Amino acid composition of parturient plasma, the intervillous space of the placenta and the umbilical vein of term newborn infants

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Abstract

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The objective of the present study was to determine the levels of amino acids in maternal plasma, placental intervillous space and fetal umbilical vein in order to identify the similarities and differences in amino acid levels in these compartments of 15 term newborns from normal pregnancies and deliveries. All amino acids, except tryptophan, were present in at least 186% higher concentrations in the intervillous space than in maternal venous blood, with the difference being statistically significant. This result contradicted the initial hypothesis of the study that the plasma amino acid levels in the placental intervillous space should be similar to those of maternal plasma. When the maternal venous compartment was compared with the umbilical vein, we observed values 103% higher on the fetal side which is compatible with currently accepted mechanisms of active amino acid transport. Amino acid levels of the placental intervillous space were similar to the values of the umbilical vein except for proline, glycine and aspartic acid, whose levels were significantly higher than fetal umbilical vein levels (average 107% higher). The elevated levels of the intervillous space are compatible with syncytiotrophoblast activity, which maintain high concentrations of free amino acids inside syncytiotrophoblast cells, permitting asymmetric efflux or active transport from the trophoblast cells to the blood in the intervillous space. The plasma amino acid levels in the umbilical vein of term newborns probably may be used as a standard of local normality for clinical studies of amino acid profiles.

The placenta, much more than a simple organ supplying nutrients to the fetus, is a highly complex metabolic organ (1). In terms of protein nutrition, a broad range of placental mechanisms have been shown to be involved in the amino acid transfer from mother to fetus. Amino acid transport is mediated by carrier proteins which in the placenta are present in the microvillous and basement membranes of the syncytiotrophoblast (2,3). Studies of transport by trophoblast membranes and of the use of functional criteria

such as substrate specificity and sodium dependence have identified 15 different active transport systems in the human placenta, 7 of which are for neutral amino acids. With the advances in the study of the molecular biology of amino acid carrier proteins, at least 25 complementary DNA clones for amino acid carriers or their subunits have been identified (3).

Cationic amino acids are actively transported by various systems on both sides of the trophoblastic cell, especially by a high capacity specific cationic system denoted y+ (3) and by three other low capacity systems called b0, + and y + L (3,4).

Among the transport systems for neutral amino acids, the A system is the most reactive with amino acids presenting linear, polar and short side chains. System 1 (previously known as system L) is more reactive with large branched and apolar chain amino acids and also with aromatic amino acids. The reactivity of the ASC system (MeAIB-insensitive L-cysteine- and L-serine-transporting electroneutral system) is more or less limited to alanine, serine, and cysteine, and, at acid pH, this system can transport aspartate and its analogues. The B or ß system is specific for amino acids such as taurine and ß-alanine. The N system transports amino acids with side chains containing nitrogen such as glutamine, asparagine and histidine. The remaining two systems are a specific system for glycine and sarcosine and a system denoted t for tyrosine transport (2,3).

Anionic amino acids are transported on both surfaces of the trophoblast by the specific system X^-_{AG} (3). Schneider and Dancis (5) observed that aspartate was concentrated 23 times and glutamate was concentrated 34 times in vesicles of the trophoblast membrane of the human placenta, whereas the other amino acids tested did not exceed a 12-fold concentration. This difference in negative umbilical arteriovenous concentration suggests uptake from the fetal circulation

(6). An explanation for these findings is the participation of the placenta, which contributes to the fetal nitrogen and amino acid balance through selective cycles in the so-called cooperative inter-organ metabolism involving anionic amino acids (1,6,7).

When mother/fetus amino acid exchanges are evaluated, the correlations between mother and fetus plasma amino acid levels are frequently analyzed. However, it is important to bear in mind that maternal blood may be affected by food ingestion, by exercise, by amino acid release from muscle into the circulation, as well as by circadian rhythm. Analysis of intervillous space blood may be a more appropriate reference point for comparison since this is an exchange interface, providing more information for a better understanding of maternal-fetal physiology (8).

In 1972, Meirelles and Matheus developed a method for blood collection from the placental intervillous space (PIVS), later modified by Camelo Jr. et al (9), which permitted a more effective evaluation of fetal-maternal exchanges by means of the correlation between the findings for maternal blood, for the PIVS and for the umbilical cord vessels. This method permits the analysis of the transfer of various nutrients and this methodology proved to be able to detect different mechanisms of passage and/or transport of different nutrients through the placenta (10-13).

The objective of the present study was to determine the similarities and differences of plasma amino acid levels in the maternal venous compartment, PIVS, and umbilical vein on the basis of the mechanisms of placental transport of the amino acids mentioned above. Our hypothesis was that the plasma amino acid levels in the PIVS should be similar to those of maternal plasma.

We selected 15 parturients with normal gestations and with no diseases identified that might compromise intrauterine growth, and with vaginal deliveries (3 by forceps and 12 normal). 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. We studied maternal blood, blood from the intervillous space of the respective placenta and umbilical vein blood from 15 term newborns, adequate for gestational age, which was calculated by the method of Dubowitz (14).

Maternal venous blood (5 ml) was collected by puncture of a peripheral forearm vein after delivery using 20% EDTA as anticoagulant. Blood from the PIVS was collected by the method of Meirelles and Matheus, modified by Camelo Jr. et al. (9). 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 EDTA. Direct dripping into the tube reduces the possibility of hemolysis. We used the Kleihäuer test modified by Sanguansemsri (9) and the samples containing contaminating fetal red blood cells in numbers exceeding 0.5% of the total were discarded (Figure 1B). Blood was collected from the umbilical vein by puncture of the vein close to the chorial plate immediately after placental detachment (Figure 1A). Blood was centrifuged and the plasma obtained was deproteinized with 10% sulfosalicylic acid (v/v, plasma/10% sulfosalicylic acid), and only the supernatant was used after centrifugation. The quantitative and qualitative free amino acid composition was determined by automatic amino acid analysis by ion-exchange chromatography with ninhydrin post-column derivatization using

an analyzer built at the Protein Chemistry Center, Faculty of Medicine of Ribeirão Preto, University of São Paulo (15).

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

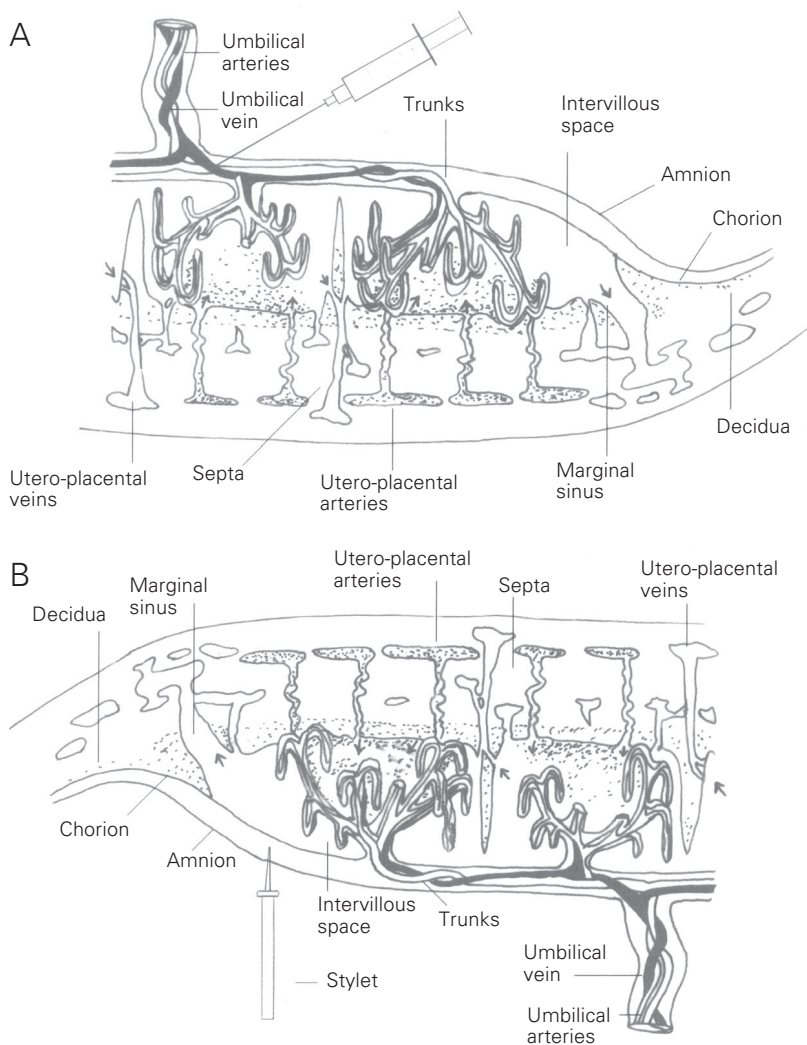The newborns were 9 females and 6 males with a gestational age of 39 ± 1 weeks (range:

Figure 1. Schematic drawing demonstrating where and how blood was obtained from two different compartments: *A*, umbilical vein puncture; *B*, chorionic plate puncture with a stylet to obtain blood from the intervillous space.

37-41 weeks) and weighing 3084 ± 392 g (range: 2570-3820 g). All infants were classified as term babies adequate for gestational age.

The amino acid levels detected in the three compartments are reported as means ± SD and are listed in Table 1. Comparison of maternal plasma and PIVS plasma amino acids demonstrated a characteristic pattern for all amino acids evaluated, except for tryptophan. PIVS values were always significantly higher than maternal plasma values, except for tryptophan that was statistically similar for maternal and PIVS plasma, with the values being 29 ± 18 and 28 ± 18 µmol/l, respectively.

Comparison of PIVS and umbilical vein amino acid levels indicated similarity for lysine, arginine, histidine, threonine, serine, alanine, valine, isoleucine, leucine, methionine, cystine, and tyrosine. In contrast, proline, glycine and aspartic acid levels were significantly lower in the umbilical vein and

tryptophan levels were significantly higher in the umbilical vein.

When maternal values were compared to those of the respective infants, a highly homogeneous pattern was again observed. Except for cystine, which did not present any difference, all other levels detected in the umbilical vein were significantly higher than maternal levels. Even tryptophan, which did not differ between maternal and PIVS plasma, was significantly higher in the umbilical vein compartment compared to the maternal one.

Lysine and arginine exhibited high concentrations in the umbilical vein compared to maternal levels. PIVS levels were significantly higher than the maternal venous compartment but similar to the levels in the fetal umbilical vein.

Neutral amino acids usually presented the same behavior as cationic amino acids when maternal levels (venous blood) were compared to fetal levels (umbilical vein), i.e., maternal levels were invariably lower than umbilical vein levels, with the only exception being cystine (32 ± 14 µmol/l in maternal venous blood and 42 ± 24 µmol/l in the umbilical vein). Although cationic and neutral amino acids are transported by different systems in the syncytiotrophoblast, the present data are consistent with the active transport mechanisms described in the literature, demonstrating a concentration of values in the direction from the mother to the fetus (2-4).

The anionic amino acids aspartic acid and glutamic acid presented significantly higher concentrations in the intervillous space compared to the umbilical vein. Studies on the transport of anionic amino acids by the syncytiotrophoblast have suggested that there is active glutamine transport from the maternal compartment to fetal blood (umbilical vein) and active glutamic acid uptake by the placenta from the fetal circulation (after fetal hepatic metabolism of glutamine), demonstrating the existence of a kind of inter-organ cooperative metabolism between

Table 1. Amino acid concentrations in maternal plasma, placental intervillous space blood and umbilical vein blood of term newborns.

Amino acids	Maternal plasma	Placental intervillous space	Umbilical vein
Lysine	104 ± 37[a,b]	252 ± 130[a]	310 ± 94[b]
Arginine	39 ± 11[a,b]	100 ± 53[a]	78 ± 29[b]
Histidine	68 ± 13[a,b]	104 ± 38[a]	108 ± 31[b]
Threonine	138 ± 54[a,b]	241 ± 118[a]	232 ± 65[b]
Serine	127 ± 57[a,b]	251 ± 136[a]	197 ± 65[b]
Proline	114 ± 52[a,b]	292 ± 343[a,c]	171 ± 87[b,c]
Glycine	119 ± 62[a,b]	435 ± 247[a,c]	291 ± 121[b,c]
Alanine	250 ± 72[a,b]	448 ± 188[a]	392 ± 102[b]
Tryptophan	29 ± 18[b]	28 ± 18[c]	40 ± 25[b,c]
Valine	111 ± 32[a,b]	208 ± 82[a]	216 ± 51[b]
Isoleucine	38 ± 16[a,b]	83 ± 41[a]	70 ± 28[b]
Leucine	64 ± 22[a,b]	162 ± 76[a]	137 ± 66[b]
Methionine	18 ± 10[a,b]	36 ± 20[a]	35 ± 24[b]
Cystine	32 ± 14[a]	64 ± 53[a]	42 ± 24
Tyrosine	35 ± 14[a,b]	67 ± 34[a]	67 ± 21[b]
Phenylalanine	44 ± 18[a,b]	93 ± 49[a]	91 ± 39[b]
Aspartic acid	24 ± 17[a,b]	302 ± 233[a,c]	100 ± 103[b,c]
Glutamic acid	168 ± 13[a,b]	594 ± 268[a]	433 ± 293[b]

Data (µmol/l) are reported as means ± SD for N = 15 samples in each group. [a]P < 0.05 comparing maternal plasma and placental intervillous space; [b]P < 0.05 comparing maternal plasma and umbilical vein; [c]P < 0.05 comparing placental intervillous space and umbilical vein (Friedman two-way analysis of variance and Wilcoxon test for correlated samples).

mother and fetus (5-7). These observations may explain the different levels between the maternal placental compartment and the umbilical vein (aspartic acid: PIVS 302 ± 233 and umbilical vein 100 ± 103 µmol/l; glutamic acid: PIVS 594 ± 268 and umbilical vein 433 ± 293 µmol/l). The present data are compatible with described mechanisms if we admit the possible existence of a passive escape from the syncytiotrophoblast cell to the blood of the intervillous space (16), not only for anionic amino acids, but also for cationic and neutral amino acids.

Studies on stable carbon isotopes with the labeling of leucine (a parameter of comparison for the placental transport of an essential amino acid), glycine and serine have identified another metabolic cycle of amino acid exchange between the placenta and the fetal liver involving serine and glycine (17). Other investigators who studied this topic (6,7) confirmed these findings. The placenta produces and supplies glycine to the umbilical vein by active transport, and glycine is metabolized by the fetal liver, where it is partially converted to serine and CO_2; serine, in turn, is actively taken up by the placenta from both the fetal and maternal circulation. In the present study glycine levels differed significantly in all compartments, with higher values in the PIVS, intermediate in the umbilical vein and lower in maternal blood. Maternal serine levels were significantly lower than PIVS and umbilical vein levels, but no significant difference was observed between the last two compartments.

The original finding of the present study was that all amino acids except tryptophan were significantly increased in the PIVS compared to maternal blood, even considering that the PIVS is a placental compartment containing maternal blood. This finding contradicts our initial hypothesis that the plasma amino acid levels in the PIVS should be similar to those of maternal plasma.

The concentrations of free amino acids in the PIVS, which were above maternal levels and also above fetal levels for some of them such as proline, glycine and aspartic acid, may be indirect indicators of the activity of the syncytiotrophoblast, which would maintain high concentrations of free amino acids inside its cells (18), permitting the asymmetrical efflux or the active transport of trophoblast cells to intervillous space blood.

Critiques of this model suggest that the concentrations in the intervillous space of amino acids from maternal arterial blood may be equal to or lower than the maternal plasma levels and much lower than the fetal levels due to the concentration by active transport at the level of trophoblast cells. However, this was not observed in the present study in the determination of plasma amino acids at the level of the intervillous space. It may be argued that blood collection after detachment of the placenta may represent a non-physiological situation, so that the altered levels in the intervillous space would not reflect the constant and stable equilibrium existing *in utero*, and that this type of maternal-fetal study may suffer distortions due to the stress caused by labor. However, this behavior observed in the intervillous space in our study seems to be limited to the amino acids. Studies on other nutrients (vitamin E and total lipids, vitamin B12 and folic acid, trace elements, and vitamin A) using the same methodology as employed in the present study have shown quite different distributions of values, compatible with differential transport mechanisms for each of these nutrients (10-13) and supporting the physiological validity of the data obtained about the amino acids.

Studies on the concentrations of free amino acids in the maternal venous blood of horses, in the umbilical venous plasma of their fetuses and in the allantochorion of their placentas have demonstrated that these concentrations are usually similar in the maternal and fetal compartments and are higher in the placental compartment (19). These observations suggest that a simple

diffusion gradient may play a partial role in the placental transfer of amino acids. One should consider the difference in placental structure between species (epitheliochorial membrane in the placenta of horses and hemochorial villous membrane in the human placenta).

Although this was not one of the objectives of the present study, the present results can be considered to be a reference standard for the plasma amino acid levels of newborns, both for the period immediately after birth and for studies of enteral and parenteral nutrition in which the adequacy of the protein sources is evaluated. Plasma amino acid levels are used on the basis of the fact that the plasma profile reflects the composition of the amino acid solution administered by the parenteral route or the amino acid composition of the feeding formula administered by the enteral route, representing an important indicator of the quality of the protein source offered. Despite the controversies about the PIVS, the plasma amino acid lev-els detected in the umbilical vein of term newborns in the present study confirm the findings of Velazquez et al. (20), among others. The situation proposed by Wu et al. (21), i.e., the use as a reference standard of amino acid profiles obtained from term new-borns aged 30 days, breast-fed and healthy, presented values fully superimposable on those of the present study, although the methodology and age range studied were different. In view of these data, the present results can be considered as a reference standard for clinical studies with amino acid profiles.

Acknowledgments

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