Response of placental amino acid transport to gestational age and intrauterine growth retardation

James C. Matthews[1,2], Mark J. Beveridge[2], Marc S. Malandro[1], Michael S. Kilberg[1]
and Donald A. Novak[2]*

[1]*Department of Biochemistry and Molecular Biology and* [2]*Department of Pediatrics, University of Florida College of Medicine,
Box 100296, Gainesville, Florida 32610-0296, USA*

Fetal N requirements increase with gestational age. During the final trimester, human fetal weight dramatically increases from approximately 1 kg at 27 weeks to 3·5 kg at term, exhibiting a growth rate of 30–35 g/d (Schneider, 1996) and an amino acid requirement of 40–60 mmol/d (Smith, 1986). The ultimate source of amino acid-N for the fetus is the maternal circulation. Any evaluation of fetal amino acid requirements, however, must take into account the requirements and metabolism of maternal amino acids by the placenta. The close interrelationship between placental and fetal amino acid absorption and metabolism is exemplified by the high NH_3 production (Holzman *et al.* 1977) and glutamine synthetase (*EC* 6.3.1.2; Dierks-Ventling *et al.* 1971) activity of the placenta. Placental cytotrophoblasts and syncytiotrophoblasts are known to catabolize large amounts of glutamate by both amino transferase and deamination pathways (Broeder *et al.* 1994). This metabolism has led to the concept that placenta is the 'functional fetal liver', until the fetus develops these metabolic capacities (Remesar *et al.* 1980; Battaglia, 1992; Hay, 1995).

The present review describes the expression of amino acid transport proteins that mediate the transfer of cationic amino acids, glutamine and glutamate into and across the placenta. Knowledge of how the normal pattern of placental amino acid transport expression is regulated may facilitate the design of nutritional strategies to address pathological states of fetal development and N metabolism associated with intrauterine growth retardation (IUGR). IUGR, which may be defined as birth weight less than the 10th percentile for gestational age, affects a large number of births annually in the USA (Golde, 1989). The deleterious effects of IUGR are not limited to the perinatal period, and may present increased risks for diabetes, coronary disease and stroke (Barker, 1994).

In primates and higher rodents, the chorio-allantoic placenta is of the haemochorial type in which maternal blood comes into direct contact with the placental villous surface. In human subjects (haemomonochorial placenta), the barrier to nutrient passage between maternal and fetal circulation is the syncytiotrophoblast (Smith *et al.* 1992), with its apical (microvillus), maternal-facing, and basal, fetal-facing, plasma membrane subdomains. In rats (haemotrichorial placenta), the apical membrane of the layer II syncytiotrophoblast and the basal membrane of the layer III syncytiotrophoblast of the chorio-allantoic placenta represent the structural and functional barriers to substrate passage between maternal and fetal circulations respectively (Davies & Glasser, 1968; Metz, 1980). The structure of the haemochorial placentas of human subjects and rats differ from the epithelio-chorial and syndesmo-chorial placentas found in the sheep, pig and cow, in which nutrients derived from the maternal circulation must traverse maternal uterine tissue before reaching the fetal circulation (Munro, 1985). These differences in placental structure must be considered when comparing experimental models of placental amino acid absorption.

Mediated amino acid transport by the placenta

Cationic amino acids

Lysine and arginine demonstrate high fetal : maternal concentrations in many species (Yudilevich & Sweiry, 1985). Cationic amino acid transport across the placenta has been documented in both human subjects and rats (Wheeler & Yudilevich, 1989; Furesz *et al.* 1991, 1995; Eleno *et al.* 1994; Malandro *et al.* 1994), and the mRNA that encode proteins associated with systems y^+ (cationic amino acid transporter 1; Albritton *et al.* 1989), $b^{o,+}$, (neutral and basic amino acid transporter; Tate *et al.* 1992; Bertran *et al.* 1992), and y^+L (heavy chain of the 4F2 surface antigen; Wells *et al.* 1992; Fei *et al.* 1995; Novak *et al.* 1997) have been detected by Northern blot analysis. Na^+-independent system y^+ activity (White & Christensen, 1982; White, 1985) has been described in human and rat placenta (Furesz *et al.* 1991, 1995; Malandro *et al.* 1994). A Na^+-dependent system $B^{o,+}$-like activity is present in the rat placental apical membrane, but not in the basal membrane, nor in the apical or basal membranes derived from human placenta (Furesz *et al.* 1991, 1995; Malandro *et al.* 1994). Rat and human placentas also contain a leucine-inhibitable Na^+-independent cationic amino acid transport activity. Originally thought to be consistent with system $b^{o,+}$ (Van Winkle *et al.* 1988; Furesz *et al.* 1991;

Malandro *et al.* 1994), this activity now appears to be system y^+L (Deves *et al.* 1992; Eleno *et al.* 1994; Novak *et al.* 1997).

Glutamine

Glutamine is the most abundant amino acid in both fetal and adult serum (Cetin *et al.* 1988; Economides *et al.* 1989). In the fetus, glutamine is extensively metabolized as a substrate for synthesis of purines and pyrimidines and oxidized for metabolic energy (Windmueller & Spaeth, 1974). Utero-placental uptake of glutamine exceeds delivery to the fetus, indicating that the placenta also metabolizes glutamine (Liechty *et al.* 1991). In hepatocytes, system N (Kilberg *et al.* 1980) is responsible for the Na^+-dependent transport of glutamine, histidine and, to a lesser extent, asparagine. Although the presence of a system N-like activity in human microvillous membrane vesicles was reported (Karl *et al.* 1989), Novak & Beveridge (1997) have suggested that this activity may instead reflect transport by system y^+L. In the latter study, glutamine transport was greater in the maternal-facing (apical) membranes than in fetal-facing (basal) plasma membranes (Novak & Beveridge, 1997).

Glutamate

Although a 'non-essential' amino acid, glutamate plays an important role in placental and fetal metabolism. *In vitro*, the human placenta is capable of absorbing 49 % of the glutamate present in fetal perfusate and is thought to metabolize 80 % of that absorbed (Schneider *et al.* 1979). In an ovine *in vivo* model, the placental trophoblast has been shown to extract nearly 90 % of circulating fetal glutamate in a single circulatory passage (Vaughn *et al.* 1995), to metabolize rapidly absorbed glutamate by decarboxylation and oxidization (Moores *et al.* 1994), and to return about 6 % of the absorbed glutamate to the fetus as glutamine by the action of placental glutamine synthetase (Battaglia, 1992). This synthesized glutamine, along with the glutamine absorbed from the maternal circulation, is released into the fetal circulation where it is utilized by the fetal liver, and other tissues, as a source of N. In the liver, the primary site of fetal glutamine metabolism, there is rapid conversion of plasma glutamine to glutamate (Vaughn *et al.* 1995). Collectively, these processes illustrate the importance of glutamate absorption by the placenta and define a feto-placental 'glutamine–glutamate cycle' (Schneider *et al.* 1979; Vaughn *et al.* 1995), which acts to shuttle amino acid-N to the fetus in the form of glutamine and to return glutamate to the placenta. Much of the returned glutamate is oxidized for metabolic fuel, thus sparing glucose for use by the fetus (Moores *et al.* 1994; Takata *et al.* 1994). Glutamate also may be used to generate NADPH for placental fatty acid and steroid synthesis (Moores *et al.* 1994; Vaughn *et al.* 1995). Another important consequence of placental glutamate uptake from the fetal circulation may be the protection of the fetus from potentially neurotoxic levels of glutamate (Broeder *et al.* 1994).

Absorption of glutamate is thought to occur by concentrative transport mechanisms, because the milli-molar placental concentrations of anionic amino acids far exceed the micromolar concentrations detected in maternal and fetal blood (Dierks-Ventling *et al.* 1971; Phillipps *et al.* 1978; Schneider *et al.* 1979). Five complimentary DNA (GLAST1, GLT1, EAAC1, EAAT4, EAAT5) thought to encode proteins capable of Na^+-dependent, D-aspartate-inhibitable glutamate–aspartate transport activity, termed system X_{AG}^- (Gazzola *et al.* 1981), have been cloned (Kanai & Hediger, 1992; Pines *et al.* 1992; Storck *et al.* 1992; Fairman *et al.* 1995; Arriza *et al.* 1997). The detection of mRNA for all glutamate transport proteins except EAAT5 in human placenta (Arriza *et al.* 1994; Fairman *et al.* 1995; Nakayama *et al.* 1996) suggests that they contribute to the system X_{AG}^- activity in placental vesicles (Moe & Smith, 1989; Hoeltzli *et al.* 1990). In the rat, over the last trimester, we have observed an increase in the steady-state mRNA levels for GLAST1, GLT1, EAAC1 and EAAT4, and determined that Na^+-dependent glutamate transport across the apical and basal plasma membrane of labyrinth tissue during the last trimester is primarily a function of system X_{AG}^- activity, in a manner that was consistent with a differential expression of GLAST1, GLT1 and EAAC1 proteins (Malandro *et al.* 1996; Matthews *et al.* 1997).

The previously mentioned studies address only transport processes at the syncytiotrophoblast layer. Maternal–fetal interactions, however, also occur elsewhere within the placenta. Maternal blood directly bathes spongiotrophoblast basophilic cells, glycogen cells and junctional giant cells (Davies & Glasser, 1968). Each of these cell types is thought to have specific functions, perhaps the best described of which is the production of endocrine hormones by the junctional giant cells of the rat and mouse placenta (Lee *et al.* 1988; Faria *et al.* 1991; Yamaguchi *et al.* 1994; Soares *et al.* 1996). Consistent with these findings, we have observed unique patterns of expression for GLT1, GLAST and EAAC1 proteins, throughout the chorio-allantoic rat placenta (Matthews *et al.* 1997).

Patterns of increased placental amino acid transport with gestational age in the rat

Fetal : maternal serum arginine and glutamine values increase with gestational age (Economides *et al.* 1989; Bernardini *et al.* 1991), which suggests that the capacity for concentrative transfer of these nutrients by the placenta increases with gestational age. To determine potential mechanisms responsible for this phenomenon, the relative increase in the activity of amino acid transport systems that recognize cationic and neutral substrates was determined in the apical and basal membranes of the rat syncytiotrophoblast (Table 1). The type and membrane-specific distribution of transporter activity at day 20 in the rat are similar to that observed for the term human placenta (Smith *et al.* 1992; Moe, 1995), except for the presence of Na^+-dependent system $B^{o,+}$ in the apical membrane (Furesz *et al.* 1991, 1995). All amino acid transport systems monitored displayed an increased transport capacity near term (day 20), as compared with the beginning of the third trimester (day 14). The concomitant and marked increase in apical Na^+-

dependent system $B^{o,+}$ and Na^+-independent systems y^+ and y^+L will facilitate an increased flux of cationic amino acids from the maternal circulation into the placenta. The subsequent high levels of cytosolic cationic amino acids will permit their downhill transfer across the basal membrane into the fetal circulation, thus meeting the increased demands of both tissues for these essential amino acids. Analogously, increases in the apical membrane transport capacity of both systems A and $B^{o,+}$, along with the increase in system y^+L capacity in the basal membrane, will ensure the potential for an increase in the translocation of glutamine (and other small neutral amino acids) into the placenta and fetus. Collectively, these observations suggest that the increased fetal : maternal serum cationic and neutral amino acid values during the final trimester of gestation, the period of greatest growth by the rat and human placenta and fetus (Schneider, 1996), is facilitated by an increased capacity for amino acid transport across both plasma membranes.

The fetal : maternal serum glutamate value also increases with gestational age (Economides et al. 1989; Bernardini et al. 1991). Recently, we have observed a small increase in system X_{AG}^- activity from day 14 to day 20 on the basal membrane of rat placenta and, in contrast, a large increase in system X_{AG}^- activity on the apical membrane (Matthews et al. 1997). When considered in terms of the glucose-sparing effect of placental glutamate absorption and oxidation, this observation suggests that the placenta primarily increases its supply of oxidizable glutamate by increasing its capacity to absorb maternal-derived glutamate.

Effect of a low-protein diet (intrauterine growth retardation pregnancy) on placental amino acid transport

Maternal factors that contribute to IUGR in human subjects include pathological conditions, alcohol, cocaine, tobacco, and malnutrition (Sastry et al. 1989). In IUGR pregnancies of unknown aetiology, the uptake of lysine by placental slices (Yamaguchi et al. 1978) and the rate of system A-mediated 2-amino isobutyric acid transport (Dicke & Henderson, 1988) was diminished in placental tissue. Although these in vitro investigations support the in vivo observation of impaired uptake of 2-amino isobutyric acid from the maternal to the fetal circulation (Sybulski & Tremblay, 1967), fetal serum levels of alanine, a primary system A substrate, are slightly higher in small-for-gestational-age fetuses than in appropriate-for-gestational-age fetuses (Economides et al. 1989).

Diminished placental blood flow in human subjects is often associated with IUGR (Bracero et al. 1989); therefore, although protein malnutrition results in smaller fetuses with poor developmental outcomes (Rosso, 1980), the relative contribution of diminished utero-placental blood flow v. specific effects on placental amino acid transport mechanisms has not been established.

In rats, maternal malnutrition-induced IUGR has been associated with (a) reduced blood flow in the placenta (Rosso & Kava, 1980), (b) reduced rates of amino acid transfer to the fetal circulation (Rosso, 1975), and (c) reduced fetal and placental weights (Rosso, 1980). Alterations in the coordinated expression of transporters on the apical and basal domains of the placental syncytium may critically reduce the trans-epithelial flux of amino acids across the placenta and (or) through the metabolic cycles that exist between the placenta and fetus. The effects of low-protein-diet-induced IUGR on the capacity of placental amino acid transport systems has been examined using a rat model (Table 2; Malandro et al. 1996). System A transport capacity was decreased by 55 % on the apical plasma membrane and 50 % on the basal plasma membrane subdomains of labyrinth trophoblasts of placentas isolated from IUGR dams. The capacity for system ASC-mediated transport of neutral amino acids, and system $B^{o,+}$ for neutral and cationic amino acids, was not altered. However, neutral and cationic amino acid transport mediated by system y^+L (Novak et al. 1997) was reduced by approximately 70 % on the basal membrane. System y^+ activity also was reduced (20 %) on the apical membrane, which was consistent with the concomitant decrease in total steady-state cationic amino acid transporter 1 mRNA isolated from the whole chorio-allantoic placenta. In basal, but not apical, plasma membranes, Na^+-dependent glutamate uptake and EAAC1 mRNA were decreased (about 50 %) in placentas isolated from IUGR dams (Malandro et al. 1996).

There was a reduction in fetal serum glutamate concentration and system X_{AG}^- activity in the basal placental membrane in the IUGR pregnancies (Malandro et al. 1996). In contrast, maternal serum glutamate concentrations and the activity of system X_{AG}^- in the apical membrane was unchanged (Malandro et al. 1996). These results may be indicative of substrate-controlled transporter localization. The specific mechanisms that sense serum amino acid availability and transduce this information into differential plasma membrane subdomain expression in the placenta have not been described. Amino acid-dependent

Table 1. Development of cationic and neutral amino acid transport capacity in rat placenta*

Gestational age (d) ... Transport system	Apical membrane		Basal membrane		Reference
	14	20	14	20	
A	+	+ + +	+ / −	+ +	Novak et al. (1996)
$B^{o,+}$	+	+ + +	−	−	Malandro et al. (1994)
y^+	+	+ + + +	+	+ +	Malandro et al. (1994)
y^+L	+ / −	+ +	+	+ + +	Malandro et al. (1994); Novak et al. (1997)

A, Na^+-dependent uptake of 2-(methylamino)isobutyric acid; $B^{o,+}$, Na^+-dependent uptake of arginine; y^+, leucine-resistant Na^+-independent arginine uptake; y^+L, leucine-inhibitable Na^+-independent arginine uptake.

*The absence (−) or presence (+) of transport activity measured in the indicated day of gestation of rat placenta. The presence of transport activity, within a transporter system, is qualitatively scaled from '+' to '+ + + +'.

Table 2. Effect of low-protein-diet-induced intrauterine growth retardation on rat placental amino acid transport (from Malandro *et al.* 1996)

Transport system... Membrane	A	ASC	$B^{o,+}$	y^+L	y^+	X_{AG-}
Apical	Decreased*	NS	NS	NS	Decreased*	NS
Basal	Decreased*	NS	NS	Decreased*	NS	Decreased*

A, Na^+-dependent uptake of 2-(methylamino)isobutyric acid; ASC, Na^+-dependent uptake of serine in the presence of 2-(methylamino)isobutyric acid; $B^{o,+}$, Na^+-dependent uptake of arginine; y^+L, leucine-inhibitable Na^+-independent arginine uptake; y^+, leucine-resistant Na^+-independent arginine uptake; X_{AG}^-, Na^+-dependent uptake of glutamate.
There was a significant difference when compared with control, *$P < 0.05$.

changes in gene expression have been documented for a number of proteins, including amino acid transporters (Shay *et al.* 1990; Hyatt *et al.* 1997), although hormone signalling is also a likely regulator of amino acid transporters in IUGR pregnancies (Warshaw, 1990).

Hormones and growth factors that are likely to mediate the effects on intrauterine growth retardation on placental amino acid transport

The development of null-mutation mice models has allowed investigation of the potential role of specific hormones and growth factors in IUGR-mediated alteration of normal placental and fetal development. Deficiency of maternal epidermal growth factor (EGF) causes severe IUGR in rats (Kamei *et al.* 1993), and null mutation of the EGF receptor causes placental disruption in homozygous fetuses (Sibilia & Wagner, 1995; Threadgill *et al.* 1995). Conversely, administration of EGF to pregnant rats has little effect on fetal size (Ali *et al.* 1990; Jansson & Skarland, 1990), and serum levels of EGF have not been clearly associated with IUGR. EGF is not produced by the fetus through most of gestation (Raaberg *et al.* 1988; Snead *et al.* 1989); knockout of transforming growth factor α, which is thought to interact with the EGF receptor during gestation, has little effect on fetal development (Mann *et al.* 1993).

In contrast, growth hormone (GH) and GH receptor are present in the fetus (Strosser & Mialhe, 1975; Garcia-Aragon *et al.* 1992; Gluckman *et al.* 1992), and decreased fetal levels of GH in human subjects may cause mild IUGR (Gluckman *et al.* 1992). However, infusion of rat dams with an antibody against GH-releasing hormone increases fetal weights, in association with elevated insulin-like growth factor (IGF)-1 and -2 levels (Spatola *et al.* 1991). Further confounding our understanding of the relationship between IUGR and GH are the observations that high doses of supplemental GH given to rat dams fed on an energy-restricted diet were associated with reduced maternal wasting and exacerbated fetal growth retardation (Chiang & Nicoll, 1991), and that transgenic mice, which constitutively secrete a large amount of GH, produce growth-retarded fetuses (Naar *et al.* 1991).

Insulin, also, is a critical fetal growth factor and fetal pancreatectomy causes profound growth retardation (Fowden *et al.* 1989). Null mutation of the fetal insulin receptor substrate-1 gene, involved in signalling from both the insulin receptor and IGF-1 receptor, produced profound IUGR (Araki *et al.* 1994), as did homozygous nonsense

mutation of the human insulin receptor (Krook *et al.* 1993). Surprisingly, null mutation of the mouse insulin receptor produces little or no effect on intrauterine growth, which suggests that the growth-promoting effects of insulin in the mouse are mediated, at least in part, through the IGF-1 receptor (Accili *et al.* 1996; Joshi *et al.* 1996). Therefore, IGF-1, IGF-2 and the IGF-1 receptor must be considered as potential effectors of IUGR, as the mRNA for all three are expressed in the developing rat placenta (Pescovitz *et al.* 1991; Zhou & Bondy, 1992; Redline *et al.* 1993).

IGF-1 mRNA expression peaks within the rat placenta at approximately day 10 of gestation, and is either absent or in low quantities after day 14 (Pescovitz *et al.* 1991; Redline *et al.* 1993). Placental IGF-2 expression begins at approximately 10 d of gestation, rising to a maximum and stable level by day 15 (Pescovitz *et al.* 1991; Zhou & Bondy, 1992; Redline *et al.* 1993). Whereas maternal serum IGF-1 levels peak at mid-gestation and then fall by approximately 50 %, maternal serum IGF-2 levels are low or undetectable throughout gestation (Gargosky *et al.* 1990). Fetal IGF-1 concentrations are correlated with fetal growth in the human subject (Lassarre *et al.* 1991; Leger *et al.* 1996).

Most *in vivo* studies have involved the administration of pharmacological doses of the hormone or growth factor to the mother; therefore, effects of fetal secretion are largely unknown. In addition, it is difficult to rule out secondary effects of the infused hormone or growth factor; for example, the regulatory effects of GH or EGF on IGF-1 production (Chernausek *et al.* 1991; Rotwein *et al.* 1993). Null mutations of growth factors or hormones and receptors of these ligands help to clarify these issues. Null mutations of IGF-1, IGF-2, and IGF-1 receptor (which mediates fetal effects of IGF-1 and, to a significant degree, IGF-2) result in severe IUGR (DeChiara *et al.* 1990; Baker *et al.* 1993; Liu *et al.* 1993; Lopez *et al.* 1996). IGF-2-knockout mice also have small placentas (DeChiara *et al.* 1990). Using null-mutation mouse models, our initial investigations indicate that IGF-2 and IGF-1 receptor differentially affect the expression profiles of specific anionic amino acid transporters through transcription and post-translation events (DA Novak, JC Matthews, MJ Beveridge, A Efstratiadis, E Dialynas, A Bartke and MS Kilberg, unpublished results).

In summary, the study of placental amino acid transport regulation is in its infancy. Some of this information is contradictory. The results of whole-tissue flux studies, using stable isotopes, has generated new theories of maternal–placental–fetal amino acid metabolism. Using newly-developed molecular reagents, several laboratories

have begun to determine the cellular distribution and relative abundance of amino acid transporter mRNA and the proteins responsible for these activities in the placenta. Likewise, studies using null-mutation and transgenic mice to mimic IUGR and other pathological states have begun to describe the hormonal or growth factor control over expression of amino acid transport in placental tissue during normal and disease states.

Acknowledgement

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