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Free amino acids in human fetal liver and fluids at 12–17 weeks of gestation

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The concentration of 23 free amino acids was measured in homogenates of fetal liver and samples of fetal plasma from 20 pregnancies between 12 and 17 weeks gestation and compared with those found in matched samples of maternal plasma and amniotic fluid. A feto-maternal plasma concentration gradient was observed for 21 amino acids indicating that the feto-maternal amino acid gradient across the placenta is established from very early in pregnancy. The amino acid concentration pattern was similar in fetal plasma and amniotic fluid but different in fetal liver, supporting the concept that it is essentially placental transport and metabolism that provides the fetus with these molecules. The highest amino acid concentration was found for glutamine in fetal plasma and glutamic acid in fetal liver. Very low concentrations of glutamic acid in fetal plasma suggest that this amino acid is actively taken up by the fetal liver. Citrulline, α-aminobutyric acid, methionine, arginine and tryptophan were not measurable in fetal liver tissue, indicating that this organ has a limited role *in utero* in the metabolism of these amino acids. Significant positive correlations were found between fetal plasma and amniotic fluid for the concentration of most amino acids whereas only the concentration of threonine was found to be positively correlated between fetal liver and plasma. These results suggest that during the second trimester passive diffusion through the unkeratinized fetal skin is the main pathway for amino acids between the fetal circulation and the amniotic cavity.

Key words: amino acid/amniotic fluid/fetus/liver/second trimester

Introduction

Studies of amino acid concentration in maternal blood and fetal fluids have increased our understanding of the interaction between maternal and fetal protein metabolism in normal and intrauterine growth-restricted pregnancies (McIntosh *et al*., 1984; Kamoun *et al*., 1985; Economides *et al*., 1989; Cetin *et al*., 1990, 1996; Jauniaux *et al*., 1994, 1998a). Comparative investigation of materno-fetal amino acid distribution has been performed from 18 weeks of gestation using fetal plasma and before 12 weeks using fetal coelomic or chorionic fluid. These studies have shown that the concentration of most amino acids is higher in embryological fluids and fetal plasma than in maternal plasma, indicating that the transplacental flux of most amino acids is against a concentration gradient (Sibley and Boyd, 1992).

The majority of amino acids incorporated into newly synthesized proteins in the rat fetus are supplied, all through gestation, by digestion of protein in extraembryonic tissue, mainly in the visceral yolk sac (Beckman *et al*., 1997). In the human, the yolk sac is only a transitory organ whose metabolic role is progressively taken over by the liver during the second month of pregnancy (Gitlin *et al*., 1972). The enzymatic mechanism necessary for the biosynthesis of an amino acid, such as phenylalanine hydroxylase activity, has been demonstrated in the first trimester human fetal liver (Jakubovic, 1971). However, the first and early second trimester fetal liver is primarily a haematopoietic organ and many liver enzymes are still immature at birth (Blackburn and Loper, 1992). Around 16 weeks, the fetal haematopoiesis is progressively transferred from the liver to the bone marrow and the liver starts producing bile pigment (Hamilton and Mossman, 1972). This is also the time of onset of hepatic glycogenesis. Data on human fetal liver amino acid uptake and metabolism during this transitional phase are not available.

We have therefore studied the distribution of free amino acids in the materno-fetal fluid system between 12–17 weeks of gestation and evaluated the amino acid pools in corresponding fetal liver tissue.

Materials and methods

Samples of fetal liver, fetal blood, amniotic fluid and maternal venous blood were obtained from 20 healthy patients at 12–17 weeks gestation during surgical termination of pregnancy under general anaesthesia for psychological reasons. All procedures were performed between 10.00 and 12.00 a.m. after an overnight fast of 12 h. Gestational age was determined from the date of the last menstrual period and confirmed by ultrasound measurement of the biparietal diameter and femur length. Only pregnancies which had been uncomplicated and with a fetal heart rate within normal range were incorporated in the study.

After written informed consent, fetal blood and amniotic fluid samples of 1.0 ml minimum were aspirated by transabdominal puncture. Amniotic fluid was first aspirated using a 22-gauge needle inserted inside the amniotic cavity through a 20-gauge guide. Subsequently, a new 22-gauge needle was re-introduced through the

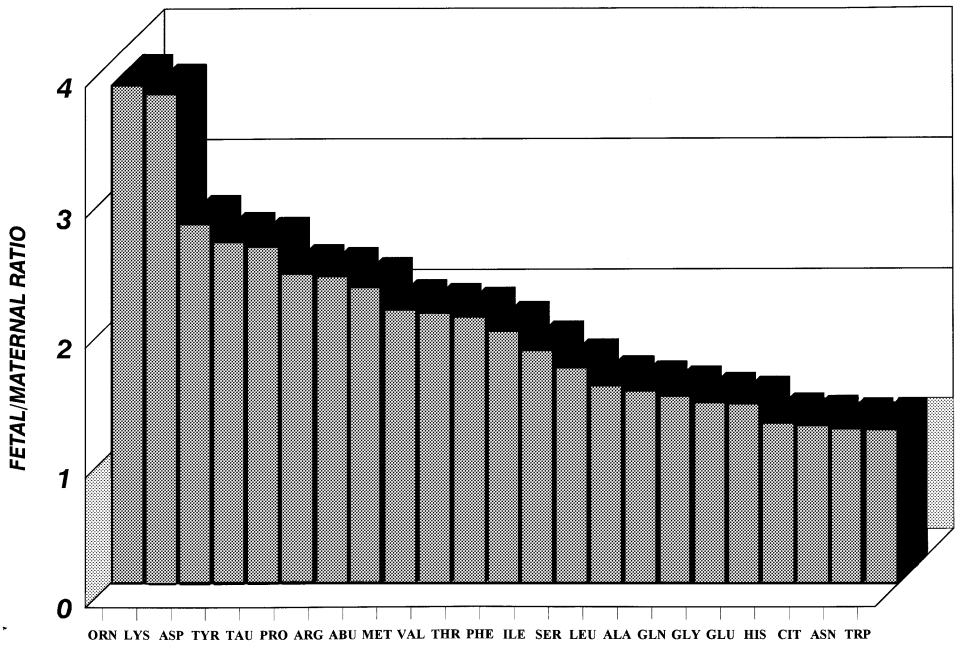

Figure 1. Feto/maternal ratio of the mean plasma concentration of 23 amino acids (standard abbreviations) at 12–17 weeks gestation.

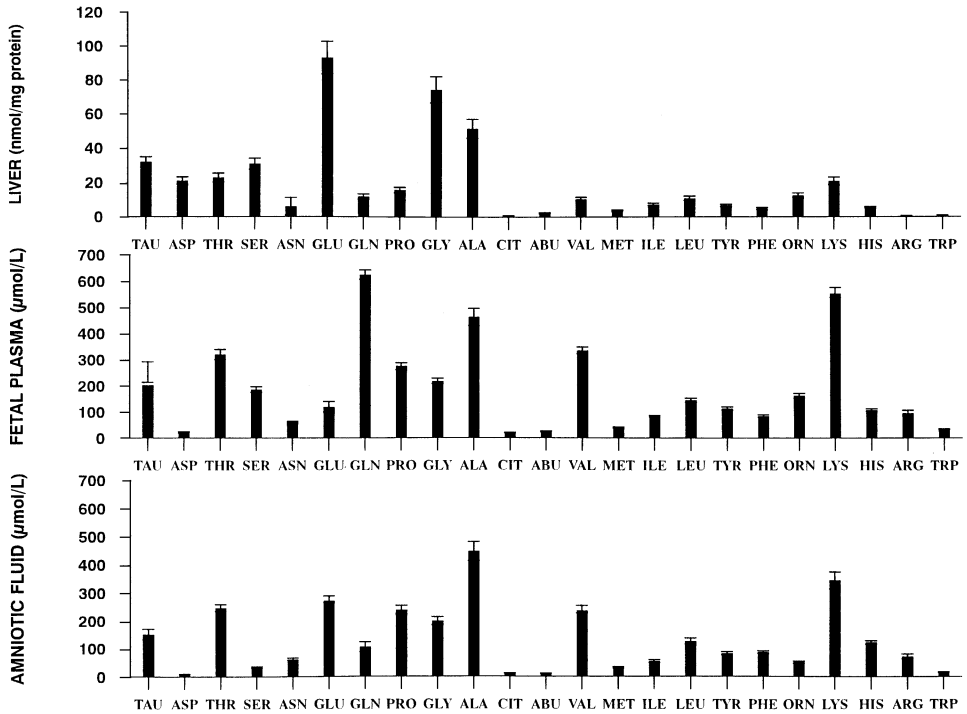

Figure 2. Mean (± SEM) concentrations of 23 amino acids (standard abbreviations) in 20 matched samples of amniotic fluid, fetal plasma and liver tissue.

guide into fetal blood via intracardiac puncture as previously described (Jauniaux *et al*., 1998b). Simultaneously, maternal blood samples were collected from an antecubital vein and centrifuged. This study was approved by the University College London Hospitals Committee on the Ethics of Human Research.

Fetal liver tissue samples were separated using a dissecting microscope and small pieces were immediately frozen in liquid nitrogen. All samples were stored for less than 6 weeks at –70°C without preservative until assayed. Samples of liver tissue were homogenized individually in five volumes of sucrose (0.25 M) containing 50 mM Tris–HCl buffer (pH 7.0), 1 mM dithiothreitol, 1 mM EDTA, 100 μg/ml phenylmethylsulphonyl fluoride, 10 μg/ml of soybean trypsin inhibitor and 2 μg/ml of aprotinin and stored at –70°C. Plasma, amniotic fluid and liver samples were each mixed with an equal volume (200 μl) of 10% (w/v) sulphosalicylic acid containing diaminobutyric acid (250 μmol/l) and left to stand at 4°C for 30 min. After centrifugation at 3000 r.p.m. for 10 min at 4°C, the pH of the supernatant was adjusted to 2.2 using LiOH 1 M. All samples were passed through a cellulose acetate centrifuge filter with 0.22 μm pore size (Micro Spin; Alltech Associates Inc., Deerfield, UK) and measured using a Biochrom 20 amino acid analyser with ninhydrin detection (Pharmacia LKB, Biochrom Ltd, Cambridge, UK). Peak

Table I. Relationships between fetal plasma (FP) and fetal liver (FL) and amniotic fluid (AF) amino acid concentrations in a series of 20 samples

Amino acid	FL versus FP		FP versus AF	
	r	*P*	*r*	*P*
Taurine	0.32	NS	0.50	< 0.05
Aspartic acid	–0.42	NS	0.08	NS
Threonine	0.65	< 0.01	0.56	< 0.05
Serine	0.08	NS	0.63	< 0.01
Asparagine	–0.34	NS	0.49	< 0.05
Glutamic acid	0.21	NS	0.44	NS
Glutamine	0.04	NS	0.20	NS
Proline	0.16	NS	–0.09	NS
Glycine	–0.22	NS	0.60	< 0.01
Alanine	0.23	NS	0.66	< 0.01
Citrulline	N/A		0.69	< 0.01
α-Aminobutyric acid	N/A		0.85	< 0.001
Valine	0.11	NS	0.72	< 0.005
Methionine	N/A		0.56	< 0.05
Isoleucine	0.25	NS	0.59	< 0.01
Leucine	–0.26	NS	0.44	NS
Thyrosine	0.21	NS	0.50	< 0.05
Phenylalanine	–0.17	NS	0.35	NS
Ornithine	0.34	NS	0.70	< 0.005
Lysine	0.19	NS	0.67	< 0.01
Histidine	–0.21	NS	0.50	< 0.05
Arginine	N/A		0.40	NS
Tryptophan	N/A		0.56	< 0.05

NS = not significant; N/A = not applicable.

integration was performed using an SP4270 integrator coupled to a personal computer equipped with WINner software (Spectra Physics, San José, CA, USA). The interassay coefficients of variation for residues were below 9%. The lower limit of detection of the method was 5 μmol/l for all amino acids, except for arginine and tryptophan for which it was 10 μmol/l.

The total protein concentration in each sample was measured by a dye-binding method (Sopachem cat. 003.0309.02; Sopar Biochem, Brussels, Belgium) with a spectrophotometric end-point. The Precimat Protein Solution (Boeringher, Mannheim, Germany) was used for the calibration curve.

Individual correlation between concentration of the different free amino acids in fetal liver, blood and amniotic fluid was calculated by the least squares method, and their slopes tested for significance by the *F* ratio test. A biomedical data processing statistical package (Statgraphics; Manugistics, Rockville, MD, USA) was used for the analysis, and results were considered statistically significant at $P < 0.05$.

Results

The feto-maternal ratio for each amino acid is displayed in Figure 1. Ornithine and lysine had ratios ranging between 3 and 4. All other amino acids had ratios ranging between 1 and 3. Glutamine, lysine, alanine, valine and threonine had the highest mean concentration in fetal plasma samples (Figure 2).

The amino acid concentration pattern was similar in fetal plasma and amniotic fluid but different in fetal liver (Figure 2). The highest fetal liver amino acid concentrations were found for glutamic acid, glycine, alanine, taurine and serine (Figure 2). Citruline, α-aminobutyric acid, methionine, arginine and tryptophan were not detectable in fetal liver tissue. Alanine had the highest median concentration in amniotic fluid samples.

Table I presents the relationships between fetal plasma and fetal liver and amniotic fluid amino acid concentrations. A significant positive correlation was found between fetal plasma and amniotic fluid concentrations of taurine, threonine, serine, asparagine, glycine, alanine, citrulline, α-aminobutyric acid, valine, methionine, isoleucine, tyrosine, ornithine, lysine, histidine and tryptophan. Only one significant positive correlation was observed between fetal liver tissue and plasma, for the concentration of threonine.

Discussion

The transport of amino acids from maternal blood to the fetal circulation occurs mainly via an active mechanism based on specific transporters located in villous syncytiotrophoblast (Sibley and Boyd, 1992). At 12–17 weeks, a feto-maternal gradient for 21 out of 23 amino acids measured was found. The amino acid pattern in maternal and fetal plasma was similar to that reported by other authors who obtained their samples by cordocentesis during the second half of pregnancy (Economides *et al*., 1989; Cetin *et al*., 1990, 1996). In particular, in this study it was observed that glutamine, lysine, alanine, valine and threonine, which are the five most abundant amino acids in fetal plasma after 18 weeks, were also the most abundant at 12–17 weeks. This indicates that the villous amino acid transporters establish a feto-maternal gradient from very early in pregnancy and that the functioning of specific transporters is similar throughout pregnancy.

The feto-maternal ratio of most amino acids at 12–17 weeks exceeded those observed in later gestational periods. It has previously been shown that the total and individual free amino acid concentrations are significantly higher in coelomic fluid samples from first trimester pregnancies than in maternal serum and that they decrease with advancing gestational age in both coelomic fluid and maternal serum (Jauniaux *et al*., 1994, 1998a). The concentration of some amino acids such as methionine and tyrosine in fetal plasma and phenylalanine and ornithine in maternal serum continues to decrease during the second and third trimesters (Cetin *et al*., 1990, 1996). Changes in fetal plasma amino acid concentrations may thus be related to variation in fetal organ amino acid uptake as pregnancy advances.

In the present study it was found that glutamic acid and glycine were in the highest concentration of any amino acids in fetal liver tissue. The glutamic acid/glutamine ratio was 7.9 compared to 0.2 in both maternal and fetal plasma. It has recently been shown (Jauniaux *et al*., 1998a) that at 7–11 weeks of gestation, the placenta also contains a proportionally high glutamic acid concentration, with a glutamic acid/glutamine ratio of 2.9. The placental transport of acidic amino acids is unique because, unlike other amino acids, they are not transferred across the human placenta (Schneider *et al*., 1979a,b). The main route of glutamic acid metabolism in the trophoblast seems to be oxidation to CO_2 and it is also metabolized to lactic acid and glucose (Broeder *et al*., 1994). The very low fetal plasma concentration of this amino acid suggests that it may be actively taken up by the fetal liver.

Data to support the production of specific amino acids within the fetal liver derive from tracer studies which cannot

possibly be performed in the human. Using a fetal lamb model, Vaughn *et al*. have recently demonstrated that maternal glutamine, which is transported across the placenta, is also taken up by the fetal liver (Vaughn *et al*., 1995) Around 40–50% glutamine is subsequently deaminated to glutamate which is then returned to the placenta, the rest is metabolized within the liver. These authors have postulated that this cycle plays a pivotal role in the provision of nitrogen to the fetus. Support for similar metabolic pathways in the human fetus is provided by the high concentration of glutamine in fetal plasma from 12 weeks of gestation. The absence of citrulline, α-aminobutyric acid, methionine, arginine and tryptophan in samples of fetal liver at 11–17 weeks suggests that the liver plays a limited role in metabolism of these amino acids in utero. By contrast, the strong positive correlation between fetal liver and plasma threonine concentrations suggests that the liver is the main source of this amino acid in the early fetus. These relationships must be further investigated using fetal liver intracellular fluid concentrations.

Amniotic fluid is technically easy to obtain and its amino acid content has been investigated from as early as 8 weeks of gestation (Queenan, 1978; Mesavage *et al*., 1985; Jauniaux *et al*., 1994, 1998a). These studies have shown that most amino acids are in lower concentration in the amniotic fluid than in maternal blood and fetal blood. The fetus swallows amniotic fluid continuously and in increasing amounts from the end of the first trimester, and thus the amniotic fluid amino acid pool may play an indirect role in fetal nitrogen metabolism. The fetal kidney starts producing urine around 10 weeks of gestation (Gulbis *et al*., 1996). Before the reabsorption capacity of proximal tubular cells is established, in the third trimester, the concentration of amino acids and proteins in amniotic fluid provides mainly evidence of fetal kidney glomerular filtration. In late pregnancy, correlation between fetal plasma and amniotic fluid amino acid concentrations can be explained by both fetal urinary excretion and diffusion from maternal circulation. However, the fetal skin only becomes keratinized around 18–20 weeks and is, therefore, highly permeable to fluids and to some dissolved solutes such as amino acids before mid-gestation (Queenan, 1978). Thus during the first half of pregnancy, passive diffusion through the large surface of the fetal skin is probably the primary mechanism for the movements of amino acids from the fetal circulation into the amniotic cavity. A significant positive correlation for most amino acid concentrations between fetal plasma and amniotic fluid, before 20 weeks, indicates that the pool of amino acids in amniotic fluid is directly influenced by the fetal plasma concentration of amino acids and supports the concept of free diffusion for most amino acids. Similar patterns for most amino acid concentrations between fetal plasma and amniotic fluid at 12–17 weeks gestation suggests also that measuring amniotic fluid amino acids may provide an indirect evaluation of placental amino acid transfer and metabolism.

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