Review

Placental transport and fetal and placental metabolism of amino acids

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Introduction

Normal fetal growth and development are dependent upon the adequate provision of oxygen and substrates from the maternal circulation. The fetal protein accretion associated with growth requires that at least the essential amino acids be transported across the placenta into the umbilical circulation. The requirement that amino acids be used for protein synthesis prompted earlier perinatal physiologists to assume that there would be little oxidation of amino acids and also very little urea production during fetal life. As we shall see, that was an erroneous assumption even for the essential amino acids. The placental mechanisms involved in amino acid transport result in both a high intracellular concentration of amino acids within the placenta and a fetal/maternal ratio greater than 1.0 for virtually all of the amino acids. The amino acids transported to the fetus can be used in three principal ways: 1) carbon and nitrogen accretion in the growing fetus, primarily in the form of protein synthesis, 2) interconversion to other substrates which are then used for carbon and nitrogen accretion, and 3) oxidative fuels for the fetus, with the production of CO_2, H_2O, and urea. In addition, as we shall see, some amino acids are not delivered to the fetus at all but instead are lost from the fetal circulation to the placenta where they may be metabolized.

This review will focus upon our current understanding of placental transport and fetal and placental metabolism of amino acids.

Placental morphology

As studies in placental metabolism and transport necessitate the use of different models, it should be recognized that there are numerous interspecies' differences in placental morphology which may affect placental function. A brief review of these differences is included below.

The critical tissue layer through which placental exchange occurs is the chorionic epithelium known as the "trophoblast." It comes in contact with the maternal (uterine) tissues and forms a velamentous "placental barrier" or "membrane" in one of three principal patterns as described by Grosser.[1] The epitheliochorial placenta found in equine, ovine, porcine, and bovine species is formed when the trophoblast is apposed to the uterine mucosal epithelium. The endotheliochorial placenta found in carnivorous species, such as the dog, is formed when the trophoblast is juxtaposed to the maternal capillaries. In man and other primates, as well as in some small mammals such as rabbits, guinea pigs and rodents, the hemochorial placenta is formed as the invasive trophoblastic tissue penetrates the uterine stroma, destroys the endometrial capillaries, and comes in direct contact with maternal blood.

In addition to the number of tissue layers, there are other factors determining the placental transport of amino acids. Baur[2] noted the difference in surface area available for exchange of molecules in compact placentas (e.g., those with cotyledons; human, ovine) and diffuse placentas (e.g., porcine). Other factors that may affect transport include the rate of maternal and fetal blood flows, the pattern of placental perfusion, and the permeability characteristics of the placental membrane. For a fuirther review of these areas, see Battaglia and Meschia.[3]

The placental trophoblast at its maternal-facing surface, which includes the microvillous brush border, is the site of amino acid absorption and transport into the trophoblast. Transport across the trophoblast involves: 1) uptake from the maternal circulation across the brush border membrane, 2) transport through the trophoblast cytoplasm, and 3) transport out of the trophoblast, across the basal cell membrane into the umbilical circulation. Permeability of the trophoblast at its maternal and fetal surfaces is likely to be determined by the concentration of specific amino acid carrier proteins on the cell membrane surface and the

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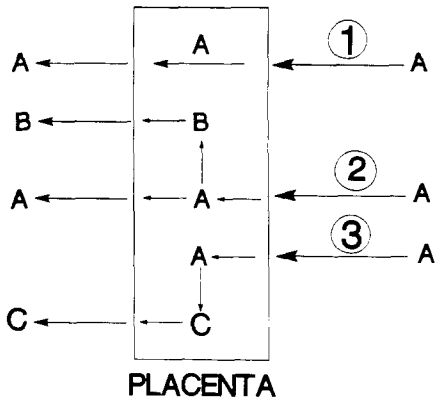

Figure 1 Potential pathways for amino acid transport and metabolism within the placenta. Each letter represents an amino acid. See text for a detailed explanation of each pathway.

affinity characteristics of these proteins. While a great deal of progress has been made in identifying the family of proteins representing the glucose transporter in various tissues, including that isolated from placental tissue, no similar progress has been made yet in isolating and characterizing amino acid transporters. *Figure 1* presents the potential pathways by which an amino acid may cross the placenta. For the essential amino acids, we presume that pathway #1 is the preferred route, with little or no metabolism of the amino acid within the placenta. However, there are no data to support this presumption at this time. The essential amino acid which has been studied most thoroughly during fetal life is leucine. Leucine transport is an example of pathway #2, in that it enters the placenta from the maternal circulation, and a significant fraction is deaminated to *a*-ketoisocaproic acid (KIC).[4] Both KIC and leucine are then transferred to the fetal circulation for further metabolism. Branched chain amino acid transaminases have high activity within the placental tissue.[5,6] Pathway #3 is included since it may be an important pathway for non-essential amino acids. For example, glycine is delivered to the fetus from the placenta in quite large amounts compared to most other amino acids, yet no significant uptake of glycine from the maternal circulation has been demonstrated. This suggests the production of glycine within the placenta. Thus, the delivery of an amino acid from the placenta into the fetal circulation does not establish that the amino acid was derived from the maternal circulation.

In the review of placental transport that follows, we will focus our attention on the known properties of the maternal-facing placental microvillous membrane in amino acid transport. Little work has been done with the basal cell membrane,[7,8] and transport at the fetal surface is poorly understood. Even less work has been done upon the metabolism of compounds within the trophoblast syncytium during nutrient transport.

Transport systems

The placental trophoblast is an epithelial tissue that cytologically resembles similar epithelia with brush border membranes. Functionally, it would be expected to possess most transport carrier mechanisms found in similar tissues. However, when compared to other epithelia (e.g., gut enterocyte and renal tubular epithelium), the pattern of transport as a whole does not mimic that seen in those tissues.

Unique characteristics for neutral amino acid uptake by the placenta were demonstrated by Smith and Johnson using a membrane vesicle preparation.[9] Both Na^+-dependent and Na^+-independent mechanisms were shown to differ in their uptake of alanine compared to studies performed in other epithelia.[10]

Currently, we view amino acid transport across the placental barrier as occurring via one or more of a number of specific transport systems. Three systems for neutral amino acids described by Christensen[11] have been described in human placental tissue by Enders.[12] These are the "A," "L," and "ASC" systems (*Table 1*). They are considered to be protein complexes within the maternal-facing plasma membrane of the trophoblast, but, as yet, have not been isolated. Their activity may be Na^+-dependent[12-15] or Na^+-independent,[9,12,13,15] and transport is an active, carrier-mediated process involving the Mg^{++}-dependent, Na^+/K^+ ATPase.[13] Inhibition of amino acid uptake occurs with varying concentrations of competing amino acids within the same transport group.[12-15] However, there is considerable overlap observed for many amino acids (alanine, threonine, serine, and glutamine). Furthermore, the presence of identified transport systems for an amino acid does not determine whether or not it is transported across the trophoblast into the fetal circulation. Nor can comparisons among amino acids of their transport into isolated microvesicles be interpreted as reflecting their relative rates of transport into the fetal circulation.

Studies using human placental slices have shown that preincubation in an amino acid-free medium will increase the uptake and accumulation of aminoisobutyric acid (AIB) in the slices.[16,17] This increase in transport has been hypothesized to be due to protein synthesis,[17,18] perhaps of specific amino acid carriers. This facilitation of amino acid transport does not occur when amino acids are added to the preincubation medium[17] or in the presence of an increased intracellular concentration of AIB.[19] It has an apparent specificity for the "A" transport system.[12,19] Thus, there may be

Table 1 Placental transport systems for amino acids

A	L	ASC
AIB	Leucine	Alanine
Alanine	Isoleucine	Serine
Glycine	Valine	Threonine
Proline	Phenylalanine	Glutamine
Serine	Alanine	
Threonine	Serine	
Glutamine	Threonine	
	Glutamine	
	Trypotphan	
	Cysteine	

some local regulation of placental amino acid transport to the fetus.

Recently, calcium depletion, blockade of calcium channels, or antagonism of calmodulin activity in human placental slices has been shown to decrease the active transport of AIB by its NA^+-dependent carrier system.[20]

The placenta is an endocrinologically active tissue, both in its production of peptide and steroid hormones and in its functional modulation by hormones. However, whereas epithelial and non-epithelial cells in numerous other organs demonstrate a regulation of amino acid uptake by insulin, and the placental microvillous membrane is richly endowed with insulin receptors, amino acid acid uptake by placenta is not influenced by insulin.[21] This suggests that the placental uptake of amino acids may be independent of fluctuations in maternal insulin concentration occurring either with normal dietary intake or in certain disease states.

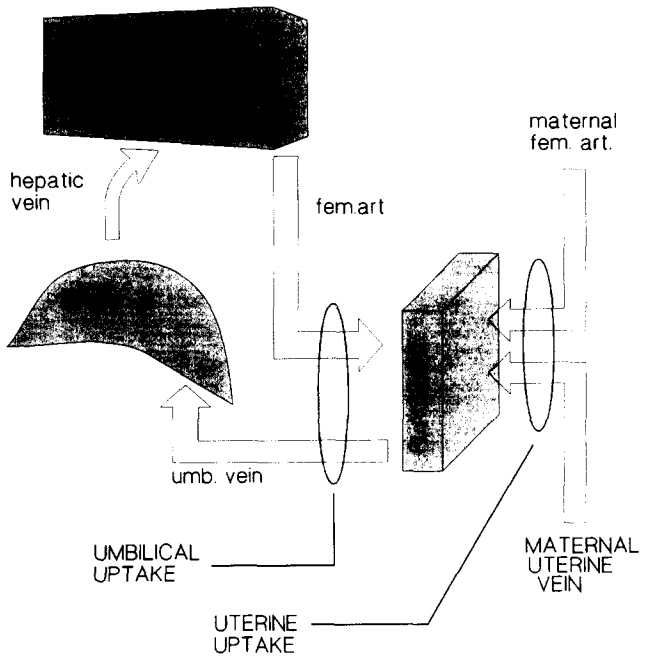

Figure 2 Fetal-placental relationships: Uterine uptake is determined by multiplying the uterine blood flow by the arterial-venous amino acid concentration differences across the uterine circulation (maternal artery—uterine vein). Similarly, the umbilical uptake is calculated by multiplying the umbilical blood flow by the AV difference across the umbilical circulation. The difference between these two values represents the amino acid uptake by the placenta and uterine wall tissues.

Calculation of AV difference across an organ such as the liver must recognize the mixed arterial input to the organ. Thus a calculated input concentration is arrived at by taking into account the concentration in each of the three vessels supplying the fetal liver (umbilical vein, hepatic artery and portal vein) and their relative contribution to total hepatic flow.

Tracer Fluxes: When tracer amino acids are infused into the fetus these calculations can be applied separately to both the substrate (tracee) and tracer concentrations so that net tracee and net tracer fluxes can be calculated.

Differences across an organ or across the uterine and umbilical circulations should be analyzed from the viewpoint of substrate concentration changes, tracer concentration changes and specific activity or MPE changes since each mode of analysis provides different information about metabolism within that tissue bed.

Types of placental preparations

Studies on placental metabolism and transport have been conducted in a variety of in vivo and in vitro models. Early work focuses on the concentration differences in maternal plasma and fetal tissue or plasma.[22-24] Such studies demonstrated higher amino acid concentrations in fetal tissue and plasma than in maternal plasma. Later, it was shown that the placenta concentrates nearly all amino acids intracellularly, presumably for transfer to the fetus.[16,25,26] The fact that amino acids are at higher concentration in fetal blood than in maternal blood, and that most amino acids are transported into the trophoblast against a large concentration gradient, led investigators to assume that all amino acids were transported to the fetus from the maternal plasma pool. It was not until the first report of the umbilical uptake of amino acids in the fetal lamb in 1976 that this hypothesis was shown to be incorrect for some of the amino acids. This was based upon the observation that there was no measurable umbilical uptake for some amino acids, such as serine and aspartate, and that glutamate was leaving the fetal circulation and entering the placenta. For these nonessential amino acids, the fetus met all requirements by amino acid production within the fetal tissues.

The umbilical uptake of amino acids has been described only in the fetal lamb.[27-30] This is surprising given the fundamental importance of this measurement and the understanding of fetal metabolism and fetal nutrition. The uterine uptake of amino acids refers to the uptake of amino acids by the pregnant uterus from the uterine circulation. Conversely, the umbilical uptake refers to the entry of amino acids from the placenta into the umbilical circulation. The uterine uptake has been measured into two species, the pregnant sheep[9,10] and the pregnant rabbit.[31] Neutral amino acids are taken up in excess of their rates of accretion into protein with the conspicuous exception of serine, which is lost from the fetal circulation into the placenta in early gestation and is neither excreted nor taken up by the umbilical circulation in late gestation. The two basic amino acids lysine and histidine are delivered to the umbilical circulation in amounts which are barely in excess of their rates of accretion. The acidic amino acids are not supplied by the placenta to the fetus in any appreciable amount. In fact, there is a significant loss of glutamate from the fetal circulation to the placenta throughout the latter half of pregnancy. Until similar measurements of umbilical uptake are made in other species, we cannot be certain to what extent these features of placental amino acid transport are peculiar to the sheep fetus and placenta or common property to most trophoblasts. Work by Pitkin[32] and Schneider[33] suggests similar characteristics of glutamate transport in the human placenta. While the umbilical uptake of amino acids is important in defining the net nitrogen and carbon delivered to the fetus as amino acids, it does not reflect the total utilization rate of these amino acids, nor does it tell us the unidirectional flux associated with this net exchange. For example, two amino acids may have

identical net umbilical uptakes expressed in μmol/kg/min, and yet one has achieved that net entry rate by no unidirectional flux from the fetal circulation to the placenta and another amino acid achieved the same net flux from the difference between two large fluxes in either direction. The important role of tracer methodology in placental physiology has been, in part, to characterize such unidirectional fluxes and also to determine pathways of utilization of amino acids. For the first amino acid in the example given above, the absence of any measurable unidirectional flux from the fetus to the placenta implies impermeability of the basal layer of the placenta; whereas, in the second case, the basal layer is permeable to the amino acid and thus, the amino acid may contribute to metabolism within the placenta. Tracer methodology can be used to define the relative magnitude of various pathways of utilization, including oxidation, conversion to other products of metabolism, or incorporation of the amino acid into protein. The next section will discuss the data that have been obtained thus far regarding pathways of utilization for some amino acids within the fetus and/or placenta.

Isolated perfused placenta

The isolated perfused placenta model requires cannulation of the umbilical circulation, via the fetal vessels, with a closed fetal perfusate system and the bathing of the maternal side in a separate medium. In some species, the maternal decidua can be cannulated adequately and an intact perfusion performed via both circulations.

These preparations have been used to study the production, metabolism, and transport of different substrates, essential amino acids, hormones, and oxygen.[34] They have allowed the determination of directionality of transport and of any asymmetry in transfer of amino acids across the fetal or maternal side.[35,36] For example, Schneider and associates[33] demonstrated a high uptake of L-glutamic acid from the maternal surfaces into the placental tissues but a very low transport across the human placenta into the fetal circulation. In contrast, L-leucine was taken up to a lesser degree by the placenta but most of it was transferred into the fetal circulation. Recently, Hibbard and associates described the active carrier-mediated nature of taurine transport in the human placenta using this model.[37]

Limitations of using this technique with the whole placenta include the requirements of an intact placenta with fetal vessels which can easily be cannulated. Perhaps only 10% of placental material available for use is suitable due to tissue damage.[38] Fetal perfusion rates must also be sufficiently high to maintain viability of the whole tissue. Use of the isolated perfused cotyledon has reduced such concerns, but care must still be given to reduce the period of ischemia to which the placenta is exposed prior to initiating the in vitro perfusion. This preparation still needs much better characterization since, in all studies, it has had a rather low rate of aerobic metabolism in contrast to the high rate of placental metabolism observed under in vivo conditions. Further studies with this preparation may lead to a better understanding of differences in trophoblast transport and metabolism among species given the limited number of species which can be studied under in vivo conditions.

Ussing chamber

The Ussing chamber consists of two pools of oxygenated bathing media separated by tissue (e.g., placental membranes), which are fixed in a flat sheet between the two pools. Early studies, employing the visceral yolk sac placenta of rabbits, demonstrated the active concentration of valine, isoleucine, phenylalanine, alanine, and methionine within and across the membrane; L-valine being concentrated to levels 76% higher on the fetal side than on the maternal side.[39] Because of the difficulties involved in maintaining placental tissue from most species as a sheet of tissue, this method is not used much at the present time for studies of amino acid transport.

Placental slices

The use of placental slices has been adapted widely for both metabolic and pharmacologic studies of the placenta. Tissue slices afford the ability to undertake multiple studies from the same placenta and allow for investigations at different stages of gestation.[34] Some of the technical problems associated with perfusion models may also be avoided. This preparation is suited particularly to studies in which production of a compound (drug metabolite, hormone, etc.) from another compound is the principal goal of the studies. Obviously, all polarity of the trophoblast is lost in tissue slices, and this severely limits the usefulness of this preparation. In contrast to other tissues in which amino acid uptake has been studied (e.g., muscle or visceral organs), the placental tissue obtained for these studies has a huge extracellular space. Thus, steps that reduce extracellular volume can be used to increase the precision with which intracellular changes can be measured.[16]

Placental slices have been employed for amino acid transport studies to demonstrate uptake kinetics and specificity,[12,16,26,40] preincubation effects,[12,16,17] determination of the active nature of the transport process,[17,40] and regulation of transport by modification of Ca^{++} availability.[20]

Membrane vesicles

A major contribution to the study of transport mechanisms was made by N.C. Smith and colleagues[41] with the introduction of a microvillous membrane preparation derived from human placental syncytiotrophoblast. These membrane vesicles consist of a plasma membrane derived from the maternal-facing microvillous brush border and are composed of phospholipid, cholesterol, sphingomyelin, and a variety of pro-

teins.[42] They are about 0.15 μm in diameter, and retain membrane-bound receptors such as those for insulin.

The vesicle preparation has allowed for further characterization of the Na^+-dependent transport mechanisms for the acidic amino acids, aspartate and glutamate.[43] It has been used extensively for amino acid transport studies, representing the maternal surface of the trophoblast.[12,14,15]

The description of amino acid transport systems using these vesicles is reasonably complete. However, there are major limitations to the extrapolation of data obtained in this in vitro system to the intact organism. First, the vesicles are composed of maternal-facing membrane and, hence, only give information regarding uptake from the maternal circulation. Secondly, they are studied in relative isolation from the hormonal milieu that constitutes the maternal, placental, and fetal environment throughout development. Finally, these vesicles do not allow for any investigation of substrate metabolism within the trophoblast, or the means by which compounds are transported within the intact cell.

Trophoblast culture

Recent developments in isolating and maintaining both murine[44] and human[45] trophoblast cells in culture have opened the door for still further studies of placental transport and metabolism. The theoretical advantages of such a system include the ability to obtain cells for culture at different time points in gestation to access changes in transport and metabolic function that are gestational age-dependent, to maintain cells for study over extended periods of time thus permitting repeated measurements, and to address inductive or maturational changes within the same cell preparation.

Fetal amino acid metabolism

It is clear from the foregoing discussion that many amino acids are delivered to the fetus in considerable excess of their protein accretion rates. This suggests that amino acids must be utilized in alternative ways such as for oxidative degradation or, for some potentially gluconeogenic amino acids, for accretion in other carbon forms such as glycogen within the fetus. The remainder of this review will be devoted to the physiologic studies of fetal amino acid uptake and utilization.

Amino acid uptake

Studies of net amino acid uptake are important in determining the bulk flow of carbon and nitrogen to the whole fetus and to individual organs. Through application of the Fick principle, amino acid uptakes have been determined across the uterine and umbilical circulations,[27,29,30,46,47] the fetal hindlimb,[48,49] and the fetal liver.[30] A net umbilical uptake (delivery from placenta to fetus) has been demonstrated for all essential and most non-essential amino acids.[27,29,30] Three exceptions for which no measurable umbilical uptake has been found are aspartate, glutamate, and serine. Glutamate is unique in that there is a net efflux from the fetus to the placenta throughout the latter half of gestation, indicating glutamate production within the fetus. Serine is of interest as well since the placental uptake varies with gestational age. At term gestation, no net flux of serine is evident while mid-gestation is characterized by a large efflux of serine from the fetus to the placenta.[3] These observations demonstrate that certain amino acids must be synthesized within the conceptus and that the fetal or placental production rate varies.

In addition to the amino acid uptake of the whole fetus, studies have been performed for individual fetal organs. The fetal liver is of interest because the majority of the umbilical blood must pass through the liver before efflux into the vena cava, permitting first pass extraction and metabolic alteration prior to entry into the systemic circulation. *Figure 2* presents the basic preparation used in such studies that isolate fetal hepatic from extra hepatic metabolism. The fetal liver demonstrates a net uptake of all the essential and most non-essential amino acids.[30] Of interest is the observation that there is a net release of both glutamate and serine from the fetal liver. While hepatic efflux of glutamate has been reported in adult animals,[50,51] the release of serine in the fed state appears to be unique to fetal metabolism.[52,53]

The biologic significance of this fetal hepatic release may be related to the placental metabolism of biochemically related amino acids. The reciprocal uptake and release of glutamate and glutamine between the placenta and the fetal liver suggest both that glutamate may be produced within the fetal liver from glutamine and that this cycle may function as a nitrogen shuttle from the placenta to the fetal liver. Similarly, the lack of a net umbilical uptake of serine, despite accretion of serine in fetal body proteins, demonstrates that the fetus must be the site of serine production. Glycine, an amino acid that is interconvertible with serine, has both a net umbilical and hepatic uptake. Thus, there may be cycling of these two amino acids between the placenta and the fetal liver. The only other fetal organ system for which studies of net amino acid uptake have been performed is the fetal hindlimb. Studies of the fetal hindlimb are important in so far as they are representative of the uptake of the whole non-visceral carcass. The site of sampling is crucial in determining the mass of tissue being studied and also in avoiding interference with the high rate of blood flow characteristic of fetal tissues. Furthermore, it is important to recognize that the composition of the tissue bed served by that circulation would vary considerably at different stages of in utero development and certainly would vary among different species at the same stage of development, particularly in the percentage of the tissue which is nonskeletal muscle tissue and in the percentage represented by adipose tissue. There has been a net uptake of all amino acids across the hindlimb when the mother is in the fed state.[48,49]

It is of interest to examine the amino acid uptake

of the fetal hindlimb during maternal fasting, since studies in adult animals have shown that skeletal muscle represents a large store of protein from which amino acids can be derived for gluconeogenesis.[54] During maternal fasting, the gluconeogenic amino acids, alanine and glutamine, demonstrate a substantial efflux from the fetal hindlimb. These data are consistent with the hypothesis that the fetus responds to a decreased supply of glucose by increased protein degradation, thereby increasing the supply of amino acids available for catabolism. Another finding consistent with this interpretation is the observation that the fetal urea production rate increases during maternal fasting.[55] Despite the obvious differences in amino acid uptake between individual organs, amino acid uptake has not been measured across other fetal organs such as the brain, kidney, and heart.

The uterine uptake of amino acids may be important clinically in the pathogenesis of several of the complications of a diabetic pregnancy, including macrosomia and hypoglycemia. In vitro studies of fetal rat pancreatic rudiments demonstrated significant beta cell hyperplasia and insulin response to increasing amino acid concentrations.[56] The response was greater with amino acids than with glucose-supplemented media. Therefore, aberrations in maternal amino acid metabolism in maternal diabetes may have an effect on the fetal beta cell and may contribute to those fetal complications presumed to be associated with fetal hyperinsulinism.

Maternal/fetal amino acid concentrations

Initial studies of maternal and fetal amino acid concentrations were performed in the fetal lamb and rat. In these studies fetal amino acid concentrations in plasma were consistently higher than in the maternal circulation.[55,57] These observations have been confirmed in the human fetus at delivery by many different investigators.[24,31,58] Through the use of cordocentesis, comparisons of plasma amino acid concentrations can be made throughout gestation. In normal fetuses the concentrations of most amino acids do not change significantly between the second and the third trimesters.[31,59,60] In contrast, differences have been noted in the concentrations of many maternal amino acids with advancing gestational age.[61-63] Unfortunately, the various studies have presented conflicting data, with both increased and decreased concentrations reported for maternal amino acids. However, throughout gestation, the fetal/maternal amino acid molar concentration ratios were greater than one, indicating that fetal concentrations remained consistently greater than those in maternal plasma.

The effects of maternal fasting on both fetal and maternal amino acid concentrations have been studied in the fetal lamb. During a maternal fast of five days, the exogenous supply of glucose to the fetus is reduced by approximately 50%, and amino acid catabolism increases as evidenced by an increased fetal urea production. Despite the increased amino acid oxidation, fetal concentrations of most amino acids remain relatively constant while others increase significantly during a maternal fast of eleven days (57). Surprisingly, it has been reported that the umbilical uptake of amino acids does not decrease during a maternal fast despite a marked decrease in the concentration of most amino acids. For example, the maternal concentration of alanine, a major gluconeogenic amino acid, is decreased by more than 50% within 72 hours of fasting, while the fetal concentration and umbilical uptake are unchanged. Therefore, while the supply of gluconeogenic amino acids decreases significantly in the maternal circulation with a prolonged fast, they remain in high concentration in the fetal circulation for utilization by the fetus. Studies of fetal plasma disposal rate of amino acids have not shown any decrease in plasma disposal rate during a maternal fast.[64]

The changes described in animals during a maternal fast of relatively short duration cannot be extrapolated to conditions associated with intrauterine growth retardation. In the latter case, there is usually a reduction in placental size and presumably in placental function. In contrast to the studies during a relatively short-term fasting period in animals, fetal amino acid concentrations have been found to be reduced in growth-retarded human fetuses and in animal models of growth retardation. In the bilateral uterine artery ligation model of growth retardation in rats, the fetal/maternal ratios of leucine, isoleucine, and valine were decreased markedly. Other amino acids were unchanged.[57,65] The limitations of human experimentation have necessitated the use of animal species for most in vivo work. However, with the advent of cordocentesis, a technique in which blood is sampled from the umbilical vessels via a needle inserted by ultrasonic guidance transabdominally in the pregnant woman,[66,67] simultaneous sampling of the human fetal and maternal circulations has been possible and is yielding informative data on amino acid concentrations at mid-gestation and late gestation.[31] In their study of normal and growth-retarded fetuses, Cetin and associates[31] used cordocentesis to demonstrate a decreased total alpha-aminonitrogen in growth-retarded fetuses weeks before delivery. This was due mainly to a reduction of the branched chain amino acids, valine, leucine, and isoleucine, and of lysine and serine. These studies suggest that altered amino acid uptake and/or metablism may play a role in the pathogenesis of intrauterine growth retardation. With the exception of this study, all other in vivo studies have been performed in animal models.

Amino acid utilization

Amino acids may be utilized within the fetus in a variety of ways, including oxidation, protein synthesis, and accretion in forms other than protein. Amino acids may also leave the fetal circulation and enter the placenta. Recently, these avenues of disposal have been investigated for a few amino acids, utilizing tracer methodology in animal models.

Amino acid utilization

Amino acids may be utilized within the fetus as metabolic fuels. The observation that most amino acids are delivered to the fetus in amounts that exceed their rates of protein accretion implies a considerable oxidation to CO_2, H_2O, and urea within the fetus. This implication is supported by the observation that there is a relatively high rate of fetal urea production, larger than that observed in the newborn or adult.[64] The oxidation rate of an amino acid can be estimated directly with the use of tracer methodology. Initial studies only documented that amino acid oxidation was taking place, but were unable to estimate the oxidation rate of a given amino acid.[68,69] By application of the Fick principle to the umbilical circulation, an accurate estimation of tracer CO_2 production rate from a given amino acid and therefore oxidation can be obtained.[70]

The only amino acid for which a fetal oxidation rate has been determined is leucine.[71,72] At term gestation, the leucine oxidation rate was measured in the late gestation fetal lamb as 6.43 ± 1.02 $\mu mol \cdot min^{-1}$. This value is somewhat higher than the rate of leucine accretion into protein. During a period of maternal fasting, the fetal leucine oxidation rate approximately doubled. These data demonstrate that there is significant fetal oxidation of even an essential amino acid and that this process increases in response to maternal fasting. When the leucine oxidation rate was measured in the mid-gestation fetal lamb, the rate of fetal leucine oxidation per gram dry weight was significantly higher than in late gestation. Therefore, even at a time when the fetus is undergoing rapid growth, significant oxidation of essential amino acids is taking place.

Protein synthesis and degradation

Much of the net amino acid uptake of the fetus is used for protein synthesis. For leucine, approximately 50–60% of tracer leucine infused into the fetal circulation is retained in fetal protein.[72] The flux of amino acids into proteins is bidirectional, as there is continuous protein degradation as well as protein synthesis. In fact, while there has been a single report professing to find little or no protein turnover in fetal life, the methodology used in that study was questionable. Other studies have demonstrated clearly a high rate of fetal protein breakdown and have found this rate to be gestational-age dependent.[71] The rate of fetal protein breakdown is higher the earlier one goes in fetal life. However, studies earlier than mid-gestation have not been carried out yet in any species. The rate of protein synthesis must exceed the rate of degradation for net protein accretion to occur.

A determination of the protein synthetic rate of the whole organism can be obtained utilizing tracer methodology. In the fetal lamb ^{14}C-Leucine and ^{14}C-Lysine infusions have both been used to estimate the rate of protein synthesis,[68,71] but other amino acids including ^{14}C-tyrosine and ^{14}C-phenylalanine have been used as well.[73-76] In the late-gestation ovine fetus, the whole body rate of protein synthesis varies widely from 15–63 g/day/kg.[68,75] The fractional synthetic rate of body proteins (the percent of body protein synthesized per day) can be calculated from the steady state specific activity of the free amino acid, the specific activity of the amino acid in body proteins, and the infusion rate. It is important to realize, however, that the calculated protein synthetic rate for the whole organism has limited meaning, since it is a sum of individual rates within that organism which may be vastly different and would not include those proteins and peptides with a short half-life and rapid turnover. The rate of protein synthesis has been measured in mid-gestation and at term gestation in the fetal lamb.[68,71] From these studies, it is clear that over the latter half of gestation, the fractional rate of protein synthesis decreases until term, with values ranging from 23% per day at mid-gestation to less than 10% at term.

Since ATP generated by oxidative phosphorylation is required for protein synthesis, changes in the fetal protein synthetic rate affect energy requirements. A comparison has been made of the changes in fetal protein synthesis to the changes in oxygen consumption that occur during gestation. During fetal life, there is a decrease in the weight specific oxygen consumption rate as the fetus matures.[77] In late gestation, the rate of protein synthesis accounts for approximately 25% of the oxygen consumption of the fetus. In mid-gestation, the rates of oxygen consumption and protein synthesis are increased but proportionately such that the higher protein synthetic rate of the mid-gestation fetus still accounts for approximately 25% of the fetal oxygen consumption.[71] During periods of fetal hypoxia, marked changes in protein synthesis and oxygen consumption may occur. Studies in the fetal lamb have demonstrated decreased protein synthetic rates during periods of decreased oxygen delivery to the fetus.[78] However, the methodology used for estimating fetal protein synthetic rate may be questioned given the inability of this methodology to detect the high rate of fetal protein breakdown. If these observations of a reduction in fetal protein synthesis due to insufficient oxygenation are valid, they may be relevant to the clinical problem of intrauterine growth retardation where conditions that limit oxygen delivery to the fetus may occur.

In addition to protein synthesis, there is a large protein turnover in the growing fetus. Evidence for a high rate of protein turnover within the fetus includes the observation that the fractional rate of protein synthesis is significantly higher than the fractional growth rate.[68]

Alterations in both protein synthesis and turnover occur with maternal fasting. During periods of maternal starvation in the fetal rat, the fractional rate of protein synthesis is maintained initially but is then inhibited with prolonged deprivation.[73,74] In contrast, the rate of protein turnover accelerates with short-term deprivation but is then decreased with prolonged fasting. This suggests that the reduced protein accretion resulting from prolonged maternal fasting may occur

as a result of decreased fractional rate of protein synthesis out of proportion to the fractional rate of protein breakdown.

In addition to being the building blocks of proteins, carbon from amino acids may also be converted to other compounds. Well-defined biochemical pathways link many amino acids to the synthesis of carbohydrates, fats, and nucleic acids. These pathways represent another avenue of amino acid disposal in the fetus. To date, little work has been done to quantify the relative contribution of these pathways for amino acid utilization.

Another route of amino acid disposal is through a unidirectional flux of amino acids from the fetal circulation into the placenta. As previously mentioned, studies of net amino acid uptake have demonstrated a net flux of all essential and most non-essential amino acids from placenta to fetus. However, tracer studies have demonstrated that this net flux is actually the sum of two opposing unidirectional fluxes that exchange amino acids between the placenta and fetus. For example, the unidirectional flux of tracer leucine from the fetus to the placenta in the mid-gestation fetal lamb is quite large.[71] The fraction of tracer leucine infused into the fetus that escaped into the placenta was inversely related to the fetal weight and reached values as high as 60%. When the placenta is large relative to the fetal weight, the efflux of amino acids from fetus to placenta may represent the largest component of the fetal disposal rate. The fate of amino acids entering the placenta includes both transport across the trophoblast to the maternal circulation and metabolism within the placenta.

Much remains to be learned about the fetal and placental metabolism of amino acids and their relationship to disease states. Animal studies have begun to show links between altered amino acid metabolism or altered delivery due to maternal malnutrition and abnormal fetal growth. The role of hormones and trophic peptides in regulating amino acid transport and metabolism urgently needs study. Many of these processes may now be studied in man with the use of stable isotopes and may help in the understanding and potential treatment of disorders of fetal growth and nutrition.

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