Placental Delivery of Amino Acids. Utilization and Production vs. Transport

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Early studies of amino acid transport across the ovine placenta suggested that amino acids taken up by the placenta from the maternal circulation are delivered to the fetus with no major loss (placental amino acid utilization) or addition (placental amino acid production) (1). Recent data indicate that for some amino acids, this suggestion is not valid, and that amino acid metabolism is an important aspect of placental function. The new information is based on several lines of evidence.

COMPARISON OF UTERINE AND UMBILICAL UPTAKE OF AMINO ACIDS

In sheep, the net flux of metabolic substrates between maternal circulation and pregnant uterus (uterine uptake) and the net flux of metabolic substrates between placenta and fetus (umbilical uptake) have been estimated using an application of the Fick principle (2). In this application, uterine uptake of any given substrate is estimated by measuring uterine blood flow (ml/min) and the concentration difference of the substrate between maternal arterial and uterine venous blood (μmol/ml). The uterine uptake (μmol/min) is then calculated as the product of uterine blood flow and concentration difference. Umbilical uptake is similarly estimated by measuring umbilical blood flow and the concentration difference of the substrate between umbilical venous and umbilical arterial blood. When uterine and umbilical uptakes are estimated in the same animal preparation, they provide information about placental metabolism. A uterine uptake that is greater than umbilical uptake indicates utilization by the uteroplacental tissues, whereas an umbilical uptake that is greater than uterine uptake indicates placental production.

The application of the Fick principle to the study of placental amino acid metabolism in vivo has been difficult to implement for the following reasons: (a) with few exceptions, the concentration differences of each amino acid across the uterine and umbilical circulations are a relatively small percentage of the arterial concentration,
Thus, a large number of measurements are required to compensate for the noise introduced by random analytic error, (b) in the chromatographic measurements of amino acid concentrations, incomplete peak separation may provide unreliable information about certain amino acids. Early attempts to measure serine uptakes were biased by this error (3), and (c) free amino acid concentrations can be measured in either whole blood or plasma. The choice of which concentration is measured affects the accuracy of the uptake estimate. For example, plasma carries glutamate at a much lower concentration than red cells and is the only blood compartment that exchanges glutamate with the placenta and other organs (4). As a result, glutamate uptake is best estimated as the product of so-called plasma flow [blood flow x (1—fractional hematocrit)] and the plasma glutamate concentration difference. Plasma concentration differences of amino acids for which plasma is not the only blood compartment of exchange must be converted to whole blood differences and then multiplied by blood flow. For leucine, conversion factors have been estimated by measuring the volume of distribution of tracer amino acid added to maternal and fetal blood incubated in vitro (5).

The interpretation of the results of uptake calculations raises theoretical concerns in addition to the practical concerns listed above. For example, the uptake calculations assume that in passing through the placenta, maternal and fetal blood do not produce free amino acids. This would not be the case if blood proteins and peptides were rapidly hydrolyzed by the placenta and became a significant source of free amino acids at the site of transplacental exchange. However, there has been no evidence thus far to suggest that processes other than placental uptake and release of amino acids play a significant role in determining the concentration differences of amino acids across the uterine and umbilical circulations.

In the last 2 years, we have begun a long-term project in which simultaneously measured uterine and umbilical blood flows and plasma concentration differences of amino acids across the uterine and umbilical circulations are used to explore placental amino acid metabolism in vivo through the application of the Fick principle. Thus far, this project has provided information about uptakes of most of the neutral and acidic amino acids in 15 pregnant sheep, studied under normal physiologic conditions and at approximately 130 days’ gestation (about 2 weeks before term). There are three notable results: (a) the branched-chain amino acids leucine, valine, and isoleucine show uterine uptakes that are significantly greater than umbilical uptakes, (b) there is a fairly large serine uptake by the pregnant uterus with no significant umbilical serine uptake. The opposite occurs with glycine: no significant uterine uptake and a large umbilical uptake, and (c) glutamate shows a large uptake by the placenta from the umbilical circulation (negative uptake). These results are confirmed and explained by other lines of evidence, including tracer studies. As placental uptake of glutamate is discussed in another chapter in this volume (Fetal Liver and the Placenta: An Interactive System), the remainder of the discussion focuses primarily on the branched-chain amino acids glycine and serine.
PLACENTAL DELIVERY OF AMINO ACIDS

PLACENTAL UTILIZATION OF BRANCHED-CHAIN AMINO ACIDS

In addition to the data already mentioned, uterine uptakes of branched-chain amino acids that are significantly greater than umbilical uptakes have been observed in two previous studies (5,6). The placenta releases into the fetal and maternal circulations the keto acids formed in the deamination of leucine (ketoisocaproic acid) (5,7,8), valine (ketoisovaleric acid) (8), and isoleucine (α-keto-β-methylvaleric acid) (8). When tracer leucine is infused into the fetus, approximately 10% is deaminated in the placenta (7). We conclude from this evidence that branched-chain amino acids entering the placenta are rapidly deaminated, and that this catabolic process reduces the flux of branched-chain amino acids from placenta to fetus.

In exploring the functional meaning of branched-chain amino acid deamination within the placenta, we note that it results in the formation of glutamate through transamination with α-ketoglutarate. Therefore, the placenta has an endogenous source of glutamate from branched-chain amino acid transamination as well as an exogenous source from the uptake of fetal glutamate. The oxidation of glutamate by placental mitochondria generates NADPH (reduced nicotinamide adenine dinucleotide phosphate), which can then be used for steroid synthesis (9). This information suggests that placental branched-chain amino acid deamination may be linked to steroidogenesis.

Through glutamate oxidation, the deamination of branched-chain amino acids ultimately results in the formation of ammonia. The uteroplacental tissues take up branched-chain amino acids at a combined rate of approximately 20 μmol/min/kg fetus and release them into the fetal circulation at the combined rate of approximately 13 μmol/min/kg fetus. This indicates a net uteroplacental branched-chain amino acid utilization rate of approximately 7 μmol/min/kg fetus. The ovine uteroplacental tissues excrete ammonia in both the maternal and fetal circulations at an estimated rate of 10 μmol/min/kg fetus (10). Therefore, the comparison of uteroplacental branched-chain amino acid utilization and ammonia excretion (7 vs. 10 μmol/min/kg fetus) suggests that deamination of branched-chain amino acids is one of the main contributors to placental ammonia production.

Recently, ovine placental transport and fetal utilization of leucine at 130 days’ gestation have been compared between control ewes and ewes in which severe intrauterine growth retardation (IUGR) had been induced by exposure to heat stress in early and midpregnancy (5). Leucine fluxes were measured by means of leucine tracers simultaneously infused into the maternal and fetal circulations. Several fluxes were reduced in the IUGR group, even when expressed per kilogram of fetus. There were significant reductions in uteroplacental utilization, fetal disposal rate, transport from mother to fetus, back flux from fetus to placenta, and decarboxylation of fetal leucine. Uteroplacental utilization and transport from mother to fetus were also significantly reduced per gram placenta. These data indicate that maternal leucine flux into the IUGR placenta was markedly reduced and that most of the reduced
flux was routed into fetal utilization through a decrease in placental leucine catabolism and a decrease in the leucine flux from fetus to placenta. It is apparent, therefore, that placental branched-chain amino acid metabolism may be altered in IUGR pregnancies and that the amount of branched-chain amino acids available for fetal consumption depends on the interplay between placental transport and placental metabolism of these amino acids.

**PLACENTAL GLYCINE PRODUCTION**

Several studies have shown a relatively large fetal uptake of glycine at the approximate rate of 5 \( \mu \text{mol/min/kg fetus} \) (11). As there is no demonstrable uptake of glycine by the pregnant uterus, the data suggest that most of the glycine delivered by the placenta to the fetus is produced within the placenta. Glycine can be formed from serine by the following reaction:

\[
\text{Serine} + \text{Tetrahydrofolate} \rightarrow \text{Glycine} + \text{Methylenetetrahydrofolate} + H_2O
\]

Therefore, the glycine data focus attention on placental serine metabolism. There are three possible sources of serine for the placenta: the fetal circulation, the maternal circulation, and serine synthesized within the placenta.

Several attempts have been made to establish whether there is a net flux of serine into the placenta from the fetal circulation. This flux has been difficult to detect, because the concentration of serine in fetal blood and plasma is very high (approximately 700 \( \mu \text{M} \)). In three studies of the sheep fetus in late gestation, the estimated net flux ranged from statistically insignificant to approximately 2 \( \mu \text{mol/min/kg fetus} \) (12). For the unpublished observations in the 15 late-gestation fetuses, the mean net flux was 1.23 ± 0.84 \( \mu \text{mol/min/kg fetus} \) and not significantly different from zero. However, two studies of amino acid uptake in the midgestational fetus were able to demonstrate a significant net flux of serine into the placenta from the fetus (12). Furthermore, the infusion of \( L-[1^{-13}C] \) serine into the fetal circulation has shown placental uptake of the tracer serine and significant placental output of \([1^{-13}C] \) glycine, both in late and midgestation (12). The combined tracee and tracer evidence indicates that fetal serine is one of the sources for the production of glycine by the placenta, although not a major source in late gestation.

The unpublished data in 15 late-gestation fetuses show a relatively large uptake of maternal serine by the pregnant uterus, equal to about 80% of placental glycine output into the umbilical circulation. This comparison suggests that in late gestation the placenta uses primarily maternal serine for the production of fetal glycine. To test the hypothesis that maternal serine is converted to fetal glycine by the placenta, we infused \( L-[1^{-13}C] \) serine intravenously into the mother and sampled maternal and fetal plasma for tracer serine and glycine enrichments. These experiments showed no enrichment of fetal serine and a significant enrichment of fetal glycine. However, the interpretation of the data was made somewhat difficult by the fact that the maternal infusion of tracer serine labeled maternal glycine, raising the question of
whether the tracer glycine transported into the fetus by the placenta had been formed in the mother rather than in the placenta. In separate experiments designed to answer this question, tracer glycine was infused into the mother. The tracer glycine infusion showed mother-to-fetus glycine transport, but at a rate that could explain only a small fraction of the fetal glycine labeling in the tracer serine experiments. Therefore, the combined results of maternal tracer serine and glycine infusion supported the hypothesis that maternal serine is used by the placenta to form fetal glycine. For a more direct proof of placental conversion of serine to glycine, we performed a different experiment, using a twin preparation (13). In this preparation, we used the local infusion of tracer serine into one uterine artery to expose the placenta of one twin (experimental placenta) to a higher concentration of tracer serine than the placenta of the other twin (control placenta). No tracer serine could be detected in either fetus. Glycine enrichments in uterine venous and umbilical arterial and venous plasma were significantly higher for the experimental placenta, thus demonstrating placental conversion of maternal serine into glycine.

The question of whether serine synthesized within the placenta is a significant source for the formation of fetal glycine remains unanswered. During the constant infusion of tracer serine into the maternal circulation, umbilical venous glycine enrichment is about 5% of maternal serine enrichment. This indicates that maternal serine entering the placenta is diluted by placental serine and that the glycine formed from the maternal serine is further diluted by placental glycine. Because the ovine placenta has a high protein turnover rate (5,14), it is unclear whether this dilution results from the release of serine and glycine by placental proteins or includes \textit{de novo} synthesis of placental serine.

In the conversion of serine to glycine, each $\beta$ carbon of serine becomes an "activated one-carbon unit" through the formation of methylenetetrahydrofolate. These carbon units are used in a large number of biosynthetic processes (e.g., purine synthesis). Therefore, the conversion of serine to glycine within the placenta may have the main function of synthesizing metabolic substrates that are important for fetal growth and depend on the availability of activated one-carbon units for their synthesis. Further studies focused on the placental utilization of $\beta$ serine carbon are needed to explore the validity of this hypothesis.

\textbf{SUMMARY}

Studies of placental amino acid metabolism, by means of \textit{in vivo} tracer methodology, have shown that the ovine placenta deaminates leucine and converts both maternal and fetal serine into fetal glycine. There is evidence that in addition to leucine, the placenta deaminates the other two branched-chain amino acids—iso-leucine and valine. The comparison of branched-chain amino acid uptake by the pregnant uterus and by the umbilical circulation (20 vs. 13 $\mu$mol/min/kg fetus) suggests that the branched-chain amino acid deamination rate by the placenta could be as high as...
7 μmol/min/kg fetus. The transamination of branched-chain amino acid with α-ketoglutarate produces glutamate, which is an oxidative substrate for placental mitochondria. In addition, the placenta oxidizes glutamate derived from fetal plasma. Glutamate oxidation by placental mitochondria has been linked to NADPH production and steroidogenesis. In the heat stress model of ovine fetal growth retardation, both placental transport and placental utilization of leucine are reduced.

Although maternal serine enters the placenta, there is no placental transport of maternal serine into the fetus. The comparison of uterine serine uptake with umbilical glycine intake indicates that in late gestation, maternal serine contributes about 80% of the glycine delivered to the fetus by the placenta. One important function of serine-to-glycine conversion is the generation of methylenetetrahydrofolate, which is a substrate for a variety of essential biosynthetic processes. It is not clear, however, what functional advantage, if any, is provided by localization of the entire conversion of maternal serine to fetal glycine in the placenta rather than the fetus.

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REFERENCES

DISCUSSION

**Dr. Rennie:** It looks as though the maternal tracer enrichment of your leucine was quite different in the IUGR and control animals—one was about 6 mol% excess and the other was about 4. Was that a reflection of the maternal turnover, or did you put different amounts of tracer in?

**Dr. Meschia:** You are quite right, there was a lower turnover of leucine in the growth-retarded than in the IUGR mother, and that correlated with nutrition. When we exposed the sheep to heat stress in a 40°C chamber for several hours, our first concern was whether we were producing growth retardation because of effects on maternal nutrition. But in fact maternal nutrition was not much affected by heat stress in early and midgestation. However, when the sheep were taken out of the chamber, there was a reduced food intake, and we attributed this to the fact that they were carrying a much smaller fetus and placenta, and the mammary glands were not so well developed.

**Dr. Soothill:** But you said in your talk that you believed this was caused by reduced transfer. But the other hypothesis is that there is reduced protein turnover and protein uptake within the fetus. How did you separate those two?

**Dr. Meschia:** First of all, there is reduced uptake of leucine by the mother, but when one looks at the concentration of leucine in maternal blood, it is not altered. The fetal leucine concentration is reduced compared with control. There is a decreased disposal rate of leucine in the fetus that is almost exclusively caused by the fact that there is less entry of leucine into the fetus and less back flux of leucine into the placenta. So it is the dysfunction of the placenta that explains the decreased disposal rate of fetal leucine.

**Dr. Jensen:** Is there any relation between oxygen delivery to the placenta/fetus and the reduced leucine disposal rate?

**Dr. Meschia:** As I have already indicated, these fetuses tend to be hypoxic, and that is because of an increased PO₂ gradient between maternal blood and fetal blood. It is not caused by decreased uterine perfusion. Although in absolute terms there is less blood flow in the growth-retarded conceptus, when one looks at uterine blood flow per kilogram of fetus or per kilogram of conceptus, then there is no difference. The fetus is hypoxic because there is a larger gradient of PO₂ between mother and fetus. In other words, the diffusing capacity of the placenta for oxygen is reduced. Oxygen consumption by the uteroplacental mass is not reduced, so this reduction of leucine utilization seems rather specific.

**Dr. Jensen:** Would that lead us to the conclusion that the transporter system for leucine and the reduced disposal rate are regulated on the fetal side rather than on the maternal side?

**Dr. Meschia:** It is extremely difficult to establish cause and effect from in vivo data. There is definitely decreased transport of leucine into the placenta. I indicated that this is probably a primary event compared with other things, such as a decrease in leucine concentration in the fetus. But I would not go so far as to claim a cause-and-effect relationship—that is really difficult to establish.
**Dr. Marini:** In your model, did you have any chance of measuring heat shock proteins in the maternal myocardium?

**Dr. Meschia:** We haven't done that yet. But we have in mind to measure heat shock proteins in the placenta to see if they appear under these conditions. We really have not yet understood the pathogenesis. What we can exclude is that this defect in transport of amino acid has anything to do with decreased blood flow. It is definitely a problem of transport.

**Dr. Godfrey:** One of the very important things to bear in mind is the likelihood that these amino acid relationships are very different in early and late pregnancy. For example, we have indirect data suggesting that indices of glycine insufficiency are telling us very different things in human early and late pregnancy. Do you have any data that might bear on that in your models?

**Dr. Meschia:** We have no data yet in early pregnancy.

**Dr. Herrera:** Is there any relationship between decreased protein synthesis and the rate of leucine transfer in the growth-retarded fetuses?

**Dr. Meschia:** We do not see significant changes in protein synthesis. I have to be very careful here. What we study, of course, is the turnover of plasma leucine, and we can estimate how much of the flux of leucine is into protein rather than oxidation. We find that the flux into protein is no different per kilogram of fetus than in the normal, although it could be 10% or 20% less, in which case it would be very difficult to establish a statistically significant difference. I want to point out that from ultrasonographic scans one can see that this growth retardation begins rather early in gestation; one can therefore assume that this severe growth retardation that one sees at the end of pregnancy is the accumulated effect of a relatively small difference in growth rates over a long time, and that is why it is not easy, in my opinion, to see changes in protein synthesis per kilogram of fetus.

**Dr. Rennie:** When we measure glycine transfer, as we can in the human situation, we do get a substantial glycine transfer from the mother to the fetus. Under IUGR conditions, although there is a substantial decrement of leucine transfer, we find there is no decrement of glycine transfer but an increased glycine back flux. So it appears that the two amino acids differ in that leucine does not get across as fast as glycine, but glycine appears not to be used so fast and therefore it comes back to the mother. I don't know whether this is a sheep difference or a model difference, because our model and yours are really very different. But I think it is fascinating that there are these differences. I wonder whether you found any differences in serine-to-glycine transfer in your growth-retarded animals. I don't think you have touched on that.

**Dr. Meschia:** We have not studied the serine-to-glycine conversion in the growth-retarded. You are quite right that we have to be very careful about the species differences. The paracellular pathway of transport seems to be absent in the sheep. So any transport of amino acid you see from mother to fetus definitely involves transporters. There is definitely a species difference here. The sheep placenta seems to act only via transporters; there is no evidence that amino acids are transported in any other way in the sheep.

**Dr. Marconi:** We have done human pregnancy studies that are quite similar to those done in the sheep by the group in Denver, infusing a bolus of leucine and glycine at the time of fetal blood sampling in normal fetuses, and we find exactly the same thing, that the transplacental passage of leucine and the fetal-maternal ratio is very close to 1, whereas the enrichment of glycine in the fetus is negligible.

**Dr. Rennie:** That is a very interesting difference, I think.

**Dr. Sibley:** We have shown that the activity of the system A transporter in the microvillous membrane of growth-retarded human placentas is about half that of normally grown fetuses.
The system A transporter will transport glycine, so we would predict a decrease in glycine transfer from those data, but there are a number of things we don't know in relating that to the *in vivo* situation, such as what the driving pulses are, what the intracellular concentrations are, and what the relative maternofetal concentrations are; but there is certainly a defect in the plasma membrane of the human placenta and in the main transporter that would transport glycine. Unfortunately, nobody has yet looked at the system L transport, which is the one that would transport leucine in the human placenta. I should also add that our system A data hold for both small-for-gestational-age (SGA) fetuses and those with severe growth retardation.

Dr. Soothill: So are you suggesting that these differences might relate to different transporter molecules?

Dr. Sibley: Yes, but this is not the whole story.

Dr. Meschia: I am aware of your results. There is no question that the literature shows more and more that in growth retardation there is a defect in transport of amino acids, such as the system A amino acids. One must bear in mind that the transport of amino acids from mother to fetus involves at least two steps—transport into the placenta and transport out of the placenta—and there could be also a problem in terms of transport out of the placenta. For instance, the sheep placenta has definitely got a system A transport, and yet we see virtually no serine and very little glycine transport into the fetus. So we have to think globally.

Dr. Marconi: I was very surprised when I saw that the reduction in umbilical uptake was mainly caused by a reduction in the direct flux of leucine from mother to fetus and not by a decreased contribution from the placental pool. How do you explain that? One would expect a similar reduction in both sources.

Dr. Meschia: The protein turnover in the placenta was expressed per kilogram of fetus, so one would have to express it per gram of placenta, but in whatever units you express it, it does not look as though the protein turnover in the placenta is really much affected, and this is consistent with the fact that the oxygen consumption of the placenta (per gram of placenta) is not really much different. I don't see a problem in combining this information with the fact that there is less leucine transported into the placenta. At this age the placenta is not growing, and there is no accretion of proteins in the placenta, so protein turnover could still go on with virtually no input of exogenous leucine.

Dr. Marconi: Yes, but as a clinician I would expect the placenta not to be working very well as an organ, but instead you tell us that the transport systems from mother to the placenta are not working, while placental metabolism reflected by protein turnover is working somehow! Is there some kind of compensation?

Dr. Meschia: I wish I knew. When you look at these placentae, you cannot see changes in oxygen consumption and yet you can see changes in leucine utilization. Perhaps this is because with leucine utilization we are looking at a single substrate.

Dr. Boyd: You have touched on a question that always worries me, and that is the matter of the denominator—whether one should be dividing by placental weight, placental surface area, fetal weight, fetal lean body mass, and so on. I haven't got any answers, but I just wonder if you could reflect for a moment on that difficulty, which really runs right across the field.

Dr. Meschia: This is very important. First of all, why did I divide the transport fluxes by kilogram of fetus? The reason for this is that if you look at a normal and a growth-retarded individual, of course the growth-retarded will have a reduction in anything you measure because it is smaller. It would be very difficult to imagine that the growth-retarded individual would consume the same amount of oxygen in absolute terms as the normal. So you have
to normalize the data. Consider oxygen consumption. If you take oxygen consumption in absolute terms, of course the growth-retarded individual has a much lower oxygen consumption than the normal, but per kilogram of fetus the oxygen consumption appears to be about the same. So I could have used oxygen consumption of the fetus as the normalizing variable and I would have exactly the same results. So the idea of doing fluxes per kilogram of fetus or per rate of fetal oxygen consumption is to exclude trivial data because of the fact that we are dealing with two different masses.

*Dr. Soothill:* I think that is a very important point in terms of the human observational data, because we have nearly always compared it with the normal cases at that gestational age, and there are important differences there.

*Dr. Owens:* When we produce experimental intrauterine growth retardation in sheep, we produce it in a different way, by restricting implantation and producing a placenta that is small because it has fewer cotyledons. In a recent study, when we looked at protein synthesis rates within that restricted placenta in late gestation, we actually found, along with reduced oxygen consumption per kilogram of placenta, a reduced rate of protein synthesis within the placenta in the most restricted placentae. So this differs a little from what you were finding, and my guess would be that the restricted placenta produced by heat stress as opposed to restricted implantation is quite different in terms of structure, although it may be exposed to a similar metabolic and endocrine milieu late in gestation. Coming back to this question of the cause of reduced leucine entry in your growth retardation model in late gestation, I wonder to what extent you have looked at structural changes within the placenta as being partly if not solely responsible for that. What I am thinking of is reductions in surface area per gram of that placenta, changes in diffusion distance perhaps, and so on. I can see clearly that people are very interested in changes in the relative abundance of relevant transporters, but I think it is also important to know what is happening structurally within the placenta.

*Dr. Meschia:* We looked years ago for histologic changes and could not establish any definite changes, but we are planning to do this again, now that we have more experience. This is a very important question. Is a gram of placenta in the growth-retarded individual the same as a gram of placenta in the normal?

*Dr. Marini:* From a clinical point of view, as neonatologists we know that the composition of an IUGR neonate is completely different from that of an appropriate gestational age preterm baby.