PAA concentrations of healthy, normally growing, 30-day-old, breast-fed, term infants[4] when administered as part of routine PN. This product includes taurine, glutamic acid, aspartic acid, N-acetyl-L-tyrosine, and higher quantities of the essential AA and histidine than standard AA solutions.

ABBREVIATIONS AA, amino acids; GT, glycyl-L-tyrosine; NAT, N-acetyl-L-tyrosine; NEC, necrotizing enterocolitis; PAA, plasma amino acid; PN, parenteral nutrition; TA, TrophAmine

In 1987, Heird et al. published the results of a multicenter study demonstrating that TA with the addition of cysteine produced a "near normal" PAA pattern in infants and children nourished by PN.[5] The infants in that study achieved adequate weight gain and positive nitrogen balance. Helms et al. reported the first clinical study comparing neonates receiving this pediatric AA formulation plus cysteine to neonates receiving a standard crystalline AA formulation without cysteine.[6] The findings suggested greater weight gain, improved nitrogen balance, and normal plasma AA concentrations (except for free tyrosine) in infants receiving TA based PN. An additional multicenter trial in low birth weight infants showed that TA was not only well tolerated in this specialized population but appeared to be more efficacious with respect to weight gain and nitrogen retention than previous AA mixtures.[7]

TA has become widely accepted by neonatal and pediatric facilities in the United States. The initial validation studies involving this AA formulation were conducted at multiple centers and enrolled only modest study patients under stringent protocols stipulating narrow age or weight ranges.[5-7] We have pooled data from our previous studies in patients receiving this formulation plus cysteine to create a single, large data set (108 patients with 355 observations) of PAA concentrations over time and across a wider range of ages. This data set represents a 10-year experience with this formulation in a highly regulated clinical and laboratory analysis environment. Over the course of this study period, our center utilized uniform clinical methodologies and PN practice standards.

We hypothesized that most PAA concentrations would be uniform across a wide range of ages in parenterally fed patients. We suspected from previous research that differences might appear in both the aromatic AA (phenylalanine and tyrosine) and in the sulfur-containing AA (methionine, cysteine/cystine, and taurine) with age.[5-8] Other studies have suggested that some nonessential AA (i.e., arginine and glutamine) appear to become important during times of stress, critical illness, and in prematurity, making them conditionally essential.[9-12] The purpose of our current study was to provide a comprehensive review of the PAA response to a pediatric AA formulation delivered over time and to a wider range of ages, and to review the literature to determine if a new pediatric-specific AA formulation is needed.

METHODS

All patients were enrolled in protocols approved by the University of Tennessee Health Science Center Institutional Review Board, and all parents or guardians gave informed consent. Patients ranged in age from preterm neonates (≥ 28 weeks gestation) to 7 years of age. The preterm neonates and infants had gastrointestinal diseases requiring surgical intervention. The patients > 12 months of age were totally PN-dependent secondary to short gut syndrome.[13,14] All patients had some type of intestinal failure or gastrointestinal illness, including necrotizing enterocolitis. None had diseases or medications known to affect AA metabolism. There were no oncology patients or patients requiring extracorporeal membrane oxygenation included in our study population. No patient was septic or was experiencing major organ dysfunction during study periods. Table 1 summarizes the clinical characteristics and nutrient intakes for the four groups.

Patients received PN via a peripheral (exclusively in the neonatal population) or a central venous catheter for a total study period ranging from 5 to 180 days (5-28 days in younger patients). In the neonatal population, peripheral PN was allowed as the volume intake accommodated sufficient protein and non-protein caloric intake for the purpose of this study. All PN solutions contained TA plus cysteine•HCl•H_2O (Abbott Laboratories, North Chicago, IL), dextrose, lipids, electrolytes, vitamins, minerals, and trace elements. The dose of cysteine was uniform at

Table 1. Clinical Characteristics and Nutrient Intakes

	Neonates	1-6 months	7-12 months	1-7 years
No. of patients	48	36	11	13
No. of observations	139	124	41	51
Gestational age (wk)	35 ± 2.90	34 ± 4.7	NA	NA
Birth weight (kg)	2.00 ± 0.56*	2.48 ± 2.07	NA	NA
Study age (months)	11.2 ± 7.0 days	2.3 ± 1.1	9.0 ± 1.3	33.0 ± 23.9
Study weight (kg)	1.76 ± 0.73	2.75 ± 1.24	6.59 ± 1.85	13.5 ± 5.09
Amino Acids (g/kg/day)	2.40 ± 0.37	2.39 ± 0.25	2.40 ± 0.25	1.80 ± 0.20
Glucose (g/kg/day)	19.4 ± 7.06	26.6 ± 3.91	26.2 ± 2.61	13.2 ± 0.21
Fat (g/kg/day)	1.96 ± 1.00	2.37 ± 0.44	2.58 ± 0.54	1.03 ± 0.33
Total Calories (kcal/kg/day)	95 ± 33	128 ± 17	127 ± 15	63 ± 13

NA, data not available
**As a referral institution, birth weights were available for only 25 infants in this group.*

40 mg/g protein (AA) for neonates and infants, but was lower for older children. The cysteine dose was capped at 1000 mg/day when the AA intake was > 25 grams per day. Thus, for the 1- to 7-year-old age group, the average daily cysteine dose was 75 mg/kg/day. The standard practice at our institution is to use TA during the first year of life. However, the use of TA in children > 1 year of age was based on a specific protocol looking at outcomes in home PN patients receiving TA. Enteral feedings were restricted to less than 10% of total protein and/or caloric intake. All patients were receiving parenterally delivered age-appropriate intakes of protein, calories, and micronutrients prior to study enrollment.

Blood for PAA data was generally obtained from each patient once or twice during each study period of 5 to 28 days (approximately once every 30 days in older patients). Neonates and infants were weighed daily, and strict intake and output records were recorded daily. Periodic monitoring of electrolytes and liver function tests were performed routinely as part of standard clinical practice.

Heparinized blood samples were obtained between 8 am and 10 am, and plasma was separated immediately. A portion of whole plasma was stored at -70°C. A second portion of whole plasma was then deproteinized with 5′-sulfosalicylic acid at 40 mg/mL of plasma and stored at -70°C until analysis. All PAA were determined on a Beckman 6300 Amino Acid Analyzer, using a 10-cm Li High Performance Column and a four buffer (lithium citrate), three temperatures, expanded physiologic program (Beckman Instruments, Inc., Palo Alto, CA) with post-column ninhydrin detection. Total and free cysteine/cystine concentrations were determined spectrophotometrically using whole plasma after reduction with dithiothreitol by a modification by Malloy of the method of Gaitonde.[15,16] Previous work has demonstrated that cysteine, cystine, and the conjugate, D-glucose-L-cysteine, are all quantified by this methodology.[17]

The target PAA ranges (95% confidence limits) were established as the mean 2-hour postprandial PAA concentrations in healthy, normally growing, 1-month-old, breast-fed, term infants.[4] This group consisted of 20 Hispanic and one African American infant, mean weight 4.45 kilograms, and were of normal weight-for-age, length-for-age, and weight-for-length measurements. PAA analyses were performed using similar analytical methods as for the study population. This PAA pattern appears to represent an appropriate range to maximize growth in both preterm and term infants.[4,18] It has been suggested that stable PAA concentrations of essential amino acids are likely of physiologic relevance in the promotion of normal growth and are maintained (in the absence of disease) within this reference range throughout childhood.[8] The investigators have additionally made comparisons with an age-matched reference population in clinic patients at our institution for study children 1-7 years old. This reference range employed the same analytical method used for the study population.[13]

The Kolmogorov-Smirnov test was used to test normal distribution of data. Amino acids

Table 2. Nonessential AA Z scores in Patients Receiving TrophAmine + Cysteine HCl

Amino acid	Breast-fed neonates[4] (µmol/L)	Neonates (n=139)	1-6 months (n=124)	7-12 months (n=41)	1-7 years* (n=51)
Alanine	386 ± 123	-1.51	-1.34	-0.89	-0.84 (-1.14)
Arginine	95.3 ± 24.9	0.14	-0.25	-0.24	-0.15 (0.1)
Asparagine	48.2 ± 15.2	-1.84	-1.57	-1.63	-1.55 (-1.94)
Aspartic acid	27.6 ± 10.8	-0.69	-1.05	-1.81	-1.65 (2.5)
Citrulline	14.4 ± 4.3	-0.74	-0.58	-1.02	-0.16 (-1.32)
Glutamic acid	134 ± 51.4	-1.48	-1.43	-1.60	-1.33 (4.27)
Glutamine	496 ± 166	-0.66	-0.91	-0.98	-0.80 (-3.11)
Glycine	226 ± 70.3	1.20	0.94	0.71	0.32 (-0.34)
Ornithine	77.3 ± 37.5	0.72	0.25	-0.35	-0.59 (-.071)
Proline	201 ± 55.6	-0.70	-1.00	-0.82	-0.34 (0.15)
Serine	159 ± 78.4	0.16	0.11	-0.30	-0.57 (-0.49)

**Z scores using a normal reference range for 2- to 12-year-old children[13]*

were normally distributed and were analyzed using parametric analyses. Means ± standard deviations were calculated for subpopulations stratified by age as follows: neonates (up to 1 month), 1-6 months, 7-12 months, and 1-7 years. To help the reader readily assess plasma amino acid concentrations of the study population compared to reference populations, Z scores were used. Calculated Z scores compared the mean plasma concentration of each AA from the study population with the respective normal control PAA concentration.[4,13] Z scores were calculated as follows: (observed mean study AA concentration minus normal control mean AA concentration) divided by the standard deviation of the normal controls.[19] Plasma AA concentrations with Z scores between ± 2 SD were considered to be within the 95% confidence limits of the plasma concentration goals.

RESULTS

A total of 108 patients, gestational ages ranging from 28 to 39 weeks, study ages ranging from 4 days to 7 years, and weighing 1.03 to 18.6 kg were studied (Table 1). All patients received age-appropriate, protocol-established intakes of protein and calories. For neonates and infants, these intakes included 2.5 to 3 gm/kg/day of protein and 50 to 110 non-protein kcal/kg/day. For older children, protein intakes ranged from 1.5 to 2 gm/kg/day with non-protein calorie intakes of 50 to 70 kcal/kg/day.

A total of 355 PAA observations were made in these 108 children. Multiple observations made in the same patient were separated by at least 3 days and were treated as independent samples. The mean plasma concentrations of nonessential and essential AA (excluding aromatic and sulfur-containing AA) were within the 95% confidence limits (Z score between ± 2) of the neonatal target range across all age groups (Tables 2 and 3, respectively). When the Helms childhood range was used to calculate Z scores for patients in the 1-7 year group, aspartic and glutamic acids were above the 95% confidence limits, and glutamine fell below. Excluding neonatal Z scores for threonine (Z score 1.48) and tryptophan (Z score -1.14), and the 1-7 year group Z scores for methionine when compared to the childhood range (+1.61), the plasma concentrations of essential AA were within ± 1 SD of the goal across all age groups (Tables 3 and 4).

Z scores for aromatic and sulfur-containing AA in patients receiving TA plus cysteine HCl are shown in Table 4. With regard to the aromatic AA, phenylalanine levels were elevated (Z score 2.18) only in neonates. Despite the presence of N-acetyl-L-tyrosine in the formulation, free tyrosine levels were low in all age groups except in children 1 to 7 years old when compared to the age-matched children. For sulfur-containing AA, both methionine and taurine were normalized across all age groups. Total cysteine/cystine values were normalized in all age groups except neonates. However, despite cysteine supplementation, plasma free cystine levels were low in all groups except neonates (Z score -1.33).

Table 3. Essential AA Z scores in Patients Receiving TrophAmine + Cysteine HCl

Amino acid	Breast-fed neonates[4] (µmol/L)	Neonates (n=139)	1-6 months (n=124)	7-12 months (n=41)	1-7 years* (n=51)
Histidine	76.2 ± 20.0	0.17	0.38	-0.25	-0.55 (-1.1)
Isoleucine	58.2 ± 14.9	0.35	0.28	0.05	0.13 (-0.02)
Leucine	111 ± 27.3	0.17	-0.23	-0.68	-0.22 (-0.45)
Lysine	156 ± 35.5	0.54	0.26	-0.57	-0.27 (-0.58)
Threonine	134 ± 29.9	1.48	0.45	-0.99	-0.28 (-0.1)
Valine	155 ± 31.3	0.29	0.04	-0.33	0.72 (-0.24)

**Z scores using a normal reference range for 2- to 12-year-old children[13]*

DISCUSSION

As hypothesized, the majority of essential and nonessential PAA concentrations were uniform across a wide range of ages in pediatric patients receiving TA. Consistent with previous studies, abnormalities in PAA concentrations existed within the aromatic and sulfur-containing AA.[6,7,14,17]

Orally ingested phenylalanine and methionine undergo enterohepatic metabolism and may be partially converted to tyrosine and cysteine (cysteine then partially converted to taurine), respectively. Phenylalanine and methionine delivered parenterally bypass this process and may result in inadequate production of tyrosine, cysteine, and taurine. Thus, children and adults solely nourished by the parenteral route likely require preformed sources of these 3 conditionally essential AA.[1,20,21]

In neonates, it has been suggested that phenylalanine hydroxylation is limited and can result in elevated phenylalanine and suboptimal tyrosine concentrations.[20] Activity of the transsulfuration pathway is either absent or decreased in neonates, resulting in elevated methionine and decreased cysteine/cystine and taurine levels.[22,23] The metabolic immaturity of the neonatal population, especially those born prematurely, requires the provision of optimal amounts of cysteine, taurine, and tyrosine in parenteral AA formulations. Unlike standard AA formulations, pediatric AA formulations contain taurine. Our data suggest that TA provides appropriate concentrations of methionine and taurine, as steady state plasma levels of both AA were normal in all age groups. The slightly elevated phenylalanine concentrations in neonates may be explained by limited hydroxylation to tyrosine in this population. Optimal provision of tyrosine and cysteine remain problematic.

Limited solubility of tyrosine led to the use of a more soluble tyrosine precursor, N-acetyl-L-tyrosine (NAT). Despite the addition of NAT, the TA formulation failed to achieve normal plasma free tyrosine concentrations.[5-7,24,25] However, plasma total tyrosine levels (free tyrosine plus NAT) were usually normalized. We found tyrosine levels to be suboptimal in all of our age groups, except in the 1-7 year old group when using the older child reference range. Previous studies have documented higher plasma levels of acetylated than deacetylated tyrosine. In addition, NAT urinary excretions were usually 25%-30% of total NAT intake.[5,7] It is possible that deacetylation during PN is not optimal (most notably in preterm neonates), thus allowing water soluble NAT to be largely excreted in urine.[25] The dipeptide glycyl-L-tyrosine (GT) has been suggested to be effectively handled by neonates, as GT concentrations in urine are either minimal or undetectable.[10] This dipeptide should be considered as a tyrosine precursor for future AA product development.

Cysteine is unstable in aqueous solution,[26] thus preventing its addition to AA mixtures at the time of formulation. Cysteine HCl can be safely added to parenteral nutrition solutions prior to administration. The addition of cysteine (as cysteine HCl) provides a slightly more acidic pH, which enhances calcium and phosphorus solubility in PN solutions but may also increase the need for acetate in order to buffer the increased acid load.[27] The 3 circulating forms of cyst(e)ine (term used when referring to all forms) include: cysteine (monomer form), cystine (dimer form), and protein-bound cysteine/cystine. Normal plasma proportions of free cystine to cysteine

Table 4. Aromatic and Sulfur AA Z scores in Patients Receiving TrophAmine + Cysteine HCl. Values greater than ± 2.0 are shown in bold.

Amino acid	Breast-fed neonates[4] (µmol/L)	Neonates (n=139)	1-6 months (n=124)	7-12 months (n=41)	1-7 years* (n=51)
Tryptophan	59.7 ± 19.6	-1.14	-0.49	-0.77	-0.26 (-0.04)
Phenylalanine	45.8 ± 11.3	**2.18**	1.14	1.06	0.98 (0.91)
Tyrosine	78.8 ± 19.0	**-2.29**	**-2.33**	**-2.48**	**-2.01** (-1.31)
Cystine	51.9 ± 8.0	-1.33	**-2.26**	**-2.58**	**-2.50** (-2.47)
Total cysteine/ cystine	153 ± 25.5	**2.09**	0.66	-0.20	0.93 (-0.93)
Methionine	35.8 ± 6.7	0.37	1.76	-0.18	-0.26 (1.61)
Taurine	83.9 ± 38.9	-0.08	-0.89	-1.26	-0.90 (-0.81)

**Z scores using a normal reference range for 2- to 12-year-old children[13]*

and of free cysteine/cystine to bound cysteine/cystine are 2:1 and 1:1, respectively.

Our finding of low free cystine levels with normal total cysteine/cystine values (in all age groups except neonates) is consistent with previous observations.[5,28] Parenteral cysteine supplementation appears to distort the normal ratios by providing a higher free or bound cysteine concentration without proportional increases in free or bound cystine. The clinical significance of this disproportionality remains unknown, but reproducing these normal ratios during PN remains problematic and will require further clinical investigation. Maintenance of plasma cystine concentrations may be important to ensure the appropriate cystine-glutamate gradient between extra- and intracellular concentrations via the active transport system Xc^-. This system exchanges intracellular glutamate for extracellular cystine and may be important for intracellular cystine availability under conditions of oxidative stress. Under these conditions, intracellular cystine may support cysteine availability and glutathione production.[29] Normal cystine levels in neonates may suggest the need for age-based cysteine dosing. Recent work in our laboratory has suggested a positive correlation between cysteine dose and plasma cystine concentrations.[30] In addition, RBC glutathione concentrations may be modestly improved with increasing cysteine dosage in PN.[30,31]

Although arginine and glutamine plasma concentrations were within the ± 1 SD of the goals across all of the age groups in our study except for the older child group when compared to the older reference range (see Table 2), increased arginine intakes should be evaluated in neonates who require PN. Some nonessential AA may become conditionally essential during critical illness or stress. Arginine and glutamine have been studied in patients who are critically ill, septic, or premature. Both arginine and glutamine are thought to play a role in the pathogenesis of necrotizing enterocolitis (NEC).[9,11] Arginine deficiency has also been noted in sepsis[12] and in neonates with pulmonary hypertension.[32] Amin et al. found a statistically significant reduction in the incidence of NEC in a group supplemented with arginine.[11] Another study compared PAA concentrations of preterm infants diagnosed with NEC to a control group to determine if there were any differences or abnormalities observed.[9] Both groups received TA as the parenteral AA source. The authors reported significantly lower levels of both arginine and glutamine in the NEC group. This observation was noted at least 7 days prior to the diagnosis of NEC.

DiLorenzo et al. have also proposed L-arginine as a potential treatment option in medically managing NEC.[33] The authors induced NEC in a short-term neonatal piglet model and provided arginine as a continuous infusion. For comparison, control piglets received a continuous infusion of N-omega-nitro-L-arginine (a nitric oxide synthase inhibitor) instead of arginine. Both groups showed extensive gut necrosis. However, there was a 74% reduction in the amount of transluminal intestinal damage sustained in the L-arginine treated group. Our investigative group had previously demonstrated elevations in glutamic acid with corresponding decreases in plasma glutamine in home parenteral nutrition patients receiving either an adult amino acid formula or TA.[13] This could have been the result of decreased synthesis of glutamine or increased

consumption of glutamine. However, we felt chronic bicarbonate wasting in short gut children was the likely cause for increased glutamine consumption, as 60 to 70% of ammonium ion excreted by the kidney comes from glutamine. These findings suggest that additional glutamine and arginine should be considered in future AA formulations. Because of glutamine instability in aqueous solution (formation of pyroglutamic acid), a dipeptide containing glutamine, such as alanylglutamine dipeptide, would seem to be a desirable direction for formulation change.

Although TA was specifically designed for the neonatal population, its use has been validated in preterm infants[6,7] and has been successfully used throughout infancy and into early childhood.[5,13,14] With the addition of cysteine, TA continues to suggest several basic advantages: the reduction of plasma methionine and glycine concentrations, the provision of optimal nitrogen for the synthesis and accretion of protein[5-7]; a more acidic pH to allow increased calcium and phosphate delivery,[34] and a potential decrease in the incidence of cholestasis in infants and children who are exclusively fed parenterally for prolonged periods.[5,35-39]

CONCLUSION

Normalization of aromatic and sulfur-containing PAA has remained problematic in pediatric patients supplemented with TA. In order to achieve optimal cellular protein synthesis in all tissues, the plasma levels of all AA must be adequate. Our experience with this pediatric AA formulation over time and across a wide range of ages suggests that adjustments in cysteine dosage are needed to normalize cystine in infants and young children, and that formulation modifications are needed to normalize free tyrosine in all age ranges except for the children 1-7 years old, and phenylalanine in neonates. While arginine is included in this pediatric AA formulation at approximately 12% wt/wt, some data suggests that a higher dosage of arginine should be considered, especially in critically ill children. For children receiving home parenteral nutrition generally as a result of short gut syndrome, we would suggest the addition of glutamine. We concur with others that at the doses that have been evaluated to date, there does not appear to be a general need for glutamine supplementation to neonatal and infant PN.[40,41]

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