Original Article

Dynamic changes in blood amino acid concentrations in preterm infants in different nutritional periods

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Background and Objectives: Neonatal nutrition is critical for the growth and development of preterm infants. Dynamic changes in the amino acid profiles in preterm infants of different gestational ages and in different nutritional periods were investigated. **Methods and Study Design:** Premature infants who received parenteral nutrition support after birth were enrolled and divided into four groups based on their gestational ages. Blood samples were collected as a dried blood spot before nutritional support, and in the total parenteral nutrition, partial parenteral nutrition, and total enteral nutrition periods. Amino acid concentrations were detected in the samples by liquid chromatography tandem mass spectrometry and compared between the different nutritional periods and gestational ages. **Results:** Samples from 124 premature infants were statistically analyzed. Concentrations of all amino acids, except glutamine, were statistically different at distinct nutritional periods. Threonine and aspartic acid concentrations gradually increased, while valine, methionine, phenylalanine, and glycine concentrations gradually decreased with the transition from TPN to TEN. At different gestational ages, significant differences were observed in the concentrations of seven amino acids only in the PPN period but not in the others. **Conclusions:** The concentrations of amino acids in preterm infants vary with nutritional period.

Key Words: preterm infant, nutrition, amino acid, mass spectrometry, metabolism

INTRODUCTION

It is essential to provide premature infants with appropriate nutrients, for the various short- and long-term benefits to their health. The European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) has published clinical guidelines for neonatal nutrition. However, a large gap has been recently noted between the recommended standards and actual intake.[1-5] Aggressive nutritional support has been shown to help reduce malnutrition and growth restriction.[6-8] On the other hand, excessive growth rates may lead to higher risks of obesity, insulin resistance, metabolic syndrome, and cardiovascular diseases in adulthood.[9-13] Therefore, a balance between the demand and intake of nutrients is critical. Several metabolomic studies have been carried out to understand the amino acid metabolism in premature infants.[14-16] It has been shown that blood amino acid profiles in premature infants change with gestational age and age at sampling.[17] A large number of very preterm infants have to be given parenteral nutrition at first and then gradually transition to enteral nutrition. The intake of amino acids change with time and may contribute to the diversity in the blood amino acid profiles. We used liquid chromatography tandem mass spectrometry (LC-MS/MS) to detect the blood amino acid concentrations in premature infants during different periods after birth, in order to demonstrate the changes during the transition between nutritional periods, to explore the effect of gestational age, and to provide clues on the relationship between nutrition and metabolism in these infants.

METHODS

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Xinhua Hospital Ethics Committee Affiliated to Shanghai Jiao Tong University School of Medicine (XHEC-C-2016-139). Parental written informed consent was obtained for all participants. The study has been registered

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at www.clinicaltrials.gov (NCT03100305).

Study participants

The study participants were premature infants (gestational age < 37 weeks) who were admitted to the neonatal intensive care unit (NICU) of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine within 24 hours after birth and received parenteral nutrition support for no less than 3 days. The infants with inherited metabolic diseases or other severe congenital abnormalities were excluded. We also excluded cases of death due to critical illnesses during the study.

Nutritional support

A nutritional strategy was formulated based on the guidelines for the clinical practice of nutrition support in Chinese neonates (published in 2013).[18] The nutrient solution used for parenteral nutrition (PN) was a compound amino acid (6%) injection (18AA-II), while the enteral diet included breast milk, fortified breast milk, Alfaré (Nestlé), preNAN (Nestlé), and Neocate (Nutricia). The ingredients of the compound amino acid (6%) injection (18AA-II) is listed in Supplementary Table 1. Nutrient solution is infused via peripherally inserted central catheters (PICC) or umbilical venous catheters (UVC).

Amino acid profiles

The peripheral blood samples were collected from the infants' heels as spots, which were dried at room temperature and stored at -20°C for further testing. The sampling times included different periods of nutrition as follows:

(1) Initial period: within 48 hours of birth before parenteral nutrition.

(2) Total parenteral nutrition (TPN): When the intake of parenteral energy reached 80 kcal/(kg•d) or before enteral nutrition, excluding minimal enteral nutrition, which means formula or breast milk intake <20 mL/(kg•d).

(3) Partial parenteral nutrition (PPN): When the intake of enteral energy reached 50% of the total energy intake.

(4) Total enteral nutrition (TEN): After parenteral nutrition support was stopped and enteral intake reached 150 mL/(kg•d).

We used LC-MS/MS by Waters xevo-TQ to determine the concentrations of amino acids including alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), methionine (Met), phenylalanine (Phe), tyrosine (Tyr), leucine (Leu), tryptophan (Trp), valine (Val), arginine (Arg), citrulline (Cit), glycine (Gly), ornithine (Orn), glutamine (Gln), histidine (His), serine (Ser), and threonine (Thr). The internal isotope standard for tandem mass spectrometry was NSK-A from Cambridge Isotope Laboratories.

Data collection

All of the clinical data were collected with the help of electronic medical records by specially trained researchers. At the time of enrolment, we recorded the history of maternal pregnancy and birth, sex, gestational age, birth weight, Apgar scores, as well as any other diagnosis. As day-age increased, both the parenteral and enteral nutrient intakes were recorded every day during hospitalization. The calculation of energy and nutrients is based on: enteral protein 4 kcal/g, enteral carbohydrate 4 kcal/g, enteral fat 9 kcal/g, parenteral amino acids 4 kcal/g, parenteral dextrose 3.4 kcal/g, parenteral lipid 10 kcal/g, transitional breastmilk 65 kcal and 1.5 g protein/100 mL, mature breastmilk 72 kcal and 1.2 g protein/100 mL.[19] We also weekly measured their length, weight, and head circumference so as to evaluate growth and development and adjusted the nutrient supplement. During the study, once the diagnosis of major inborn error or inherited metabolic disease was confirmed, the infants with those complications were excluded.

Statistical analysis

The data of the study were analysed using SPSS 22.0. Based on their gestational age, the premature infants were divided into 4 groups: extreme preterm infants (EPI, gestational age < 28 weeks), very preterm infants (VPI, gestational age ≥28 weeks and <32 weeks), moderately preterm infants (MPI, gestational age ≥32 weeks and <34 weeks) and late preterm infants (LPI, gestational age ≥34 weeks and <37 weeks). The blood amino acid profiles were compared between different gestational ages as well as across nutritional periods. Categorical variables were described as n, % and compared by χ^2 or the Fisher's exact test. Continuous variables with parametric data distribution were described as $x \pm s$ and compared by using the Student's t-test or analysis of variance (ANOVA), and nonparametrically distributed data were shown as M (P25, P75) and evaluated by using the Wilcoxon test or Kruskal-Wallis test. $p<0.05$ was considered statistically significant.

RESULTS

Based on the inclusion and exclusion criteria, a total of 125 patients were enrolled from January 1, 2017, to December 31, 2017. A total of 124 cases was statistically analysed (Figure 1).

Population characteristics

The mean gestational age of the premature infants was 31.8 ± 2.6 weeks, and the birth weight was 1.53 ± 0.33 kilograms. There were 12, 48, 39 and 25 infants in the EPI, VPI, MPI, and LPI groups, respectively. Between the different gestational age groups, there were significant differences in the duration of hospitalization, the caesarean section rate, Apgar scores, and prevalence of some neonatal diseases, but no statistical difference was found in the history of maternal pregnancy (Table 1).

We collected 410 blood samples of which 92 were from the initial period, and 105, 123 and 90 from the TPN, PPN, and TEN periods, respectively. The duration of the TPN and PPN periods were significantly different among the different gestational age groups. In addition, there were significant differences in the intake of energy, amino acid and lipid during parenteral nutrition, as well as the enteral lipid intake during PPN period among different gestational age groups, probably caused by the faster speed of milk addition in infants with large gestational ages (Table 2).

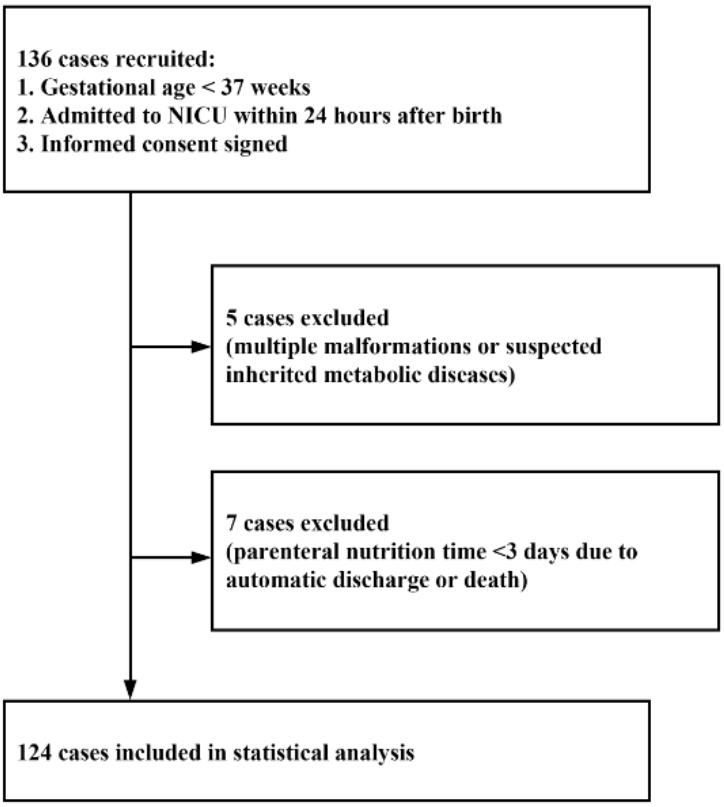

Figure 1. Selection and exclusion of cases.

Table 1. Population characteristics

	EPI	VPI	MPI	LPI	Significance
Number of cases	12	48	39	25	-
Duration of hospitalization (d)	60.3±32.8	40.1±16.0	22.6±10.0	17.8±15.8	<0.001
Gestational age (wk)	26.6 ±1.0	30.5 ±1.0	32.8 ±0.6	35.2±0.9	<0.001
Birth weight (kg)	0.95±0.20	1.40±0.23	1.67±0.24	1.82±0.15	<0.001
Boy (n, %)	7, 58.3%	29, 60.4%	20, 51.3%	12, 48.0%	0.721
Caesarean section (n, %)	4, 33.3%	29, 60.4%	32, 82.1%	25, 100.0%	<0.001
Apgar score					
1 min	6.5 (5.25, 9)	9 (8, 10)	9 (8, 10)	10 (9, 10)	<0.001
5 min	8.5 (8, 10)	10 (9, 10)	10 (9, 10)	10 (10, 10)	0.008
Disease diagnosis (n, %)					
Jaundice	10, 83.3	46, 95.8	37, 94.9	24, 96.0	0.527
Pneumonia	10, 83.3	43, 89.6	35, 89.7	19, 76.0	0.411
Atrial septal defect	7, 58.3	33, 68.8	30, 76.9	23, 92.0	0.082
Hypoproteinaemia	11, 91.7	33, 68.8	12, 30.8	3, 12.0	<0.001
Anaemia	10, 83.3	31, 64.6	8, 20.5	6, 24.0	<0.001
Patent ductus arteriosus	3, 25.0	15, 31.3	10, 25.6	3, 12.0	0.353
Respiratory failure	9, 75.0	18, 37.5	4, 10.3	1, 4.0	<0.001
Electrolyte disorder	7, 58.3	10, 20.8	6, 15.4	1, 4.0	0.002
Respiratory distress syndrome	3, 25.0	17, 35.4	5, 12.8	0, 0.0	0.002
Asphyxia	6, 50.0	5, 10.4	5, 12.8	1, 4.0	0.007
Bronchopulmonary dysplasia	6, 50.0	9, 18.8	2, 5.1	0, 0.0	<0.001
Acidosis	4, 33.3	6, 12.5	4, 10.3	0, 0.0	0.017
History of maternal pregnancy (n, %)					
Hypertension	1, 8.3	7, 14.6	9, 23.1	6, 24.0	0.477
Gestational diabetes mellitus	2, 16.7	4, 8.3	4, 10.3	2, 8.0	0.857
Intrahepatic cholestasis of pregnancy	0, 0.0	1, 2.1	0, 0.0	0, 0.0	0.591
Fetal growth restriction	0, 0	1, 2.1	3, 7.7	4, 16.0	0.091
Fetal distress	1, 8.3	2, 4.2	6, 15.4	3, 12.0	0.320
Multiple pregnancies	4, 33.3	11, 22.9	11, 28.2	7, 28.0	0.876
Placenta previa	0, 0.0	6, 12.5	2, 5.1	0, 0.0	0.061
Abruptio placentae	0, 0	1, 2.1	4, 10.3	0, 0	0.094
Premature rupture of membranes	3, 25.0	11, 22.9	13, 33.3	3, 12.0	0.278
Umbilical cord abnormalities	1, 8.3	6, 12.5	3, 7.7	5, 20.0	0.523

EPI: extreme preterm infants (gestational age <28 weeks); VPI: very preterm infants (gestational age ≥28 weeks and <32 weeks); MPI: moderately preterm infants (gestational age ≥32 weeks and <34 weeks); LPI: late preterm infants (gestational age ≥34 weeks and <37 weeks).

Table 2. Nutritional support

	EPI	VPI	MPI	LPI	Significance
Duration (d)					
TPN period	8 (6.25, 12.5)	4 (3, 8)	2 (2, 5)	1 (0, 2)	<0.001
PPN period	33.5 (24, 52.5)	15 (10, 25)	8 (7, 13)	7 (5.5, 10.5)	<0.001
TPN period					
Energy [kcal/(kg·d)]	58.6±10.2	57.1±12.8	52.7±13.7	46.4±13.6	0.007
Amino acid [g/(kg·d)]	2.30±0.32	2.25±0.52	2.08±0.58	1.89±0.62	0.044
Lipid [g/(kg·d)]	1.74±0.53	1.66±0.39	1.55±0.43	1.32±0.47	0.010
PPN period					
Energy (parenterl) [kcal/(kg·d)]	45.1±5.0	46.1±8.7	43.8±7.8	44.0±9.2	0.614
Energy (enteral) [kcal/(kg·d)]	53.8±8.2	58.5±13.9	62.3±10.0	57.1±13.7	0.177
Amino acid (parenteral) [g/(kg·d)]	1.80±0.19	1.84±0.37	1.73±0.31	1.76±0.35	0.473
Protein (enteral) [g/(kg·d)]	1.40±0.25	1.55±0.39	1.70±0.27	1.56±0.38	0.073
Lipid (parenteral) [g/(kg·d)]	1.47±0.26	1.39±0.31	1.36±0.26	1.29±0.27	0.311
Lipid (enteral) [g/(kg·d)]	2.50±0.48	2.85±0.73	3.16±0.53	2.93±0.73	0.029
TEN period					
Energy [kcal/(kg·d)]	113±27	118±13	120±15	119±10	0.675
Protein [g/(kg·d)]	3.08±0.78	3.20±0.43	3.26±0.46	3.28±0.28	0.632
Lipid [g/(kg·d)]	5.53±1.55	5.70±1.07	6.13±1.02	6.21±0.60	0.081

EPI: extreme preterm infants (gestational age <28 weeks); VPI: very preterm infants (gestational age ≥28 weeks and <32 weeks); MPI: moderately preterm infants (gestational age ≥32 weeks and <34 weeks); LPI: late preterm infants (gestational age ≥34 weeks and <37 weeks); TPN: total parenteral nutrition; PPN: partial parenteral nutrition; TEN: total enteral nutrition.

Comparison of amino acid profiles at in different nutritional periods

With the exception of glutamine, concentrations of all amino acids varied statistically in the different nutritional periods. Compared with the initial period, concentrations of about half of the amino acids (leucine, methionine, tryptophan, histidine, arginine, glutamic acid, aspartic acid, and serine) increased significantly after nutritional support, while phenylalanine, tyrosine, glycine, and alanine concentrations decreased. Concentrations of most amino acids showed differences between the TPN, PPN, and TEN periods (Table 3). While threonine and aspartic acid concentrations gradually increased with the change in nutritional period, valine, methionine, phenylalanine, and glycine concentrations gradually decreased during the transition from TPN to TEN (Figure 2).

Comparison of amino acid profiles between different gestational age groups

In each nutritional period, we compared the amino acid concentrations among infants of different gestational ages. Significant differences among gestational age groups were noted in the concentrations of tyrosine and threonine in initial period, aspartic acid in TPN period, and citrulline in TEN period. Moreover, there appeared to be a significant difference in seven amino acids in PPN period in different gestational age groups. Some amino acids such as valine, methionine, and alanine were found in lower

Table 3. Amino acid profiles in distinct nutritional periods (μmol/L, M)

	Initial period	TPN period	PPN period	TEN period	Comparison between before and after (*p*-value)	Comparison among TPN, PPN, and TEN (*p*-value)
EAA						
Leu	90.4	113.3	111.9	111.7	<0.001	0.451
Val	81.0	97.0	86.1	73.7	0.448	<0.001
Met	21.7	28.4	27.2	23.4	0.005	<0.001
Phe	63.4	61.4	51.1	49.2	<0.001	<0.001
Thr	63.5	54.9	64.3	68.5	0.825	0.002
Trp	25.0	27.7	27.1	29.8	<0.001	0.004
His	28.3	36.5	33.8	35.9	<0.001	0.417
CEAA						
Arg	5.15	7.14	8.29	8.43	0.001	0.501
Gln	5.79	6.24	6.33	4.90	0.360	0.637
Tyr	65.2	55.2	48.4	62.3	0.003	<0.001
Gly	368	342	291	287	<0.001	<0.001
NEAA						
Ala	183	156	144	164	<0.001	<0.001
Glu	205	227	219	257	<0.001	<0.001
Asp	27.1	28.92	29.8	35.9	<0.001	<0.001
Ser	72.4	83.4	79.4	89.0	0.001	0.137
Others						
Cit	11.4	11.1	9.6	10.7	0.017	0.004
Orn	33.9	54.9	56.8	53.4	<0.001	0.539

TPN: total parenteral nutrition; PPN: partial parenteral nutrition; TEN: total enteral nutrition.

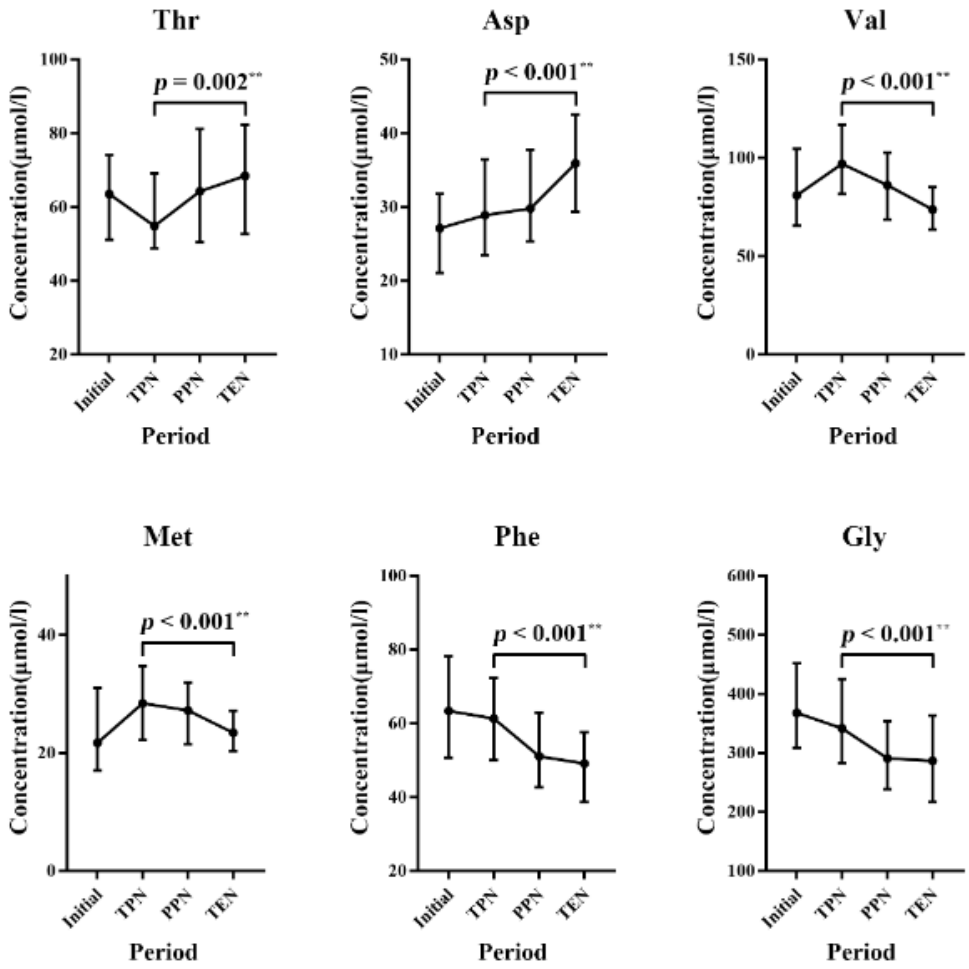

Figure 2. Changes in amino acid concentrations with the transition of nutritional periods. TPN: total parenteral nutrition; PPN: partial parenteral nutrition; TEN: total enteral nutrition.

concentrations in preterm infants with smaller gestational ages (Table 4, Figure 3).

DISCUSSION

Significant differences in amino acid profiles in different nutritional periods

When a premature infant is born, it is necessary to provide exogenous amino acids as soon as possible to compensate for the sudden interruption in the nutrient supply through the placenta. Though it is not clear how the amino acid profiles would look in the absence of nutritional management, we found that some of the amino acids were present in higher concentrations after receiving nutritional support, which may be due to the intake of nutrients. Compared with initial period, we found a decrease in phenylalanine, tyrosine, glycine, and alanine concentrations after the start of receiving nutritional support, suggesting the difference in nutritional and metabolic status between fetal and neonatal periods.

Though enteral nutrition is the preferred mode of providing nutrients to preterm infants, due to the immaturity in their digestion and absorption, some of them (mostly the EPIs and VPIs) have to depend on parenteral nutrition before gradually establishing total enteral nutrition. Our findings indicate that the amino acid profiles change with the nutritional period, the causes of which are mainly intake and utilization (Figure 4). A study by Morgan et al. found that increasing amino acid intake results in higher concentrations of some essential amino acids.[20] In addition, various paths of nutrition lead to differences in the utilization of amino acids by the portal-drained viscera (PDV). It has been shown that PDV accounts for 20%-35% of the systemic energy consumption. During the period of intestinal feeding, the nutrient requirements of PDV are first satisfied, which could result in lower blood concentrations of amino acids.[21] Although the blood amino acid profiles can be significantly influenced by various nutritional factors, insufficient attention has been paid to this issue in research and clinical practice.

The changes in amino acid concentrations during the transition between nutritional periods

During the transition from TPN and PPN to TEN, while threonine and aspartic acid concentrations gradually increased, valine, methionine, phenylalanine, and glycine concentrations decreased. Threonine is an essential amino acid. Studies have shown that 70%-80% of threonine is first taken up and utilized by the intestine during enteral nutrition,[22] which may cause a significant decrease in the blood threonine concentration during enteral nutrition. However, the opposite result occurs in the study. Therefore, it can be inferred that the intake of threonine during enteral nutrition may be too high, or the intake during

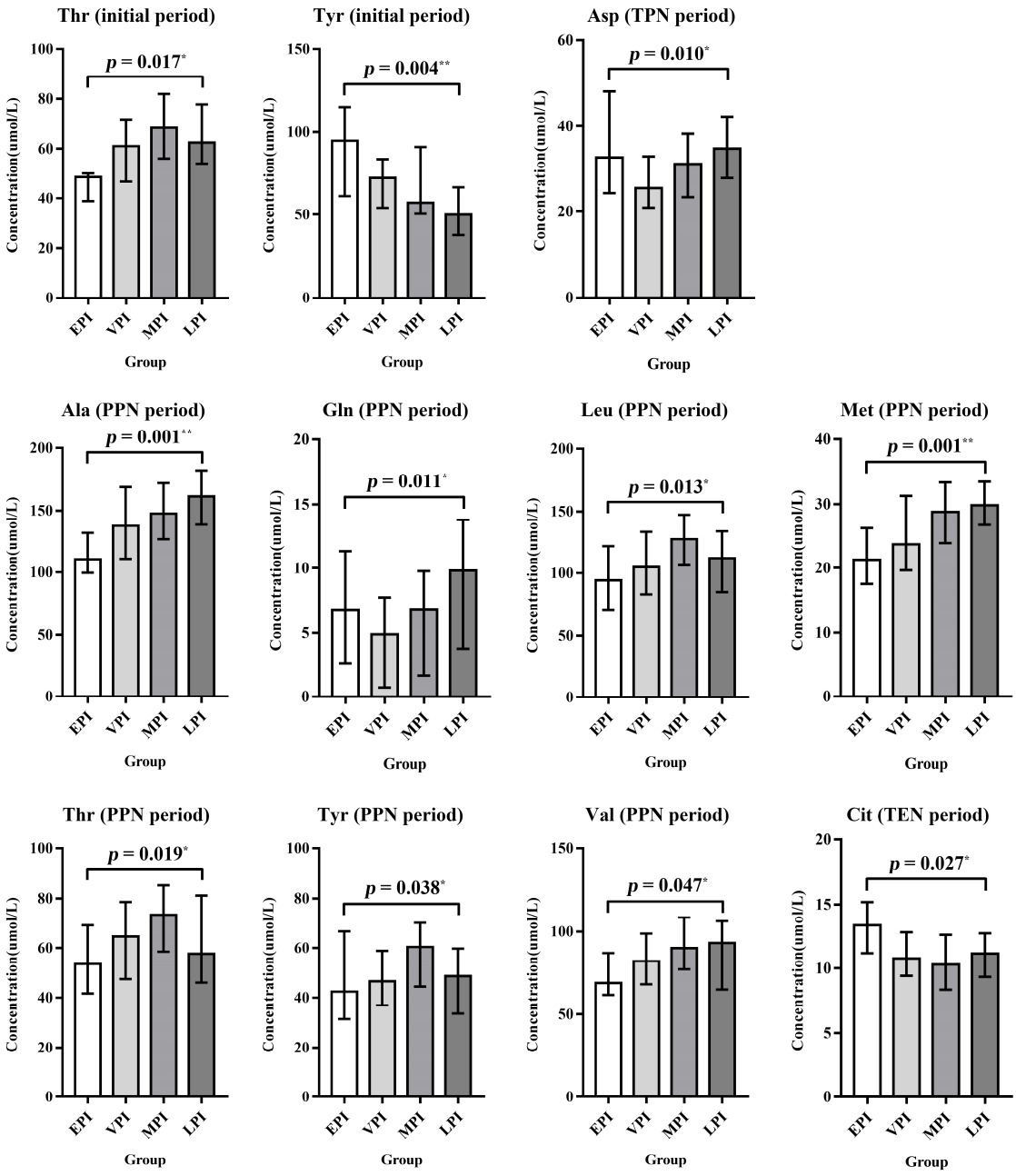

Figure 3. Comparison of amino acids among different gestational age groups in each nutritional period. EPI: extreme preterm infants (gestational age < 28 weeks); VPI: very preterm infants (gestational age ≥28 weeks and <32 weeks); MPI: moderately preterm infants (gestational age ≥32 weeks and <34 weeks); LPI: late preterm infants (gestational age ≥34 weeks and <37 weeks); TPN: total parenteral nutrition; PPN: partial parenteral nutrition; TEN: total enteral nutrition.

parenteral nutrition may be insufficient, or both. Some commercially available formulas are reported to provide much higher amounts of threonine, up to twice as much as the requirement.[23] Threonine is involved in the synthesis of secretory glycoproteins, which contribute to the intestinal barrier function,[24] but excessive intake leads to reduce mucin synthesis and results in changes in the villus architecture.[25] Although preterm infants have the ability to regulate plasma threonine concentrations through amino acid oxidation, the increased concentration of threonine in preterm infants also needs to be noticed. At the same time, it is also necessary to pay attention to insufficient threonine intake of preterm infants during parenteral nutrition.

Aspartic acid is a non-essential amino acid and a precursor of asparagine, which takes part in the urea cycle, purine and pyrimidine synthesis, and gluconeogenesis. During enteral nutrition, almost all of the aspartic acid is first metabolized by PDV. A stable isotope tracing study has shown that a high proportion of labelled aspartic acid is recovered in the exhaled gas, indicating it has a high first-pass metabolic uptake fraction similarly to threonine, but the difference is that more aspartic acids may be oxidized by the tricarboxylic acid cycle.[26] Similar to threonine, the change in aspartic acid concentrations also seems to be related to the imbalance between intake and demand. Early studies have hypothesized that high aspartic acid concentrations in the cerebrospinal fluid is associated with neonatal seizures and intracranial haemor-

Table 4. Comparison of amino acid profiles among EPI, VPI, MPI and LPI group (*p* value)

	Initial period	TPN period	PPN period	TEN period
EAA				
Leu	0.705	0.101	0.013	0.895
Val	0.822	0.297	0.047	0.881
Met	0.969	0.125	0.001	0.987
Phe	0.323	0.379	0.154	0.419
Thr	0.017	0.414	0.019	0.867
Trp	0.560	0.362	0.194	0.412
His	0.807	0.744	0.842	0.223
CEAA				
Arg	0.475	0.211	0.239	0.131
Gln	0.517	0.129	0.011	0.612
Tyr	0.004	0.478	0.038	0.893
Gly	0.059	0.689	0.880	0.622
NEAA				
Ala	0.733	0.214	0.001	0.441
Glu	0.409	0.496	0.258	0.264
Asp	0.115	0.010	0.156	0.082
Ser	0.154	0.107	0.710	0.461
Others				
Cit	0.098	0.132	0.190	0.027
Orn	0.789	0.153	0.585	0.177

EPI: extreme preterm infants (gestational age <28 weeks); VPI: very preterm infants (gestational age ≥28 weeks and <32 weeks); MPI: moderately preterm infants (gestational age ≥32 weeks and <34 weeks); LPI: late preterm infants (gestational age ≥34 weeks and <37 weeks); TPN: total parenteral nutrition; PPN: partial parenteral nutrition; TEN: total enteral nutrition.

rhage. However, whether high blood aspartic acid concentrations lead to neurotoxicity or not, is not known.

Valine is an essential branched-chain amino acid (BCAA). A study in newborn piglets has shown that the requirement for BCAAs during parenteral nutrition is 56% of what is required during enteral nutrition, though the utilization pathway has not been studied well.[27] It should be pointed out that many studies have found that increasing the total amount of amino acids during parenteral nutrition can significantly increase the plasma valine concentrations, suggesting the intake is relatively sufficient.[20, 28] Our previous retrospective analysis (not yet published) supports this finding as well, indicating that excessive intake of valine cannot be well utilized in preterm infants.

Methionine is an essential amino acid and an important methyl donor in cell metabolism. The pathway of sulphur transfer effect to synthesize cysteine has been proven to be enhanced by parenteral nutrition.[29] Paradoxically, the methionine concentration at the TPN period was higher in our study, which is most likely due to an excessive intake during the parenteral nutrition.

Phenylalanine is an aromatic essential amino acid that, in addition to its role in protein synthesis, is irreversibly hydroxylated to tyrosine and participates in the regulation of adrenaline, norepinephrine, and dopamine. The requirements for phenylalanine during enteral nutrition in term and preterm infants have been reported to be 58 mg/kg/d and 80 mg/kg/d, respectively under conditions of sufficient tyrosine intake.[30] Nevertheless, in clinical practice, the demand for phenylalanine is more dependent on the need for hydroxylation to tyrosine. Pencharz et al. have shown that the addition of sufficient tyrosine can reduce the phenylalanine requirement by about 75%.[31] Because of the low solubility and content of tyrosine in parenteral nutrient solution, high intake of phenylalanine is common and necessary, which often results in its high blood concentrations in the TPN and PPN periods. There is currently no evidence that indicates poor phenylalanine hydroxylation in premature infants, suggesting that phenylalanine supplementation is relatively safe in infants with no congenital metabolic diseases.[32]

Glycine is a conditionally essential amino acid, and its chemical structure is quite simple. It can be synthesized by serine, threonine, choline, and glyoxylate.[33] The metabolism of glycine during parenteral and enteral nutrition remains unclear and needs to be clarified in the future. The changes in glycine concentrations may be related to different paths of nutrition or other undiscovered reasons.

In summary, the dynamic changes in the concentrations of amino acids reflect the transition to total enteral nutrition. Meanwhile, it seems to be inevitable due to the differences in amino acid metabolism and the preparation of the nutrient solutions. It should be pointed out that the study has limitations. Considering the amount of liquid, preterm infants sometimes cannot receive recommended intake of energy and nutrients, which may have an impact on the results. Further studies that apply the mass spectrometry to clinical nutrition in premature infants are needed to understand the relationship between amino acid profiles and prognosis of future growth and development.

Effect of gestational age on blood amino acid profiles

Many studies have confirmed significant differences in the amino acid profiles of neonates with different gestational ages.[34-35] Wilson et al. analysed the results of newborn screenings and observed that blood arginine, leucine, ornithine, phenylalanine, and valine concentrations were inversely related to gestational age, while glycine concentrations showed a direct relation.[15] To further explore the effect of gestational age, we analysed the amino acid profiles at each nutritional period and found that the effect of

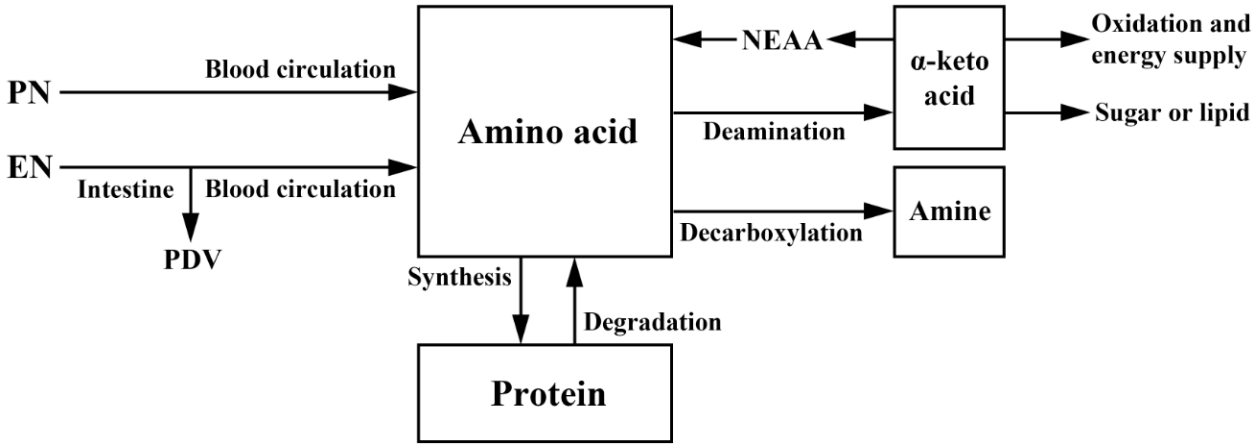

Figure 4. Amino acid metabolism.

gestational ages on amino acid concentrations vary with the nutritional period. In contrast to some other studies,[36-37] our results showed that the amino acid profiles were not conspicuously different between the different gestational age groups, irrespective of the nutritional period, suggesting comparable metabolism of amino acids across gestational ages. However, a significant influence of the gestational age on amino acid concentrations was seen in the PPN period, and there was an upward trend generally along with the gestational age. In view of clinical practice, we speculate several possible causes for this observation. First, the metabolism of amino acids is more complicated and varied in both parenteral and enteral nutrition. Second, the intake of amino acids and proteins are constantly changing with changes in the consumption of milk with gestational age. Finally, during the long duration of the PPN period, several diseases and interventions are likely to have an impact, especially in small preterm infants. Though there is not enough evidence to confirm the effect of diseases and interventions on amino acid metabolism, their confounding effects cannot be ignored.

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AUTHOR DISCLOSURES

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Supplementary table 1. The ingredient list of the compound amino acid (6%) injection (18AA-II)

Ingredient	Content (g/100 mL)
Isoleucine ($C_6H_{13}NO_2$)	0.49
Leucine ($C_6H_{13}NO_2$)	0.84
Lysine acetate ($C_6H_{14}N_2O_2 \cdot C_2H_4O_2$)	0.69
Methionine ($C_5H_{11}NO_2S$)	0.20
Phenylalanine ($C_9H_{11}NO_2$)	0.29
Threonine ($C_4H_9NO_3$)	0.25
Tryptophan ($C_{11}H_{12}N_2O_2$)	0.12
Valine ($C_5H_{11}NO_2$)	0.47
Cysteine hydrochloride ($C_3H_7NO_2S \cdot HCl$)	<0.02
Histidine ($C_6H_9N_3O_2$)	0.29
Tyrosine ($C_9H_{11}NO_3$)	0.14
Alanine ($C_3H_7NO_2$)	0.32
Arginine ($C_6H_{14}N_4O_2$)	0.73
Proline ($C_5H_9NO_2$)	0.41
Serine ($C_3H_7NO_3$)	0.23
Aspartic acid ($C_4H_7NO_4$)	0.19
Glutamic acid ($C_5H_9NO_4$)	0.30
Glycine ($C_2H_5NO_2$)	0.22
Taurine ($C_2H_7NO_3S$)	0.015