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ORIGINAL ARTICLE

Plasma and urine amino acid pattern in preterm infants on enteral nutrition: impact of gestational age

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Abstract Plasma and urine amino acids were determined by ion-exchange chromatography in 80 healthy preterm infants divided into three groups: (1) 23 0/7–28 0/7, (2) 28 1/7–32 0/7 and (3) 32 1/7–35 0/7 weeks of gestation. Samples were collected from days 5 to 57 of life, when infants were exclusively orally fed. Infants with evidence of underlying diseases were excluded. Concentrations of most plasma amino acids increased with gestational and maturational age; urinary excretion followed an opposite course. Few amino acids depended on postnatal age. Plasma amino acids did not correlate inversely to their counterparts in urine indicating that plasma amino acids do not simply reflect kidney function. Some amino acids in blood and urine were linked to nutrient intake and body weight. Our data clearly indicate the heterogeneity of the preterm cohort; therefore, gestational age-matched reference values have to be used for diagnostic purposes in preterm infants.

Keywords Amino acids · Preterm infants · Gestational age · Maturation · Enteral nutrition

Introduction

Amino acids are key metabolites in many pathways of intermediary metabolism as well as constituents of exogenous nutritional compounds. The pattern of amino acids in body fluids gives account of the physiological and pathophysiological status of different metabolic pathways. Identification and measurement of free amino acids in plasma and urine is crucial for the diagnosis and treatment of inborn errors of amino acid metabolism.

The interpretation of abnormalities of amino acid profiles in blood and urine is difficult. An increase or decrease of single amino acids can be due to various aetiologies. Early diagnosis of disturbed amino acid metabolism or transport is very important, as some of these conditions can be treated, resulting in the prevention of (further) clinical symptoms. In those disorders, which cannot be treated, early diagnosis in an index-patient may enable prenatal diagnosis in subsequent siblings.

Primary aminoacidopathies can be due to genetically determined transport disorders or enzyme deficiencies in amino acid metabolism or degradation. Secondary aminoacidopathies result from abnormal or deficient nutrition, intestinal dysfunction, organ pathology or other metabolic diseases like organic acidurias. Levels of amino acids in plasma and urine are significant indicators of protein metabolism and nutritional status. Subtle increases or deficits in amino acids may compromise normal growth and development. Several studies have indicated that premature infants often develop severe nutritional deficits during the first weeks after birth (Clark et al. 2003;

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Coverston and Schwartz 2005; De Curtis and Rigo 2004; Ehrenkranz et al. 2006; Embleton et al. 2001). For the evaluation of pathological states, knowledge of reference ranges for amino acids in urine and plasma in a healthy age-matched population is essential.

Normative values for plasma and urine amino acid concentrations have been reported in adults, infants and older children (Armstrong and Stave 1973; Brodehl and Gellissen 1968; Caballero et al. 1991; Dickinson et al. 1965; Fukuda et al. 1984; Gregory et al. 1986; Lepage et al. 1997; Liappis et al. 1990; Lindblad et al. 1978; Picaud et al. 2001; Szajewska et al. 2001). Small sample size and different types of nutrition (especially enteral vs. parenteral and diverse hydrolysate formulas) are limiting factors for interpreting and comparing the results of those studies. Only very few data from premature infants with a gestational age less than 27 weeks do exist. The design of most of these studies is characterized by comparison of amino acid profiles under different feeding regimens (Boehm et al. 1993; Clark et al. 2007; Cooke et al. 1992; Janas et al. 1987; Maggio et al. 2005; Moro et al. 1999; Polberger et al. 1990; Rigo and Senterre 1987; Schanler and Garza 1987; te Braake et al. 2005; Tikanoja et al. 1982). However, data are sparse describing the gestational age-specific distribution and developmental changes of plasma and urine amino acid concentrations in preterm infants. Establishing "normal" amino acid profiles in premature infants would allow the identification of infants with abnormal values and to evaluate the impact of those abnormal values on important health outcomes like liver function, growth and neurodevelopmental outcome.

We determined amino acids in plasma and urine from 80 premature infants who were divided into three groups (1) 23 0/7 to 28 0/7, (2) 28 1/7 to 32 0/7 and (3) 32 1/7 to 35 weeks of gestation. We further analyzed these data by testing correlations between metabolite values, gestational, maturational and postnatal age at sample collection, birth weight, daily nutrient intake and other clinical parameters.

Materials and methods

We conducted a non-randomized prospective clinical trial. Preterm infants hospitalized at our neonatal intensive care unit without evidence of renal, gastrointestinal, muscular or metabolic diseases were consecutively included in the study as soon as they were completely fed by special preterm formula (Beba FG 16%, Nestlé nutrition, Frankfurt am Main, Germany)—and/or enriched human milk (FM 85, Nestlé nutrition). Most of them received a mixture of human as well as formula milk. In group 1, four babies, in group 2 three and in group 3 eight babies were fed only with formula milk. None of our babies received parenteral nutrition when blood and urine was taken. Thus, all infants were in a physiological nutritional state with a positive energy balance resulting in adequate weight gain (15–17 g per kilogram body weight per day) at the time of sample collection. All infants included were in a stable respiratory and cardiovascular condition without any catecholamine and no insulin substitution. Neither Apgar scores nor the presence of respiratory distress in the past precluded an infant from inclusion in the analysis.

Urine and blood samples were not always taken simultaneously on the same day, therefore tubular reabsorption (% TAA) could not be calculated. Blood (0.5 mL EDTA tubes) and urine samples (collection bags for infants) were obtained from preterm infants of both sexes ranging from 23.4 to 34.7 weeks of gestation before the next meal on days 5–57 of life. Further analysis of blood and urine samples was performed directly after collection; otherwise urine samples were frozen at −20°C and analyzed within few days after collection.

Plasma and urine amino acid concentrations were determined after deproteinization with sulphosalicylic acid (5%) and sodium dodecylsulfate (0.5%). Amino acid profiles in blood and urine were analyzed by ion-exchange chromatography with an amino acid autoanalyzer (LC 3000, Eppendorf, Biotronik, Germany). Briefly, this analyzer utilizes cation-exchange chromatography with a step buffer elution and with post-column ninhydrin derivatization. Absorbance of amino acid–ninhydrin complexes was detected at 570 nm for primary amino groups and at 440 nm for proline and hydroxyproline. Amino acid concentrations were automatically calculated on the basis of both the calibrators and the internal calibrator. We quantified 28 amino acids in plasma and 35 in urine. Creatinine concentration in urine was analyzed by a standard kinetic Jaffé procedure.

Reference values for blood and urine amino acids of term infants used in our laboratory are based on results described elsewhere (Liappis et al. 1990; Parvy et al. 1988). In preliminary experiments, we could not find significant differences between amino acids in serum and plasma; these results are in line with a previously published study (Oepen and Oepen 1963).

The study was approved by our University Hospital Ethics Committee (14 July 2006), and informed consent was obtained from the parents prior to enrolment.

Statistical analysis

Parametric statistical analyses were performed with SPSS statistical software (SPSS, Chicago, IL, USA; version 15.0). First, a descriptive analysis was undertaken to assess

the quality of the data, and to identify missing data. For group comparison, analysis of variance (ANOVA, followed by post hoc tests for pairwise comparison) and unpaired t tests were performed on amino acids in urine and blood, verifying for differences in gestational and maturational age, chronological age, sex and other clinical characteristics. For correlations, Pearson's bivariate correlation coefficients were calculated. P values less than 0.05 were considered statistically significant.

Results

Eighty preterm infants (45 boys and 35 girls born at 23.4–34.7 weeks of gestation, birth weight 465–2720 g) were consecutively included in this prospective study. The study population was divided into three groups according to gestational age: group (1) 23 0/7–28 0/7, group (2) 28 1/7–32 0/7 and group (3) 32 1/7–35 0/7 weeks of gestation. Blood and urine samples were collected as soon as the infants were exclusively enterally fed, i.e. from day 5 to 57 of postnatal life. In 15 children, blood and urine samples were not obtained simultaneously the same day.

The postnatal age at blood and urine sample collection was significantly higher in groups 1 and 2 in comparison to group 3 (for blood $p < 0.001$ and 0.021 and for urine $p < 0.001$ and 0.05), respectively, and correlated inversely with gestational age ($r = -0.741$, $p < 0.001$ and $r = -0.706$, $p < 0.001$). The time intervals between terminating parenteral nutrition and blood/urine sample collection were as follows [mean ± standard deviation (SD)]: for blood collection: in group (1) 19.9 ± 11.8, in group (2) 9.5 ± 4.9 and in group (3) 4.3 ± 2.4 days. For urine collection: in group (1) 19.8 ± 12.1, in group (2) 9.3 ± 5.5 and in group (3) 5.8 ± 2.8 days. This time span was highest in group 1 ($p < 0.001$). Maturational age at blood and urine collection (gestational age plus postnatal age) was significantly different between the three age groups; highest values of maturational age were found in group 3 ($p < 0.001$). Birth weight and actual weight at sample collection differed significantly between the three groups ($p < 0.001$). Clinical characteristics of the study population are summarized in Table 1.

The daily nutritional intake of protein, fat, carbohydrates (g per kg body weight and day) and energy (kJ per kg body weight and day) was calculated for each infant (Table 2). Assumed contents (g and kJ per 100 mL milk) of carbohydrates, fat, protein and energy of enriched human and formula milk are: 10.3 g carbohydrates, 3.8 g fat, 2.5 g protein and 355.9 kJ for breast milk plus supplement (FM 85, Nestlé nutrition; 5 g per 100 mL breast milk) and 8.6 g carbohydrates, 4.2 g fat, 2.3 g protein and 339.1 kJ for Beba FG 16% (Nestlé nutrition).

At blood collection, in groups 1 and 2, nutritional intake per kilogram bodyweight of carbohydrates ($p < 0.001$ and 0.01) and energy ($p = 0.001$ and 0.002) was significantly higher in comparison to group 3. Fat and protein intake did not differ between the three groups.

At urine collection, nutritional intake of carbohydrates was higher in group 1 than in groups 2 and 3 ($p = 0.043$ and 0.001). In groups 1 and 2 compared to group 3, nutritional intake of energy ($p = 0.002$ and 0.027) was significantly higher. Fat and protein intake did not differ between the three groups. Nutrient intake was independent of gender.

In blood, only leucine, valine, tyrosine and glutamine correlated inversely with energy and histidine with protein intake ($p < 0.05$). In urine, alanine, hydroxyproline and proline correlated with energy intake ($p < 0.05$); only proline excretion was linked to protein intake.

No differences of clinical parameters at the time of sample collection could be found between formula fed infants and those who got a mixture of fortified breast milk and special preterm formula milk in this study. Some blood and urine amino acids including essential amino acids from infants fed only special formula milk differed from those who got a mixture of both milks (Table 3).

Blood amino acids

In plasma, argininosuccinic acid and homocystine could not be detected in any infant. Means, SD (standard deviation), 5th and 95th percentiles of all detected amino acids in blood are shown in Table 4; means and SD of selected plasma amino acids are presented in Fig. 1. No significant gender- related differences between values of blood amino acids except for arginine (females 66.3 ± 37.3 µmol/L, males 47.8 ± 26.6 µmol/L; $p = 0.014$) could be found.

According to gestational age, we found the following distributions with significant differences between the three gestational age groups ($n = 75$ blood samples in total, 28 plasma amino acids were analyzed): alanine, aspartic acid, citrulline, glutamate, phenylalanine, taurine and cystathionine did not increase significantly with gestational age, see Table 4 for further details. For correlations of plasma amino acids with gestational and postnatal age, see Table 4; 19 plasma amino acids correlated positively with gestational and ten inversely with chronological postnatal age, $p < 0.05$. Twenty plasma amino acids correlated positively with maturational age (Table 4).

Several plasma amino acids correlated positively with birth weight and inversely with actual weight at sample collection ($p < 0.05$), data are not shown.

For Fischer's ratio (isoleucine + leucine + valine)/(phenylalanine + tyrosine) differences between the three age groups were not significant; it did not correlate with

Table 1 Distribution of all included preterm infants according to their age, sex and weight

Groups (weeks of gestation)	f (*n*)	m (*n*)	GA (weeks)	BW (g)	MA (weeks at blood collection)	MA (weeks at urine collection)	Postnatal age (days at blood collection)	Postnatal age (days at urine collection)	Weight (g, at blood collection)	Weight (g, at urine collection)	Total (*n*)
Group 1											
23 0/7 to 28 0/7	12	14	25.7 ± 1.2 (23.4–28)	792.7 ± 176.8 (465–1,230)	29.9 ± 1.7 (26.4–33.3)	29.9 ± 1.7 (26.4–33.3)	29.5 ± 13.6 (13–57)	28.9 ± 13.4 (15–57)	1,022.2 ± 199.3 (745–1,455)	1,031.9 ± 205.5 (745–1,455)	26
Group 2											
28 1/7 to 32 0/7	16	14	30.7 ± 1.1 (28.6–32)	1,420.7 ± 324.7 (955–2,180)	32.9 ± 1.1 (30.9–34.9)	32.8 ± 1.2 (30.9–34.7)	16.0 ± 5.7 (7–28)	15.9 ± 6.6 (7–37)	1,571.6 ± 299.1 (1,010–2,090)	1,551.1 ± 331.7 (1,010–2,360)	30
Group 3											
32 1/7 to 35 0/7	7	17	32.2 ± 0.7 (32.1–34.7)	1,890.8 ± 366.4 (1,310–2,720)	34.6 ± 0.7 (33.6–36.1)	34.5 ± 0.7 (33.6–36.0)	9.9 ± 3.5 (5–21)	10.6 ± 3.2 (7–19)	1,897.1 ± 338.2 (1,320–2,625)	1,903.2 ± 346.7 (1,320–2,625)	24

The postnatal age at blood and urine sample collection was significantly higher in groups 1 and 2 in comparison to group 3 (for blood $p < 0.001$ and 0.021 and for urine $p < 0.001$ and 0.05). Maturational age at blood and urine collection (gestational age plus postnatal age in weeks) was significantly different, highest values of maturational age were found in group 3 ($p < 0.001$). Birth weight and actual weight at sample collection differed significantly between the three groups ($p < 0.001$)

Infants at blood collection: group (1) $n = 26$, group (2) $n = 28$, group (3) $n = 21$. Infants at urine collection: group (1) $n = 23$, group (2) $n = 28$, group (3) $n = 19$

Values are mean ± SD and ranges

SD standard deviation, *f* female, *m* male, *GA* gestational age, *BW* birth weight, *MA* maturational age

Table 2 Enteral nutrient intake of three groups of preterm infants at blood and urine sample collection

Groups (weeks of gestation)	At blood sample collection				At urine sample collection			
	Carbohydrates (g/kg per day)	Fat (g/kg per day)	Protein (g/kg per day)	Energy (kJ/kg per day)	Carbohydrates (g/kg per day)	Fat (g/kg per day)	Protein (g/kg per day)	Energy (kJ/kg per day)
Group 1								
23 0/7 to 28 0/7	16.9 ± 1.9; $n = 26$ (12.4–20.6)	7.1 ± 0.6 (6–9)	3.8 ± 0.3 (3.2–4.3)	610.9 ± 39.8 (489.9–674.1)	16.9 ± 1.8; $n = 23$ (13.8–20.6)	7.2 ± 0.6 (6–9)	3.8 ± 0.2 (3.2–4.2)	612.1 ± 31.8 (510.8–674.1)
Group 2								
28 1/7 to 32 0/7	15.8 ± 1.9; $n = 28$ (12.5–20.2)	7.1 ± 0.6 (6–8)	3.8 ± 0.25 (3.3–4.2)	596.6 ± 63.6 (484.04–766.2)	15.9 ± 1.9; $n = 28$ (12.5–20.2)	7.1 ± 0.6 (6–8)	3.8 ± 0.3 (3.3–4.3)	596.2 ± 60.3 (494.04–766.2)
Group 3								
32 1/7 to 35 0/7	14.3 ± 1.9; $n = 20$ (10.9–18.2)	6.5 ± 0.7 (5–8)	3.6 ± 0.4 (3.0–4.3)	543.4 ± 59.9 (414.5–628.02)	14.9 ± 1.9; $n = 18$ (11.4–18.2)	6.7 ± 0.6 (6–8)	3.6 ± 0.4 (3.0–4.2)	560.6 ± 57.4 (447.9–644.8)

At blood collection in groups 1 and 2, nutritional intake of carbohydrates ($p \leq 0.001$ and 0.01) and energy ($p = 0.001$ and 0.002) was significantly higher compared to group 3. Fat and protein intake did not differ between the three groups

At urine collection nutritional intake of carbohydrates was higher in group 1 than in groups 2 and 3 ($p = 0.043$ and 0.001). In groups 1 and 2 in comparison to group 3, nutritional intake of energy ($p = 0.002$ and 0.027) was significantly higher. Fat and protein intake did not differ between the three groups

Values are mean ± SD and ranges. *SD:* standard deviation

SD standard deviation

Table 3 Differences between blood and urine amino acids in infants fed only special preterm formula (0) and a mixture of formula and breast milk (1)

	Breast milk	*N*	Mean	SD	*p* value
Plasma amino acid (μmol/L)					
Alanine	0	13	336.23	101.68	0.004
	1	62	253.47	89.85	
Alpha-amino-butyric acid	0	13	13.62	17.92	0.008
	1	62	6.16	5.72	
Histidine*	0	13	96.08	18.97	<0.001
	1	62	74.32	18.90	
Methionine*	0	13	36.38	12.54	0.014
	1	62	29.16	8.60	
Proline	0	13	216.23	49.44	0.006
	1	62	167.34	58.41	
Serine	0	13	154.15	34.31	0.012
	1	62	125.00	37.77	
Threonine*	0	13	384.92	121.59	<0.001
	1	62	258.42	90.00	
Valine*	0	13	130.54	39.54	0.031
	1	62	105.94	36.16	
Glutamine	0	13	548.77	107.31	0.042
	1	62	450.53	163.38	
Urine amino acid (mmol/g creatinine)					
Arginine	0	13	0.08	0.12	0.027
	1	57	0.18	0.13	
Cystine	0	13	0.71	0.42	0.006
	1	57	1.20	0.59	
Isoleucine*	0	13	0.61	0.32	0.001
	1	57	0.33	0.24	
Lysine*	0	13	2.12	1.58	0.025
	1	57	3.81	2.56	
Methionine*	0	13	0	0	0.029
	1	57	0.11	0.18	

In blood those amino acids had higher concentrations in infants who were fed exclusively with formula milk

In urine excretion of amino acids were higher in infants who were fed with a mixture of formula and breast milk except for isoleucine. Methionine excretion was not detectable in infants who did not get breast milk

Values are mean ± SD

SD standard deviation

gestational age, maturational age, postnatal age, birth weight and actual weight at sample collection (Table 4). As expected, Fischer's ratio depended critically on blood levels of branched chain amino acids and tyrosine but not phenylalanine. It additionally adhered to alanine and glutamine, $p < 0.05$.

Several blood amino acids correlated to each other (data not shown, $p < 0.05$).

Amino acids in urine

In urine, argininosuccinic acid, asparagine and homocystine could not be detected in any infant. Means, SD, 5th and 95th percentiles of all detected amino acids in urine are shown in Table 5; means and SD of selected urine amino acids are presented in Fig. 2. No significant gender related differences between values of amino acid excretion except for 1-methylhistidine (females 0.06 ± 0.62 mmol/g creatinine, males 0.09 ± 0.61 mmol/g creatinine; $p = 0.04$) could be found.

According to gestational age, we found the following distributions with significant differences between the three gestational age groups ($n = 70$ urine samples in total, 35 amino acids in urine were analyzed): beta-amino-butyric acid, aspartic acid, glutamate, isoleucine, 3-methylhistidine, 1-methylhistidine, methionine, tryptophane, valine, cystathionine and gamma-amino-butyric acid did not decrease significantly with gestational age, see Table 5 for further details. For correlations of 16 urine amino acids with gestational age (inversely) and of 11 urine amino acids with postnatal age at sample collection (positively) see Table 5. Inverse correlations of 13 urine amino acids with maturational age are shown in Table 5 as well.

Excretion of several urine amino acids correlated inversely with birth weight and positively with weight at urine sample collection, data are not shown, $p < 0.05$.

As in blood, excretion of several urine amino acids correlated to each other (data not shown, $p < 0.05$).

Plasma amino acids did not correlate inversely to their counterparts in urine.

Comparison of urine creatinine excretion in spot urine showed no significant difference between the three groups.

Discussion

The design of most studies concerning blood and urine amino acid profiles in preterm infants is characterized by comparison of amino acid profiles during different feeding regimens (Boehm et al. 1993; Clark et al. 2007; Cooke et al. 1992; Maggio et al. 2005; Moro et al. 1999; Polberger et al. 1990; Rigo and Senterre 1987; Schanler and Garza 1987; Szajewska et al. 2001; Tikanoja et al. 1982). We explored the effect of immaturity on amino acid patterns in blood and urine in preterm infants on enteral nutrition (mixture of fortified breast milk and special preterm formula milk). Reference values for blood and urine amino acids of term infants used in our laboratory are based on results described before (Liappis et al. 1990; Parvy et al. 1988). As the aim of our current study was to explore developmental changes of amino acids within the preterm cohort, we did not compare these profiles to commonly known reference values from a term cohort.

Table 4 Blood amino acid concentrations divided into three gestational age groups and correlations to gestational, postnatal and maturational age

Plasma amino acid	Group	Mean ± SD 5th–95th percentile (μmol/L)	Group	Mean ± SD 5th–95th percentile (μmol/L)	*p* value	Pos. corr. to GA ($p < 0.05$)	Inv. corr. to postnatal age ($p < 0.05$)	Pos. corr. to MA ($p < 0.05$)
Alpha-amino-butyric acid	1	5.35 ± 4.9	2	6.4 ± 6.7	n.s.	+		+
		0–18.2	3	11.5 ± 13.3	0.023			
	2	6.4 ± 6.7	3	11.5 ± 14.3	n.s.			
		0–25.6		0–65.0				
Arginine	1	37.7 ± 26.0	2	72.5 ± 36.4	<0.001	+		+
		3.7–88.8	3	54.2 ± 21.6	0.06			
	2	72.5 ± 36.4	3	54.2 ± 21.6	0.038			
		23.5–161.3		14.7–103.4				
Cystine	1	28.4 ± 13.4	2	40.4 ± 14.1	0.001	+	+	+
		4.5–65.35	3	41.6 ± 11.6	0.002			
	2	40.4 ± 14.1	3	41.6 ± 11.6	n.s.			
		16.9–71.4		25.3–70.8				
Glycine	1	176.8 ± 69.5	2	224.9 ± 53.8	0.003	+	+	+
		68.8–319.5	3	236.1 ± 42.2	0.001			
	2	224.9 ± 53.8	3	236.1 ± 42.2	n.s.			
		146.5–338.4		116.0–312.3				
Histidine*	1	69.7 ± 18.1	2	79.3 ± 23.4	n.s.	+		+
		41.4–103.9	3	86.9 ± 15.4	0.004			
	2	79.3 ± 23.4	3	86.9 ± 15.4	n.s.			
		48.8–128.8		57.6–114.0				
Hydroxy- proline	1	54.1 ± 19.9	2	87.4 ± 54.2	0.002	+		+
		10.85–103.8	3	75.5 ± 27.7	0.059			
	2	87.4 ± 54.2	3	75.5 ± 27.7	n.s.			
		11.7–232.7		19.6–140.3				
Isoleucine*	1	51.04 ± 14.6	2	60.8 ± 15.9	0.025	+	+	+
		25.4–81.3	3	62.7 ± 16.3	0.013			
	2	60.8 ± 15.9	3	62.7 ± 16.3	n.s.			
		38.3–95.6		39.9–110.1				
Leucine*	1	89.6 ± 25.0	2	107.9 ± 26.7	0.015	+	+	+
		57.5–147.0	3	117.1 ± 29.9	0.001			
	2	107.9 ± 26.7	3	117.1 ± 29.9	n.s.			
		70.8–161.9		73.9–201.1				
Lysine*	1	166.2 ± 48.4	2	196.1 ± 52.7	0.025	+		+
		73.8–265.1	3	197.1 ± 39.6	0.031			
	2	196.1 ± 52.7	3	197.1 ± 39.6	n.s.			
		124.7–294.6		133.5–282.7				
Methionine*	1	24.5 ± 7.1	2	33.4 ± 10.4	<0.001	+	+	+
		11.4–40.9	3	33.7 ± 8.3	0.001			
	2	33.4 ± 10.4	3	33.7 ± 8.3	n.s.			
		15.7–55.6		18.6–55.3				
Ornithine	1	80.0 ± 38.8	2	103.5 ± 35.0	0.017	+		+
		19.4–171.4	3	111.7 ± 30.9	0.003			
	2	103.5 ± 35.0	3	111.7 ± 30.9	n.s.			
		45.5–164.8		48.6–171.2				

Table 4 continued

Plasma amino acid	Group	Mean ± SD 5th–95th percentile (μmol/L)	Group	Mean ± SD 5th–95th percentile (μmol/L)	*p* value	Pos. corr. to GA ($p < 0.05$)	Inv. corr. to postnatal age ($p < 0.05$)	Pos. corr. to MA ($p < 0.05$)
Proline	1	149.3 ± 55.5	2	193.5 ± 72.1	0.006	+	+	+
		72.8–280.7	3	185.1 ± 28.9	0.035			
	2	193.5 ± 72.1	3	185.1 ± 28.9	n.s.			
		115.2–374.8		146.5–252.6				
Serine	1	105.0 ± 36.5	2	145.1 ± 40.4	<0.001	+	+	+
		58.4–182.6	3	141.0 ± 19.7	0.001			
	2	145.1 ± 40.4	3	141.0 ± 19.7	n.s.			
		78.8–217.2		99.7–170.2				
Threonine*	1	229.9 ± 96.3	2	310.5 ± 111.0	0.005	+		+
		67.5–402.9	3	302.6 ± 94.1	0.017			
	2	310.5 ± 111.0	3	302.6 ± 94.1	n.s.			
		131.9–580.6		209.5–567.0				
Tryptophane*	1	26.4 ± 10.1	2	33.9 ± 9.8	0.007	+		+
		12.1–52.2	3	31.9 ± 9.5	n.s.			
	2	33.9 ± 9.8	3	31.9 ± 9.5	n.s.			
		17.4–49.8		10.2–52.2				
Tyrosine	1	67.3 ± 25.7	2	108.0 ± 41.6	<0.001	+	+	+
		32.4–115.7	3	121.2 ± 43.4	<0.001			
	2	108.0 ± 41.6	3	121.2 ± 43.4	n.s.			
		53.5–202.2		53.1–220.1				
Valine*	1	93.6 ± 36.7	2	109.6 ± 31.2	n.s.	+	+	+
		12.6–158.5	3	131.1 ± 37.9	0.001			
	2	109.6 ± 31.2	3	131.1 ± 37.9	0.041			
		58.8–172.2		81.8–237.9				
Alpha-amino adipic acid	1	1.2 ± 1.7	2	2.5 ± 2.2	0.018			
		0–5.65	3	1.2 ± 2.1	n.s.			
	2	2.5 ± 2.2	3	1.2 ± 2.1	0.025			
		0–7.6		0–5.9				
Glutamine	1	356.3 ± 144.9	2	494.1 ± 136.5	0.001	+	+	+
		126.8–681.6	3	569.9 ± 118.3	<0.001			
	2	494.1 ± 136.5	3	569.9 ± 118.3	0.023			
		236.8–741.6		328–833				
Alanine	1	248.19 ± 133.4	2	264.54 ± 69.02	n.s.	+		+
		105.1–602.3	3	296.48 ± 66.71	n.s.			
	2	264.54 ± 69.02	3	296.48 ± 66.71	n.s.			
		146.4–406.0		180.7–434.3				
Aspartic acid	1	12.12 ± 12.25	2	8.96 ± 4.81	n.s.			
		4.0–51.45	3	12.1 ± 10.81	n.s.			
	2	8.96 ± 4.81	3	12.1 ± 10.81	n.s.			
		4.0–21.8		2.4–53.1				
Citrulline	1	21.27 ± 8.52	2	26.46 ± 11.21	n.s.			+
		5.7–40.55	3	25 ± 13.68	n.s.			
	2	26.46 ± 11.21	3	25 ± 13.68	n.s.			
		4.9–51.1		8.3–60.9				

Table 4 continued

Plasma amino acid	Group	Mean ± SD 5th–95th percentile (μmol/L)	Group	Mean ± SD 5th–95th percentile (μmol/L)	*p* value	Pos. corr. to GA ($p < 0.05$)	Inv. corr. to postnatal age ($p < 0.05$)	Pos. corr. to MA ($p < 0.05$)
Glutamate	1	67.77 ± 81.59	2	48.68 ± 20.57	n.s.			
		13.1–337.8	3	76.48 ± 56.78	n.s.			
	2	48.68 ± 20.57	3	76.48 ± 56.78	n.s.			
		27.3–102.4		28.5–288.9				
Phenylalanine*	1	47.15 ± 12.03	2	43.32 ± 10.09	n.s.			
		29.7–76.0	3	51.81 ± 11.95	n.s.			
	2	43.32 ± 10.09	3	51.81 ± 11.95	n.s.			
		25.9–64.1						
Taurine	1	127.62 ± 98.84	2	115.61 ± 46.84	n.s.			
		38.0–404.7	3	138.19 ± 73.13	n.s.			
	2	115.61 ± 46.84	3	138.19 ± 73.13	n.s.			
		55.7–245.9		80.3–363.2				
Cystathionine	1	0.35 ± 0.79	2	0.18 ± 0.55	n.s.			
		0–2.65	3	0.24 ± 0.7	n.s.			
	2	0.18 ± 0.55	3	0.24 ± 0.7	n.s.			
		0–2.0		0–2.8				
Fischer's ratio	1	2.13 ± 0.66	2	1.90 ± 0.54	n.s.			
		1.09–3.5	3	1.88 ± 0.51	n.s.			
	2	1.90 ± 0.54	3	1.88 ± 0.51	n.s.			
		1.07–3.04		1.1–3.2				

$N = 75$ blood samples in total. Values are mean ± SD and 5th–95th percentile

SD standard deviation, *n.s.* not significant, *GA* gestational age, *MA* maturational age, + sign. correlation ($p < 0.05$)

* Essential amino acids

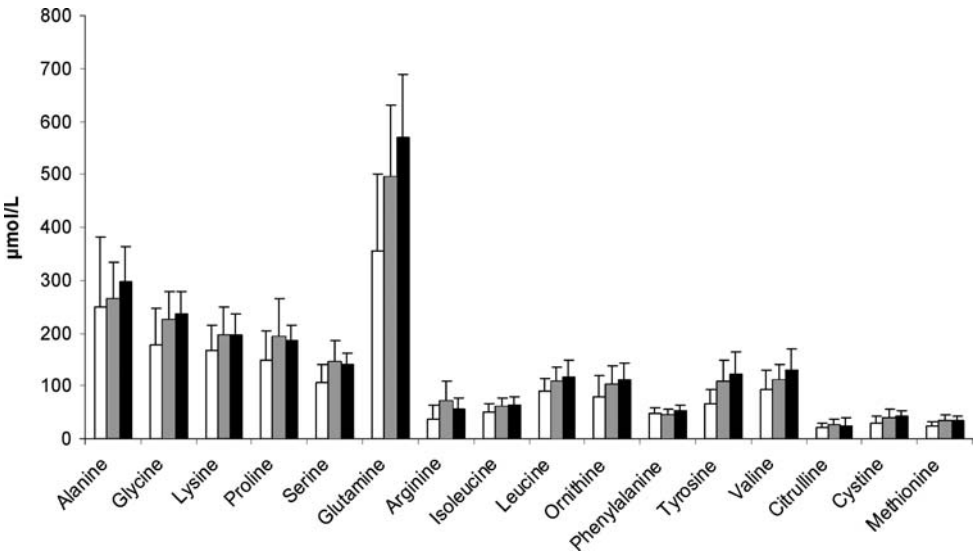

Fig. 1 Selected plasma amino acid concentrations (μmol/L) of 75 preterm infants divided into three gestational age groups. Presented are means and SD; group 1 is *white*, group 2 *grey* and group 3 *black*

Developmental changes of plasma amino acids are not limited to the neonatal period as shown by Lepage et al. They investigated the age-specific distribution of plasma amino acid concentrations in a healthy paediatric population from birth to 18 years of life (Lepage et al. 1997). Some amino acids tended to decrease during the first years of life, some did not. Some showed steadily increasing concentrations throughout infancy. Changes over time in blood amino

Table 5 Urine amino acid concentrations divided into three gestational age groups and correlations to gestational, postnatal and maturational age

Urine amino acid	Group	Mean ± SD 5th–95th percentile; (mmol/g creatinine)	Group	Mean ± SD 5th–95th percentile; (mmol/g creatinine)	*p* value	Inv. corr. to GA ($p < 0.05$)	Pos. corr. to postnatal age ($p < 0.05$)	Inv. corr. to MA ($p < 0.05$)
Alanine	1	5.4 ± 2.0	2	3.5 ± 1.3	<0.001	+	+	+
		1.5–9.1	3	2.3 ± 0.8	<0.001			
	2	3.5 ± 1.3	3	2.3 ± 0.8	0.012			
		1.8–6.4		0.80–4.2				
Beta-alanine	1	0.22 ± 0.55	2	0.03 ± 0.05	0.037	+	+	
		0–2.06	3	0.03 ± 0.07	n.s.			
	2	0.03 ± 0.05	3	0.09 ± 0.09	n.s.			
		0–0.16		0–0.20				
Alpha-amino-butyric acid	1	0.06 ± 0.05	2	0.01 ± 0.04	0.002			
		0–0.1	3	0.03 ± 0.06	n.s.			
	2	0.01 ± 0.04	3	0.03 ± 0.06	n.s.			
		0–0.1		0–0.20				
Arginine	1	0.20 ± 1.7	2	0.18 ± 0.12	n.s.			+
		0–0.48	3	0.09 ± 0.07	0.01			
	2	0.18 ± 0.12	3	0.09 ± 0.07	n.s.			
		0–0.40		0–0.20				
Citrulline	1	0.36 ± 0.27	2	0.19 ± 0.15	0.002	+	+	+
		0–0.86	3	0.07 ± 0.07	<0.001			
	2	0.19 ± 0.15	3	0.07 ± 0.07	0.04			
		0–0.56		0–0.20				
Cystine	1	1.35 ± 0.68	2	1.14 ± 0.5	n.s.	+		+
		0.4–2.9	3	0.79 ± 0.5	0.002			
	2	1.14 ± 0.5	3	0.9 ± 0.5	0.044			
		0.29–2.4		0.20–2.1				
Glycine	1	15.6 ± 6.2	2	14.8 ± 6.12	n.s.	+		
		6.9–28.7	3	10.7 ± 4.3	0.007			
	2	14.8 ± 6.12	3	10.7 ± 4.3	0.019			
		6.5–27.2		4.4–21.0				
Histidine*	1	4.1 ± 1.7	2	2.8 ± 1.3	0.001	+	+	+
		1.02–7.3	3	2.1 ± 1.0	<0.001			
	2	2.8 ± 1.3	3	2.1 ± 1.0	n.s.			
		1.1–6.2		0.1–4.2				
Hydroxyproline	1	6.37 ± 3.3	2	6.4 ± 2.3	n.s.		+	
		0.24–13.1	3	4.4 ± 1.8	0.015			
	2	6.4 ± 2.3	3	4.4 ± 1.8	0.009			
		3.8–13.1		0–7.8				
Leucine*	1	0.39 ± 0.2	2	0.39 ± 0.22	n.s.			
		0.1–0.80	3	0.25 ± 0.1	0.017			
	2	0.39 ± 0.22	3	0.25 ± 0.1	0.017			
		0.045–0.96		0–0.40				
Lysine*	1	4.6 ± 2.9	2	3.6 ± 2.3	n.s.	+		+
		1.4–11.5	3	2.0 ± 1.4	0.001			
	2	3.6 ± 2.3	3	2.0 ± 1.4	0.02			
		1.1–9.6		0.50–5.7				

Table 5 continued

Urine amino acid	Group	Mean ± SD 5th–95th percentile; (mmol/g creatinine)	Group	Mean ± SD 5th–95th percentile; (mmol/g creatinine)	*p* value	Inv. corr. to GA ($p < 0.05$)	Pos. corr. to postnatal age ($p < 0.05$)	Inv. corr. to MA ($p < 0.05$)
Ornithine	1	0.68 ± 0.7	2	0.48 ± 0.42	n.s.	+		+
		0.02–2.1	3	0.28 ± 0.26	0.005			
	2	0.48 ± 0.42	3	0.28 ± 0.26	n.s.			
		0.15–1.8		0–0.90				
Phenylalanine*	1	0.23 ± 0.12	2	0.16 ± 0.14	0.04	+		+
		0.02–0.48	3	0.11 ± 0.08	0.01			
	2	0.16 ± 0.14	3	0.11 ± 0.08	0.04			
		0–0.56		0–0.30				
Proline	1	4.5 ± 2.1	2	3.9 ± 2.3	n.s.	+	+	
		1.2–8.8	3	2.2 ± 1.6	0.001			
	2	3.9 ± 2.3	3	2.2 ± 1.6	0.006			
		1.3–9.2		0–6.1				
Serine	1	5.4 ± 2.9	2	3.9 ± 2.2	0.027	+		+
		0.16–10.8	3	2.6 ± 1.6	<0.001			
	2	3.9 ± 2.2	3	2.6 ± 1.6	n.s.			
		0.19–9.4		0.20–6.2				
Taurine	1	4.6 ± 2.6	2	4.1 ± 1.9	n.s.			+
		0.8–10.3	3	3.1 ± 1.7	0.023			
	2	4.1 ± 1.9	3	3.1 ± 1.7	n.s.			
		0.75–7.8		0–6.6				
Threonine*	1	7.2 ± 5.2	2	4.8 ± 3.1	<0.001	+	+	+
		1.6–20.5	3	2.8 ± 2.3	n.s.			
	2	4.8 ± 3.1	3	2.8 ± 2.3	n.s.			
		1.2–12.3		0.1–8.6				
Tyrosine	1	0.62 ± 0.4	2	0.59 ± 0.42	n.s.			
		0.04–1.7	3	0.38 ± 0.24	0.044			
	2	0.59 ± 0.42	3	0.38 ± 0.24	n.s.			
		0.05–1.4		0–1.0				
Alpha-aminoadipic acid	1	0.22 ± 0.14	2	0.17 ± 0.14	n.s.	+	+	
		0–0.40	3	0.14 ± 0.11	0.042			
	2	0.17 ± 0.14	3	0.14 ± 0.11	n.s.			
		0–0.40		0–0.40				
Glutamine	1	3.3 ± 2.0	2	2.8 ± 1.7	n.s.	+		
		0.32–8.2	3	1.9 ± 1.2	0.012			
	2	2.8 ± 1.7	3	1.9 ± 1.2	n.s.			
		0.33–6.9		0–4.3				
Phospho-ethanolamine	1	0.27 ± 0.29	2	0.09 ± 0.11	0.001	+		+
		0–0.9	3	0.07 ± 0.16	0.002			
	2	0.09 ± 0.11	3	0.07 ± 0.16	n.s.			
		0–0.30		0–0.60				
Beta-amino-butyric acid	1	0.13 ± 0.34	2	0.10 ± 0.15	n.s.			
		0–1.34	3	0.07 ± 0.11	n.s.			
	2	0.10 ± 0.15	3	0.07 ± 0.11	n.s.			
		0–0.46		0–0.30				

Table 5 continued

Urine amino acid	Group	Mean ± SD 5th–95th percentile; (mmol/g creatinine)	Group	Mean ± SD 5th–95th percentile; (mmol/g creatinine)	*p* value	Inv. corr. to GA ($p < 0.05$)	Pos. corr. to postnatal age ($p < 0.05$)	Inv. corr. to MA ($p < 0.05$)
Aspartic acid	1	0.161 ± 0.12	2	0.161 ± 0.16	n.s.		+	
		0–0.48	3	0.147 ± 0.11	n.s.			
	2	0.161 ± 0.16	3	0.147 ± 0.11	n.s.			
		0–0.67		0.10–0.50				
Glutamate	1	0.309 ± 0.5	2	0.289 ± 0.39	n.s.			
		0–2.0	3	0.211 ± 0.41	n.s.			
	2	0.289 ± 0.39	3	0.211 ± 0.41	n.s.			
		0–1.1		0–1.8				
Isoleucine*	1	0.313 ± 0.3	2	0.414 ± 0.26	n.s.			+
		0–0.96	3	0.426 ± 0.26	n.s.			
	2	0.414 ± 0.26	3	0.426 ± 0.26	n.s.			
		0–0.92		0–0.80				
3-Methyl-histidine	1	0.35 ± 0.21	2	0.29 ± 0.18	n.s.			
		0–0.68	3	0.35 ± 0.14	n.s.			
	2	0.29 ± 0.18	3	0.35 ± 0.14	n.s.			
		0–0.5		0–0.60				
histidine	1	0.09 ± 0.07	2	0.08 ± 0.06	n.s.			
		0–0.20	3	0.07 ± 0.06	n.s.			
	2	0.08 ± 0.06	3	0.07 ± 0.06	n.s.			
		0–0.2		0–0.20				
Methionine*	1	0.083 ± 0.15	2	0.129 ± 0.18	n.s.		+	
		0–0.4	3	0.047 ± 0.16	n.s.			
	2	0.129 ± 0.18	3	0.047 ± 0.16	n.s.			
		0–0.56		0–0.70				
Tryptophane*	1	0.23 ± 0.29	2	0.21 ± 0.28	n.s.			
		0–1.0	3	0.18 ± 0.20	n.s.			
	2	0.21 ± 0.28	3	0.18 ± 0.20	n.s.			
		0–0.86		0–0.60				
Valine*	1	0.161 ± 0.18	2	0.093 ± 0.14	n.s.	+	+	
		0–0.58	3	0.079 ± 0.11	n.s.			
	2	0.093 ± 0.14	3	0.079 ± 0.11	n.s.			
		0–0.51		0–0.50				
Cystathionine	1	0.37 ± 0.29	2	0.32 ± 0.31	n.s.			
		0–0.98	3	0.29 ± 0.24	n.s.			
	2	0.32 ± 0.31	3	0.29 ± 0.24	n.s.			
		0–1.2		0–0.60				
Gamma-amino-butyric acid	1	0.02 ± 0.09	2	0.00 ± 0.00	n.s.			
		0–0.34	3	0.04 ± 0.16	n.s.			
	2	0.00 ± 0.00	3	0.04 ± 0.16	n.s.			
		0–0		0–0.70				

$N = 70$ urine samples in total. Values are mean ± SD and 5th–95th percentile

SD standard deviation, *n.s.* not significant, *GA* gestational age, *MA* maturational age, + sign. correlation ($p < 0.05$)

* Essential amino acids

acids could also be demonstrated by Clark et al. investigating effects of two different doses of amino acid supplements on blood amino acid levels in premature infants. During the first 4 weeks, some amino acid levels decreased, few increased and were relatively constant (Clark et al. 2007). Maggio et al. (2005) studied the effects of a preterm

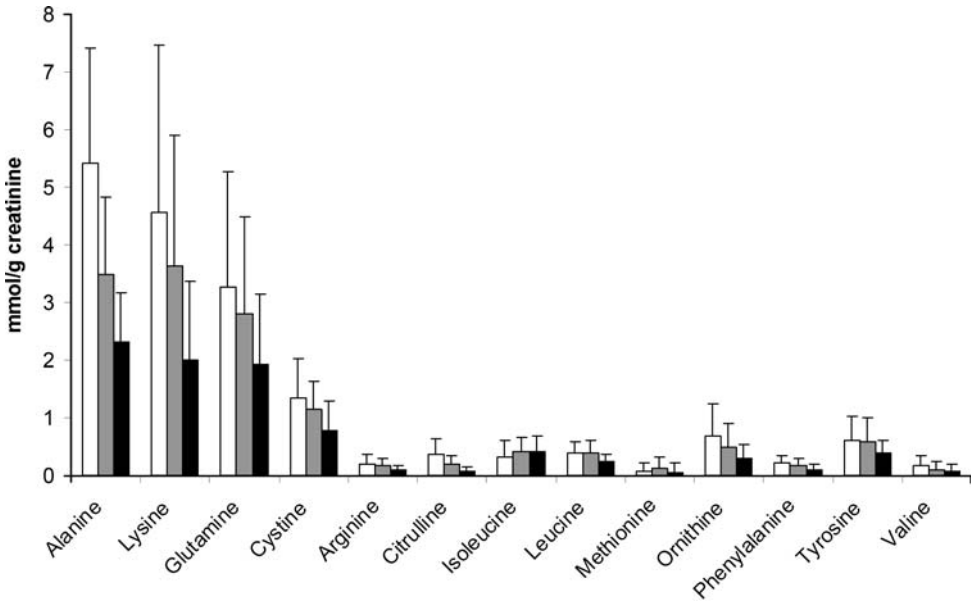

Fig. 2 Selected urine amino acid concentrations (mmol/g creatinine) of 70 preterm infants divided into three gestational age groups. Presented are means and SD; group 1 is *white*, group 2 *grey* and group 3 *black*

formula with hydrolysed cow's milk proteins on urinary and plasma amino acid levels. Renal excretion of essential amino acids tended to increase in hydrolysed formula fed infants during the first 2 weeks of life.

We explored the effect of immaturity on amino acid patterns in preterm infants on enteral nutrition as this reflects the common clinical setting for selective screening. As common, most infants received a mixture of enriched breast and formula milk; therefore differences of amino acid profiles between formula and breast-fed infants could not be detected in our study. Absolute nutrient intake and other clinical parameters did not differ between these two feeding regimens, therefore significant influence on amino acid metabolism (especially due to differences in protein intake) should be excluded. We found that infants who got a mixture of fortified breast milk and special preterm formula milk had some lower plasma amino acids with a higher excretion in urine (Table 3).

In our prospective study, we could show developmental changes in plasma and urine amino acid concentrations: substantial differences of blood and urine amino acid concentrations between three gestational age groups of our cohort were found. In total, 73% of plasma and 50% of urine amino acids that were detected correlated significantly with gestational age. Plasma amino acids increased and excretion decreased with maturation (Tables 4, 5). Arginine, cystine, glycine, histidine, hydroxyproline, leucine, lysine, ornithine, proline, serine, threonine, tyrosine and glutamine have shown this pattern in parallel in urine and plasma. Concentrations of some amino acids in blood and urine tended to decrease (in blood) and increase (urinary excretion) within the first days of postnatal life, see Tables 4 and 5. A limiting factor for interpretation of the relation of amino acids to postnatal life is the distribution of this time span between the three gestational age groups. As expected, the postnatal age at sample collection (when infants were exclusively enterally fed) was highest in the most premature infants (Table 1). High postnatal growth rates in preterm infants may increase protein requirements leading to reduced free amino acids in blood in this period. As protein intake did not differ between the three groups at blood and urine collection, contribution of amino acids from the diet would not greatly influence their concentrations in plasma and urine. Except for arginine in blood and the excretion of 1-methyl-histidine, no gender-specific amino acid pattern could be found.

Amino acids measured in plasma reflect the extracellular free amino acid pool of the organism. This pool can be influenced by protein and energy intake, maturity of metabolizing enzymes and protein turn over. Using stable isotope tracer methods, several groups have shown that preterm infants have high rates of proteolysis, which is not influenced by the administration of glucose or intravenous amino acids (Denne 2007; Hertz et al. 1993; Poindexter et al. 2001).

According to these observations, amino acids in blood and their excretion in urine were strongly related to birth weight and weight at sample collection of the preterm cohort.

Plasma amino acids did not correlate inversely to their counterparts in urine, indicating that homeostasis of plasma amino acids is not only elementary controlled by the kidneys. Development of glomerular and tubular renal function is delayed in preterm infants and proximal tubular maturation seems to be related to gestational age rather than birth weight (Jones and Chesney 1992). A possible

explanation for the development and discrepancy of age-dependent amino acid concentrations in plasma and urine in preterm infants could be that renal secretion predominates over amino acid reabsorption in the proximal tubule. This reabsorption mechanism could still be immature and contribute to lower plasma and higher urinary amino acid concentrations in very preterm infants. The tubular reabsorption of certain amino acids and the secretion of organic acids, hydrogen ions and potassium is a function of postnatal age, being relatively immature at birth, especially in the preterm infant (Jones and Chesney 1992). Apart from renal immaturity, immature metabolism in different organs may be responsible for developmental changes in amino acid profiles. For example immaturity of the liver may play a role as many amino acids are metabolised in this organ. Blood levels of methionine correlated with those of serine, ornithine, phenylalanine and tyrosine, in urine with cystathionine. Those amino acid levels are known to be linked to liver function and maturity. Serine, the major source of one carbon units, plays—together with folate—an important role in methylation of homocysteine to methionine. Furthermore, serine was linked to cystine and glycine in blood and urine, whereas serine serves as precursor for both amino acids.

The Fischer's ratio did not differ between the three gestational age groups (Table 4), whereas branched chain amino acids and tyrosine but not phenylalanine in blood increased with gestational age. In blood, branched chain amino acids correlated inversely with energy intake, a phenomenon that we could already observe in patients suffering from phenylketonuria on special protein restricted diet (Illsinger et al. 2005). Increased levels of branched chain amino acids are probably due to catabolism resulting in low insulin levels. Elevated levels of tyrosine are well known in premature infants.

Immaturity of the urea cycle in liver and kidney may be responsible for changes of amino acids involved in this pathway. Arginine adhered with citrulline, ornithine and glutamine levels in plasma; ornithine results from arginine that is metabolized by the enzyme arginase in the liver, glutamine serves as "ammonia buffer" and is consequently related to the urea cycle. Arginine and citrulline have diverse functions in the organism; they are key metabolites of the urea cycle eliminating ammonia. Arginine, an amino acid that is nutritionally essential for the fetus and neonate, is crucial for the synthesis of molecules with important regulatory function (including creatine, nitric oxide, and polyamines). As in blood, urinary citrulline excretion was linked to ornithine and glutamine levels. Furthermore, arginine excretion was related to the excretion of cystine and the dibasic amino acid lysine. As known in patients suffering from cystinuria, excretions of cystine, ornithine, lysine and arginine were associated to each other.

Citrulline is a non-protein amino acid synthesized from glutamine/glutamate and proline only in the intestine (Wu et al. 2004). Apart from its role in ammonia detoxification plasma concentrations of citrulline have been suggested as an indicator for intestinal mass or adaptation in preterm infants (Crenn et al. 2000; Wu et al. 2004).

Data are sparse regarding concentrations of amino acids in plasma and urine according to gestational age and developmental changes in preterm infants. Our study presents gestational age-specific values for concentrations of plasma and urinary amino acids. A common tendency for plasma and urinary amino acids could be observed: plasma amino acid concentrations tended to increase, whereas urinary excretions decreased with maturation. These observations could be found for several chemical groups of amino acids like neutral, basic, aromatic, sulphur as well as for heterocyclic amino acids in blood and urine (Tables 4, 5).

Many parameters, including plasma and urine amino acids, show an age-related distribution of their concentrations. However, plasma levels do not simply reflect urinary amino acid excretion. Specific age-matched reference values should be used for amino acids within the preterm population. Such data may help to provide a basis for diagnosing (inborn) metabolic abnormalities in preterm paediatric patients.

Conclusion

Our results emphasize the importance of comparing patients' amino acid data with gestational age-matched reference data in the premature. It is concluded that the assessment of the metabolic and nutritional state in preterm infants by amino acid analysis has to be based on age-specific values.

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