Fetal Liver and the Placenta: An Interactive System

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Fetal physiologists have centered their attention on the endocrine system when considering the potential interaction of one fetal organ with another or one fetal organ with the placenta. Our own studies in pregnant sheep showed an impressive correlation between placental size and the size of the fetal liver (Fig. 1). This observation—which is not a characteristic of the fetal brain, for example—suggests that the growth of the two organs is interrelated. In our earlier studies of carbohydrate metabolism, it rapidly became clear that the function of the fetal liver and the placenta must be integrated, at least with respect to glucose metabolism. In a series of studies (1-3), we showed that the placental delivery of glucose to the fetus was a function of the transplacental glucose gradient. The fetal hepatic production of glucose was also a function of the placental delivery of glucose. Only when the latter fell to low levels did the rate of fetal gluconeogenesis become significant (3). This makes good sense, of course, because if the fetal liver constantly produced glucose, it would decrease the transplacental gradient by raising fetal glucose concentration. This would then lead to reduced umbilical uptake. Hence, fetal hepatic glucose production, under normal circumstances, would be counterproductive, as it would substitute fetal carbon sources for maternal glucose carbon. These studies made us curious as to whether a similar interaction between fetal hepatic metabolism and placental transport and metabolism also existed for amino acids.

AMINO ACID TRANSPORT AND METABOLISM

The more we have studied different groups of amino acids, the more it has become clear that fetal hepatic metabolism and placental metabolism must be viewed as an integrated system. There has been a considerable body of evidence attesting to the organ-specific aspects of amino acid metabolism during adult life. The presumption has been that such specificity would apply to early postnatal life. Our studies have shown that this is also true of fetal development. In this chapter, I shall review three
aspects of fetal hepatic and placental metabolism of amino acids: (a) their net uptake and/or release from both organs; (b) serine and glycine exchange; and (c) glutamine and glutamate exchange.

FETAL HEPATIC AND PLACENTAL AMINO ACID UPTAKE OR RELEASE

The uptake or release of amino acids from the placenta to the fetal circulation for the ovine fetus is reviewed in Meschia's chapter in this volume (Placental Delivery of Amino Acids). The estimate of net amino acid exchange has required updating as improvements in amino acid methodology have come along. The reasons for this rest with several facts peculiar to this stage of development, and these have been discussed in some detail by Meschia. The high rate of perfusion of the fetal liver and placenta is coupled with the fact that some amino acids are present at extremely high concentrations in the ovine fetus, further leading to a low extraction coefficient (e.g., threonine, with a fetal concentration of 366 μM and an umbilical extraction coefficient of 0.05). A specific analytic problem was posed by the particular pattern of umbilical arteriovenous differences for glutamine and glutamate, in that glutamine enters the umbilical circulation from the placenta in large amounts, resulting in a high umbilical venous concentration, whereas glutamate is almost totally cleared from the fetal plasma across the placenta, resulting in very low umbilical venous concentrations. By some modifications in the HPLC methodology, this difficulty can be overcome (4).

Some years ago, we measured the concentration differences of amino acids across
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FIG. 2. Comparison of arteriovenous differences (μM) of individual amino acids and ammonia across fetal left hepatic lobe and umbilical circulation. From Marconi et al. (5), with permission.

the fetal hepatic circulation and the umbilical circulation. Figure 2 summarizes the data from that study (5). For most amino acids, there is a large uptake into the fetal liver from the placenta. However, some differences from postnatal life are striking. We have already commented on the absence of a net glucose release from the fetal liver, despite large hepatic uptakes of alanine, glutamine, and lactate. In postnatal life, a large uptake of these compounds would be associated with hepatic glucose production. In addition, glutamate and serine are produced in the fetal liver and taken up from the fetal circulation and delivered to the placenta and other fetal organs. In one sense, glutamate production by the fetal liver serves as an alternative to hepatic glucose production, although the combined uptake of lactate, alanine, and glutamine far exceeds net glutamate output. The uterine uptake of amino acids can be measured, although with considerably less precision, given the fact that uterine blood flow is almost twice umbilical flow (see Placental Delivery of Amino Acids). One of the striking comparisons is between uterine glycine uptake, which is not measurable, and umbilical glycine uptake, which is quite large. Taken together, these observations point to a high rate of placental glycine production.

FETAL HEPATIC AND PLACENTAL SERINE-GLYCINE EXCHANGE

We have studied serine (6,7) and glycine (8,9) exchange between the fetal liver and placenta, as well as their fetal plasma fluxes. These were the first studies that clearly demonstrated to us that the supply of amino acids to the fetus is not only a
function of transplacental transport but also of placental production or fetal production. In fact, for serine and glycine, the ovine fetal requirements are almost solely met by production within the placenta or fetus. Figure 3 presents a summary in diagrammatic form of the conclusions from these studies, in which stable isotopic tracer methodology was used for in vivo investigation in the late-gestation fetal lamb. An impressive organ specificity is shown by these amino acids. Fetal serine oxidation represents 7.9 ± 0.5% of infused L-[U-14C]serine and occurs primarily in the carcass (approximately 80% of the total fetal CO2 production). By contrast, glycine oxidation represents 11.3 ± 0.5% of the L-[l-14C]glycine infused, and approximately 70% of total fetal oxidation can be accounted for by the hepatic rate of oxidation (8). Stoichiometrically, the molar ratio of fetal hepatic serine production and CO2 production from glycine is approximately 1:1, consistent with the combined actions of serine hydroxymethyltransferase and glycine cleavage system, both of which have high activity in the fetal liver (8). Thus, in the fetal liver, glycine uptake is utilized, in part, for serine production and for oxidation. Two pathways of fetal serine utilization have been established from these studies: (a) uptake and utilization by the placenta for glycine production, and (b) uptake and oxidation in the fetal skeletal tissues.

In the placenta, there is an uptake of serine from both the maternal circulation and the fetal circulation. Clearly, therefore, all the fetal requirements for serine must be met by fetal production. Within the placenta, some of the serine is utilized for glycine production as well as for CO2 production. The consequence of this serine
to glycine flux is the production of methylenetetrahydrofolate (MeTHF). This compound is also produced from glycine oxidation within the placenta. The fate of the MeTHF within the placenta is not known, although it may contribute not only to nucleic acid synthesis but also to homocysteine-methionine interconversion.

Figure 4 presents the relationships between fetal serine concentration, fetal plasma disposal rate, fetal serine oxidation, and fetal glycine derived from fetal plasma serine (6). Clearly, an increase in fetal serine concentration increases the disposal rate and the flux to CO₂ and glycine production.

To test if maternal plasma serine was also utilized for placental production of glycine, we carried out a series of studies in twin pregnancies (9). L-[1-¹³C]serine was infused into the maternal artery supplying only one uterine horn. The fetus and placenta of that horn were the “experimental” horn, with the other uterine horn and its fetus and placenta serving as a “control.” Despite almost 300 minutes of infusion that led to a maternal uterine venous enrichment of approximately 18%, there was no detectable serine enrichment in either fetal circulation. However, glycine enrichments were higher in the uterine and umbilical veins of the experimental horn compared with either the maternal and fetal arterial enrichments, or compared with the venous enrichments in the control horn. These results established that maternal plasma serine is also utilized for placental glycine production and that there is no detectable transplacental transport of serine for the ovine placenta.

**FIG. 4.** A: Relates the fetal plasma glycine-serine enrichment ratio to fetal plasma serine concentration. B: Presents the fetal plasma serine disposal rate vs. fetal plasma serine concentration. C: Presents the serine flux to CO₂ per kilogram of fetal weight vs. the fetal plasma serine disposal rate (units as in B). Data from Cetin et al. (6), with permission.
The studies outlined above were carried out in late gestation, at approximately 120 days. However, we have studied fetal serine fluxes at midgestation as well (7). At this stage, one has a relatively large placenta (410 ± 20 g) and a relatively small fetus (145 ± 12 g). The fetal plasma serine disposal rate is enormous (61.8 ± 4.0 μmol/min/kg fetal weight), owing to a large uptake of serine from the fetal circulation into the placenta (approximately 80% of the total L-[1-13C]serine infused). In none of the studies was it possible to demonstrate a significant transfer of fetal plasma serine or glycine into the maternal circulation. Thus, these amino acids are effectively trapped within the fetus or placenta, or both.

From these studies we can conclude that the fetal requirements for some nonessential amino acids are met primarily—if not entirely—by fetal or placental production rather than by transplacental transport, and that there is significant organ specificity to amino acid metabolism in fetal life, just as there is in postnatal life.

FETAL HEPATIC AND PLACENTAL EXCHANGE OF GLUTAMINE AND GLUTAMATE

Among amino acids, the most striking example of the fetal liver and placenta functioning as an integrated organ system is given by the interorgan fluxes of glutamine and glutamate. Even in the early studies, which did not utilize stable isotope tracer methodology but were simply directed at determining the net uptake or release of these amino acids across the fetal liver and placenta, certain striking features were observed (5). The glutamine taken up from the placenta into the fetal circulation was used by the fetal liver, and the glutamate produced by the fetal liver was used by the placenta. The fetal plasma glutamate was virtually cleared (60% extraction) across the fetal umbilical circulation. This striking interorgan exchange was then explored using stable isotope methodology (10).

L-[1,2-13C2]glutamine was infused into the fetal circulation. The entry of glutamine from the placenta accounted for approximately 60% of the fetal disposal rate. Approximately 45% of the glutamine taken up by the fetal liver was released from the fetal liver as glutamate, and the rate of fetal hepatic production accounted for almost the total fetal glutamate production. In adult humans, it has been estimated that on a daily protein diet containing one mole of amino acids, the molar ratio of hepatic glucose production to O2 uptake would be about 0.15. Therefore, if the adult liver had to oxidize the glucose produced from amino acids completely, its O2 consumption would increase by 90%. As 4.5 moles of O2 are required to oxidize glutamate completely, the fetal liver would require an increase in O2 consumption of 60% to 70% to oxidize the glutamate produced. Thus, the unique characteristic of the fetal liver—namely, a large net glutamate production—can be regarded as an excellent accommodation to the virtual absence of gluconeogenesis during fetal life.

In another study, L-[2,3,3,4,4-2H5]glutamate was infused into the fetal circulation (11). The fetal plasma disposal rate was 11.9 ± 1.3 μmol/min/kg fetal weight.
Given the low fetal arterial concentration of plasma glutamate (about 50 μM), this represents an extraordinary fetal plasma clearance of 200 ± 8 ml/min/kg fetus, which is greater than umbilical plasma flow. The placental extraction of fetal plasma glutamate was almost 90%. Most of the glutamate was oxidized, about 40% in the fetus and about 40% in the placenta. There was a small (6%) amount of the placental glutamate uptake returned to the fetus as glutamine, but the major disposal was placental oxidation.

The impact of the large placental uptake of fetal plasma glutamate can be seen when the fetal plasma clearances of six amino acids that have been studied using comparable methodology and over the same gestational age range are compared (11) (Table 1).

In summary, we have presented the following hypothesis: During fetal life, fetal hepatic gluconeogenesis is uncoupled from amino acid oxidation. By this view, the release of glutamate from the fetal liver is a consequence of such uncoupling. In addition, our data have shown that the rate of placental uptake and oxidation of glutamate is supply-limited. This led us to a second hypothesis: Fetal modulation of placental metabolic activity depends on glutamate oxidation. Glutamate oxidation generates NADPH (reduced nicotinamide adenine dinucleotide phosphate), a required cofactor for steroidogenesis. Thus, it is possible that endocrine effects on fetal hepatic amino acid metabolism might subsequently affect the placenta indirectly. Klimek et al. (13) have demonstrated a link between human placental mitochondrial oxidation of glutamate and synthesis.

From such hypotheses, we were led to question whether, associated with the profound endocrine changes during parturition, there were demonstrable changes in fetal hepatic glutamine and glutamate metabolism. The study we have recently concluded (4) used a fetal infusion of dexamethasone to induce parturition, which occurred at 47 ± 4 hours from the start of the fetal infusion. After 24 hours of dexamethasone infusion, there was a significant reduction in fetal hepatic glutamate output to one quarter of preparturition values. This led to a decrease in fetal glutamate concentration and in net placental glutamate uptake to one fifth of preparturition values.

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<th>Table 1. Comparison of mean fetal arterial plasma concentrations, disposal rates, and clearances for five amino acids</th>
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<td><strong>Arterial plasma concentration, μM</strong></td>
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values. These changes are presented in Fig. 5. Thus, steroid-induced parturition is associated with profound changes in fetal hepatic glutamate metabolism. These changes may play a role in integrating changes in fetal hepatic and placental metabolism during parturition.

SUMMARY

The evidence we have accumulated for amino acid exchange and metabolism clearly demonstrates that the fetal liver and placenta function as an integrated system of organs. This is a striking characteristic with regard to serine-glycine interactions and glutamine-glutamate interactions. Their importance is at least in part a function of controlling one-carbon pools (serine-glycine) and NADPH supply (glutamine-glutamate). Functionally, the production of glutamate in the fetal liver serves as an alternative to glucose production for the release of carbon derived from amino acids.
ACKNOWLEDGMENT

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REFERENCES


DISCUSSION

Dr. Chard: To what extent can some of these things be extrapolated to the human? For example, it is not my understanding that in the human the most oxygenated blood goes to the liver.

Dr. Battaglia: There is a ductus venosus in the human as well, and a large fraction of umbilical venous blood is shunted through it. The question for the liver is what kind of blood perfuses it. It is perfused by that fraction of umbilical venous blood that is not shunted through the ductus. It is not receiving any appreciable component from portal venous drainage, and that is the big difference from postnatal life and would apply in the human. As I said, there are clearly going to be species differences in metabolism, but so far the evidence for glutamate
being important in the placenta seems to be supported by a number of studies in the human placenta as well. I think the evidence is good. I am also encouraged by the fact that it would not matter whether you studied sheep or human mitochondria from the heart, as they both use glutamate preferentially. I believe that would be true of small bowel in different species as well. So I don’t think these cycles are going to be confined to just the ovine species.

Dr. Schneider: In the isolated perfused human placenta, we showed many years ago that there is uptake of glutamate from the fetal circulation and the glutamate is largely metabolized in the placenta. This has been confirmed in blood samples obtained from the umbilical vein and artery at the time of cesarean section. This all supports the view that there is a similar mechanism of glutamate clearance from the umbilical circulation by the placenta in the human.

Dr. Battaglia: The other difference I mentioned relating to the liver was that in fetal life there is no net glucose release. We can’t measure net glucose release in the human fetus, but certainly our group and others have looked in the human for evidence of gluconeogenesis and have been unable to demonstrate it. So it seems that those key features of development are similar.

Dr. Milliez: What could be the link between glutamine and parturition? Do you think it is just a coincidence or is there a direct causal relationship?

Dr. Battaglia: We don’t know the answer to that. I think this will be linked with parturition and with steroidogenesis in the placenta. The effect may be mediated through glutamate transport into the placenta being turned off. I don’t want to focus only on glutamate metabolism; its transport 
per se 
may be important. But yes, I think that glutamate deprivation of the placenta probably will prove to be important in parturition.

Dr. Chard: But on the same vein, where you showed progesterone going down, presumably you might show other phenomena there, such as estrogens going up and cortisol going up in the same samples. Cortisol might be an even better candidate to control your metabolic processes.

Dr. Battaglia: First of all, let’s get rid of the estrogen issue—it’s a log order different from progesterone. Steroid output as a whole is decreasing; whether you take progesterone alone or subtract the tiny amount of estrogen, you are not going to affect that statement. As far as cortisol is concerned, I don’t know. We haven’t studied other tissues in the fetus, and I don’t know if there is an effect on the adrenal. There is certainly a possibility of other organ effects. Glutamate is an important signaling compound, that is clear enough, even from studies in postnatal life, and it could affect a multitude of tissues.

Dr. Chard: So your suggestion is that the glutamate is affecting the progesterone, not the reverse.

Dr. Battaglia: What I showed you are the data. The data clearly show, and this is the first demonstration for any aspect of hepatic metabolism, that glutamate fetal hepatic output is changing radically during parturition. I also showed you a correlation with progesterone output by the uterus. I don’t know if they are causally related at this stage, but it raises a lot of interesting questions.

Dr. Owens: I am interested in your views on what might be the molecular mechanisms involved in altered steroid exposure modulating hepatic glutamate production. Do you think this is steroid-responsive elements altering gene expression for the relevant enzymes, or do you rather see other endocrine factors or local factors being involved, such as altered IGF-2 or IGF-1 production within the liver?

Dr. Battaglia: We have been talking about that a lot. There is a suggestion in adult hepatocyte cultures that growth hormone triggers a release of glutamate, and that made us interested in whether ovine placental lactogen could be playing such a role, but we have no data for
this. To some extent, in vivo studies are too laborious and expensive to address endocrine regulation, so you have to do it with in vitro cultures.

Dr. Rennie: I think it has been shown that there are at least three different membrane transporters for glutamate that are important in the placenta. There is EAC-1, which is the excitatory amino acid transporter present in the brain; there is the GLUT-1 (GLT-1), which is also present in the brain, and there is the XAG transporter. And those transporters show differential regulation according to the degree of intrauterine growth retardation (IUGR), but the transcription and the protein expression of those transporters go down dramatically in IUGR in rat placenta. And yet the expression of other amino acid transporters does not change to anything like the same extent, so it is obviously crucial.

Dr. Battaglia: There is a problem for us with studying IUGR in this model, because this is a difficult preparation and involves fetal surgery. Many of those fetuses will not tolerate that. The heat stress fetuses will survive if you leave them alone, but if stressed, they don't survive. So we haven't done these kinds of flux studies in growth-retarded animals.

Dr. Marini: The fetus is nourished in the pulsatile way because the mother is eating. Is the level you achieve in the mother equal to the level you can achieve if you feed the animal with a normal meal?

Dr. Battaglia: You are thinking as a clinician. These are ruminants, so you are not going to get anything like the postprandial swings you get in primates. Furthermore, the maternal arterial concentration changes with feeding are quite small for amino acid concentrations.