Brazilian Journal of Medical and Biological Research (2007) 40: 971-977
ISSN 0100-879X

Plasma amino acids in pregnancy, placental intervillous space and preterm newborn infants

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Research supported by CAPES.

Publication supported by FAPESP.

Received August 17, 2006
Accepted March 6, 2007

Abstract

Plasma amino acid levels have never been studied in the placental intervillous space of preterm gestations. Our objective was to determine the possible relationship between plasma amino acids of maternal venous blood (M), of the placental intervillous space (PIVS) and of the umbilical vein (UV) of preterm newborn infants. Plasma amino acid levels were analyzed by ion-exchange chromatography in M from 14 parturients and in the PIVS and UV of their preterm newborn infants. Mean gestational age was 34 ± 2 weeks, weight = 1827 ± 510 g, and all newborns were considered adequate for gestational age. The mean Apgar score was 8 and 9 at the first and fifth minutes. Plasma amino acid values were significantly lower in M than in PIVS (166%), except for aminobutyric acid. On average, plasma amino acid levels were significantly higher in UV than in M (107%) and were closer to PIVS than to M values, except for cystine and aminobutyric acid ($P < 0.05$). Comparison of the mean plasma amino acid concentrations in the UV of preterm to those of term newborn infants previously studied by our group showed no significant difference, except for proline ($P < 0.05$), preterm > term. These data suggest that the mechanisms of active amino acid transport are centralized in the syncytiotrophoblast, with their passage to the fetus being an active bidirectional process with asymmetric efflux. PIVS could be a reserve amino acid space for the protection of the fetal compartment from inadequate maternal amino acid variations.

Key words
- Amino acid analysis
- Placenta
- Umbilical cord
- Intervillous space
- Maternal venous blood
- Premature infants

Introduction

The systems of amino acid transport in the mammalian placenta have been identified and characterized according to functional criteria, basically by using *in vitro* preparations (1,2). At least 15 different active amino acid transport systems have been identified in the human placenta, 7 of which involving neutral amino acids. With the advances in molecular biology studies, at least 25 clones of complementary DNA for amino acid or amino acid subunit carrier proteins have been identified in trophoblastic cells (3,4).

Classically, *in vivo* studies have been conducted to analyze the relationship between plasma amino acid levels of mother and fetus (5,6). However, these relationships

can be altered by the modifications provoked in the maternal circulation by food ingestion, exercise, amino acid release from muscle into the circulation, and the circadian rhythm (7).

An interesting alternative would be the study of plasma amino acid levels in the intervillous space of the placenta. Since the maternal compartment represents the exchange interface with the syncytiotrophoblast, it provides more information about or a better understanding of the maternal-fetal physiology related to protein metabolism (7). However, the placental intervillous space continues to be a poorly understood compartment from a functional viewpoint regarding the transport of nutrients between mother, placenta and fetus. To our knowledge, there are no studies on the plasma amino acid concentrations obtained *in vivo* for the placental intervillous space in preterm gestations.

The objective of the present study was to determine if there are associations between the plasma amino acid concentrations of the maternal venous compartment, the placental intervillous space and the umbilical vein in preterm deliveries.

Material and Methods

According to the Declaration of Helsinki, all participating women gave written informed consent to participate in the study, which was approved by the Ethics Committee of the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo.

We randomly selected 14 parturients with preterm gestations. Gestational age (GA) was assessed by the method of Dubowitz et al. (8), a good method for preterm GA assessment based on a combination of detailed physical and neurologic parameters, after the first 12 h life, with the newborn in stable condition. The adequacy for gestational age was assessed by the method of Alexander et al. (9), considering the 3rd percentile as the limit between adequate and small for gestational age. Blood was obtained from a maternal peripheral vein, from the intervillous space of the placenta and from the umbilical vein of the newborn infants in order to determine the plasma amino acid levels.

Maternal venous blood (5 mL) was collected by puncture of a peripheral forearm vein after delivery using 20% EDTA as anticoagulant. Blood from the placental intervillous space was collected by the method of Meirelles and Matheus, modified by Camelo Jr. et al. (10). After placental detachment, the retroplacental clot was removed and the basal plate was closed with the membranes. The placenta was placed inside a plastic bag, which was lifted to a height that would permit the investigator to observe it, with the chorial plate looking down, and a region of the chorial plate with no fetal vessels was identified. The plastic bag was sectioned with a scissors and the chorial plate was perforated at that site with a stylet. Blood (5 mL) was allowed to drip freely and directly into the collecting tube containing dried 20% EDTA. Direct dripping into the tube reduces the possibility of hemolysis. We used the Kleihäuer test modified by Sanguansermsri (11), and the samples containing contaminating red blood cells in numbers exceeding 0.5% of the total were discarded. Blood (5 mL) was collected from the umbilical vein by puncture of the vein close to the chorial plate immediately after placental detachment.

Blood was centrifuged for 15 min at 8500 *g*, and the plasma obtained was deproteinized with 10% sulfosalicylic acid (v/v, plasma/10% sulfosalicylic acid). Only the supernatant was used after a second centrifugation. The samples were filtered through a 0.45-µm Millipore membrane and applied to the amino acid analyzer.

The quantitative and qualitative free amino acid compositions were determined by automatic amino acid analysis by ion-exchange chromatography, with ninhydrin post-column derivatization using an automatic analyzer at the Protein Chemistry Cen-

ter, School of Medicine of Ribeirão Preto, University of São Paulo, São Paulo, SP, Brazil (12). Aliquots were applied to the cationic ion-exchange column of the analyzer packed with PC-6A amino acid analysis resin (Pierce Chemical Co., Rockford, IL, USA) (short column for basic amino acids and tryptophan, long column for neutral and acidic amino acids), and eluted by pH and ionic strength gradient. After chromatographic separation, the amino acids eluted from the column reacted with ninhydrin for 15 min at 100ºC and the products of these reactions were detected colorimetrically at two different wavelengths, i.e., 440 nm for proline and 570 nm for the remaining amino acids and recorded graphically. The peaks were identified based on the retention time of each residue and the height of the peaks was used to determine the calculation factors. Differences between duplicates were ≤8%. Tryptophan was not determined.

Data were analyzed statistically by the Student *t*-test, Friedman two-way analysis of variance by ranks, Wilcoxon test for 2 x 2 comparison of correlated samples, and the Mann-Whitney test for 2 x 2 independent samples. The level of significance was set at 5%.

Results

We successfully obtained blood samples from a peripheral vein of 14 mothers and from the intervillous space of the placenta and umbilical vein of their preterm newborns.

Table 1 presents the main characteristics of the study group. Mean mother age was 26.8 years and only 3 mothers were primigestae. Six mothers delivered by the vaginal route, 1 delivery required a forceps and 7 were cesarean sections. Five of the cesarean sections were indicated due to acute fetal suffering, but all the delivered babies were in good condition at the 5th min, with an Apgar score ≥8. The mean Apgar score was 8 at the 1st min and 9 at the 5th min. GA ranged from 30 to 36 weeks (mean ± SD = 34 ± 2 weeks) and all newborns were considered to be adequate for gestational age.

Plasma amino acid levels in the three compartments studied are listed in Table 2. All amino acid values were higher in the

Table 1. Maternal and newborn infant characteristics.

Characteristics	
Maternal age (years, mean ± SD)	26.8 ± 5.6
Cesarian section	7 (50%)
Gestational age (weeks)	34 ± 2 (30-36)
Infant weight (g, mean ± SD)	1827 ± 510
Female/male ratio	7 (50%)/7 (50%)
Adequate for gestational age	14 (100%)
Apgar score (1st min ≤3)	2
Apgar score (5th min >6)	14

Table 2. Amino acid concentrations in maternal plasma, placental intervillous space (PIVS) and umbilical vein of 14 preterm newborn infants.

Amino acids (µM)	Maternal plasma	Placental intervillous space	Umbilical vein
Lysine	151 ± 72[a,b]	334 ± 94[a,d]	371 ± 91[b]
Arginine	41 ± 15[a,b]	112 ± 38[a,c]	93 ± 39[b,c]
Ornithine	38 ± 25[a,b]	59 ± 26[a,c]	81 ± 23[b,c]
Histidine	81 ± 26[a,b]	120 ± 27[a]	110 ± 35[b]
Threonine	144 ± 68[a,b]	261 ± 72[a]	252 ± 100[b]
Serine	100 ± 38[a,b]	231 ± 56[a,c]	197 ± 68[b,c]
Proline	174 ± 58[a,b,d]	283 ± 70[a,d]	276 ± 181[b,d]
Glycine	123 ± 44[a,b]	388 ± 133[a,c]	286 ± 106[b,c]
Alanine	239 ± 97[a,b]	402 ± 153[a]	359 ± 132[b]
Glutamine	173 ± 92[a,b]	270 ± 149[a]	234 ± 106[b]
Valine	122 ± 44[a,b]	225 ± 56[a]	213 ± 64[b]
Isoleucine	41 ± 28[a,b]	84 ± 45[a]	68 ± 38[b]
Leucine	71 ± 37[a,b]	161 ± 74[a]	135 ± 70[b]
Methionine	20 ± 9[a,b]	34 ± 11[a]	32 ± 12[b]
Cystine	32 ± 20[a]	49 ± 28[a,c]	36 ± 26[c]
Taurine	41 ± 25[a,b]	843 ± 379[a]	469 ± 336[b]
Tyrosine	39 ± 15[a,b]	79 ± 22[a]	73 ± 27[b]
Phenylalanine	45 ± 16[a,b]	92 ± 31[a]	81 ± 30[b]
Aspartic acid	20 ± 13[a,b]	243 ± 139[a,c]	97 ± 75[b,c]
Glutamic acid	206 ± 142[a,b]	813 ± 403[a,c]	483 ± 291[b,c]
Aminobutyric acid	18 ± 9	26 ± 20	22 ± 16

Data are reported as means ± SD. Measurements were made in duplicate and differences were ≤8%.
[a]P < 0.05 comparing maternal plasma and PIVS; [b]P < 0.05 comparing maternal plasma and umbilical vein; [c]P < 0.05 comparing PIVS and umbilical vein; [d]P < 0.05 comparing umbilical vein in term newborn infants (Ref. 13) versus preterm newborn infants (Friedman two-way analysis of variance and Wilcoxon test for correlated samples).

placental intervillous space than in maternal plasma, and this increase was statistically significant for all amino acids except aminobutyric acid. The same pattern was observed when maternal blood and umbilical cord blood were compared, i.e., all umbilical vein values were higher than the maternal values, with the difference being statistically significant for all amino acids except cystine and aminobutyric acid. When the placental intervillous space and cord blood were compared, however, a quite diverse scenario was observed. The values were similar, except for ornithine, which was the only cationic amino acid whose values in the umbilical vein were significantly higher. Arginine, another cationic amino acid, showed significantly higher values in the intervillous space compared to the umbilical cord. The values for serine, glycine and cystine (neutral amino acids) and for aspartic and glutamic acids (anionic amino acids) showed similar patterns. The remaining amino acids did not show a significant difference between the placental intervillous space and the umbilical vein.

Data from preterm deliveries were compared to previously published data from term newborn infants (13). Figure 1 compares mean plasma amino acid concentrations in the umbilical vein of preterm newborns (N = 14) to those of term newborn infants (N = 15), presented in decreasing order and reported as µmol/L ($P < 0.05$; preterm > term for proline was the only difference).

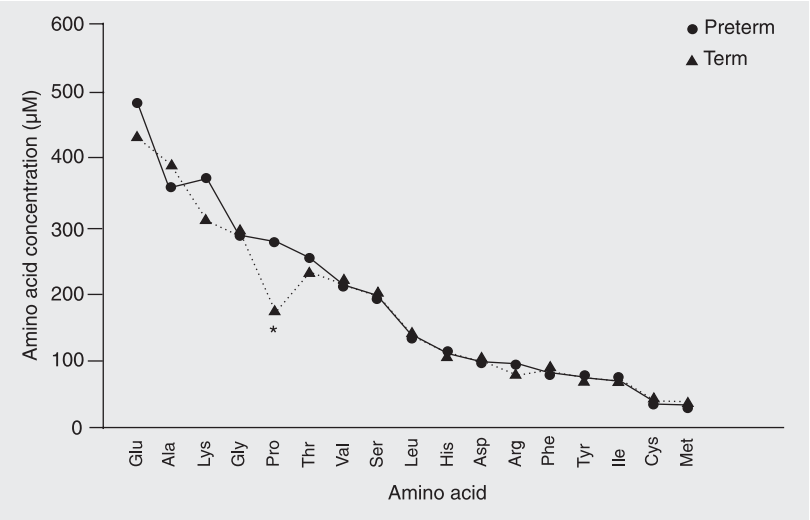

Figure 1. Mean plasma amino acid concentrations in the umbilical vein of preterm newborns (N = 14) and term newborns (N = 15) (Ref. 13). Data are reported as µM. *$P < 0.05$ (preterm > term) (Mann-Whitney test for independent samples).

Discussion

Numerous studies have been conducted on protein metabolism and on the mode of amino acid transfer from mother to fetus, with good reviews about these mechanisms available in the literature (3,4,14-21). Unfortunately most of these studies have been conducted *in vitro*. The possibility of obtaining blood from the placental intervillous space has created a unique opportunity to study maternal-fetal inter-relations *in vivo* (10).

Obtaining blood immediately after detachment of the placenta provides an accurate measure of the composition of the peripartum blood from the placental intervillous space. With this method of collection it is possible to obtain intraplacental blood with practically no direct contamination with fetal blood, thus permitting the individualized study of the three compartments, i.e., the maternal, intervillous space and fetal compartments (10). Previous studies have demonstrated that the nutrient concentrations (vitamin E, vitamin B_{12}, folate, zinc, and copper) in the three compartments and their interactions vary widely according to the nutrients studied (22-25). On this basis, the objective of the present study was to assess the amino acid concentrations in the three compartments for a better understanding of maternal-fetal relations.

The study was conducted on larger preterm infants, born in relatively good condition, and adequate for gestational age. These inclusion criteria were used to obtain a group of children without many other factors that might interfere with the amino acid

equilibrium between mother and fetus. Regarding the method for the collection of placental blood, with very small placentae, sample contamination and hemolysis frequently occur, impairing the determinations (10). Pathological situations such as maternal diabetes (26), intrauterine growth restriction (2) and hypoxia (27) can significantly alter the activity of placental transporters.

Perinatal hypoxia can interfere with placental amino acid transport, as observed in the experimental study of Nelson et al. (27); however, the eventual interference depends on the intensity and duration of hypoxia. Since system A is the most compromised by a hypoxic insult and this system preferentially transports alanine, serine and proline, we checked the plasma levels of these amino acids in the umbilical vein, and observed that all of them were within the range considered normal for healthy newborns in the literature (28), with no statistically significant differences from the umbilical vein of term newborn infants previously studied by our group, except for proline (Figure 1) (13).

Lindblad and Baldesten (5) reported the plasma amino acid concentration determined *in vivo* at delivery, from maternal vein to umbilical vein. Velázquez et al. (6) described the same parameter nine years later, determining the amino acid levels in maternal arterial and venous plasma, arterial and venous cord blood plasma and placental tissue. Considering only the maternal venous compartments and cord blood, the range of amino acid concentrations was reasonably close to those observed here, but these investigators did not study the placental intervillous space. Kamoun et al. (29) studied fetal amino acid concentrations obtained by cordocentesis in 28 gestations between 20 and 33 weeks of gestational age and compared them to adult amino acid levels. Even though some amino acids in fetal plasma agreed with the previous studies and ours, others (alanine, arginine, citrulline, cystine, glutamic acid (+ glutamine), glycine, isoleucine, and leucine) were significantly lower in fetal than in adult plasma. No association was observed between gestational age and the concentration of any of the amino acids. Probably the differences were due to the fact that the authors were not able to separate arterial and venous umbilical blood, and the levels of almost 2/3 of the amino acids are higher in venous than in arterial blood.

Interestingly, the values detected in the present study were similar to those detected in a previous study in which amino acid concentrations were also analyzed in the three compartments, but in term newborns (13). The differences in the data between preterm and term infants were very few. Only lysine in the placental intervillous space and proline in the three compartments presented statistically significant differences. Figure 1 shows the comparison of plasma amino acid concentrations in the umbilical vein between term and preterm babies. The data for term newborn infants have been used as a normality range (reference values) compared with the plasma amino acid levels of preterm newborn infants in nutritional studies (30).

Although intervillous space blood is of maternal origin, the plasma amino acid values of this compartment were, on average, 166% higher than those of the maternal venous compartment, except for aminobutyric acid. The same finding had also been obtained for term newborns (13). Theoretically, plasma amino acids would be expected to present the same concentrations in maternal blood and in the placental intervillous space, but this is not what is observed. Concentration of amino acids occurs inside the placenta despite the continuous flow of maternal blood towards the intervillous space. Active transport mechanisms probably are involved, creating a sort of reserve or space for amino acid concentrations in order to protect the fetal compartment from inadequate maternal amino acid variations.

On the other hand, the plasma amino acid levels of the umbilical vein were closer to those of the placental intervillous space than to those of the maternal venous blood (Table 2). While the values for the umbilical vein were usually slightly lower than those for the intervillous space, they were, on average, 107% higher than the values for the maternal venous compartment, except for cystine and aminobutyric acid. The same was also detected in the study of term newborns (13).

When the plasma amino acid values for the intervillous space were compared to those for the umbilical vein, a significant difference was observed for arginine and ornithine (cationic), serine, glycine and cystine (neutral), and aspartic and glutamic acids (anionic). Only for ornithine were the umbilical vein values higher than those of the intervillous space. Thus, it seems that, in general, the amino acid transport from the intervillous space to the fetus is an active bidirectional process with asymmetrical efflux (18,31). It should be emphasized that these compartments maintain an important continuous interrelation. This pattern of dependence was suggested in a study by Józwik et al. (17), which showed convergence of uterine and umbilical uptake in the low-maternal amino acid concentrations, as was the case for branched-chain amino acids.

Proteolysis has been reported to occur during labor in rats, possibly changing amino acid concentrations in the umbilical arteries more than in the umbilical vein (32). However, this phenomenon would not be sufficient to explain the high amino acid concentration observed in the intervillous space of preterm placentas.

We have demonstrated that the amino acid concentration in the placental intervillous space is much higher than in maternal plasma and is similar to that occurring in fetal plasma. This leads to the assumption that the mechanisms of active transport operate especially at the intraplacental level centralized in the syncytiotrophoblast, with passage to the fetus being an active bidirectional process with asymmetrical efflux (18, 31). The placental intervillous space could be a sort of reserve or space for amino acid concentration protecting the fetal compartment from inadequate maternal amino acid variations.

Acknowledgments

We are grateful to the Protein Chemistry Center, School of Medicine of Ribeirão Preto, University of São Paulo, and to Gilberto Padovan for the amino acid analyses.

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