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Transport and Metabolism of Amino Acids in Placenta

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In all mammalian species, the 20 amino acids of the genetic code are required for net protein accretion. The nutritional supply of amino acids for growth is defined as the net umbilical uptake of amino acids, representing the net transfer from maternal circulation, through the placenta and then to the fetus, of essential and nonessential amino acids. In considering the primary role of the placenta in the delivery of amino acids to the fetus for metabolism, it is important to consider the multiplicity of factors that may affect these overall delivery rates, including the activity and location of amino acid transporter systems, changes in these systems as gestation advances, effects of changes in placental surface area, uteroplacental blood flows, and maternal concentrations of amino acids. In this review, we discuss placental amino acid transport, the systems and their associated proteins, umbilical uptake data in animal and human studies, and amino acid transport in fetal growth restriction. Additionally, we discuss the current pool of thought concerning the mechanisms of placental amino acid transport as generated through in vitro vesicle studies and how they relate to the in vivo fluxes of animal studies. Finally, we discuss fetoplacental amino acid metabolism and specifically interorgan exchange.

Key Words: Amino acid; placenta; transport systems; transporter proteins; vesicles; fetus; FGR.

Introduction

The placenta plays a critical role in the delivery of amino acids to the fetus, which are essential for fetal growth. The understanding of amino acid transport systems and metabolism, within the placenta, is of great importance in our understanding of how this specialized organ functions. The subject of placental transport and metabolism of amino acids has been reviewed many times in recent years *(1,2)*, largely because of the information that has become available with respect to the transporter proteins that convey specificity to the trophoblast membranes for amino acid transport *(3,4)*.

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The study of placental amino acid transport has involved the use of many different animal models *(5–13)*, and one of the goals of comparative physiologic studies is to identify principles that would apply equally well to understanding human placental amino acid transport and metabolism. There are major differences across species in terms of placental structure and function. However, three observations suggest that qualitatively the results obtained in sheep studies occur in similar fashion for the human placenta:

1. Both serine and glutamate are taken up from fetal circulation into the placenta, where they undergo metabolism, serine into glycine and glutamate oxidized to CO_2 *(14,15–20)*.
2. The rapidity of transplacental transport of amino acids follows an order that, thus far, appears to be similar in the two species *(21,22)*.
3. In fetal growth restriction (FGR) for both human and sheep pregnancies, the transplacental flux of some essential amino acids (leucine, threonine, and phenylalanine *[12,13,22]*), is significantly reduced.

For comparative purposes, one needs to be aware of differences among species in placental morphology, fetal body composition, and placental and fetal growth rates that determine the accretion rate of amino acids. Understanding these differences in relation to amino acid transport across the placenta is a new and expanding area of investigation.

This review examines the placental transport and metabolism of amino acids, with a special emphasis on unifying and interpreting in vivo data, principally generated in the sheep model, and in vitro data.

Placental Amino Acid Transport Systems and Their Proteins

The transport of amino acids across the trophoblast involves three fundamental steps. First, the uptake from the maternal circulation across the microvillous membrane; second, the transport through the trophoblast cytoplasm; and third, the transport out of the trophoblast, across the basal membrane into the umbilical circulation. Transport systems are required for the two steps that involve transport across plasma membranes, i.e., the maternal microvillous and fetal-facing basal membrane surfaces. Amino acid transporter nomenclature was first defined by the studies of Christensen *(23–25)* and Van Winkle *(26)*, who examined a wide range of nonepithelial and epithelial cell types. Transport systems

were arranged based, first, on whether they were sodium dependent or sodium independent; and, second, on whether systems preferred cationic, zwitterionic, or anionic substrates.

In recent years, both in vivo *(28–32)* and in vitro *(33–45)* studies have brought out the complexity of the placenta as an organ for amino acid transport and metabolism. The concentration of most amino acids, including some of the essentials, is higher in fetal than in maternal plasma *(28,31, 46)*, consistent with a process of active transport of amino acids across the placenta. An increased fetal-to-maternal ratio of plasma amino acid concentrations has been documented for humans *(47,48)*, primates *(49)*, rats *(50)*, guinea pigs *(51–53)*, sheep *(28,31,32,54)*, and cows *(56)*. Although the fetal/maternal concentration ratio is >1.0 for most amino acids measured, there are significant differences among species. For example, the fetomaternal ratio in human whole blood for the cationic amino acids lysine and histidine is consistently greater than 1.0, whereas in sheep it is <1.0 *(55)*.

Mammalian transport systems have been characterized over the years by such general properties as ion dependence, kinetics, substrate specificity, competitive inhibition, and regulation of activity. This process has led to the description of multiple transport systems for neutral, cationic (basic), and anionic (acidic) amino acids *(57,58)*. It is now becoming possible to move to a more precise description of the protein chemistry of the transporter system. The ability to clone, sequence, and study the expression of transporters has led to an explosion of data concerning the molecular basis of amino acid transport systems and their potential in vivo function *(3,4,27)*. Some placental transporters exist as a monomeric protein, while other transport systems exist as heterodimeric proteins *(27,59,60)*. Those monomeric transport systems induce amino acid uptake in a manner similar to the amino acid uptake associated with permeases described in microorganisms *(61–64)*, although the regulation of placental membrane acquisition of monomeric systems has not yet been studied. Heterodimeric systems involve the grouping together of two proteins to facilitate amino acid transport. In placental tissues, monomeric transport systems are believed to include y^+ *(65)* and most likely the X_{AG}^- system, while heterodimeric transport systems include System L *(66,67)*, y^+L *(68)*, and the sodium-independent Asc system *(67,69)*. Those systems that operate in a heterodimeric manner are generally made up of a common heavy chain, specifically one of the type II membrane glycoproteins, 4F2hc (comparable to the CD98 surface antigen, CD98hc *[70,71]*), the neutral and basic amino acid transport (NBAT, equivalent to rBAT or D2 *[72–75]*) protein, or as-yet undefined proteins *(76)*, which associate with one of a variety of light chain transport proteins, providing a range of amino acid transport systems *(60,67,68,70,77)*. There is also evidence that these heavy chains may interact with themselves and each other to form homodimers and/or heterodimers before interacting with a specific light chain to induce activity *(78)*.

Heavy-chain mRNA is ubiquitously expressed *(79)*. The 4F2hc protein is expressed in placental tissues throughout pregnancy, whereas the NBAT protein appears to be expressed only in first- and early second-trimester placentae and not in term tissues *(71,78,80)*, nor in the human choriocarcinoma cell line JAR *(80)*. During human and rat pregnancy, whole-tissue studies have demonstrated 4F2hc predominantly in the microvillous membrane, with the content increasing as gestation advances *(66,71,78)*. However, 4F2hc has not been detected in the human basal membrane *(66,78)*, despite reports of 4F2hc mRNA in rat placental basal membrane preparations *(71)*, and evidence that 4F2hc plays a role in basal membrane amino acid transport in other polar tissues, including the intestine and kidney *(75)*.

The glycosylated 4F2hc protein is composed of a single transmembrane region with a large C-terminal extracellular domain *(70,81,82)*. Because mammalian substrate transporters appear to have multiple transmembrane helices, it is unlikely that the 4F2hc protein is capable of inducing amino acid transport alone *(83)*. It is postulated that 4F2hc is in fact the modulator of heterodimeric transport systems and acts as a guidance molecule, translocating the nonglycosylated light chains to the plasma membrane, from an intracellular pool, after which disulfide linkage between 4F2hc and the light-chain occurs *(60,84–86)*. The subcellular location of the light-chain proteins and their interaction with 4F2hc protein is not well defined. Presently, the model of interaction involves an intracellular pool of light chains that is available for incorporation with the membrane-bound heavy chain *(65,70)*. Following incorporation into the membrane, functional transport then occurs, as both heavy and light chains and their interaction are required for transporter function *(60,80,87)*. The expression of the heavy chain, and not that of the light chains, is best correlated with placental amino acid transport system activity *(65,70)*.

Figure 1 represents a summary of traditional amino acid transport systems described for the maternal and basal membranes of the trophoblast, showing that each system is distinct, but exhibits some overlapping substrate specificity. This complexity is furthered by the fact that traditionally identified amino acid transport systems, such as the neutral amino acid system, System N, actually transports both neutral (glutamine) and cationic or basic (histidine) substrates. Hence, some transport systems are represented more than once and others such as the β system, traditionally associated with neutral amino acids, transports taurine and is shown in the anionic section of Fig. 1. Figure 1 also shows the reported light-chain proteins for the individual transporter systems. Identification of individual transporter proteins in the trophoblast will be expanded considerably as sequence data for the specific transporters permit rapid identification of other members of the same family of transporters. Thus, the information provided in Fig. 1 should be regarded as an interim report in a rapidly changing field.

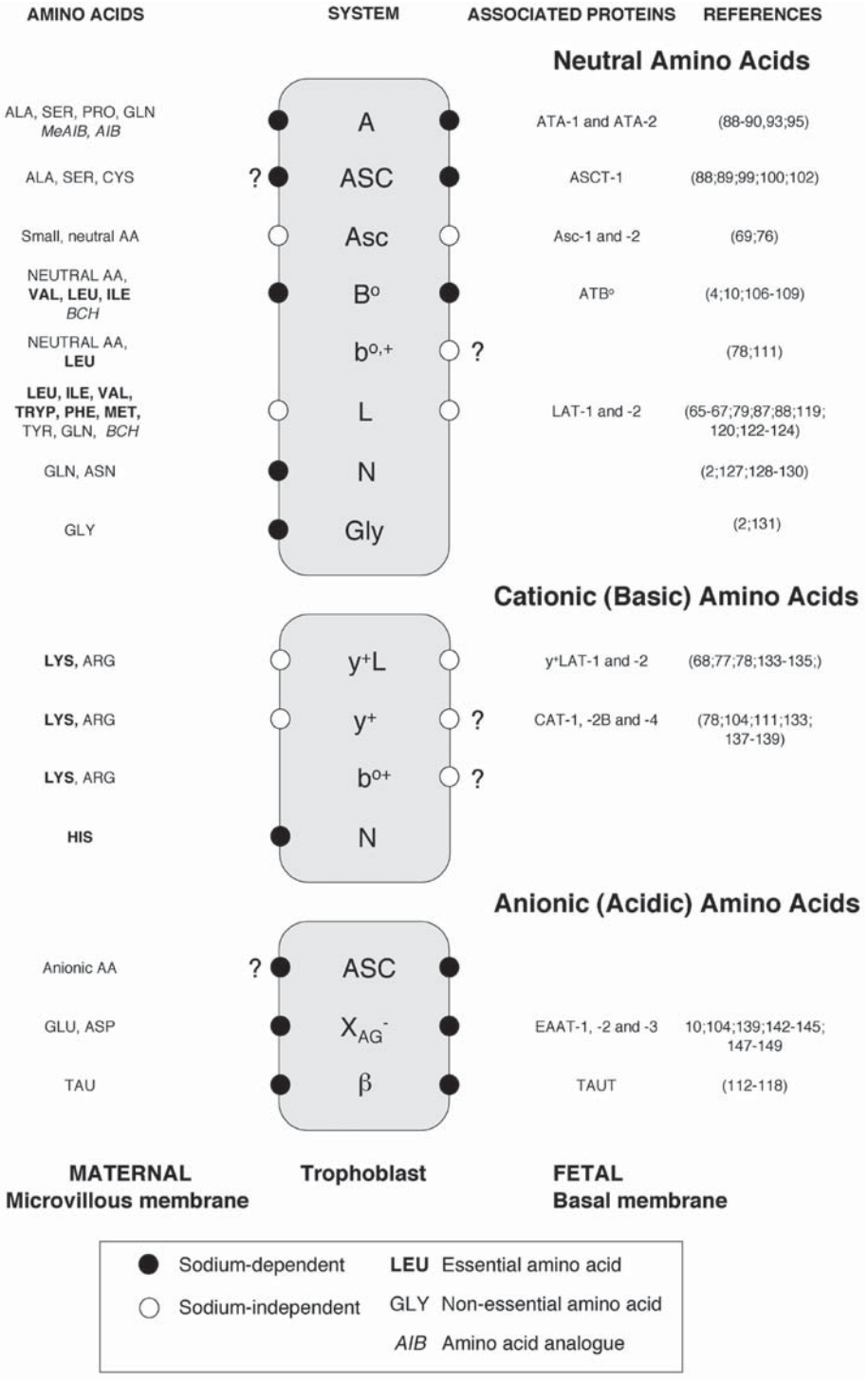

Fig. 1. Schematic representation of sodium-dependent (●) and sodium-independent (○) amino acid transport systems for both microvillous (maternal) and basal (fetal) membranes of the trophoblast. Associated light-chain proteins are also contained within the body of the figure. Amino acid substrates are listed on the left and references on the right. AIB, α-aminoisobutyric acid; BCH, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid.

Neutral Amino Acid Transport System and Their Proteins

System A is a sodium-dependent, unidirectional transporter with a characteristic affinity for N-methylated substances, and activity has been demonstrated in both microvillous and basal membrane *(88–90)*. Its activity is increased as an adaptation to cellular depletion of amino acid substrates, similar to that described for System X_{AG}^- *(91,92)*. Currently, four subtypes of the amino acid System A have been cloned: amino acid transporter A-1 (AT A-1), ATA-2, ATA-3, and SA-1.

The ATA-1 (or GlnT *[93,94]*) system has a limited tissue distribution, being expressed predominantly in the human placenta and heart, and is responsible for the transport of small short-chain neutral amino acids, such as alanine, serine, methionine, asparagines, and glutamine *(93)*. The functional characteristics of ATA-2 are similar to those of ATA-1, though found in a wider range of tissues, including the placenta *(93, 95)*. ATA-3 appears to have a unique tissue distribution predominantly in hepatic and skeletal muscle tissues *(96,97)*. SA-1 expression in placental tissues has not been reported *(98)*.

System ASC has been described for neutral amino acid transport, specifically alanine, serine, and cysteine, as well as for some anionic amino acids, though it is intolerant to the *N*-methylated substrates, such as methylaminoisobutyric acid (MeAIB) *(88,89,99,100)*. It is a sodium-dependent system localized at the basal membrane, though studies utilizing BeWo cells suggest that System ASC transporters may be present on the microvillous membranes as well, but after differentiation and syncytial formation, such activity is lost *(101)*. The ASCT-1 (SATT) *(83)*, a component of the ASC transport system, has been localized to human placental tissue, but its expression is very low *(102)*. Another component of the ASC system, ASCT-2, has also been cloned *(103)*, but expression in placental tissues has not yet been reported. Note that there are possible species differences in amino acid system location and activity. Whereas ASC activity is absent in human microvillous preparatations, ASC has been reported in the microvillous membrane of the rat placenta *(104)*. Additionally, differences between human and guinea pig placenta have also been reported: whereas human placenta possesses both System A and ASC, in the guinea pig, only System ASC is present *(2,5,105)*. A sodium-independent group of transporters (Asc) is also reported to exist in placental tissue *(69,76)*. The sodium-independent transport of small neutral amino acids is carried out by the Asc system, comprised of the proteins Asc-1 and Asc-2, both of which have been identified in placental mRNA *(69,76)*. Asc-1 forms a heterodimeric complex with 4F2hc *(69)*, whereas Asc-2 appears to interact with a presently unknown heavy chain *(76)*.

In addition, the broad-scope sodium-dependent transporter B^o is responsible for neutral amino acid transport including branched-chain amino acids (BCAAs), and has been localized to rat microvillous and human basal membranes *(4,10,106–108)*. ATB^o is proposed to represent the B^o system in the human placental choriocarcinoma cell line, JAR, and isolated human placental Poly(A)$^+$ mRNA *(107, 109)*, although its mode of activity, whether monomeric or heterodimeric, is still undetermined. The B title represents this system's broad substrate preference, and the capital status indicates its sodium dependence. The superscript "o" defines it as accepting zwitterionic or neutral amino acids, which distinguishes it from the $B^{o,+}$ system which also accepts cationic amino acids *(107,110)*. These systems are differentiated from the $b^{o,+}$ system, which also transports neutral and cationic amino acids, but in a sodium-independent manner. This latter system has been reported in basal membrane preparations *(111)*, though recently this localization has been questioned with its activity attributed instead to the y^+L system *(78)*.

The β-amino acid taurine utilitizes the System β transport system. Studies have demonstrated that this β transport system is a sodium-dependent transporter, found on both membranes *(112–117)*. The β transporter for taurine has exhibited activity in placenta microvillous membrane and basal membrane vesicles *(112–117)*, although basal membrane activity was only approx 6% of that in the microvillous membrane *(115)*. Presently, this activity is associated with an mRNA encoding TAUT, in placental cell lines and human placental Poly(A)$^+$ mRNA *(118)*.

System L (or l) transporters are sodium independent and have been localized to both trophoblast membranes *(88, 119,120)*. They play a major role in the transport of essential amino acids, having a high affinity for leucine and the other BCAAs *(2)*, and exhibit *trans*-stimulation *(4)*. Light-chain proteins associate with 4F2hc to form the functional L transport system *(80,87)* catalyzing the uptake of neutral amino acids in a sodium-independent manner. Two light-chain proteins have been reported to determine L system activity, system L amino acid transporter-1 (LAT-1) and LAT-2. The 4F2hc/LAT-1 transport activity displays a narrow specificity toward neutral amino acids, uninfluenced by pH, while 4F2hc/LAT-2 activity has a broad specificity and is stimulated by decreasing pH *(121)*. Both LAT-1 and LAT-2 have been reported in placental samples *(65–67,79, 87,122–124)*. Immunologic and functional studies suggest that at term, the light chain LAT-1 is located predominantly in the microvillous membrane and the syncytiotrophoblast layer of the villi *(66,124)*. Furthermore, the L transport system phenotype associated with that of the placental microvillous membrane is found to occur when the LAT-1 and not the LAT-2 catalytic subunit is coexpressed with the 4F2hc *(125)*. Human LAT-2 mRNA has been identified in the choriocarcinoma BeWo cell line and placental villous tissues *(67,125)*. It is localized to the basal membrane, and not the microvillous membrane, as is the case for LAT-1 *(87,125,126)*. These studies suggest that the localization of system activity may be further defined through specific light-chain location.

System N transports neutral amino acids but differs from Systems A and ASC in that it displays a preference for amino acids containing nitrogen-bearing side chains, transporting only glutamine, asparagine, and histidine *(2,127,128)*. While its activity has been reported in placental tissues, others have attributed this transport activity, in the rat placenta, to a combination of System A, possibly system ASC, and $B^{o,+}$ and y^+L *(10)*. Recently, cDNAs encoding two subtypes of System N—SN1 and SN2—have been described *(129,130)*, but expression of these subtypes in placental tissues has not yet been reported. Finally, a further neutral amino acid system responsible for nonsystem A transport of gly-

cine has been reported. The glycine transporter system, System Gly, is sodium dependent and its localization has been suggested to occur on the microvillous membrane *(2,131)*. Two glycine transporters are reported to exist: GLYT-1 and GLYT-2 *(132)*. GLYT-2 is specific to the central nervous system, while GLYT-1 is expressed in peripheral tissues *(27)*, but its placental localization has not yet been reported.

Cationic or Basic Amino Acid Transport Systems and Their Proteins

The cationic amino acid transport System y^+L is considered a low-capacity, high-affinity system that exchanges cationic amino acids for neutral amino acids, in the presence of sodium, and its activity is localized to both the microvillous membrane and basal membrane *(78,133–135)*. One pro-posed hypothesis is that this transporter system in the basal membrane is the major supply route of cationic amino acids to the fetus through the uptake of neutral amino acids from fetal circulation, in exchange for cationic amino acids from the placenta *(78)*. Because neutral amino acids are also required by the fetus, it is not yet apparent how their umbilical uptake is maintained under this exchange system. Placental y^+L system activity is determined by which light chain, System y^+L–amino acid transporter-1 (y^+LAT-1) or y^+LAT-2, forms a heterodimer with the membrane-bound 4F2hc. Human placental preparations contain y^+LAT-1 mRNA *(68,77)*, and when this mRNA is coexpressed with 4F2hc in *Xenopus laevis* oocytes, amino acid uptake characteristics similar to that of System y^+L are displayed *(77)*. y^+LAT-2 has been described in a number of tissues *(136)*, though currently not the placenta. Presently, it is unclear if System y^+LAT light-chain proteins display a tissue distribution pattern similar to that displayed by the LAT proteins.

The sodium-independent, low-affinity, electrogenic, high-capacity y^+ transport system is a high-capacity system that transports cationic amino acids such as arginine, lysine, and ornithine. It is considered to be the major cationic transport system in placental tissues *(78,104,137)*, localized to the microvillous membrane *(78,133,138,139)* and possibly to the basal membranes *(111)*. System y^+ activity is dependent only on the expression of monomeric proteins, unlike systems L and y^+L, which require expression of both a common heavy chain and a related light chain *(65,77,121)*. Currently, there are three known mRNAs that encode for proteins related to this cationic amino acid transport system in placental tissues: CAT-1, CAT-2B, and CAT-4 *(78,137,140)*. Recently, kinetic and substrate inhibition studies and reverse transcriptase polymerse chain reaction studies localizing CAT-4 mRNA in human placental tissue suggest that y^+ activity is limited to the microvillous membrane and not the basal membrane *(78)*. A role for the related $b^{o,+}$ system in placental tissues has been reported *(111,138,141)*; however, as mentioned earlier, further vesicle studies suggest no functional evidence for this system in either term microvillous or basal membrane *(78)*. The question of its existence and role during the earlier stages of pregnancy remains unanswered.

Anionic or Acidic Amino Acid Transport Systems and Their Proteins

The placenta takes up the anionic amino acids glutamate and aspartate from the maternal and fetal circulations, but does not actively transfer these amino acids from mother to fetus. Recent studies utilizing isolated human and rat placental membrane vesicles have revealed the presence of a high-affinity transport system for aspartate and glutamate via the sodium/potassium-dependent X_{AG}^- system within both the microvillous and basal plasma membranes *(10,104, 139,142–145)*. This system may mediate the concentrative uptake of anionic amino acids from the maternal and fetal circulations into the placenta. Five cDNAs encoding proteins capable of mediating high-affinity placental sodium-coupled transport have been reported, and three of these have been cloned from both rat and human placenta: excitatory amino acid transporter-1 (EAAT-1) (GLAST1), EAAT-2 (GLT1), and EAAT-3 (EAAC1) *(10,144,146)*. EAAT-1 and EAAT-3 have been detected in human placental tissue *(147,148)*, while these two and EAAT-2 have been detected in rat placenta tissues *(144,149)*. The regulation of these proteins appears to be under the control of growth hormone and insulin-like growth factor family members *(149)*, and upregulation of the system has been demonstrated through cell density and nutrient deprivation studies in muscle and placental cell lines *(91,144)*. These latter studies demonstrate that EAAT-1 and EAAT-3 play a key role in the basal anionic amino acid transfer, while EAAT-2 may be involved in conditions of amino acid depletion *(144)*.

Umbilical Uptake of Amino Acids: The End Point of Placental Amino Acid Transport

In vivo amino acid transport is the algebraic sum of multiple fluxes across the two plasma membranes of the trophoblast, and only a few in vivo fluxes have been measured. Table 1 lists those amino acids whose in vivo placental clearances have been measured in sheep pregnancy. Most of those amino acids have large bidirectional fluxes across both the maternal microvillous and fetal-facing basal membrane surfaces of the trophoblast. Placental production and/or utilization of an amino acid plays a role in determining the magnitude of the flux into the fetal circulation. Another factor contributing to the magnitude of this in vivo flux is placental protein turnover. In late gestation, in most species, there is little further growth of the placenta and, thus, little net protein accretion. However, protein turnover must be considered in the interpretation of tracer studies since metabolic cycling (e.g., alanine-pyruvate-lactate) for these processes significantly alters intracellular tracer/tracee ratios.

In vivo it is the umbilical uptake that represents the nutritional supply of amino acids to the fetus. Of the 20 amino

Table 1
Comparison of Mean Transplacental Clearance Rates from Maternal and Fetal Plasma[a]

Amino acid	Placental clearance (in mL of maternal plasma) (mL/[$kg_{fetal\ wt}$ · min])	Placental clearance (in mL of fetal plasma) (mL/[$kg_{fetal\ wt}$ · min])
Glutamate		200 ± 8.0 *(19)*
Tyrosine		21 *(222)*
Alanine		52 *(9)*
Glycine		31 *(14)*
Serine		55 *(18)*
Leucine	13.6 ± 1.3	55 *(174)*
	14.2 ± 1.4	
Methionine	17.1 ± 3.0	
Phenylalanine	15.6 ± 1.8	
Isoleucine	14.4 ± 2.1	
Valine	12.5 ± 2.0	
Tryptophan	7.0 ± 2.4	
Threonine	4.1 ± 0.8	
Histidine	3.7 ± 0.7	
Lysine	2.9 ± 0.6	

[a]Data are compiled from refs. *19* and *21*. Maternal clearance rates are compiled solely from ref. *21*, and fetal clearance rate references are represented in parentheses.

acids in the genetic code, 9 are essential amino acids in that their carbon skeletons cannot be synthesized by the fetus or placenta. It follows that there must be a significant umbilical uptake of these 9 amino acids. This is not necessarily a requirement for the 11 remaining amino acids. Figure 2 presents a comparison of the umbilical uptakes of 18 of the 20 amino acids in the genetic code with their net rates of accretion in late sheep pregnancy *(150)*. No data are available for cysteine or cystine. Note that all amino acids other than glutamate (Glu), aspartate (Asp), and serine (Ser), have a significant uptake from the placenta into the fetal circulation. For Glu, Asp, and Ser, there is a significant uptake by the placenta from the fetal circulation.

In considering the end point of placental amino acid transport—umbilical amino acid uptake—there are four questions that might be posed in the regulation of the umbilical uptake of amino acids:

1. What are the changes in ontogeny that occur for the activity of amino acid transporter proteins on the two surfaces of the trophoblast?
2. How do changes in effective placental surface area (including microvillous density) affect placental amino acid transport capacity?
3. How do changes in uterine and umbilical blood flows affect amino acid delivery into the fetal circulation?
4. What is the relationship between umbilical uptake and maternal concentration of each amino acid?

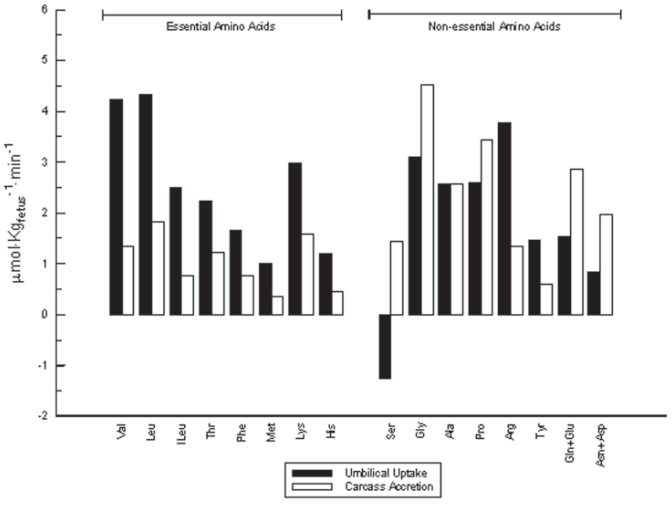

Fig. 2. Comparison of mean umbilical uptakes and estimated normal fetal accretions of amino acids. For the pairs of amino acids (glutamine + glutamate) and (asparagine + aspartate), individual accretion rates are not known. (Adapted from ref. *150*.)

Changes in Ontogeny of Placental Amino Acid Transport Systems

What are the changes in ontogeny that occur for the activity of amino acid transporter proteins on the two surfaces of the trophoblast? This first question relates to how transporter proteins change within the plasma membranes of the two surfaces of the trophoblast as a function of gestational age. Such changes could amplify the changes in surface area if transporter density increases along the plasma membrane surface. The ontogeny studies of transporter expression in the placental plasma membranes currently do not provide an overall description of the impact of gestational age on transport characteristics. It is clear, however, from studies of heterodimeric systems, that heavy-chain expression within the placenta changes during development. Accompanying the maturational changes in trophoblast surface area, amino acid transport systems are reported to have differing expression and transport parameters through gestation *(66,78,90,138,151,152)*.

Furthermore, the functional properties of transport systems may also change as gestation advances. For example, first-trimester microvillous membrane has increased transport activity compared to term placental vesicles *(152)*, and 4F2hc protein levels differ between early/midpregnancy and term placenta *(66,78)*. Early in gestation, the K_m of the microvillous high-affinity System y^+L is significantly less than in term preparations *(78)*. Such studies highlight the complex interactions that occur between developing microvillous membrane and basal membrane, within the trophoblast and between the two circulations, to facilitate an increase in nutrient delivery to the growing fetus as gestation advances. However, more detailed studies of the activity of the monomeric and heterodimeric systems as a function of gestational age in normal and FGR pregnancies need to be conducted.

Changes in Effective Placental Surface Area

How do changes in effective placental surface area (including microvillous density) affect placental amino acid transport capacity? This second question relates amino acid transport to the surface area changes occurring during gestation. As pregnancy advances, the increasing nutrient demands of the developing conceptus must be met through an appropriate increase in placental nutrient transport. This increase is facilitated through alterations in placental perfusion and changes in membrane exchange surface area. Between the 16th week of pregnancy and term, human fetal weight increases approx 20-fold, whereas the peripheral villous surface area increases only 9-fold *(153–156)*. More important, the multiplication factor of 9 actually decreases in late gestation *(156)*. It should be emphasized, however, that there are only a few careful ontogenetic studies of how total surface area, including microvillous surface, changes over gestation *(154,156,157)*. The increases in total surface area of the placenta alone cannot account for the exponential fetal growth occurring over this time period. These data suggest that fetal growth is supported not by changes in villous surface area alone but, rather, that the total transport capacity of the trophoblast is likely to be determined by changes in microvillous surface area, placental permeability, the concentration of specific amino acid carrier proteins on the cell membrane surface, as well as the affinity characteristics of these proteins and the circulating amino acid concentrations at both the maternal and fetal surfaces.

The observations of differences in amino acid transport activity in FGR placenta highlight the important point that the activity of transport systems must be considered in conjunction with the other contributors to amino acid flux. In studies comparing normal and FGR placentae, there are reported reductions in total villous surface area *(157,158)*, suggesting that morphometric changes contribute to the overall reduction in placental amino acid transport capacity, although the composition and permeability of placental plasma membrane vesicles is not altered *(159)*. There have been clinical studies comparing estimated surface area for normal and FGR placentae that reported significant reductions in placental surface area *(160,161)*, which is not surprising given the large reduction in placental mass that accompanies FGR pregnancies. Other studies of FGR placentae have shown decreased diameter of terminal villi and increased extracellular matrix *(162)*. Thus, at the present time, it appears that reductions in surface area for exchange as well as in specific transporter number and activities, and decreased perfusion may all contribute to the reduction in amino acid transport in FGR pregnancies.

Effects of Uterine and Umbilical Blood Flow on Amino Acid Uptake

How do changes in uterine and umbilical blood flows affect amino acid delivery into the fetal circulation? This third question of the relationships among the two circulations perfusing the placenta and umbilical amino acid uptake is one that has not yet been directly addressed by in vivo studies relating uterine and umbilical blood flows to amino acid uptake. This question may be especially important for human pregnancies since recent studies have shown that umbilical blood flow may be reduced by as much as 70% in FGR pregnancies *(163,164)*. Fortunately, however, there are two studies that examined the relationship of the umbilical uptakes of O_2 and glucose as a function of uterine blood flow in normal sheep pregnancies *(165,166)*. Both studies showed a similar relationship—that there is a range of uterine flow over which umbilical uptake of each is relatively unaffected by reductions of flow. Essentially, this provides a margin of safety to the fetus so that delivery of nutrients is preserved despite reductions in uterine flow.

Recent studies in human pregnancies have shown that umbilical blood flow increases during gestation in a pattern similar to fetal growth rate. The relationship between blood flow and body size is quite good, albeit not perfect. When blood flow is expressed per kilogram of fetal weight, it falls slightly throughout gestation. This pattern of decline is similar to the changes in fetal heart rate in human pregnancies, which decreases as gestation advances. Heart rate is closely linked to metabolic rate. Thus, the changes in blood flow and in fetal metabolic rate appear to be reasonably well linked in human pregnancies. The decrease in umbilical blood flow per kilogram of fetal weight in human pregnancies does not present a problem in terms of supplying amino acids to the fetus as it grows. This is owing to the fact that changes in blood flow are compensated for by an increase in placental surface area, and presumably an increase in the number of membrane-bound transporters available for delivery of amino acids.

However, further reductions in flow have a profound effect on umbilical uptake of O_2 and glucose. For the umbilical circulation, there has been only one study that addressed this question, at least in terms of O_2 uptake *(167)*. This study showed a similar relationship to that found for uterine flow, as depicted in Fig. 3. Note that there is a margin of safety depicted by the data, but there is also a critical point below which umbilical O_2 uptake is profoundly affected. From these studies in sheep pregnancy, it is reasonable to postulate similar relationships in human pregnancy. Thus, for both uterine blood flow and umbilical blood flow, there is probably a fairly wide range over which changes in flow have only minor effects on amino acid transport. Once this limit is exceeded by larger reductions in flow, amino acid uptake should also be significantly reduced.

Relationship Between Maternal Concentration and Umbilical Uptake

What is the relationship between umbilical uptake and maternal concentration of each amino acid? This fourth question has received some study in vivo in both human and sheep pregnancies by the infusion of amino acids into

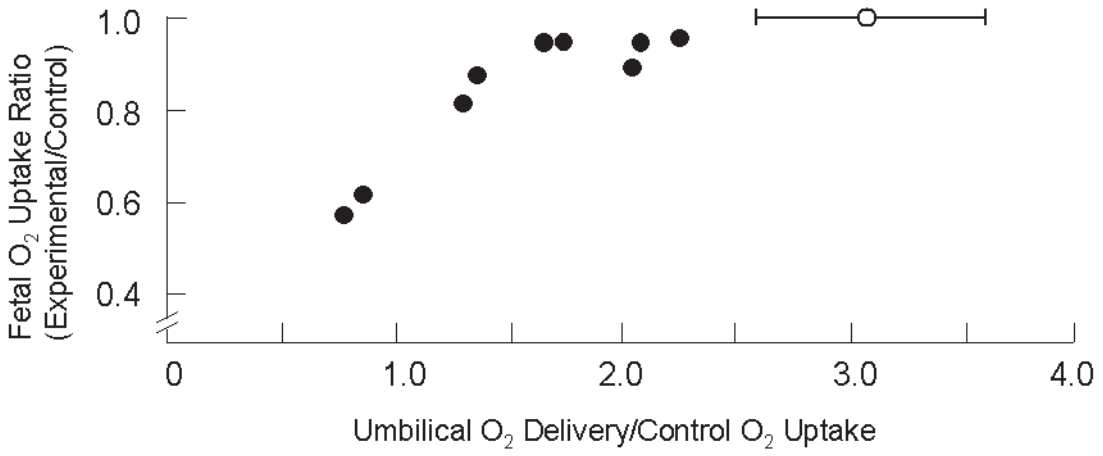

Fig. 3. Changes in fetal O_2 uptake and fetal arterial blood pH in relation to umbilical O_2 delivery-to-control O_2 uptake ratio. In the control period (represented by ○), the ratio averaged 3.09, and ranged between 2.6 and 3.62. Note that O_2 uptake and pH remained virtually constant for O_2 delivery-to-control O_2 uptake ratios >1.6 *(166)*.

the maternal circulation with measurements of their umbilical uptake. How do maternal concentrations of amino acids relate to their umbilical uptakes? is a key question in fetal nutrition. This question is important because it would help define the extent to which changes in maternal amino acid concentrations could impact the fetal delivery of amino acids. The relationship between maternal concentration and umbilical uptake is a complex one. In sheep studies, the flux from the maternal to the fetal circulations has been studied for all nine essential amino acids *(21,168)*. A recent study demonstrated striking differences among the nine amino acids for the placental clearance of the essential amino acids *(21)*. The clearance was most rapid for five amino acids: the three BCAAs, phenylalanine, and methionine (see Fig. 4). For these five, differences in transplacental flux are directly attributable to the differences in the maternal concentrations that are normally maintained for each of them. Several studies in normal sheep pregnancies have examined the relationship of umbilical uptake to maternal concentration utilizing maternal infusions of amino acids *(168–170)*. These studies have shown that uptake can be increased by increasing maternal concentration, but the relationship is different for each amino acid.

In normal human pregnancies *(171)*, and in pregnancies complicated by FGR *(172)*, the fetal uptake of most amino acids can be increased by increasing the maternal concentration significantly. However, it is also true that the uptake of certain amino acids does not increase when an infusion of many amino acids is given to the mother, presumably as a reflection of competitive inhibition. In sheep studies, it has been shown that whenever the concentration of an amino acid is increased in the maternal circulation by the infusion of the single amino acid into the mother, the umbilical uptake of the amino acid increases. This has been shown for alanine, leucine, threonine, and glycine *(12,13,16,173)*. However, because of competitive inhibition effects, when a large number of amino acids are given to the mother simultaneously (as would be the case clinically), the umbilical uptake of some amino acids increases but not that of all amino acids.

Animal and Clinical Studies of Amino Acid Uptake In Vivo

The in vivo studies of placental transport capacity for amino acids are subdivided into those that present data from various animal species and those that present clinical data. For the most part, in vivo studies of amino acid flux have been carried out in sheep pregnancies.

Fetoplacental Amino Acid Uptake in Animal Studies

In normal sheep pregnancies, stable isotopic methodology has been used extensively to examine the multiple fluxes of an amino acid, which together determine the net delivery of the amino acid into the fetal circulation. As an example of the contribution of various fluxes to net umbilical uptake, let us consider two amino acids, leucine and alanine, both of which have large net umbilical uptakes. Figure 5 presents the leucine fluxes measured in normal pregnancy for late gestation. Note that the umbilical uptake is largely determined by three fluxes of approximately equal magnitude. One is the transplacental flux from the maternal circulation into the fetal circulation, another is the back flux from the fetal circulation into the placenta, and the third represents the leucine flux derived from placental protein turnover. There is significant deamination of the BCAAs within the placenta leading to a net flux of α aminoisocaproic acid (KIC) into the fetal and maternal circulations *(13,174)*. Figure 6 presents similar data for alanine. Note that the transplacental flux now represents a relatively minor component and alanine production within the placenta a much larger component. This is not unexpected given the magnitude of the

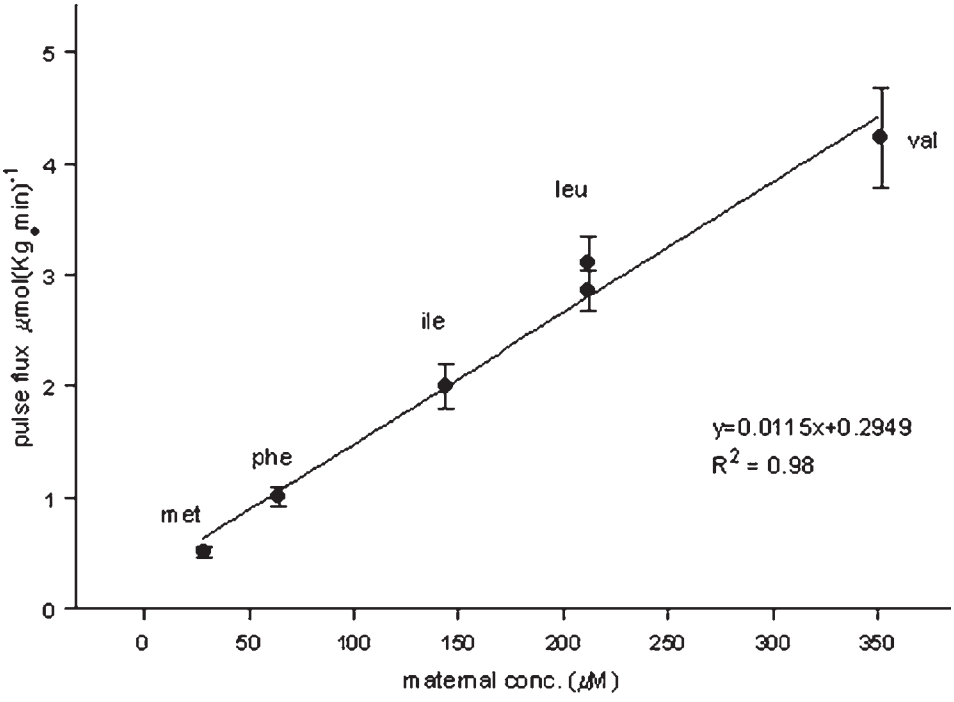

Fig. 4. Pulse flux calculated over first 10 min after bolus vs maternal plasma arterial concentration for five essential amino acids that have high and similar pulse flux clearances *(21)*. The two leucine data represent two separate studies *(13,21)*.

alanine ↔ pyruvate ↔ lactate exchange within the placenta *(9,173)*. For most amino acids that have been studied, there are significant bidirectional fluxes at both the maternal microvillous and fetal basal membrane surfaces of the placenta.

The umbilical uptake of amino acids representing the nutritional supply to the fetus exceeds the requirements for net accretion in normal pregnancies *(150)*. This observation, well established for normal sheep pregnancy both at mid-gestation and at term, is supported by the fact that there is extensive oxidation of amino acids in the fetal tissues. The oxidation rate of an amino acid in normal pregnancy attests to the margin of safety in the supply of amino acids to the fetus. The quantitation of the umbilical uptake is quite precise, but there are more difficulties in defining the uterine uptake of amino acids. In sheep pregnancy, uterine blood flow is approximately two times the umbilical blood flow. Thus, if an amino acid is delivered to the uterus in an amount equal to its entry into the fetal circulation, the arteriovenous difference is only half that of the umbilical arteriovenous difference. The very small coefficients of extraction across the uterine circulation make quantitation of the uterine uptake less precise than that of the umbilical uptake. Obviously, a comparison of uterine and umbilical uptakes is important because the difference between the two defines the net utilization or net production of an amino acid by the placental tissues. The one study to address this issue with large enough numbers to provide confidence in the comparison of the two amino acid uptakes found that there was evidence of net placental utilization of five amino acids and net placental production of three amino acids *(150)*; this is depicted in Fig. 7.

The question of how the umbilical supply of amino acids changes during gestation has been addressed by one study carried out in normal pregnant sheep at midgestation *(175)*. This study showed that in relationship to fetal dry weight, the umbilical uptake of amino acid nitrogen was approximately four times higher than it was in late gestation (18.0 vs 4.6 mg/d/g fetal dry wt). Thus, at midgestation as well as in late gestation, amino acids are supplied to the fetus in amounts that exceed their rates of accretion. Again, as in late gestation, this finding receives support from the evidence demonstrating a high fetal amino acid oxidation rate at midgestation *(175–177)* and a high rate of fetal oxygen consumption *(178)*.

Clinical Human Studies of Amino Acid Uptake In Vivo

Normal human pregnancies have been studied using techniques adapted to the clinical setting. For example, in animal studies, measurement of the umbilical uptake of amino acids is relatively straightforward, requiring only measurement of the umbilical blood flow and the arteriovenous concentration difference for the amino acids. In human pregnancies, the umbilical blood flow can be measured in utero with Doppler techniques *(163)*, but the arteriovenous difference can be measured only at the time of delivery, when both cord vessels can be sampled. To circumvent this problem, studies have taken advantage of the fact, well established in animal studies, that fetal O_2 consumption is quite constant among fetuses of the same gestational age, even though the umbilical blood flow can vary considerably. The reason for the constancy of the O_2 consumption measurement is that

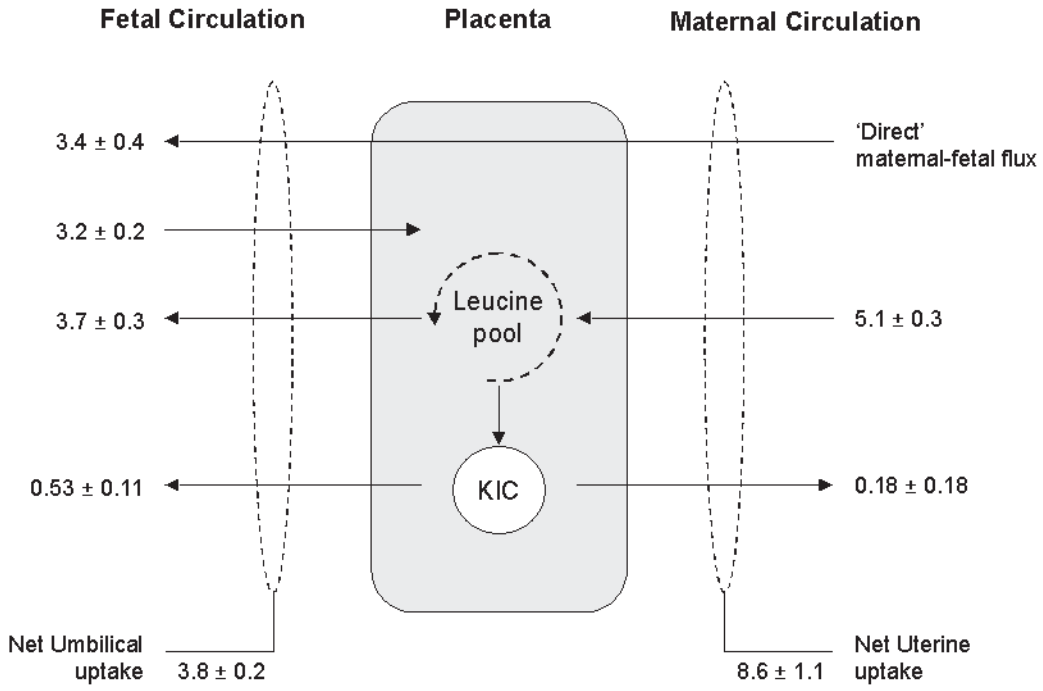

Fig. 5. The unidirectional fluxes of leucine into and out of the placenta ($mmol \cdot min^{-1} \cdot kg^{-1}_{fetal\ wt}$) measured in vivo under steady-state conditions are presented. The data are abstracted from refs. *13* and *221*. Note that the three major fluxes that together determine the umbilical uptake are of approximately equal magnitude.

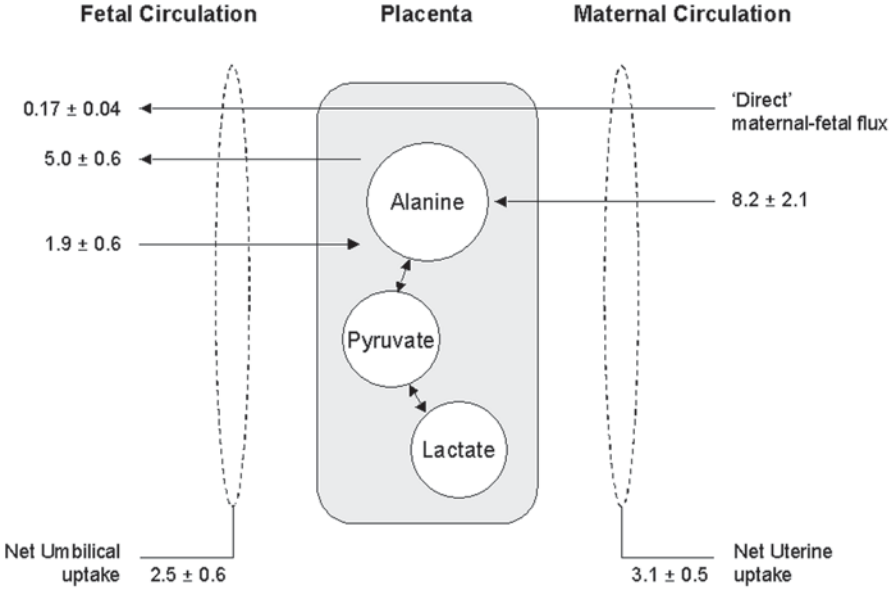

Fig. 6. The unidirectional fluxes of alanine into and out of the placenta ($mmol \cdot min^{-1} \cdot kg^{-1}_{fetal\ wt}$) measured in vivo under steady-state conditions are presented *(173)*.

as the flow changes, there are compensating changes in the arteriovenous difference in O_2 content *(179)*. Thus, clinical studies have relied on measuring the ratio of arteriovenous difference in amino acid concentration/arteriovenous difference in O_2 content *(171)*. These studies have shown that in normal human pregnancies, the uptake of amino acids exceeds the requirements for accretion *(180)*, similar to observations made in pregnancies in other species. This suggests a relatively high rate of fetal amino acid oxidation. Estimates of a high rate of urea production during late pregnancy *(181)* support the conclusion of extensive fetal oxidation of amino acids. Further, it has been shown that the fetal uptake of amino acids could be increased in normal pregnancies *(171)*, and in FGR pregnancies *(172)*, by

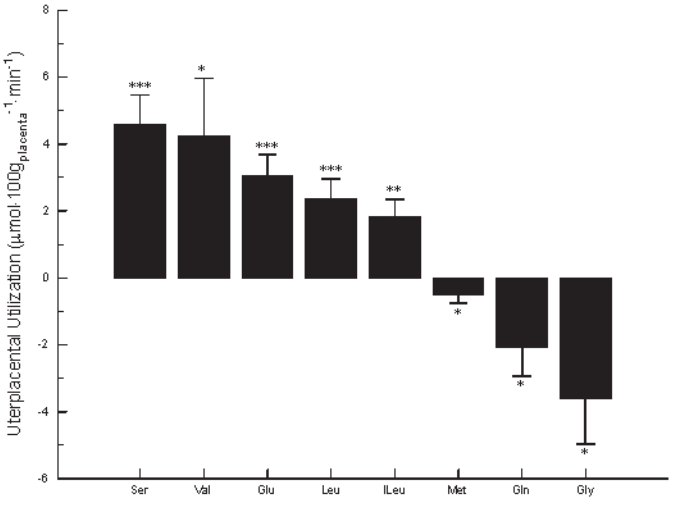

Fig. 7. Uteroplacental utilizations that were significantly different from zero, expressed per 100 g of placenta and ranked by magnitude (negative utilization = production). Bar graphs are means ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. The p values were determined by Student's t-test for paired samples. (Adapted from refs. *150*.)

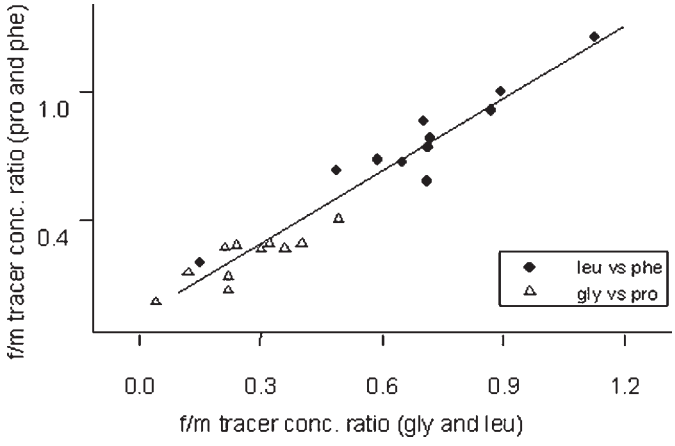

Fig. 8. The F/M plasma tracer concentration ratios are plotted with the values for glycine (gly) and leucine (leu) along the abscissa and those for proline (pro) and phenylalanine (phe) along the ordinate. The regression is highly significant, $(F/M)y = -0.0511 + 1.1274(F/M)x$, $R^2 = 0.94$, $p < 0.001$. (Adapted from ref. *22*.)

elevating the maternal concentrations of amino acids with an infusion of amino acids. However, as mentioned earlier, this was not true of all amino acids, presumably owing to competitive inhibition. The fact that the umbilical uptake of a few amino acids, including some essential amino acids, could not be increased by the infusion of a mix of amino acids presents a problem, which would need to be overcome if one attempted to treat FGR pregnancies by a maternal amino acid infusion.

Stable isotopes have also been used in human pregnancies to study the steady-state metabolism of amino acids in vivo *(182,183)*. The stable isotope of leucine was infused into the maternal circulation until a steady-state enrichment was achieved in the maternal circulation *(183)*. In normal pregnancies, the fetal/maternal enrichment (F/M) ratio was approx 0.8. This study also clearly demonstrated a significant reduction in the F/M ratio in FGR pregnancies, and this reduction correlated with a classification of clinical severity based on umbilical artery velocimetry data and FHR *(183)*. Thus, a problem definable for leucine transport correlated with circulatory changes in FGR pregnancies.

Clinical studies of amino acid transport have also been carried out using the same non-steady state-protocol employed in the sheep studies *(15,22)*. In this protocol, stable isotopically labeled amino acids were given as a bolus infusion into the maternal circulation within a 10-min window of cord sampling. One study compared the F/M ratio for leucine and glycine in normal human pregnancies as a function of the time interval from the bolus infusion to cord sampling *(15)*. There was a much lower rate of glycine transport compared to leucine; the F/M ratio for glycine was only 16% of the F/M ratio for leucine. As discussed previously, the sheep studies had led to the hypothesis that exit from the placenta into the fetal circulation was a major factor in determining the rate of transplacental transport *(21,184)*. Those studies showed that the five amino acids that utilize exchange transporters had the most rapid transplacental flux (Fig. 4). To investigate these activities in normal and FGR human pregnancies, a bolus study was done comparing phenylalanine, leucine, glycine, and proline *(22)*. The study found that there were no significant differences in the F/M ratio for leucine vs phenylalanine nor for glycine vs proline, although the latter pair had significantly lower F/M enrichment ratios than the former (Fig. 8). There were also lower F/M ratios in the FGR pregnancies compared with normal pregnancies, in agreement with the steady-state study of leucine flux.

Amino Acid Transport Systems and Amino Acid Fluxes in FGR

In clinical studies, the concentration of amino acids has been observed to be lower in the fetal and maternal plasma of pregnancies complicated by FGR *(55,185,186)*, suggesting alterations in amino acid delivery. This depression in amino acid concentrations is true regardless of whether fetal concentrations were determined at the time of delivery or many weeks prior to delivery. Recently, the same investigators of these studies demonstrated that lower amino acid concentrations were found in FGR pregnancies even if the fetus had normal fetal heart rate and velocimetry measurements, suggesting that this may antedate other clinical pathologic findings *(187)*. Studies in human FGR demonstrate that System A may be impaired *(188)*. An in vitro study utilizing microvillous membrane vesicles from the placen-

tae of appropriate-for-gestational age (AGA) and small-for-gestational age (SGA) babies demonstrated markedly lower activity (by 63%) of the A system transporters in the SGA compared to the AGA membrane vesicles *(189)*, suggesting a positive association between fetal growth and System A activity. In human FGR, the transport of taurine from maternal to fetal circulation is also reduced, thereby affecting fetal taurine concentrations *(115)*. In addition, studies of maternal protein deprivation in rats have demonstrated a downregulation of placental amino acid transport, specifically System A as well as Systems X_{AG}^- and y^+ *(139,189)*. Inhibition of System A transport in rat pregnancies has been purported to affect fetal weight, demonstrating a role for System A transport in fetal growth *(190)*. Regarding System X_{AG}^- transport, the basal membrane activity of EAAT-1 is reduced in an FGR rat model, which could impact placental glutamate uptake from the fetal circulation *(139)*. Recently, our own studies in a sheep model of hyperthermia induced FGR have shown reduced fetal glutamate concentrations and a reduced umbilical coefficient of extraction, pointing to possible problems in both the fetal liver and placenta in FGR *(191)*.

The microvillous membrane System y^+ and y^+L activity is not altered in human FGR *(135,192)*, although basal membrane transport of lysine is reduced, as represented by reduced mediated lysine uptake and reduced V_{max} for the y^+L system *(135)*. Reductions in the uptake of leucine in both microvillous and basal membrane preparations of FGR placentae highlight possible alterations in the L transport system *(135)*. These changes in basal membrane transport properties could be an important adaptive response by the trophoblast, limiting the back flux of amino acids from the fetal circulation to the placenta. In sheep pregnancies, FGR can be established by the exposure of pregnant sheep early in gestation to relatively high (equivalent to summertime) environmental temperatures *(193,194)*. This model has been studied from the perspective of the placental vascular and circulatory changes *(195)*, as well as aspects of oxygen and glucose transport *(196)*. Studies in this FGR model in late gestation have shown lower fetal amino acid concentrations suggestive of decreased placental flux. Stable isotopic studies with leucine *(13)* and threonine *(12)* have established the following characteristics for amino acid flux in this FGR model: (1) a decreased transplacental flux of both leucine and threonine from the maternal plasma to the fetal circulation; (2) a decreased fetal concentration of these amino acids, which, in turn, leads to (3) decreased back flux from the fetal circulation into the placenta; and (4) decreased rate of fetal oxidation for both amino acids. The result of the latter two changes is to spare the reduced fetal supply of these amino acids for protein synthesis. These data suggest that changes in basal membrane function may have occurred. At steady state, the F/M of leucine in normal human pregnancies is approx 0.8, a much higher ratio than the determined 0.4 ratio in sheep *(13,183)*. However, in both species, this ratio is significantly lower in FGR pregnancies. In the sheep FGR model, the reduction in F/M ratio is due to a significant reduction in transplacental leucine flux *(13)*, which is probably also the mechanism in human FGR pregnancies. Furthermore, the magnitude of the reduction in leucine ratio correlates with a clinical classification of FGR severity based on a completely different set of clinical data: fetal arterial velocimetry and fetal heart rate data *(187)*. In addition, recent clinical studies have shown that the leucine and phenylalanine F/M ratio is significantly lower in human FGR compared with normal pregnancies *(22,183)*.

Linking In Vitro Vesicle and In Vivo Flux Studies

The majority of studies investigating placental amino acid transport have taken the form of in vitro studies utilizing various forms of placental membrane preparations. Studies of in vivo transport and metabolism of amino acids are fewer, owing to the difficulties of establishing adequate biologic preparations and the unavoidable complexity of in vivo systems. Both in vitro and in vivo studies are required to fully understand placental dynamics responsible for the active transport of amino acids and the nutritional end point of umbilical uptake. In vivo studies, of necessity, include the interaction of perfusion and permeability and, by design, study placental transport at the amino acid concentrations that actually exist in the fetal and maternal circulations.

Uptake of Amino Acids Across Microvillous Membrane

The concept of active transport of amino acids has been corroborated by a variety of experiments. Studies of placental tissue cultured in vitro with nonmetabolizable amino acid analogs, such as AIB, as well as other amino acids, have shown tissue concentrations to be at levels several times greater than maternal concentrations *(146,197–202)*. Placental tissues have also been found to have extremely high levels of the amino acids taurine, glutamate, and aspartate, as well as moderately high levels of alanine, glycine, serine, glutamine, and threonine *(48)*, when compared to maternal or fetal concentrations. Additionally, in vivo experiments have examined the uterine and umbilical uptakes of analogs of amino acids and a limited number of amino acids that have differing transport system affinities following infusion into the maternal circulation *(16,173,184)*. The amino acid analog MeAIB, which has affinity for the System A transporter, enters the placenta from the uterine circulation in large amounts. AIB and aminocyclopentane-1-carboxylic acid (ACP), which utilize sodium-independent exchange transporters, also enter the placenta. Both glycine and alanine steady-state experiments have also demonstrated transplacental fluxes in vivo *(16,173)*. These in vitro and in vivo results support the idea that transport across the microvillous membrane, into the trophoblast, represents a major active transport step in maternal to fetal amino acid transport.

However, if we consider the transport of glycine and leucine, it becomes clear how many details are still unknown. One of the major transporters of glycine is System A, which in human vesicle studies concentrates glycine at high concentrations in the trophoblast. This is supported by placental tissue measurements *(4,48)*. In vivo studies have shown that the high tissue concentrations occur, without significant net uterine uptake of glycine. Both in vivo and in vitro studies have documented that serine is utilized for the production of glycine *(14–18,203)*. Furthermore, when L-[1-^{13}C] glycine was infused to steady-state enrichments in the maternal circulation, no significant dilution of uterine venous glycine enrichment was noted, which is not consistent with a large unidirectional glycine flux from the trophoblast into the maternal circulation *(16)*. Still much concerning the actual mechanisms of this transport loop remain to be determined.

For example, present in vitro models, do not address the contribution the amino acid alanine may make to these transplacental fluxes. Alanine, a nonessential neutral amino acid like glycine, is transported through System A. It may act as a neutral exchange amino acid for both other neutral amino acids such as leucine and cationic amino acids. Studies conducted in pregnant sheep demonstrate that increasing maternal alanine concentrations increase uterine and umbilical alanine uptake *(173)*. However, at normal maternal alanine concentrations, the transplacental flux of alanine is quite small. Most of the alanine that is delivered to the fetus is produced within the placenta *(173)*, as part of the alanine ↔ pyruvate ↔ lactate exchange. The contributions of these other amino acids to transport activities is still unknown.

Amino Acid Transport Across Fetal-Facing Basal Membrane

Let us now consider the in vitro and in vivo explanations of the basal membrane transport of amino acids. There exist two schools of thought concerning this movement. One line of thought is that once concentrated within the trophoblast, amino acids diffuse down their concentration gradient *(4)*. The other idea is that amino acids are actively transported across the basal membrane, which is supported by the identification of specific basal membrane transporters and by in vivo data, which demonstrate that the placenta may in fact be impervious to the passive movement of amino acid analogs. Specifically, in studies concerning a maternal injection of two nonmetabolizable amino acids (MeAIB and ACP), MeAIB, after entering the microvillous, was essentially trapped within the placenta, unable to enter the fetal circulation, whereas ACP entered the fetal circulation rapidly *(184)*.

The transport between placenta and the fetal circulation of two amino acids, serine and alanine, represents another difficult area in terms of reconciling the disparities between in vitro and in vivo data. Both serine and alanine are presumed to utilize similar transport systems on the basal membrane, the ASC system, yet their net fluxes are in opposite directions. Vesicle studies, alone, have not yet replicated these phenomena. Presumably these differences may be accounted for by the fact that alanine may utilize exchange transporters on the basal membrane, such as the y^+L system for exit from the placenta into fetal circulation *(89)*. However, serine, which shares many of the transport characteristics with alanine, is taken up by the placenta from the fetal circulation, in a similar manner to that of glutamate *(17, 150)*, presumably by basal-bound, inward-facing trophoblast system ASC or A transporters. Yet the net uptake of serine into the placenta from the fetal circulation is much less than the entry rate of alanine and other amino acids into the fetal circulation *(18,173)*.

Explanations of transplacental fluxes using in vivo steady-state methodologies are complex experiments, with little control over the individual components of the systems being investigated. These experiments yield much information concerning actual fluxes, which should be used to plan in vitro experiments, designed to examine specific components of the in vivo system. In vitro experiments in which many details can be controlled have difficulty taking into account the role of other transporters, placental amino acid metabolism, amino acid back flux, and actual physiologic amino acid concentrations. However, much of the incompleteness of both forms of investigation will be overcome with time, and a fuller, more representative picture of amino acid transport will become apparent. The possibility of placenta amino acid uptake being presented in the form of $\mu mol \cdot cm^{-2} \cdot h^{-1}$ of placental membrane, as has been recently done in the rabbit ileum *(204)* is exciting, and such data, together with calculated surface area, could lead to calculated fluxes that can be more readily correlated with in vivo fluxes.

Placental Metabolism of Amino Acids

The placenta has the capacity for utilization, production, and interconversion of amino acids, all of which can profoundly affect the quantity of an amino acid delivered into the fetal circulation. Given the fact that the placenta has an extremely high metabolic rate, comparable to brain tissue, the utilization of amino acids as metabolic fuels, particularly glutamate, is an important metabolic characteristic of this organ *(205)*. Alanine, aspartate, glutamate, and glycine have all been shown to be oxidized by placental mitochondria *(206)*. However, given the much larger net uptake of glutamate by the placenta from the fetal circulation, it is likely that this amino acid is the predominant metabolic fuel for the placenta among the amino acids *(19)*. There is also a small but significant production of glutamine from glutamate within the placenta. This aspect of placental glutamate metabolism accounts for the fact that the umbilical uptake of glutamine is significantly greater than the placental uptake of glutamine from the uterine circulation *(150)*.

Basically, five amino acids show a significant net utilization by the placenta: serine; glutamate; and the three BCAAs —leucine, isoleucine, and valine. The three BCAAs are trans-

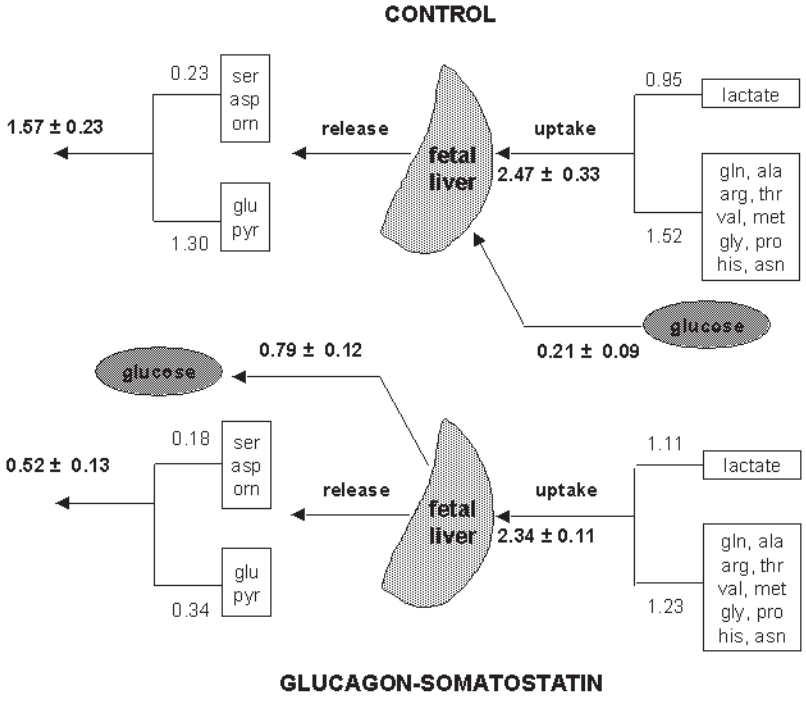

Fig. 9. Fetal hepatic uptake and output of glucose carbon and glucogenic substrate carbon under normal physiologic conditions (control) and during a fetal glucagon somatostatin infusion. Each number represents a substrate carbon-to-oxygen uptake ratio. (Adapted from ref. *213*.)

aminated to their α ketoacids. The α ketoacids are not utilized for oxidation in the placenta owing to very low activity of the α ketoacid decarboxylases *(207)*. Instead, the α keto-acids are released into the umbilical and uterine circulations and contribute to placental ammonia production. Since they can be transaminated in fetal tissues, the nutritional supply of BCAAs into the fetal circulation is represented by the sum of the BCAA uptakes and the umbilical uptakes of their corresponding α ketoacids.

Another important example of placental amino acid cycling is that of alanine ↔ pyruvate ↔ lactate. The interconversion of alanine, pyruvate, and lactate is very extensive in the placenta *(9,173)*. There is a large placental uptake of alanine from the uterine circulation and release into the fetal circulation. Although there is no evidence of net placental utilization of alanine, there is a net uptake of pyruvate from the fetal circulation into the placenta and a net efflux of lactate into the fetal circulation *(173)*. Sheep *(208)*, rabbit *(209)*, and guinea pig *(6,210)* placentae in vivo have all been shown to have a net release of lactate into both the uterine and umbilical circulations. By contrast, pyruvate is taken up from the fetal circulation into the placenta. Under normal conditions, the fetal liver is characterized by a net release of pyruvate into the fetal circulation, and this pyruvate release is maintained under hypoxic conditions *(211)*. The large alanine ↔ pyruvate ↔ lactate exchange in the placenta leads to only a small fraction of the net umbilical uptake of alanine coming from transplacental flux of alanine from the maternal plasma into the fetal plasma. Taken together, the alanine and lactate uptake in the umbilical circulation is quite large even when corrected for the flux of pyruvate into the placenta from the fetal circulation.

Fetoplacental Amino Acid Metabolism

Glutamine and Glutamate Metabolism

In postnatal life the carbon from many amino acids can be utilized for hepatic glucose production, but in fetal life, hepatic gluconeogenesis is minimal *(212)*, suggesting that amino-acid-derived carbon in the fetal liver is either utilized for other metabolic functions (for example, glycogen deposition) or released from the liver in another form. The fetal hepatic production of the amino acid glutamate provides a mechanism whereby carbon can be shifted to the placenta for oxidation as well as providing a means of reducing fetal hepatic O_2 requirements *(19)*. The placental oxidation of this fetal-generated glutamate not only provides energy for the placenta, but also generates NADPH, which can be utilized for steroidogenesis *(206)*. Under control conditions in late pregnancy, glutamate and pyruvate represent approx 82% of the placental carbon-to-oxygen uptake (Fig. 9). Under glucagon-somatostatin treatment, simulating post-

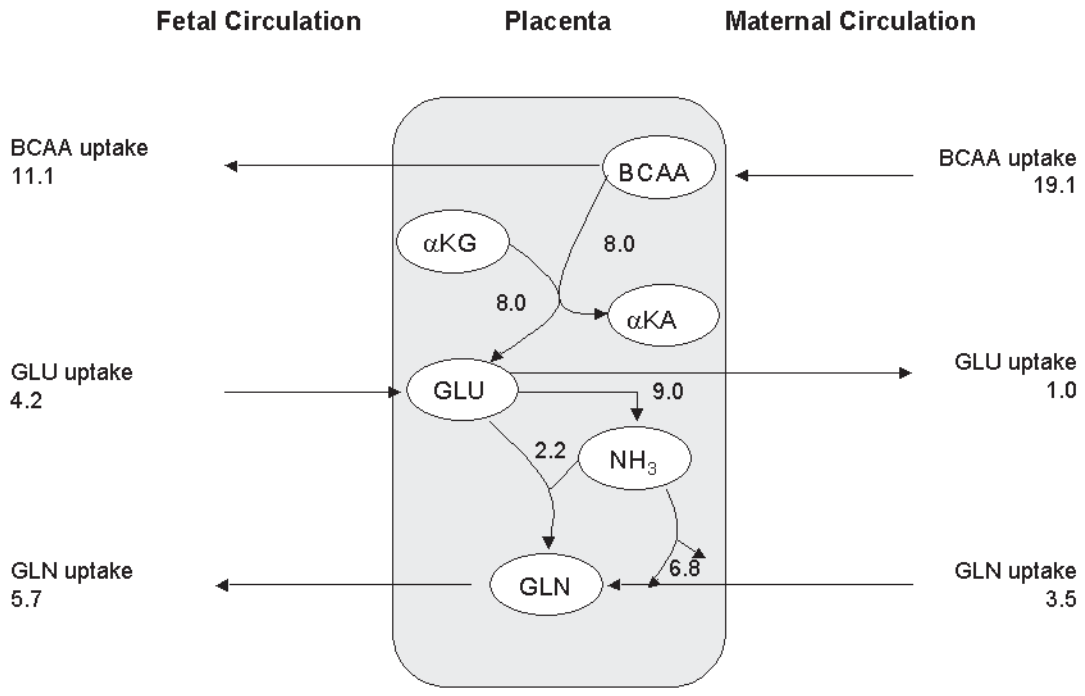

Fig. 10. Schematic diagram of contribution of BCAA deamination and fetal glutamate uptake to placental glutamate (GLU). Oxidation of placental glutamate produces ammonia, which can be either used in synthesis of glutamine (GLN) or excreted into uterine and umbilical circulations. Values are in millimoles/(kilograms of fetal weight·minutes). αKG, α-ketoglutarate; αKA, branched-chain keto acids. (Adapted from ref. *150*.)

natal life, fetal hepatic gluconeogenesis is stimulated and glutamate output significantly depressed *(213)*, demonstrating a important role for glutamate during fetal life.

Additionally it has been shown that glutamate and its amino acid pair, glutamine, display important interorgan exchange between the fetal liver and the placenta *(214)*, in sheep pregnancy. The metabolism of these amino acids within the placenta is linked to both BCAA transamination and placental ammonia production. Glutamate is taken up from the fetal circulation by the placenta without significant uterine uptake. The glutamate taken up is almost completely oxidized by the uteroplacental tissues, with approx 6% returned to the fetus as glutamine *(19)*. Figure 10 presents a schema describing the magnitude of the fluxes for BCAA, glutamate, ammonia, and glutamine in late gestation pregnancy in sheep. Similar data are not yet available in human pregnancy, although a net uptake of glutamate from the fetal circulation into the placenta has been reported *(215)*. Note that the net balance of fetal uptake of glutamine and release of glutamate is quite small since the two fluxes are almost of equal magnitude.

In the fetal liver, the converse is true. There is a large uptake of glutamine and release of glutamate. Approximately half of the glutamine entering the fetal liver exits as glutamate, and the production rate of glutamate by the fetal liver accounts for the total fetal glutamate production. This hepatic glutamate efflux is vital to the fetus since it effectively substitutes for glucose efflux in postnatal life. Fetal hepatic and placental metabolism of glutamate and glutamine change radically under other conditions besides a fetal glucagon infusion. Parturition, either spontaneous *(216)* or dexamethasone induced *(217)*, is associated with decreased fetal hepatic glutamate output and placental glutamate uptake. In addition, in sheep pregnancies complicated by FGR, fetal glutamate concentrations are very low and placental uptake is reduced *(191)*. In human pregnancies, it has been shown that fetal hepatic blood flow can be markedly reduced *(164)*, which may affect fetal hepatic metabolism of these and other amino acids.

Fetoplacental Serine and Glycine Metabolism

Serine utilization within the placenta is every bit as complicated as that of glutamate and glutamine. There is a significant net uptake of serine from both the maternal and fetal circulations into the placenta *(17)*. The net placental uptake of serine from the fetal circulation establishes the fact that all fetal serine requirements must be met by fetal serine synthesis, not by placental transport *(14,16,18)*. Glycine, by contrast, is delivered to the fetal circulation in relatively large amounts, but does not originate from placental transport from the maternal circulation *(16)*. Instead, placental serine uptake from both circulations is utilized for glycine produc-

tion, which then exits the placenta into the fetal circulation. The fetal oxidation of glycine is almost entirely accounted for by oxidation in the fetal liver *(218)*. Fetal hepatic glycine oxidation and serine production occur through the combined actions of the enzyme systems glycine oxidase and serine hydroxymethyltransferase (SHMT). The latter enzyme has two isoforms, a cytosolic and a mitochondrial. In sheep fetal liver, it is the cytosolic SHMT that changes during gestation, increasing as gestation advances, and accounts for the bulk of the fetal hepatic SHMT activity *(219)*. Fetal serine oxidation, in contrast to glycine, is largely in extrahepatic tissues *(220)*. The end result of both fetal hepatic and placental metabolism of serine and glycine is that there is a net hepatic release of serine and CO_2 and a net placental production of glycine and methylenetetrahydrofolate. The latter can be used in purine synthesis and/or remethylation of homocysteine to methionine.

Methionine and Homocysteine Metabolism

Two other amino acids, methionine and homocysteine, play a role in fetoplacental nutrient exchange. It was a surprising finding that in late gestation pregnancies in sheep, there is a net production of methionine by the uteroplacental tissues *(150)*. Methionine is an essential amino acid because its carbon skeleton cannot be synthesized in mammals. Thus, this result implies a net uptake of homocysteine from the maternal circulation into the placenta although there are no data as yet to support this hypothesis. Remethylation of homocysteine to methionine requires methylenetetrahydrofolate, which is a byproduct of placental glycine production from serine. These issues need to be studied further utilizing tracer methodology; this is the only example of a net production of an essential amino acid by the placenta.

Conclusion

We have highlighted those contributions made by in vivo studies of amino acid uptake from the placenta into the fetal circulation and by in vitro studies of specific transporter systems and their activities.

This review has discussed placental amino acid transport, the systems and their associated proteins, umbilical uptake data in animal and human studies, and amino acid transport in FGR. Additionally, the current pool of thought concerning the mechanisms of placental amino acid transport as generated through in vitro vesicle studies and how these relate to the in vivo fluxes of animal studies is also discussed. In the last part of this review we have demonstrated the importance of fetoplacental cycling and metabolism, in both terms of utilization rates of amino acids within the placenta and their interconversion in contributing to the fetal uptake of amino acids and to the provision of nitrogen for placental metabolism. Furthermore, it is clear that an understanding of placental amino acid transport and metabolism requires recognition of the important interaction between the placenta and fetal liver.

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