FETAL HEPATIC AND PLACENTAL AMINO ACID METABOLISM

EXPERIMENTAL STUDIES IN LATE OVINE GESTATION

FOETAAL HEPATISCH EN PLACENTAIR AMINOZUUR METABOLISME

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PROF. DR. P.W.C. AKKERMANS M.A.

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DOOR

MICHELLE TIMMERMAN

GEBOREN TE ROTTERDAM

Promotiecommissie

Promotores	Prof. dr. H.C.S. Wallenburg
	Prof. dr. F.C. Battaglia
Overige leden	Prof. dr. Th. J. M. Helmerhorst
	Prof. dr. H.G. van Eijk
	Dr. L.L.H. Peeters

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LIST OF ABBBREVIATIONS

A	maternal common iliac artery
V	uterine vein
α	fetal aorta
γ	umbilical vein
h	left hepatic vein
h_i	left hepatic input
Ht	hematocrit
Q	blood flow
C	concentration
F	fractional contribution
I	infusion rate
R_{acc}	rate of accumulation
dpm	desintegrations per minute
Δaa	amino acid plasma concentration difference
MPE	molar percent enrichment
r_0	blanc ion abundance ratio
r_s	steady state ion abundance ratio
DR	disposal rate
$R_{P,m}$	maternal to placental flux
$R_{f,P}$	placental to fetal flux
$R_{P,f}$	fetal to placental flux
$R_{f,m}$	direct maternal to fetal flux
$R_{GLU,GLN}$	glutamate derived from glutamine
$R_{CO2,GLN}$	CO_2 derived from glutamine
N	nitrogen
BCAA	branched chain amino acids
oGDH	ovine glutamate dehydrogenase
CS	citrate synthase

LIST OF AMINO ACID ABBREVIATIONS

Essential amino acids

Threonine	Thr
Phenylalanine	Phe
Methionine	Met
Lysine	Lys
Histidine	His
Valine	Val
Leucine	Leu
Isoleucine	ILe

Non-essential amino acids

Serine	Ser
Glycine	Gly
Alanine	Ala
Proline	Pro
Arginine	Arg
Ornithine	Orn
Tyrosine	Tyr
Glutamine	Gln
Glutamate	Glu
Asparagine	Asn
Aspartic acid	Asp
Citrulline	Cit
Taurine	Tau

Chapter 1

INTRODUCTION AND OBJECTIVES

For a long time, the study of the metabolism of nutrients by the placenta attracted much less attention than that by the fetus. This scant interest was probably due to the commonly held view of the placenta as an organ with minimal metabolic needs, serving only as a means of transport between the maternal and fetal circulations. This concept changed when it was demonstrated that in late ovine gestation only about half of the oxygen taken up from the uterine circulation is actually delivered to the fetus whereas the other half is used by the placenta itself, which implies a high metabolic rate approximately equal to that of the brain (64, 87). Further quantitative in vivo studies on net substrate fluxes across both fetal and maternal circulations of the uteroplacental unit demonstrated that placental metabolism plays a significant role in the nutritional demands of pregnancy.

In obstetrics the main focus of scientific interest has been on the significance of reduced uteroplacental and umbilical perfusion with impaired oxygenation and uptake of nutrients as the pathophysiological mechanism of fetal growth restriction. But in small-for-gestational age (SGA) fetuses, not only total aminonitrogen levels are reduced, there are also marked differences between adequate-for-gestational age (AGA) and SGA fetuses with respect to fetal concentrations of leucine, isoleucine and valine (13, 14, 18). A possible role for placental amino acid metabolism in fetal growth restriction is apparent from in vivo experiments in ovine gestation with restriction of fetal growth induced by heat-stress. These studies showed a significantly reduced uterine uptake of essential amino acids such as threonine and leucine by the placenta expressed per gram of placenta (1, 76). This finding is supported by the observation of a significantly reduced amino acid transport activity by microvillous vesicles from placentas obtained from patients with fetal growth restriction (20, 58). Moreover, under circumstances of a reduced uterine

uptake of amino acids, placental amino acid utilization has been shown to be reduced in favor of fetal uptake, possibly as an adaptive mechanism. (1, 76).

Therefore, a basic understanding of placental amino acid metabolism is physiologically important and may be clinically relevant since placental metabolism could play an important role in determining which amino acids are supplied to the fetus, and at which rates. No data are available in the accessible literature on the relationship between maternal concentrations of amino acids and fetal uptake. Furthermore, it may be of clinical importance with respect to human pregnancy to investigate if the supply to the fetus of a specific amino acid can be increased by raising its maternal concentration and, if so, the effect this would have on placental metabolism. A suitable amino acid for such an experiment would be alanine. It accounts for 7% of the total nitrogen delivered to the ovine fetus, and since it is readily interchangeable between lactate and pyruvate, it is important in carbohydrate as well as protein metabolism.

Although a continuous supply of gluconeogenic substrates, including gluconeogenic amino acids like glutamine, glycine and alanine as well as lactate, is delivered to the fetal liver by the umbilical vein directly from the placenta, no net fetal hepatic gluconeogenesis appears to exist under normal physiological conditions (3, 28, 33). It is known that net hepatic glucose release would curtail exogenous supply of glucose by the placenta because glucose is transported from mother to fetus by a concentration gradient (82). Instead, the fetal liver shows a net release of the amino acids glutamate and serine. The hepatic release of glutamate and serine could be a means of delivering nutrients to the fetus without raising fetal glucose concentration. But at present there are no data to support or refute that hypothesis.

In late ovine gestation there is a selective interorgan cycling of glutamine and glutamate between the fetal liver and the placenta (93). Glutamine is taken up by the placenta from the maternal circulation and released into the fetal circulation where it is taken up by the fetal liver. The fetal liver releases glutamate, nearly half of which is derived from the glutamine taken up (93). Virtually all of the glutamate is extracted by the placenta from the umbilical circulation and oxidized (69).

Parturition marks the end of gestation. In several species, including the sheep, progesterone concentration falls and estradiol 17-β concentration increases prior to parturition under the influence of a fetal cortisol surge. (52). Ovine parturition can be induced by mimicking the fetal cortisol surge by fetal administration of glucocorticoids (51). During dexamethasone administration net fetal hepatic glutamate and serine outputs fall significantly (4). Because placental glutamate delivery is directly dependent on fetal hepatic release, it also decreases significantly in the days prior to parturition (69). The fall in placental uptake is accompanied by a reduction in the placental progesterone production, which has been demonstrated to be crucial in the maintenance of pregnancy. It has been suggested that placental glutamate oxidation could play a pivotal role in the availability of placental nicotinamide adenine dinucleotide phosphate (NADPH), necessary for steroidogenesis since the placenta lacks an active pentose phosphate pathway. (11, 42).

Glucocorticoids increase the activity of gluconeogenic enzymes and promote glycogen deposition (5). The marked fall in net fetal hepatic serine and glutamate output that accompanies dexamethasone-induced parturition (4) suggests that previously released carbon may now be redirected towards glycogen synthesis. This would mean that the metabolism of these amino acids within the fetal liver undergoes major changes under the influence of dexamethasone. No data is available regarding these metabolic changes in spontaneous parturition. One study showed an increased activity of glutamate dehydrogenase in the fetal rat liver during glucorticoid administration (72). This is the enzyme that converts glutamate into α ketoglutarate, a citric acid cycle intermediate. This observation supports the hypothesis that under the influence of glucocorticoids glutamate carbon may be redirected towards entry into the citric acid cycle, rather than released into the circulation.

Based on the considerations presented above the following objectives of this thesis were formulated:

-    To assess net placental amino acid utilization and production in late ovine gestation.

- To determine fetal and placental uptake of the non-essential amino acid alanine as a function of maternal concentration in late ovine gestation.
- To investigate fetal hepatic glutamine and glutamate metabolism in late ovine gestation and its changes under the influence of dexamethasone.
- To assess the changes in fetal hepatic and placental glutamine and glutamate exchange under the influence of the endogenous cortisol surge associated with spontaneous as compared with dexamethasone-induced ovine parturition.
- To determine whether the demonstrated fall in net fetal hepatic glutamate output during dexamethasone-induced parturition can be explained by up-regulation of fetal hepatic glutamate dehydrogenase mRNA expression and/or by a change in enzyme activity.

All studies were performed in chronically instrumented ewes in late gestation.

Chapter 2

MATERIALS AND METHODS

This chapter presents an overview of the surgical and experimental techniques and of the analytical methods used in the experiments in the thesis.

2.1 BIOLOGICAL PREPARATION

Experimental research in fetal-maternal physiology has mainly been performed in pregnant sheep and goats. The popularity of these animals for research is based on several characteristics. One is the relatively large fetal size that allows implantation of catheters in various vessels, and the accompanying relatively large blood volume, which imposes limited restrictions on repetitive blood sampling. Another advantage is the capability of these animals to endure extensive surgical trauma with a relatively small risk of fetal death and preterm delivery. The development of the chronic fetal lamb preparation, as used in the experiments described in the thesis, makes it possible to study maternal, fetal and placental metabolism under physiological conditions (62).

The animals used in all studies reported in this thesis were mixed breed Columbia-Rambouillet ewes. Because of controlled breeding gestational age was accurate within approximately 24 hours. The ewes were fed a standard diet ad libitum of alfalfa pellets with mineral supplements, and they had free access to water and salt prior to and during experiments.

2.1.1 Surgical procedures

Surgery was performed after a 48-hour fast with free access to water. Preoperatively, the ewe received 500 mg ampicillin and 500 mg gentamycin intramuscularly. The initial sedation was obtained with pentobarbital (65 mg/ml) given through a catheter placed in a jugular vein by direct puncture. Once the animal was sedated, spinal analgesia was induced under sterile conditions with 1% pontacaine as a 2

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ml intrathecal dose. During surgery general anesthesia was maintained by intermittent intravenous infusion of pentobarbital via the jugular catheter. The fetus was anesthetized with pentobarbital via the maternal circulation. During surgery the ewe received a continuous infusion of isotonic glucose solution in a total amount of one liter.

2.1.2 Catheterizations

The ewe was placed on the operating table in supine position. The uterus was exposed via a midline laparotomy to provide access for placement of maternal and fetal catheters, as summarized in Fig. 2.1. A polyvinyl catheter was placed in the uterine vein (Fig. 2.1., V) draining the pregnant horn via small branches of the venous tree at the tip of the horn, and advanced approximately 17 cm into the common uterine vein. The position of the catheter tip in the main venous trunk was verified by palpation. An approximately 7-9 cm incision was made in the uterine wall and a fetal hindlimb was delivered. The fetal pedal artery was exposed through a small incision on the anterior surface of the knee and catheterized (Fig. 2.1., α). The catheter was advanced approximately 17 cm in order to position the tip in the fetal abdominal aorta. Next, a fetal forelimb was exteriorized, an incision was made on the medial site of the knee joint, and the brachial vein was located and catheterized (Fig. 2.1.,II). The tip of the catheter was advanced approximately 10 cm into the fetal axillary vein. This catheter served as the fetal infusion catheter. After closure of the skin both limb catheters were secured to the skin, and an amniotic catheter was tied to one of these catheters to allow the administration of antibiotics into the amniotic cavity. Subsequently, the umbilical cord was exposed through the uterine incision at its site of insertion. A catheter was placed in the common umbilical vein (Fig. 2.1., γ) through one of the two umbilical veins. The catheter was secured at the puncture site with tissue adhesive (910 FS Bonding Adhesive, Permabound Int., NJ) and with a 5.0 silk suture through the amniotic membrane and vessel wall. The end of the catheter was sutured twice to the fetal skin of the abdominal wall, with a loop under the amniotic membrane covering the cord to allow fetal growth.

A left fetal hepatic venous catheter was placed through a right side thoracotomy at the level of the seventh rib to expose the intrathoracic segment of the inferior vena cava. The neuromuscular blocker pancuronium (Pavulon) was administered intravenously to
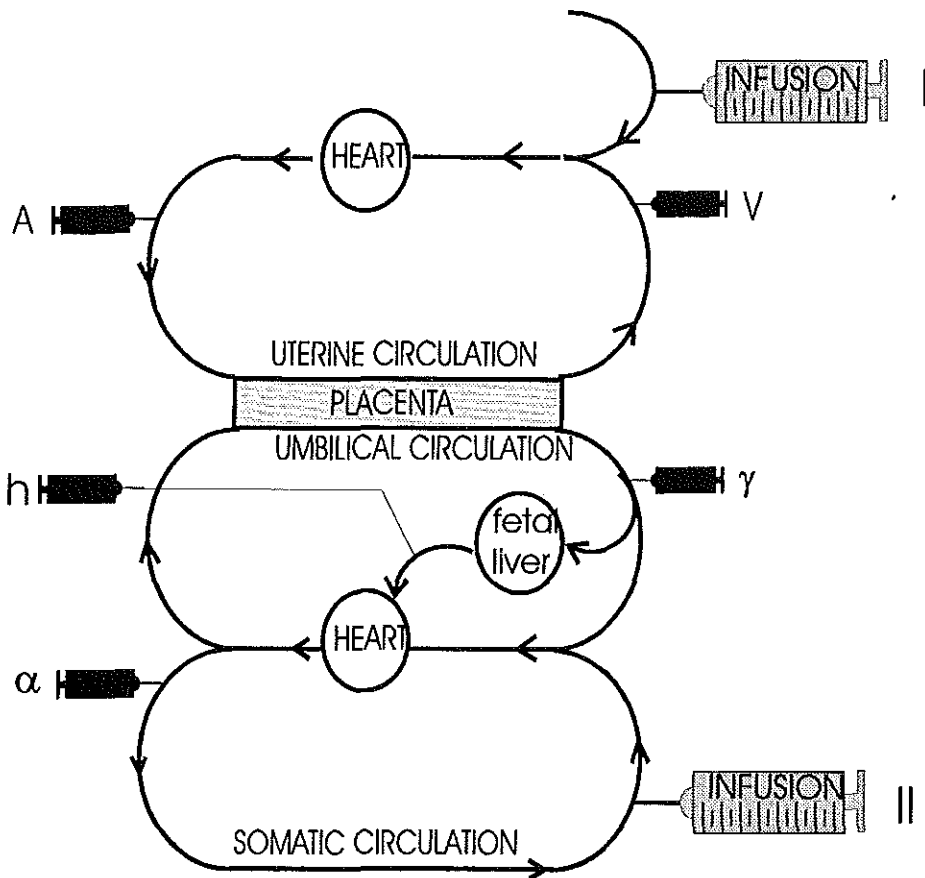
Figure 2.1 Schematic diagram of the fetal and maternal circulations and catheter placements in the vessels supplying and draining both sides of the placenta and fetal liver.

A: maternal common iliac artery, V: uterine vein,

α: fetal aorta, γ: umbilical vein, h: left hepatic vein.

Infusion catheters: I: maternal infusion catheter in common iliac vein, II: fetal infusion catheter in brachial vein.

the fetus in a dose of 0.3 mg, 10 minutes prior to the insertion of the hepatic catheter, in order to reduce abdominal tone resulting in less protrusion of the diaphragm into the

thoracic cavity. A intravenous catheter/needle unit, 2.1 mm, 5.1 cm, (Insyte®, Becton Dickinson, Sandy, Utah) was placed in the inferior vena cava 1.0 cm above the diaphragm, through which the catheter was guided into the portal sinus and the left hepatic vein, with the tip positioned 4 cm distal to the point of entry (Fig. 2.1., h). The distinction between the position of the catheter in the left or right hepatic vein was based on the angle between the catheter and the inferior vena cava. The distinction between placement of the catheter in the left hepatic vein and in the ductus venosus was based on the fact that, when the catheter was placed in the ductus venosus, it could be advanced far beyond the approximately 5 cm that were possible when positioned in the left hepatic vein. The left hepatic venous catheter was secured by suturing the edges of the insertion opening around the catheter without constricting the vena cava, and by fixation to the inside of the ribcage. The fetal ribs were approximated and the fetal skin was closed with 3.0 silk sutures. The uterine and the abdominal midline incisions were closed with 3.0 silk sutures for the uterus and 1.0 for the abdomen.

The maternal femoral artery and vein (Fig. 1.1, A, I) were identified through an incision in the left groin and polyvinyl catheters were advanced approximately 10 cm into the common iliac artery and vein, respectively. All catheters were tunneled subcutaneously to a pouch on the ewe's left flank.

2.1.3 Postoperative care

The first postoperative day the ewe received 250 mg phenylbutazone intravenously as an analgesic to promote recovery. All catheters were flushed at least every other day with heparinized saline (30 U/ml). During the first three days after surgery, 500 mg ampicillin was administered into the amniotic cavity through the amniotic catheter.

At least five days were allowed for full recovery as indicated by a return to normal food intake and by a normal fetal O_2 content and glucose concentration.

The ewes were kept in separate carts in a quiet room, always in the company of at least one other ewe. A day and night cycle was maintained. The experiments were performed in the same room with the ewe standing in her cart with food and water ad libitum.

At the end of the experiments, the ewe was killed by intravenous injection of pentobarbital sodium in 10% alcohol (Sleepaway, Fort Dodge, IA). Autopsy was performed to determine the weights of the fetus, fetal liver, placenta and uterus, and to confirm all catheter positions.

All studies were approved by the University of Colorado Health Sciences Center Animal Care and Use Committee.

2.2 ANALYTICAL METHODS

General analytical methods are described here, special methods applied to specific experiments are reported in the respective chapter.

Hemoglobin and O_2 saturations were measured spectrophotometrically (OSM-3, Radiometer, Copenhagen, Denmark). The blood O_2 content was calculated from the hemoglobin content, Hb(Fe), expressed as O_2 capacity, multiplied by the O_2 saturation.

Plasma concentrations of tritiated water (^3H_2O) were measured on triplicate aliquots in a scintillation counter and converted to blood ^3H_2O concentrations on the basis of hematocrit values (92). Respiratory gas tensions and pH were measured with an ABL330 Radiometer. Total CO_2 concentration in blood was calculated using the Henderson-Hasselbach equation and a nomogram from Van Slyke and Sendroy (91).

The $^{13}CO_2$ enrichment was measured in samples of 0.5 ml whole blood, which were collected in vacuum tubes and analyzed using tandem gas chromotography ion ratio mass spectrometry.

Plasma glucose and lactate concentrations were determined in duplicate by means of a glucose/lactate analyzer (YSI Model 2700 Select and Dual Standard).

Plasma insulin concentrations were determined with the Rat insulin RIA kit, respectively (Linco Research Inc., MO).

Plasma cortisol was determined with the Corti-cote kit (Becton Dickinson and Company, NY). Inter- and intra-assay coefficients of variation for the low quality control were 11.2% and 6.6%, respectively, and for the high quality control 4.9% and 3.4%, respectively.

Progesterone was measured using a validated (10) Coat-a-count progesterone RIA kit (Diagnostic Product Corporation, Los Angeles, CA). Inter- and intra-assay coefficients of variation for the low quality control were 9.2% and 6.0%, respectively, and for the high quality control 6.7 and 3.4%, respectively.

Catecholamines were determined by radioimmunoassay by the Nichols Institute, California.

Plasma samples for determination of amino acid concentrations were frozen at −70°C within 5 minutes until analysis, when they were thawed quickly and deproteinized with 15% sulfosalicylic acid containing 0.3 μmol/l norleucine as internal standard. The pH was adjusted to 2.2 with 1.5 M LiOH. After centrifugation, the supernatant was analyzed with a Dionex high performance liquid chromotography (HPLC) amino acid analyzer, (Dionex Corp., Sunnyvale, California). The same HPLC column was used for all samples from an individual animal. Reproducibility within the same column had a mean value of ± 2%. Samples from all vessels drawn simultaneously were loaded to run within 12 hours. Amino acid concentrations were measured after reaction with ninhydrin at 570 nm, except for proline, which was measured at 440 nm wavelength.

Chapter 3

PRODUCTION AND UTILIZATION OF AMINO
ACIDS BY OVINE PLACENTA IN VIVO

3.1 INTRODUCTION

Amino acid uptake by the pregnant uterus and by the fetus is a vital aspect of maternal and fetal nutrition. Uterine uptake defines amino acid supply to both placenta and fetus by the maternal organism, whereas umbilical uptake defines amino acid supply to the fetus. Therefore, the comparison of uterine and umbilical amino acid uptakes provides basic information about uteroplacental amino acid metabolism *in vivo*. Uterine and umbilical uptakes of individual amino acids are not necessarily equal, because amino acids can be produced or utilized by the uteroplacental tissues. Although these tissues include the myometrium, there is evidence that their metabolic activity represents primarily placental metabolism, since at least 80% of uteroplacental glucose utilization is by tissues that exchange glucose with the umbilical circulation (64).

A test of the hypothesis that placental metabolism plays a physiologically important role in determining which amino acids are supplied to the fetus and at what rates requires simultaneous measurements of uterine and umbilical blood flows, and of amino acid concentration differences across the uterine and umbilical circulations.

Although such measurements have been feasible in sheep for more than a decade, there has been only one study attempting to quantify uterine and umbilical amino acid uptakes simultaneously in a chronic sheep preparation (48). This study and others in which uterine (36, 70) and umbilical (8, 46, 47) amino acid uptakes were

measured in separate animals have shown that whole blood amino acid concentration differences across the uterine and umbilical circulations are relatively small, hence difficult to measure. An additional difficulty is that fetal sheep blood contains some compounds, e.g. methionine sulfone and N-methyl-lysine, which are present in high concentrations and may interfere with the chromatographic separation of physiologically important amino acids. Thus, in the previous comparison of uterine and umbilical uptakes (48), glycine concentrations were not measured despite the significant contribution that glycine makes to umbilical uptake of amino acids (15).

One reason for the relatively small whole blood concentration differences of amino acids across the uterine circulation is that the amino acids carried by adult ovine red cells do not contribute significantly to the rapid amino acid exchange between body organs and circulation (54). The exchange is virtually limited to the plasma compartment. Therefore, the accuracy of uterine amino acid uptake estimates can be improved by calculating uterine uptake as the product of uterine arterial - venous plasma concentration differences times uterine plasma flow, provided that plasma is separated from red cells within a few minutes of sampling. At the onset of this study, we had evidence that the umbilical uptake of glutamate is best estimated as the product of umbilical venous - arterial plasma concentration difference times umbilical plasma flow (69), but it was not clear whether this method of calculation could be extended to other fetal amino acids.

In the first part of this chapter, we present *in vitro* evidence that the exchange of amino acids between fetal red cells and plasma is sufficiently slow to allow the use of plasma concentrations and plasma flows for estimating umbilical amino acid uptakes. In the second part, we present the results of simultaneous measurements of uterine and umbilical amino acid uptakes in 18 pregnant sheep using the plasma concentration difference times plasma flow calculation. These measurements demonstrate significant placental metabolism of several amino acids.

3.2 MATERIALS AND METHODS

3.2.1 Surgery and animal care

Eighteen Columbia-Rambouillet ewes pregnant of a single fetus were used. Surgery was performed at 120 - 127 days of gestation. Preoperative preparation, surgical techniques and animal care were performed as described in chapter 2.

3.2.2 Study Design

In vitro study

An in vitro study was performed to determine whether a decrease in plasma amino acid concentrations causes a net flux of amino acids from red cells to plasma. Fetal sheep blood (131 days gestation) was drawn in a syringe containing ethylene-diamino-tetra-acetate (EDTA). The hematocrit was measured and used to calculate how much plasma should be withdrawn from the blood to increase its hematocrit to approximately 50%. The blood was centrifuged briefly, the calculated amount of plasma was withdrawn and the red cells were mixed again with the remaining plasma. Duplicate 3 ml samples of this high hematocrit blood were incubated at two different temperatures, 0°C and 38°C. At time zero, each blood sample was mixed with 1 ml isotonic saline with the same temperature as the sample. The saline contained known quantities of $1-^{14}$C-glutamate and Evans blue as two internal indicators for calculating plasma volume before and after dilution. One ml aliquots of the diluted blood were removed at 0.5, 2 and 5 minutes, stored in ice and then centrifuged for 10 minutes in a refrigerated centrifuge. The diluted plasma samples were used to measure the concentrations of $1-^{14}$C-glutamic acid, Evans blue and amino acids. Three ml undiluted plasma samples were carried through the same incubation routines as the blood samples and were used to measure the initial amino acid concentrations. These concentrations and the changes in plasma volume were used to calculate expected amino acid plasma concentration changes in the absence of any appreciable amino acid efflux from the red cells.

In vivo study

Studies were performed at 125 - 134 days of gestation. Blood samples were drawn from the maternal common iliac artery, uterine vein, fetal aorta and umbilical vein for determination of tritiated water concentration, oxygen saturation, glucose concentration, lactate concentration, and amino acid concentrations as baseline data. Then, a constant

tritiated water infusion was started into the fetal pedal vein. Sixty minutes after the start of the infusion, at steady state, four sets of samples were collected simultaneously from the maternal common iliac artery, uterine veins, fetal aorta and the common umbilical vein.

At the end of the experiment, the ewe and the fetus were killed by intravenous injection of pentobarbital sodium and autopsy was performed.

3.2.3 Analytical methods

All samples were analyzed for tritiated water, hemoglobin, hematocrit, O_2 saturation, glucose, lactate and amino acid concentrations. The analytical methods were as described in chapter 2.

3.2.4 Calculations

Blood flows and uptakes

Umbilical (Q_f) and uterine (Q_m) blood flows were calculated using the steady-state transplacental diffusion method, with tritiated water as the blood flow indicator (64). When tritiated water is infused into the fetal compartment, it either accumulates or escapes via the placenta - the transplacental diffusion rate - assuming the loss through the fetal kidneys in the time period of the study is negligible. This can be written as:

Infusion rate (dpm/ml) = I =

transplacental diffusion rate (dpm/ml) + accumulation rate (dpm/ml)

The rate of accumulation in the fetus $(R_{acc})_f$ or in the placenta $(R_{acc})_P$ can be determined from the slope of the tritiated blood concentration (dpm/ml/min) in the fetal arterial blood and its distribution volume. The volume of distribution for tritiated water is equal to the total water content which accounts for 80% of fetal and placental weight. Therefore:

$(R_{acc})_f$ = slope · 80% of fetal weight

$(R_{acc})_P$ = slope · 80% of placental weight

Umbilical (Q_f) and uterine (Q_m) blood flows (ml/min) can then be calculated using the following equations:

$$Q_f = (R_{inf} - (R_{acc})_f) \div (C_\alpha - C_\gamma)$$

$$Q_m = (R_{inf} - (R_{acc})_f - (R_{acc})_P) \div (C_V - C_A)$$

where C_a, C_γ, C_V and C_A represent the tritiated water concentration (dpm/ml) in the fetal aorta, umbilical vein, uterine vein and maternal common iliac artery respectively.

Whole blood tritiated water values were calculated from plasma tritiated water values as follows:

$$Fetal = [(0.626 \cdot Ht_f + 0.96(1 - Ht_f)] \div 0.96 \cdot plasma\ dpm$$

$$Maternal = [(0.626 \cdot Ht_m = 0.92(1 - Ht_m)] \div 0.92 \cdot plasma\ dpm$$

where 0.0626 is the fraction of water in red cells, 0.96 and 0.92 are the fractions of water in fetal and maternal plasma, respectively, and Ht represents the hematocrit fraction.

Umbilical amino acid uptakes were calculated using the Fick principle, as the product of umbilical plasma flow ($Q_f = Q_f \cdot (1 - fractional\ fetal\ hematocrit)$) and the plasma concentration differences of each amino acid (Δaa) between umbilical vein (γ) and fetal aorta (α). Similarly, uterine uptakes were calculated using uterine plasma flow ($Q_m = Q_m \cdot (1 - fractional\ maternal\ hematocrit)$) and the plasma concentration differences (Δaa) between maternal common iliac artery (A) and uterine vein (V):

$$umbilical\ uptake = Q_f \cdot (\Delta aa)_{\gamma-\alpha}$$
$$uterine\ uptake = Q_m \cdot (\Delta aa)_{A-V}$$

Accretion rates

The umbilical uptake of each amino acid was compared with its normal fetal accretion rate. The fetal accretion rate was calculated from data on amino acid concentration per gram of nitrogen of the fetal carcass (61) and an estimated fetal nitrogen accretion rate of 848 mg $N \cdot Kg_{fetus}^{-1} \cdot day^{-1}$. This estimate is the product of N content per Kg_{fetus} (21 g/Kg) and the fractional rate of N accretion 0.0404 per day at 130 days gestation. Both N content and fractional accretion rate were derived from

measurements of fetal weight and body composition at different gestational ages (7). Note that the N fractional accretion rate is greater than the fractional fetal growth rate (~ 0.032) because N content per unit wet weight increases with gestation (7).

3.2.5 Statistical analysis

All data are expressed as means ± SE. Differences between two groups (maternal vs. fetal, uterine vs. umbilical, etc.) were tested using Student's t test for paired samples. Two-tailed p values were considered significant at $p < 0.05$.

3.3 RESULTS

3.3.1 In vitro study

The degrees of plasma dilution calculated on the basis of $1\text{-}^{14}C$-glutamate and Evans blue were virtually identical and were expected to cause a decrease in plasma amino acid concentrations to 62.6% of the initial value. Initial and post-dilution average hematocrits were 44% and 33%, respectively. With the exception of alanine, the observed concentrations did not deviate significantly from expected and showed no detectable change between the 0.5, 2, and 5 minute samples. Furthermore, the observed concentrations at two different temperatures were not significantly different from one another. The alanine data indicated a significant (p< 0.02) efflux of this amino acid from red cells at 38°C. The expected alanine concentration change was 192 μM (514 to 322 μM). At 38°C, the observed change was 188 μM at 2 minutes and 174 μM at 5 minutes; this is not a rapid efflux. On the assumption that the rate of efflux is proportional to the plasma concentration change, failure to cool and centrifuge the blood for 5 minutes after sampling would cause approximately a 10% underestimate in the umbilical uptake of alanine. On the basis of these results, we selected to calculate all amino acid uptakes as the product of plasma concentration differences times plasma flows.

3.3.2 In vivo study

The eighteen animals had normal values of fetal and placental wet weight, uterine and umbilical blood flows, hematocrit, hemoglobin, O_2 saturations, maternal and fetal glucose and lactate concentrations, and uterine and umbilical uptakes of O_2, glucose and lactate (7). These data are summarized in Tables 3.1 and 3.2. Mean concentrations of amino acids in maternal arterial, uterine venous, fetal arterial and umbilical venous plasma are presented in Table 3.3. The table also presents the mean percent concentration differences across the uterine and umbilical circulations for each amino acid. Each difference was calculated using the highest value of the arterial - venous difference as the reference value. For example, the umbilical percent change of glutamine was calculated as $100 \ (\gamma - \alpha)/\gamma$ and the percent change of glutamate as $100 \ (\gamma - \alpha)/\alpha$. All uterine concentration differences were less than 11%. Differences across the umbilical circulation were more variable. Extraction of fetal plasma glutamate by the placenta created a 72% concentration change across the umbilical circulation. Maternal and fetal arterial plasma amino acid concentrations are graphically compared in Fig. 3.1. Among the essential amino acids, valine, threonine, phenylalanine and methionine had significantly higher fetal concentrations. Among the non-essential amino acids, fetal serine concentration was nine times higher than the maternal concentration.

Table 3.1 Gestational age, fetal and placental weights and uterine and umbilical blood flows in 18 ewes. Values are means ± SE.

Gestational age, days	
Surgery	123 ± 0
Study	130 ± 1
Fetal weight, grams	2909 ± 101
Placental wet weight, grams	305 ± 14
Blood flows, ml·Kg$_{fetus}^{-1}$·min^{-1}	
Uterine	576 ± 36
Umbilical	217 ± 8

Table 3.2 Hematocrits, hemoglobin, O_2 saturations and utilizations, glucose concentrations in maternal and fetal blood and glucose uptakes, and lactate concentrations in maternal and fetal blood and lactate uptakes. Values are means ± SE.

Hematocrit, %	
Maternal artery	33.5 ± 0.5
Fetal artery	36.2 ± 1.0
Hemoglobin,, O_2 capacity, mM	
Maternal artery	6.36 ± 0.11
Fetal artery	6.33 ± 0.16
O_2 Saturations, %	
Maternal artery	93.1 ± 0.4
Fetal artery	55.3 ± 2.3
O_2 utilization, $\mu mol \cdot Kg_{fetus}^{-1} \cdot min^{-1}$	
Uterine	588 ± 24
Umbilical	365 ± 8
Glucose concentration, $\mu mol \cdot ml^{-1}$	
Maternal artery	2.89 ± 0.15
Fetal artery	0.97 ± 0.05
Glucose uptake, $\mu mol \cdot Kg_{fetus}^{-1} \cdot min^{-1}$	
Uterine	86.3 ± 6.8
Umbilical	41.7 ± 1.4
Lactate concentrations, $\mu mol \cdot ml^{-1}$	
Maternal artery	0.55 ± 0.07
Fetal artery	1.90 ± 0.07
Lactate uptake, $\mu mol \cdot Kg_{fetus}^{-1} \cdot min^{-1}$	
Uterine	-23.1 ± 5.0
Umbilical	24.8 ± 1.76

Table 3.3 Amino acid concentrations (nmol/ml) in maternal arterial (A), uterine venous (V), umbilical arterial (α), and umbilical venous (γ) plasma. Values are means ± SE.

	A	V	α	γ	% Concentration difference*	
					Uterine	Umbilical
Essential						
Val	331.8 ± 28.8	310.2 ± 28.6	473.1 ± 43.7	504.5 ± 44.3	6.5	6.2
Leu	241.4 ± 18.6	223.1 ± 18.4	184.2 ± 16.2	216.2 ± 17.1	7.6	14.8
ILe	158.8 ± 11.2	147.3 ± 11.2	100.9 ± 9.8	119.3 ± 10.4	7.2	15.4
Thr	208.0 ± 22.1	201.5 ± 22.4	381.0 ± 38.4	396.0 ± 38.4	3.1	3.8
Phe	72.7 ± 4.7	68.6 ± 4.6	113.0 ± 9.5	125.4 ± 10.0	5.6	9.9
Met	35.4 ± 3.8	34.0 ± 3.6	88.4 ± 9.3	95.8 ± 9.7	4.0	7.7
Lys	172.0 ± 20.6	161.7 ± 20.7	71.8 ± 13.6	95.0 ± 14.6	6.0	24.4
His	55.0 ± 3.2	51.4 ± 3.7	55.0 ± 8.2	64.2 ± 9.2	6.5	14.3
Non-essential						
Ser	80.9 ± 6.4	72.5 ± 5.9	682.9 ± 51.7	673.2 ± 50.6	10.4	-1.4
Gly	436.8 ± 25.0	439.7 ± 26.3	478.3 ± 48.2	497.0 ± 47.8	-0.7	3.8
Ala	145.8 ± 8.9	139.1 ± 8.8	326.9 ± 15.8	345.4 ± 15.5	4.6	5.4
Pro	101.3 ± 15.3	90.9 ± 14.4	140.4 ± 11.6	160.8 ± 13.6	10.3	12.7
Arg	197.4 ± 32.9	183.5 ± 35.4	102.2 ± 16.0	130.6 ± 14.3	7.0	21.7
Orn	109.6 ± 37.9	100.5 ± 34.9	81.7 ± 11.2	84.7 ± 10.0	8.3	3.5
Tyr	82.6 ± 6.0	78.4 ± 5.9	129.2 ± 9.1	139.9 ± 9.1	5.1	7.6
Gln	249.5 ± 11.0	239.0 ± 11.2	413.2 ± 11.9	454.6 ± 13.8	4.2	9.1
Glu	79.8 ± 5.9	82.3 ± 5.8	42.1 ± 2.4	11.9 ± 1.1	-3.0	-71.7
Asn	45.4 ± 5.5	43.2 ± 5.6	42.9 ± 3.6	50.1 ± 4.5	4.8	14.4
Asp	8.2 ± 0.9	8.5 ± 0.6	17.5 ± 1.7	17.5 ± 1.2	-3.5	0.0
Cit	222.2 ± 27.6	219.1 ± 28.3	149.1 ± 8.9	149.1 ± 9.2	1.4	0.0
Tau	83.6 ± 6.0	83.8 ± 6.0	89.0 ± 23.8	88.7 ± 23.1	-0.2	-0.3

*Each change calculated using the highest value of the arterial-venous difference as the reference value. A negative uterine change indicates net amino acid flux from pregnant uterus to maternal circulation. A negative umbilical change indicates net amino acid flux from fetal circulation to placenta.
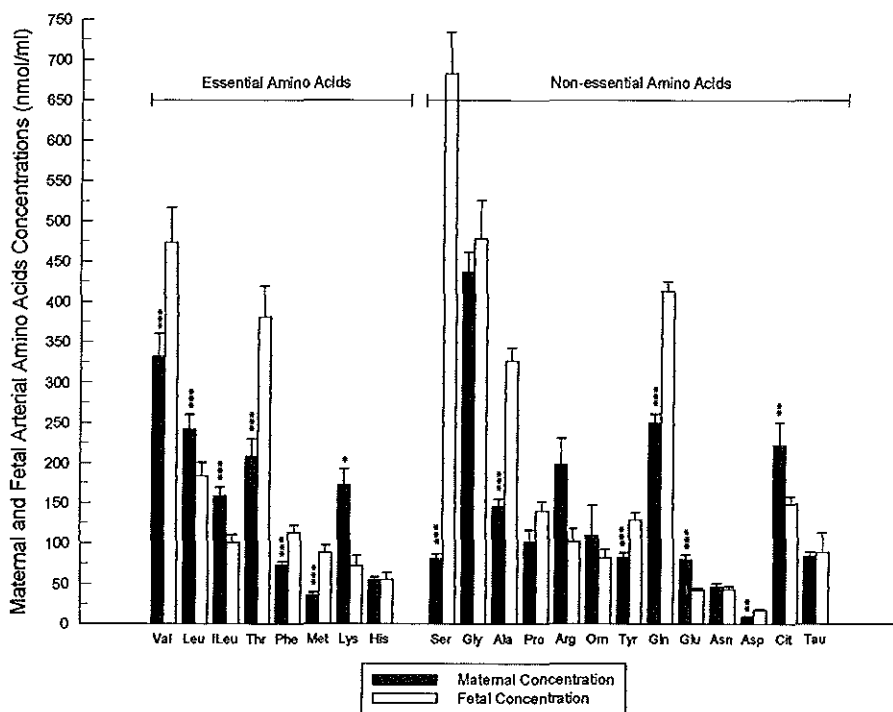
Fig 3.1 Comparison between maternal and fetal arterial amino acid plasma concentrations. Bar graphs are means ± SE. Statistically significant differences between maternal and fetal concentrations are marked by asterisks (* p< 0.05, ** p< 0.01, *** p< 0.001).

Uterine and umbilical uptakes expressed as $\mu mol \cdot Kg_{fetus}^{-1} \cdot min^{-1}$ are presented in Fig. 3.2. Both uptakes are also expressed in nitrogen equivalents (mg $N \cdot Kg_{fetus}^{-1} \cdot day^{-1}$) in Table 3.4. Both the uterine and umbilical uptakes of all essential amino acids were significantly greater than zero. The umbilical uptakes of glutamate and serine were negative, i.e., there were uptakes of fetal glutamate and serine by the placenta. There was a small efflux of glutamate into the uterine circulation which was of borderline significance ($p = 0.046$). The uterine uptake of glycine was not significantly different from zero. Uterine uptakes normalized for maternal arterial concentration, i.e., expressed as plasma clearances, are shown in Figure 3.3. The data are grouped into neutral, basic and acidic amino acids to emphasize the role of amino acid transporters in uterine uptake.

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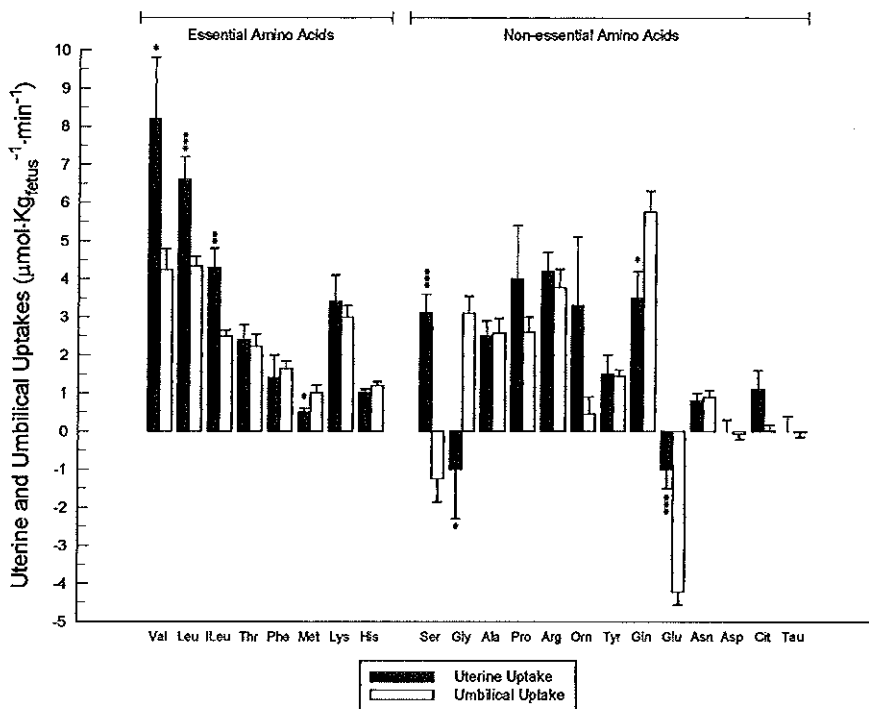
Figure 3.2 Comparison between uterine and umbilical uptakes of amino acids. Bar graphs are means ± SE. Statistically significant differences between uterine and umbilical uptakes are marked by asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$).

Eight amino acids demonstrated a significant difference between uterine and umbilical uptakes. The net uteroplacental utilization rates of these amino acids, normalized for placental weight, are presented in Fig. 3.4. Serine had the highest net utilization rate and glycine the highest net production rate. Most of the serine utilized was maternal serine and most of the glycine produced was delivered into the umbilical circulation. There was net uteroplacental utilization of the branched chain amino acids (BCAA), i.e., valine, leucine and isoleucine, taken up from the maternal circulation, and of the glutamate taken up from the umbilical circulation. Uteroplacental utilization of BCAA represented approximately 40% of their uterine uptake. Concomitant with BCAA

and glutamate utilization there was net glutamine production, so that umbilical glutamine uptake included glutamine synthesized by the placenta. Finally, there was weak statistical evidence for a relatively small uteroplacental production of methionine ($p =$ 0.047).

Umbilical uptake of each essential amino acid was in excess of its estimated normal fetal accretion rate (Figure 3.5). Among the non-essentials, umbilical arginine uptake was remarkably large. It was almost three times the estimated accretion and made the largest contribution to the umbilical uptake of amino acid nitrogen (Table 3.4). The combined glutamine and arginine uptakes represented approximately 45% of the total amino acid nitrogen delivered by the placenta to the fetus.
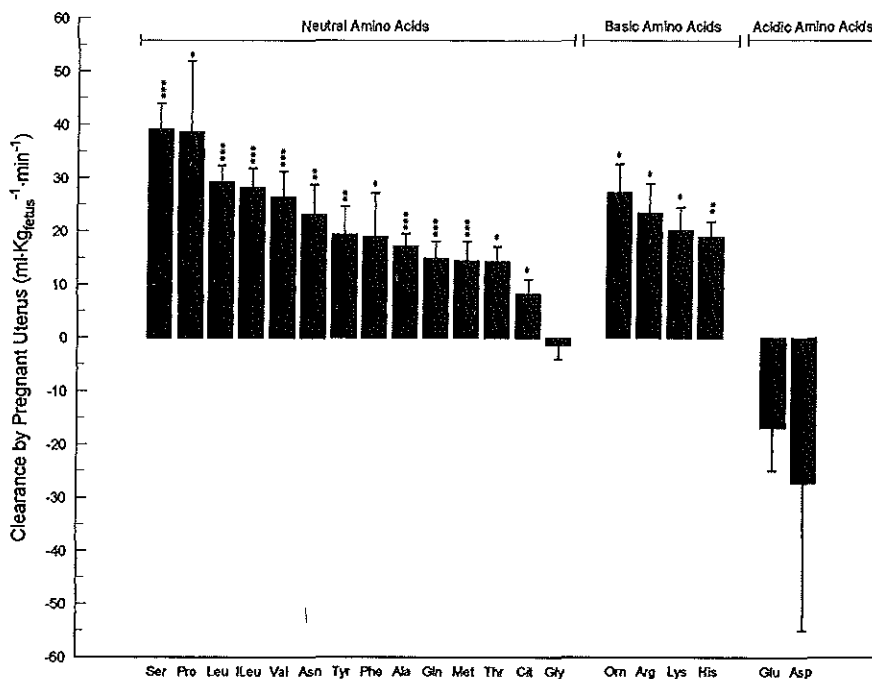
Figure 3.3 Uterine uptakes of amino acids normalized for maternal arterial concentrations, expressed as plasma clearances. Bar graphs are means ± SE. Statistically significant differences from zero are marked by asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$).
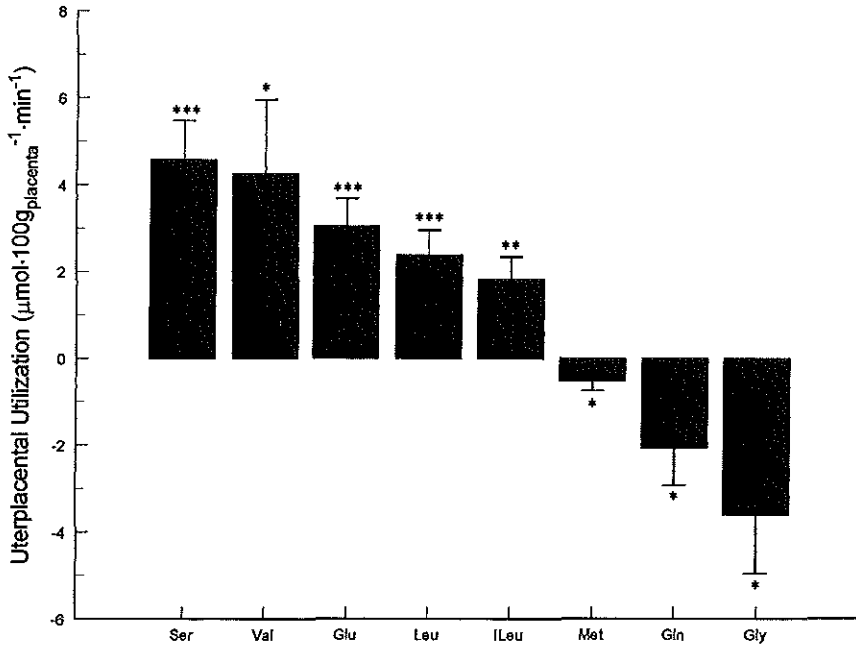
Figure 3.4. Uteroplacental utilizations which were significantly different from zero, expressed per 100 g placenta and ranked by magnitude (negative utilization = production). Bar graphs are means ± SE. Levels of statistical significance are marked by asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$).

3.4 DISCUSSION

This discussion addresses two separate, but related, issues. The first is the contribution of red cells to the exchange of amino acids between the placenta and the uterine and the umbilical circulations. The second issue, which is the primary focus of the study, addresses the conclusions that can be drawn about placental amino acid metabolism from the simultaneous measurement of uterine and umbilical amino acid uptakes.

Table 3.4. Amino acid nitrogen uptakes and food nitrogen intake (mg N·Kg$_{fetus}^{-1}$·day^{-1}). Values are means ± SE.

Essential	Uterine	Umbilical
Val	165.86 ± 31.39	85.54 ± 10.97
Leu	133.66 ± 11.74	87.35 ± 5.04
ILe	85.94 ± 10.09	50.15 ± 3.41
Thr	47.96 ± 9.07	44.98 ± 6.11
Phe	28.22 ± 11.23	33.12 ± 3.94
Met	9.88 ± 2.30	20.12 ± 4.28
Lys	136.79 ± 29.73	120.47 ± 12.45
His	62.04 ± 8.02	72.48 ± 6.42
Non-essential		
Ser	63.33 ± 10.08	−25.16 ± 12.18
Gly	−19.29 ± 25.68	62.42 ± 9.25
Ala	49.46 ± 7.43	51.91 ± 7.79
Pro	80.61 ± 27.93	52.40 ± 8.00
Arg	335.75 ± 41.74	305.05 ± 38.52
Orn	134.77 ± 70.66	18.34 ± 18.10
Tyr	30.78 ± 9.52	29.41 ± 3.10
Gln	142.70 ± 28.43	231.95 ± 22.31
Glu	−20.34 ± 9.42	−84.98 ± 6.76
Asn	31.35 ± 6.23	36.22 ± 6.69
Asp	0.08 ± 5.89	−1.30 ± 2.98
Cit	68.37 ± 30.02	0.62 ± 9.59
Tau	−0.05 ± 8.74	−0.35 ± 2.75
SUM	1567.87	1190.74
Food Nitrogen Intake		
4195 ± 434		
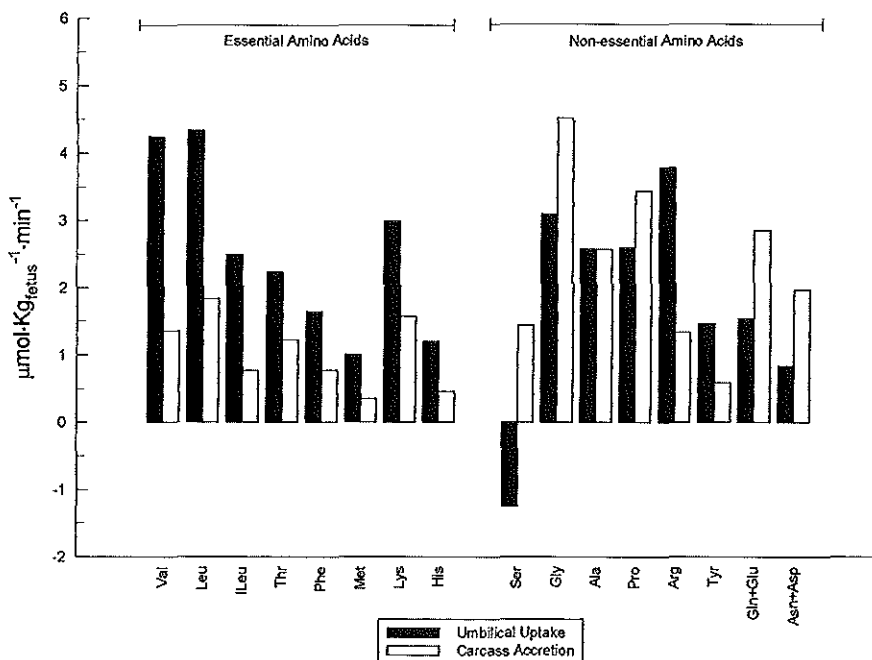
Figure 3.5 Comparison between mean umbilical uptakes and estimated normal fetal accretions of amino acids. For the pairs of amino acids (glutamine + glutamate) and (asparagine + aspartate) individual accretion rates are not known.

3.4.1 Plasma-red cell exchange

Adult sheep erythrocytes have a sodium-dependent transport system for neutral and basic amino acids (101). Although this transport system is important for erythrocyte viability (101), it transports amino acids quite slowly in comparison to the rate of amino acid exchange between blood and tissues. The characteristics of erythrocyte amino acid transport change during development, as shown by the finding that reticulocytes possess sodium-dependent glycine transporters which are lost during maturation (9). Nevertheless, fetal and adult erythrocytes do share some properties such as their impermeability to glutamate (93). The present study shows that a large decrease in the concentration of fetal plasma amino acids is not followed by rapid, net transport of neutral and basic amino acids from red cells to plasma. This *in vitro* evidence indicates

that there is no mechanism, in either adult or fetal red cells, for the fast loading and unloading of amino acids which would make them suitable for amino acid shuttling among organs.

An alternative approach to the study of the role of erythrocytes in amino acid exchange is a comparison of plasma uptakes (i.e., plasma concentration differences times plasma flow) with blood uptakes (i.e., whole blood concentration differences times blood flow) across the circulation of different organs. If erythrocytes do not contribute to the exchange, plasma and blood uptakes should be equal. According to a recent study, there is no significant difference between plasma and blood uptakes of most amino acids across the splanchnic and hepatic circulations of sheep (54). These data support the conclusion that there is no major involvement of erythrocytes in the amino acid exchange between blood and tissues (54). *In vivo* data do not lend themselves to a more precise conclusion, because the comparison of plasma and blood uptakes depends upon the accuracy of comparing small percent changes in concentration.

A consequence of amino acid uptake being limited primarily to plasma amino acids is that, at normal hematocrits, plasma concentration differences across organs are approximately 1.5 times the whole blood concentration differences. For this reason plasma differences can be determined more precisely. An additional consideration is that the transporter molecules located on the maternal and fetal surfaces of the placenta are exposed to plasma amino acid concentrations. Plasma concentration measurements allow estimation of transport rates in relationship to the concentrations that are relevant to transport. A theoretical possibility that has been discussed in the physiological literature (54) is direct amino acid exchange between erythrocytes and the intracellular amino acid pools of some organs. However, the possibility of such an exchange is difficult to confirm. It presupposes a close tissue-red cell interaction for which there is no known mechanism. Another theoretical concern is that amino acid uptake calculations assume no production of free amino acids within the blood as it circulates through the organ. This assumption would not be the case if the organ stimulated rapid hydrolysis of blood proteins and peptides. In practical terms, measurements of uterine and umbilical plasma amino acid uptakes provide the best quantitative evidence about rates of placental amino acid transport and metabolism *in vivo*. Given the complexity of the placental system with

its perfusion by two different circulations, this evidence needs to be considered in the light of all the available information.

3.4.2 Net placental, uterine and umbilical uptakes

Uterine and umbilical uptakes of amino acids can be examined with emphasis on either metabolic pathways or transport systems. From the metabolic point of view, an obviously important distinction is between essential and non-essential amino acids. In the present study, all the essentials demonstrated both uterine and umbilical uptakes, with each umbilical uptake being approximately 1.8 to 3.3 times greater than the estimated normal accretion rate. This finding establishes significant fetal catabolism of all the essential amino acids. Fetal oxidation of leucine (56, 76), threonine (1), and lysine (60) has been verified by tracer methodology. Among the essentials, the three branched chain amino acids (BCAA) form a unique group. In the present study, they showed the highest uterine uptake and demonstrated net uteroplacental utilization. Uteroplacental utilization of BCAA was demonstrated in a previous study in which uterine and umbilical blood uptakes were compared (48). The ovine placenta releases into the fetal and maternal circulations the keto acids that are formed in BCAA deamination (56, 83). Since BCAA deamination results in the formation of glutamate via transamination with α-ketoglutarate, it is likely that placental uptake of fetal glutamate and uteroplacental BCAA utilization represent two separate mechanisms for supplying glutamate to the placenta. Previous studies from this laboratory have demonstrated that approximately 70% of the fetal glutamate taken up by the placenta is rapidly oxidized (69), and that the placenta excretes ammonia (8, 35). The present study shows for the first time significant net glutamine production by the placenta, in agreement with the evidence that glutamine synthetase is present in this organ (73). Some of the glutamine delivered to the fetus by the placenta is converted back to glutamate by the fetal liver which produces most of the glutamate consumed by the placenta (93). This establishes a glutamate-glutamine shuttle, which promotes oxidation of glutamate in the placenta and fetal hepatic utilization of the amide group of glutamine. We may infer from the present set of observations that the quantitative relationship of placental glutamate supply and utilization, under normal

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physiological conditions, is as follows. BCAA deamination and placental uptake of fetal glutamate supply the placenta with approximately 11.2 $\mu mol \cdot Kg_{fetus}^{-1} \cdot min^{-1}$ of glutamate (8.0 from BCAA deamination and 3.2 from glutamate net uptake). Approximately 2.2 μmol of the glutamate combine with ammonia to produce glutamine that is delivered to the fetus. The remainder (approximately 9 μmol) becomes available for oxidation and provides the placenta with approximately 6.8 μmol of ammonia nitrogen (i.e. 9.0-2.2) that can be channeled either into synthetic processes via additional glutamine formation or into excretion. From separate measurements of uterine (35) and umbilical (8) ammonia uptakes, it can be estimated that total ammonia excretion by the late gestation ovine placenta is about 10 $\mu mol \cdot Kg_{fetus}^{-1} \cdot min^{-1}$, with most of the excretion being into the uterine circulation. Late gestation umbilical uptake of ammonia nitrogen is approximately 2 $\mu mol \cdot Kg_{fetus}^{-1} \cdot min^{-1}$ (8). Placental glutamate oxidation is likely to serve several functions. Glutamate output by the fetal liver allows the fetus to uncouple the oxidation of glucogenic amino acids from gluconeogenesis (69). Glutamate oxidation by placental mitochondria generates NADPH and therefore plays a role in biosynthetic reactions such as steroidogenesis (42). The role of BCAA in placental glutamate metabolism as explained above is schematically presented in Fig. 3.6.

Placental conversion of maternal and fetal serine into glycine has been demonstrated by experiments of serine tracer infusion into the maternal (17, 68) and fetal (8) circulations. In the present study, umbilical glycine uptake was represented entirely by glycine produced within the placenta; all of the serine taken up by the pregnant uterus was utilized by the uteroplacental mass. Uterine serine uptake could account for most of the placental glycine production. In conjunction with the tracer studies, these data point to the conclusion that one of the major metabolic functions of the ovine placenta is glycine production from serine and that the major source of placental glycine is maternal serine. The conversion of serine to glycine channels the β carbon of serine via the production of methylentetrahydrofolate into synthetic reactions that require activated one carbon units, such as purine synthesis. Since purine synthesis requires also the utilization of glycine and of the amide group of glutamine, it represents an aspect of placental metabolism that could link together in a common function all the major findings about placental amino acid production and utilization. Measurements of placental nucleotide

metabolic rates *in vivo* are needed in order to assess the quantitative importance of this linkage.

FETAL   PLACENTA   MATERNAL

BCAA UPTAKE
11.1

BCAA UPTAKE
19.1

GLU UPTAKE
4.2

GLU UPTAKE
1.0

GLN UPTAKE
5.7

GLN UPTAKE
3.5
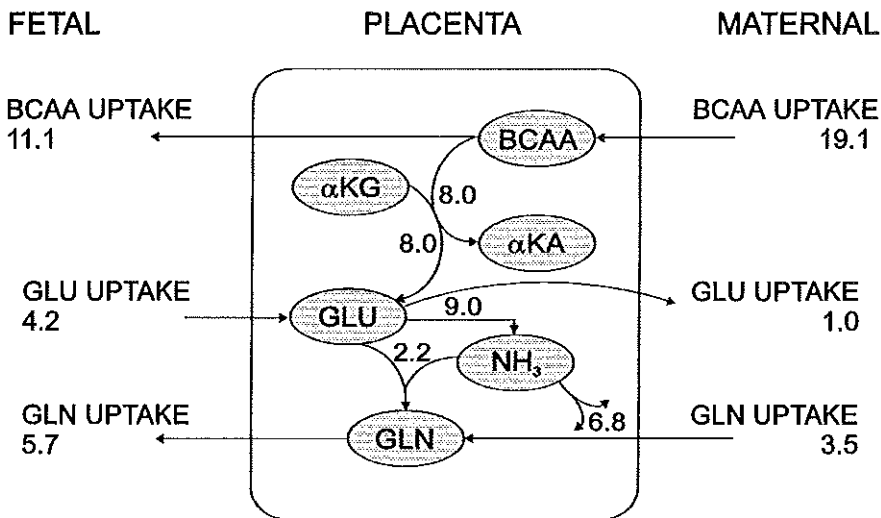
Figure 3.6 Schematic diagram of contribution of branched chain amino acids (BCAA) deamination and fetal glutamate uptake to placental glutamate (GLU). Oxidation of placental glutamate produces ammonia which can be used either in the synthesis of glutamine (GLN) or excreted into the uterine and umbilical circulations. The values are in $\mu mol \cdot Kg_{fetus}^{-1} \cdot min^{-1}$. $\alpha KG = \alpha$-Ketoglutarate. αKA=branched chain keto acids. Fetal uptake of placental glutamine (2.2 $\mu mol \cdot Kg_{fetus}^{-1} \cdot min^{-1}$) may underestimate placental glutamine synthetic rate because the placenta is likely to utilize the amide group of glutamine.

An unexpected finding was a small but statistically significant production of methionine, an essential amino acid, by the placenta. Sheep plasma contains methionine sulfone and methionine sulfoxide, which suggests that reduction of methionine sulfoxide to methionine within the ovine placenta may be a source of fetal methionine. Methionine sulfoxide can be utilized instead of methionine to sustain the growth of weaning rats (2).

It should be noted that amino acids may participate in important metabolic reactions within the placenta without showing net placental utilization or production, either because the reaction rates are not sufficiently rapid or because utilization and

production go on simultaneously. For example, the placenta is capable of producing nitric oxide and citrulline from arginine (12). One possible outcome of this reaction would be net arginine utilization and net citrulline production, similar to that described for the small bowel (97). However, uterine and umbilical uptakes of arginine were virtually equal. The net uteroplacental utilization of citrulline which was found of borderline significance ($p = 0.06$). This suggests that there may be approximately equal rates of citrulline production from arginine and arginine synthesis from citrulline, a hypothesis that requires tracer studies for confirmation.

The catabolism of amino acids in both the placenta and fetus make the nitrogen requirements of the pregnant uterus considerably greater than the requirements estimated from fetal accretion. In the present study, the nitrogen uptake represented by the combined uptake of all the measured amino acids was approximately 1568 mg $N \cdot Kg_{fetus}^{-1} \cdot day^{-1}$ for the uterus, and 1191 mg $N\ Kg_{fetus}^{-1} \cdot day^{-1}$ for the fetus. These uptakes are about 84% and 40% greater than the estimated 848 mg $N \cdot Kg_{fetus}^{-1} \cdot day^{-1}$ fetal accretion rate. Uterine nitrogen uptake was also a large fraction, approximately one third, of the maternal nitrogen dietary intake. The 377 mg $N \cdot Kg_{fetus}^{-1} \cdot day^{-1}$ difference between uterine and umbilical amino acid nitrogen uptakes is somewhat higher than the uteroplacental ammonia excretion of about 200 mg $N \cdot Kg_{fetus} \cdot day^{-1}$, estimated from measurements of uterine (35) and umbilical (8) ammonia uptakes. It is uncertain whether this discrepancy represents mostly experimental error or as yet unidentified placental utilization of amino acid nitrogen.

Transport mechanisms interact with metabolic activity in providing the amino acid uptakes that are measured *in vivo*. The relationship between amino acid transport by the maternal circulation and uterine amino acid uptake is defined by the percent concentration changes of amino acids across the uterine circulation, which represent the uptake / circulatory supply ratios. As demonstrated in Table 3.3, these ratios were quite small for each amino acid, including those with the highest uptakes. Therefore, under normal physiological conditions, transport by the maternal circulation is not a limiting factor for the uterine uptake of amino acids. The uterine clearance of neutral amino acids was variable. This variability is probably related to the presence of several transport systems for neutral amino acids on the maternal surface of the placenta, each having

highest affinity for different amino acids (84). The virtual absence of glycine uptake by the pregnant uterus, despite a very high maternal plasma glycine concentration, suggests that the sodium dependent GLY system is not expressed in the ovine placenta.

Placental transport systems for cationic amino acids have not been studied in detail. The sodium independent y^+ system, which does not discriminate between arginine and lysine, has been found on the basal membrane of the human trophoblast (84). The present study shows relatively high and similar uterine clearances of ornithine, arginine and lysine, with transport of arginine and lysine into the fetal circulation.

The absence of glutamate uptake by the ovine pregnant uterus is puzzling because a sodium-dependent transporter for glutamate on the maternal surface of the human trophoblast has been reported (84). This led to the prediction that there should be placental uptake of maternal glutamate. The finding that the ovine placenta does not verify this prediction may reflect a species difference. Another possibility is that, within the population of cells exchanging glutamate between pregnant uterus and maternal blood, uptake by one group is masked by efflux from another group.

The placental uptake of glutamate and serine from the fetal circulation presupposes the presence on the fetal surface of the placenta of transporters that pump these amino acids into the placenta from the umbilical circulation. A sodium-dependent, high affinity, electrogenic transporter for anionic amino acids, which resembles the X^-_{AG} system, has been shown to be present on the basal surface of the human trophoblast (84) which is consistent with the *in vivo* data. Its electrogenic property suggests that transport of fetal glutamate into the placenta may serve to regulate intracellular Ca^+ as has been reported for glutamate transport in pituitary cells (94). Placental uptake of fetal serine is more difficult to explain than glutamate uptake, because serine shares transport systems with other neutral amino acids that have net flux from placenta to fetus. The placental uptake of fetal serine results from bidirectional serine fluxes between placenta and fetus (17). Similarly, the umbilical uptakes of leucine (76) and alanine (30) result from bidirectional fluxes, but in contrast to serine, there is a net umbilical uptake of these amino acids. The neutral amino acid transporters on the fetal surface of the placental epithelium include sodium-dependent transport systems that promote the flux of fetal neutral amino acids into the placenta (84).

The *in vivo* data highlight the difficulty of attempting to predict the direction of net amino acid uptake from the study of transporters in isolated placental membrane vesicles. One possible explanation consistent with both *in vivo* and *in vitro* data is that the neutral amino acid exchange between placenta and fetus involves transport systems which mediate fluxes of opposite sign, and that serine interacts strongly with one or more of the sodium-dependent transport systems on the fetal surface of the placenta. The finding that the sodium dependent ASC transport system, for which serine has relatively high affinity, is present on the fetal surface of the human trophoblast (84) supports this hypothesis. In addition, the high serine concentration of fetal plasma may promote placental uptake of fetal serine by sodium-independent exchangers via counter-transport with other amino acids.

Chapter 4

RELATIONSHIP OF FETAL ALANINE UPTAKE AND PLACENTAL ALANINE METABOLISM TO MATERNAL PLASMA ALANINE CONCENTRATION

4.1 INTRODUCTION

Placental transport of amino acids is a complex process in which specificity, activity and location of amino acid transporters within the placenta (84) and placental amino acid metabolism play a role in determining which amino acids are supplied to the fetus and at what rates. The importance of placental metabolism in this context has become apparent in the comparison of serine and glycine transport by the ovine placenta. The placenta takes up serine from the maternal circulation but does not release serine into fetal blood. Maternal serine is used by the placenta for the production of glycine which is then taken up by the fetus via the umbilical circulation (68).

There are no studies on the role of placental metabolism in determining the supply of alanine to the fetus. In sheep, alanine is transported into the uteroplacental unit (36, 48) and from the placenta to the fetus (30, 47, 59). Although this suggests alanine transport across the placenta, it is not known whether the alanine delivered to the fetus by the placenta is derived directly from the maternal circulation or represents a product of placental alanine turnover. Transport of maternal alanine to the fetus has been suggested by a study in which the intravenous infusion in pregnant sheep of a solution of several amino acids, including alanine, caused a significant increase in fetal plasma alanine concentration (57). However, the increase in fetal concentration was only 12 percent of the increase in maternal concentration, and no attempt was made to demonstrate that uterine and umbilical alanine uptakes had actually increased. The present study was

designed to address two questions concerning alanine transport by the ovine placenta. First, what fraction of the alanine flux from placenta to fetus represents direct alanine transport from the maternal to the fetal circulation? Second, what is the effect of an increase in maternal plasma alanine concentration upon placental and fetal alanine uptake and utilization?

4.2. MATERIALS AND METHODS

4.2.1 Surgery and animal care

Twelve pregnant Columbia-Rambouillet ewes were studied, each with a single fetus. Surgery was performed at 120-128 days of gestation. Surgical techniques and animal care are described in chapter 2.

4.2.2 Study design

The following experimental protocol was used to study eight sheep at 127-134 days of gestation (Fig. 4.1). Maternal and fetal blood samples were drawn for tritium and alanine enrichment blanks. Then, a fetal infusion of L-[1-^{13}C]alanine in a dose of 0.83 ± 0.09 $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$ and tritiated water in a dose of 0.22 $\mu Ci \ min^{-1} \cdot Kg_{fetus}^{-1}$ was started via the fetal venous catheter. Simultaneously, a maternal venous infusion of L-[3,3,3-D$_3$] alanine, (CIL, Andover, MA) $0.17 \pm 0.02 \ \mu mol.min^{-1} \cdot Kg_{fmother}^{-1}$ was started.

At steady-state, four sets of samples were collected simultaneously from the maternal artery, uterine vein, fetal artery and the common umbilical vein at 120, 150, 180 and 210 min (S1-S4) from the start of the infusions. Each sampling represented the loss of approximately 10 ml of fetal blood. Fetal blood loss was corrected by transfusing the fetus, between sampling, with an equal amount of blood from a donor sheep. This part of the experiment is referred to as the control period. Then, the maternal infusate was replaced by one containing a mixture of L-[3,3,3,-D$_3$]alanine and reagent grade L-[^{12}C]-alanine (1000 $\mu mol/ml$) in order to raise maternal alanine concentration four to fivefold. At steady-state, two hours after the start of the new maternal infusion, four sets of blood samples were collected at 330, 360, 390 and 420 min from the beginning of the study (S5-S8), following the same procedure used in collecting the control samples. This part

of the experiment is referred to as the experimental period. After collection of the last set of samples, the ewe and the fetus were killed. Necropsy was performed to obtain fetal, placental and uterine weights, and to verify catheter positions.

To help in the interpretation of the tracer data generated by these experiments, four additional animals were studied. In two, an identical protocol was followed, with the exception that L-[3,3,3-D$_3$] alanine was infused into the fetus. In the other two animals, the study was limited to the control period, and the L-[1-^{13}C] alanine infusion into the fetus was combined with the infusion of L-[U-^{13}C] alanine into the ewe.

FETAL INFUSATE

Tritiated water

L- [1-^{13}C] Alanine

MATERNAL INFUSATE 1

L- [3,3,3-D$_3$] Alanine

MATERNAL INFUSATE 2

L- [^{12}C] Alanine

CONTROL			EXPERIMENTAL		
S1 S2 S3 S4			S5 S6 S7 S8		

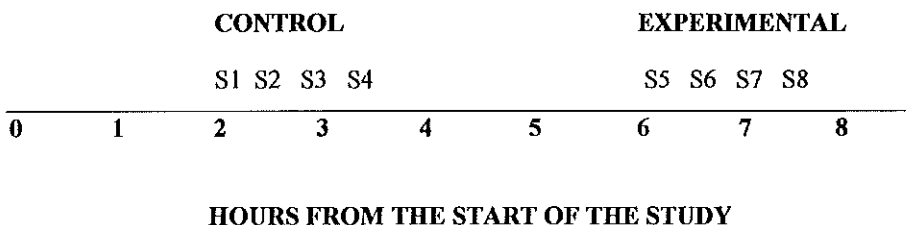| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

HOURS FROM THE START OF THE STUDY
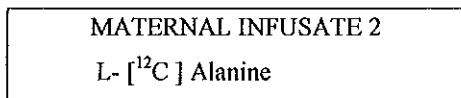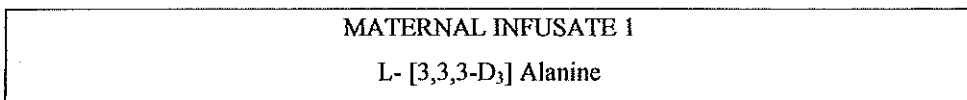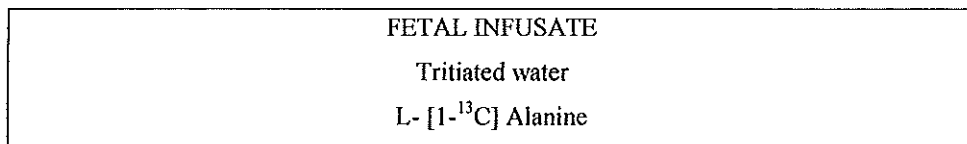
Figure 4.1 Flow chart of study design.

4.2.3 Analytical methods

All samples were analyzed for hemoglobin, hematocrit, O_2 saturation, glucose, lactate, tritiated water and amino acid concentrations, and plasma alanine enrichments. Maternal and fetal arterial samples were analyzed for plasma insulin. Analytical methods were as described in chapter 2.

For mass spectrometry, amino acids were first separated on 0.2 ml AG50 cation exchange resin (BioRad Mesch 100-200). For the determination of the alanine enrichments, plasma (0.2 ml) was mixed with 300 µl 30% acetic acid (Fisher Scientific, Pittsburgh, PA) and applied to the column. After washing the resin with 2 ml distilled water, the amino acids were eluted with 750 µl NH_4OH and lyophilized. Tri-t-butyldimethylsilyl derivatives were formed with 200 µl acetonitrile containing 15% N-methyl-N(t-butyldimethylsilyl)trifloroacetamide and 1.5% t-butyldimethylchlorosilane (Aldrich Chemicals, Milwaukee, WI) at 100°C for 30 min. Tandem gas chromatography mass spectrometry (GCMS) was performed on an HP-5790 gas chromatograph with a 30 m DB-17 0.025 mm ID 0.25 µm film thickness fused silica capillary column (J and W Scientific, Folsom, CA) with helium as the carrier gas. The selected condition was 200°C initial port temperature, an initial column temperature of 120°C with a 5°C/min ramp to 150°C, resulting in an alanine peak at ~7 min. The ion clusters for the alanine M-57 peak were monitored at M/Z 260, 261, 262 and 263.

The enrichments were calculated using the increase in the isotope peaks after correcting for the natural abundance. The molar percent enrichments (MPE) were calculated using steady state (r_S) and blank (r_O) ion abundance ratios:

$$MPE = [100(r_S - r_O)] \div [1 + (r_S - r_O)]$$

The blank ion abundance ratios were calculated with the deconvolution equation:

$M+1cor = (M+1) - ((M) \cdot (M+1nat/Mnat)) = (a)$

$M+2cor = (M+2) - ((M) \cdot (M+2nat/Mnat) + (a) \cdot (M+1nat/Mnat)) = (b)$

$M+3cor = (M+3) - ((M) \cdot (M+3nat/Mnat) + (a) \cdot (M+2nat/Mnat) +$

$(b) \cdot (M+1nat/Mnat)) = (c)$ etc.

4.2.4 Calculations

Blood flows and uptakes

Umbilical and uterine blood flows, Q_f and Q_m respectively, were calculated by the steady-state transplacental diffusion method using tritiated water (92). The uterine and umbilical uptakes of oxygen, glucose and lactate were calculated by application of the Fick principle:

$$\text{uterine uptake} = Q_m \cdot (A\text{-}V)_{blood}$$

$$\text{umbilical uptake} = Q_f \cdot (\gamma\text{-}\alpha)_{blood}$$

where A, V, γ and α refer to maternal arterial, uterine venous, umbilical venous and umbilical arterial concentrations, respectively.

The uterine and umbilical uptakes of alanine were similarly calculated using plasma flows and plasma alanine concentrations:

$$\text{uterine alanine uptake} = Q_m(1\text{-}Ht_m) \cdot (A\text{-}V)_{plasma}$$

$$\text{umbilical alanine uptake} = Q_f(1\text{-}Ht_f) \cdot (\gamma\text{-}\alpha)_{plasma}$$

where Ht_m and Ht_f represent the maternal and fetal fractional hematocrits, respectively. These equations are based on the assumption that in sheep, the rapid amino acid exchange between circulating blood and body tissues is virtually limited to an exchange between the tissues and the plasma compartment as reported in 3.3.1. This observation is supported by observations in vivo (54) and by measurements of amino acid fluxes between red cells and plasma in vitro (101).

The alanine molar percent enrichments (MPE) were calculated using steady state (r_S) and blank (r_O) ion abundance ratios:

$$MPE = [100(r_S - r_O)] \div [1 + (r_S - r_O)]$$

Disposal rates.

The maternal plasma alanine disposal rate (DR_m) was calculated as follows:

$$Dr_m = \left(\frac{100}{{}^m MPE_A} \cdot {}^m C \cdot {}^m I \right) - {}^m C \cdot {}^m I$$

where ${}^m MPE_A$ is the maternal arterial plasma enrichment of the maternally infused tracer at steady state, ${}^m C$ is the concentration of the tracer in the maternal infusate ($\mu mol/ml$) and ${}^m I$ is the infusion rate of the maternal infusate (ml/min).

The fetal plasma alanine disposal rate (DR_f) was similarly calculated:

$$DR_f = \left(\frac{100}{^f MPE_\alpha} \cdot {}^f C \cdot {}^f I \right) - {}^f C \cdot {}^f I$$

where $^f MPE$ is the steady state fetal arterial plasma enrichment of the tracer infused into the fetus, $^f C$ is the concentration of the tracer in the fetal infusate ($\mu mol/ml$) and $^f I$ is the infusion rate of the fetal infusate (ml/min). We note that these equations do not include the disposal rate of the naturally occurring isotopes (75).

Since the DR_m and DR_f calculations are based on steady state measurements, they also represent estimates of the rate of appearance of alanine in maternal and fetal plasma, respectively

Tracer alanine concentrations.

Plasma tracer alanine concentrations were calculated as total plasma concentrations times MPE divided by 100.

Placental alanine fluxes.

The numbers that follow the equations below refer to fig. 4.2. The maternal tracer concentration differences across the uterine circulation $(A-V)_{mat.\ tracer}$ and the MPE of the maternal tracer in maternal arterial plasma
($^m MPE_A$), were used to calculate the flux of maternal alanine into the placenta from the maternal circulation ($R_{P,m}$):

$$R_{P,m} = [(A-V)_{mat.\ tracer} \cdot \text{uterine plasma flow}] \div 0.01\ ^m MPE_A \quad (1)$$

The fetal tracer concentration differences across the umbilical circulation, $((\alpha-\gamma)_{fetal\ tracer})$, and the MPE of the fetal tracer in umbilical arterial plasma, $(^f MPE_\alpha)$, were used to calculate the flux of fetal alanine into the placenta from the fetal circulation ($R_{P,f}$):

$$R_{P,f} = [(\alpha-\gamma)_{fetal\ tracer} \cdot \text{umbilical plasma flow}] \div 0.01\ ^f MPE_\alpha \quad (2)$$

COMPONENTS OF UMBILICAL UPTAKE OF AN AMINO ACID
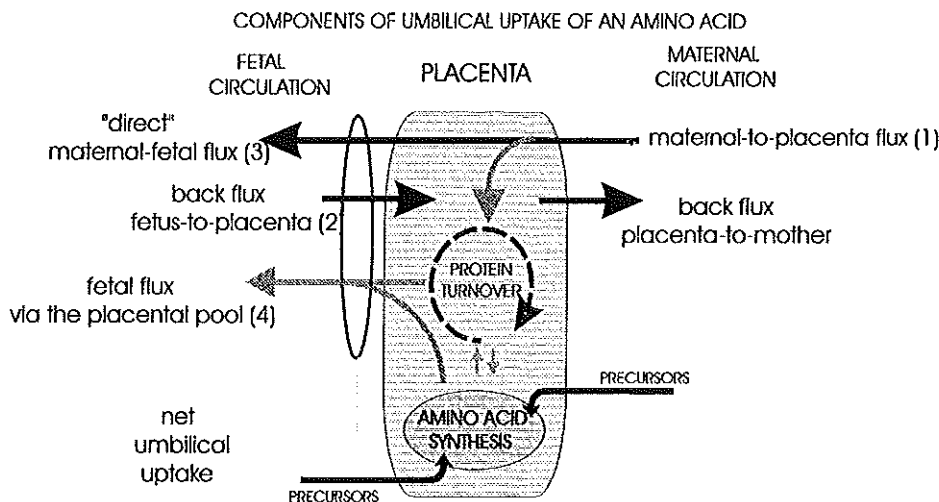
Fig. 4.2 Schematic diagram of placental alanine fluxes. Numbers are explained in the text.

Fetal alanine disposal rate and the fetal/maternal MPE ratio of the maternal tracer (^{m}MPE$_\alpha \div ^m$MPE$_A$) were used to calculate the direct flux of maternal alanine into the fetal systemic circulation ($R_{f,m}$):

$$R_{f,m} = DR_f (^m MPE_\alpha \div ^m MPE_A) \tag{3}$$

The flux of alanine to the fetus from the placenta ($R_{f,P}$) was calculated as the sum of $R_{P,f}$ and umbilical uptake:

$$R_{f,P} = R_{P,f} + \text{net umbilical alanine uptake} \tag{4}$$

The placental to fetal alanine flux consists of the direct alanine flux and the fetal alanine flux via the placental pool.

4.2.5 Statistical analysis

The data were analyzed using the SAS statistical program (Statistical Analyses System, SAS Institute, Inc., Cary, North Carolina). Each sheep provided two averaged data points, control and experimental, for each variable. Differences between study periods were tested using Student's t test for paired samples. Two-sided p values were considered significant at $p < 0.05$. Because of paired measurements for the same sheep, control and experimental, a general linear regression model could not be applied to the

variables of interest. Instead, the paired lines representing control and experimental data for each sheep were analyzed. Student's *t* test was applied to each slope of the paired lines to detect statistical significance between control and experimental periods. In addition, a general linear model program for repeated measures was applied to the data (102). This program gave the same average line as the line which was calculated by averaging each slope and intercept for all paired lines.

4.3 RESULTS

Mean gestational age was 131 ± 1 days, fetal and placental wet weights were 3064 ± 156 and 321 ± 19 grams, respectively. Table 4.1 presents blood flows, oxygen, glucose and lactate uptakes for the 10 sheep infused with alanine. In response to the alanine infusion, umbilical glucose uptake increased significantly and was associated with significant increases in maternal and fetal glucose concentrations.

4.3.1 Alanine concentrations and uptakes

The infusion of alanine into the maternal circulation elevated maternal plasma alanine concentration approximately fourfold and increased mean fetal plasma alanine concentration by 36% (Table 4.2). These concentration changes were associated with a mean sixfold increase in uterine alanine uptake and a twofold increase in umbilical alanine uptake. The analysis of individual changes in uterine and umbilical uptakes showed that the two changes were significantly correlated to the changes in maternal plasma concentration (Fig. 4.3). Uterine alanine uptake was similar to umbilical uptake in the control period (2.5 vs. 3.1 $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$) but became significantly greater than umbilical uptake during the alanine infusion (15.5 vs. 6.9 $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$ ($p < 0.01$). Therefore, net utilization of alanine by the uteroplacenta increased markedly in response to maternal alanine infusion, from -0.6 to 8.6 $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$ ($p < 0.01$).

Table 4.1 Oxygen data, blood flows and glucose and lactate concentrations and uptakes in 10 ewes before (control) and during (experimental) maternal alanine infusion. Values are means ± SE.

	Control	Experimental	P-value
Maternal hematocrit, %	32 ± 1	32 ± 1	NS
Fetal hematocrit, %	37 ± 1	36 ± 1	NS
Maternal hemoglobin, O_2 capacity, mM	6.1 ± 0.2	6.0 ± 0.1	NS
Fetal hemoglobin, O_2 capacity, mM	6.5 ± 0.2	6.4 ± 0.2	NS
Maternal arterial O_2 saturation, %	94.8 ± 0.6	94.5 ± 0.7	NS
Umbilical arterial O_2 saturation, %	54.3 ± 2.9	48.1 ± 3.4	<0.01
Maternal arterial plasma glucose, mM	3.71 ± 0.06	4.01 ± 0.07	0.001
Fetal arterial plasma glucose, mM	0.99 ± 0.07	1.06 ± 0.07	<0.01
Maternal arterial plasma lactate, mM	0.59 ± 0.06	0.63 ± 0.05	NS
Fetal arterial plasma lactate, mM	1.76 ± 0.06	1.80 ± 0.09	NS
Uterine blood flow, $ml \cdot min^{-1} \cdot Kg_{fetus}^{-1}$	595 ± 52	551 ± 46	NS
Umbilical blood flow, $ml \cdot min^{-1} Kg_{fetus}^{-1}$	212 ± 12	216 ± 16	NS
Uterine O_2 uptake, $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$	596 ± 28	540 ± 30	NS
Umbilical O_2 uptake, $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$	360 ± 11	375 ± 14	NS
Uterine glucose uptake, $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$	90 ± 8	82 ± 10	NS
Umbilical glucose uptake, $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$	40 ± 3	46 ± 3	<0.01
Uterine lactate uptake, $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$	-16 ± 3	-19 ± 2	NS
Umbilical lactate uptake, $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$	23 ± 2	25 ± 2	NS

4.3.2 Alanine disposal rates and placental fluxes

Maternal and fetal plasma alanine enrichments approximated steady state conditions in both the control and experimental periods for each alanine isoptomer (Fig. 4.4). Maternal alanine disposal rate increased significantly in response to the alanine infusion (Table 4.2). The increase in maternal disposal rate was approximately equal to the alanine infusion rate (9.9 ± 1.1 vs. 8.4 ± 1.0 $\mu mol \cdot min^{-1} \cdot Kg_{mother}^{-1}$, $p=0.325$). The enrichment of maternal tracer was significantly less in the uterine vein than in the maternal artery ($p < 0.001$) (Fig. 4.4). This observation, coupled with the uterine uptake data, demonstrated bidirectional alanine fluxes between the maternal circulation and the

Table 4.2 Alanine concentrations, uptakes and fluxes. Values are means ± SE.

	n	Control	Experimental	P-value
Maternal arterial plasma alanine conc., μM	10	155 ± 14	629 ± 78	< 0.001
Fetal arterial plasma alanine conc., μM	10	303 ± 17	413 ± 30	< 0.001
Uterine alanine uptake, μmol·min^{-1}·Kg$_{fetus}$$^{-1}$	10	2.5 ± 0.6	15.5 ± 3.1	< 0.001
Umbilical alanine uptake, μmol·min^{-1}·Kg$_{fetus}$$^{-1}$	10	3.1 ± 0.5	6.9 ± 0.8	< 0.001
Net uteroplacental alanine utilization, μmol·min^{-1}·Kg$_{fetus}$$^{-1}$	10	-0.6 ± 0.8	8.6 ± 2.7	< 0.001
Maternal plasma alanine disposal rate, μmol·min^{-1}·Kg$_{mother}$$^{-1}$	8	6.1 ± 0.9	16.0 ± 1.6	< 0.01
Fetal plasma alanine disposal rate, μmol·min^{-1}·Kg$_{fetus}$$^{-1}$	8	19.6 ± 1.3	22.0 ± 1.2	< 0.01
MPE ratios of maternal tracer times 100				
Fetal arterial/maternal arterial	8	1.0 ± 0.2	3.5 ± 0.8	0.01
Umbilical venous/maternal arterial	8	1.5 ± 0.1	4.4 ± 0.9	0.01
Alanine flux to uteroplacenta, from mother ($R_{P,m}$) μmol·min^{-1}·Kg$_{fetus}$$^{-1}$	8	8.2 ± 2.1	32.8 ± 7.1	< 0.01
Direct alanine flux to fetus, from mother ($R_{f,P}$) μmol·min^{-1}·Kg$_{fetus}$$^{-1}$	8	0.17 ± 0.04	0.71 ± 0.20	< 0.02
Alanine flux to fetus, from placenta ($R_{f,p}$) μmol·min^{-1}·Kg$_{fetus}$$^{-1}$	8	5.0 ± 0.6	7.3 ± 1.3	0.06
Alanine flux to placenta, from fetus ($R_{P,f}$) μmol·min^{-1}·Kg$_{fetus}$$^{-1}$	8	1.9 ± 0.6	0.3 ± 1.3	NS

pregnant uterus. Similarly, the fetal alanine tracer data demonstrated bidirectional alanine exchange between placenta and fetus (Table 4.2 and Figure 4.5). The flux of alanine to the fetus from the placenta ($R_{f,P}$) was 26% of fetal plasma alanine entry rate in the control period (i.e., 5.0 vs. 19.6) and 33% in the experimental period (i.e., 7.3 vs. 22.0). However, a very small portion of $R_{f,P}$ represented direct flux of maternal alanine into the fetus ($R_{f,m}$). In the control period, $R_{f,m}$ was 0.17 ± 0.04 μmol·min^{-1}·Kg$_{fetus}$$^{-1}$). This flux was approximately 3% of $R_{f,P}$ (0.17 vs. 5.0) and 0.9% of the fetal plasma alanine entry rate (0.17 vs. 19.6). In the experimental period, the direct flux of maternal alanine into the fetus increased significantly to 0.71 ± 0.2 μmol·min^{-1}·Kg$_{fetus}$$^{-1}$, but remained small in

Figure 4.4 Plasma enrichments for both L-[1-^{13}C] alanine (fetal tracer) and L-[3,3,3-D$_3$] alanine (maternal tracer) in control and experimental periods are molar percent enrichments (MPE, means ± SE) plotted against time. α = umbilical artery, γ = umbilical vein, A = maternal common iliac artery, V = uterine vein.

comparison to the other alanine fluxes, approximately 10% of $R_{f,P}$ (0.71 vs. 7.3) and 3% of the fetal plasma alanine entry rate (0.71 vs. 22.0). Fetal alanine DR increased significantly in the experimental period (Table 4.2) and by a value which was virtually equal to the increase in $R_{f,P}$ (2.4 vs. 2.3 ± μmol·min^{-1}·Kg$_{fetus}^{-1}$).

The increase in $R_{f,m}$ caused by maternal alanine infusion was significantly correlated to the increase in maternal alanine flux to the uteroplacenta ($R_{P,m}$) ($p < 0.01$). However, the slope of the regression line relating $R_{f,m}$ to $R_{P,m}$ was about one tenth of the slope relating umbilical and uterine uptakes (Fig. 4.5). The increase in umbilical uptake was approximately 29% of the increase in uterine uptake, whereas the increase in direct

alanine flux to the fetus from the mother was only 2.2% of the increase in alanine flux into the pregnant uterus.

Figure 4.3 Umbilical and uterine alanine uptakes (top and middle) and uteroplacental alanine utilization (bottom) versus maternal alanine concentration.

4.3.3 Dependence of results on the choice of tracers

Recycling of alanine through pyruvate and lactate pools makes the disposal rate of deuterium labeled alanine more rapid than the disposal rate of ^{13}C labeled alanine because the deuterium label is selectively lost in the recycling (99). This characteristic of the deuterium label requires validation of the assumption that in estimating $R_{f,m}$ we could use the fetal disposal rate of L-[1-^{13}C] alanine to calculate the fetal disposal rate of maternal L-[3,3,3-D_3] alanine. Two fetal sheep infused with L-[3,3,3-D_3] alanine yielded fetal plasma alanine disposal rates equal to 19.5 and 31.3 $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$,

Table 4.3 Summary of results in 2 sheep infused intravenously with L-[U-^{13}C] alanine in the mother and L-[1-^{13}C] alanine in the fetus.

	Sheep #1	Sheep #2
Fetal age, days	128	134
Fetal weight, grams	2897	3946
Placental weight, grams	393	434
Arterial plasma alanine concentration, mM		
Maternal	158	150
Fetal	270	295
Umbilical alanine uptake, $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$	2.9	3.7
Fetal plasma alanine disposal rate, $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$	19.4	21.0
MPE ratio, maternal tracer times 100		
Fetal arterial/maternal arterial	3.8	4.3
Umbilical venous/maternal arterial	5.9	5.5
Alanine flux to fetus, $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$		
Direct from mother ($R_{f,m}$)	0.7	0.9
From placenta ($R_{f,P}$)	8.1	6.1

respectively. Compared with the fetal disposal rates measured with L-[1-^{13}C] alanine (mean 19.6, range 14.6 to 23.6), these data indicate that the disposal rates of the two tracers are sufficiently similar for the purpose of estimating the contribution of $R_{f,m}$ to placental or fetal alanine fluxes.

A second issue related to the metabolism of alanine tracers is that rapid alanine recycling within the placenta would cause the transplacental flux of deuterium labeled maternal alanine to be less than the transplacental flux of ^{13}C-labeled maternal alanine. In other words, ^{13}C labeling of maternal alanine would include in the calculation of the $R_{f,m}$ flux maternal alanine molecules that had undergone deamination and reamination within the placenta. To explore the magnitude of this effect, we performed two studies using maternal infusion of L-[U-^{13}C] alanine. The relevant results are summarized in Table 4.3. In this table, the fetal/maternal MPE ratios of maternal tracer and the $R_{f,m}$ flux are all greater than the corresponding mean values in Table 4.2 by more than four standard deviations, thus indicating a greater transplacental flux of the ^{13}C labeled tracer. Even so, the labeling of maternal alanine with ^{13}C confirms that the direct flux of maternal alanine into the fetus is a small fraction of the alanine flux to the fetus from the

placenta. In the two animals of Table 4.3, the direct flux of maternal alanine across the placenta was only 9% and 15%, respectively, of the alanine flux from placenta to fetus.

Figure 4.5 Relation of umbilical (Umb) alanine uptake to uterine (Utn) alanine uptake and flux of maternal alanine into the fetus ($R_{f,m}$) to flux of maternal alanine into the uteroplacenta ($R_{up,m}$).

4.3.4 Effect of alanine infusion on other amino acids

In response to the alanine infusion, the increase in maternal plasma alanine concentration was accompanied by a significant increase in the maternal concentrations of glutamate and glutamine and a significant decrease in the concentrations of most other amino acids (Fig. 4.6). In fetal plasma, the increase in alanine concentration was accompanied by a significant increase in the concentrations of glutamine and serine, and a small decrease in the concentrations of several amino acids. This decrease was significant only for leucine, tyrosine and methionine. Concomitant with these changes in amino acid concentrations there were significant increases in maternal plasma insulin

(from 22.5 ± 3.9 to 28.2 ± 4.2 µU/ml, P< 0.001) and fetal plasma insulin (from 18.1 ± 2.3 to 21.1 ± 2.8 µU/ml, $p < 0.05$). The uterine and umbilical uptakes of amino acids other than alanine did not show any detectable change, with the exception of a significant decrease in the uterine uptake of leucine (from 5.7 ± 0.8 to 4.4 ± 0.7 µmol·min^{-1}·Kg$_{fetus}$$^{-1}$, $p < 0.05$).
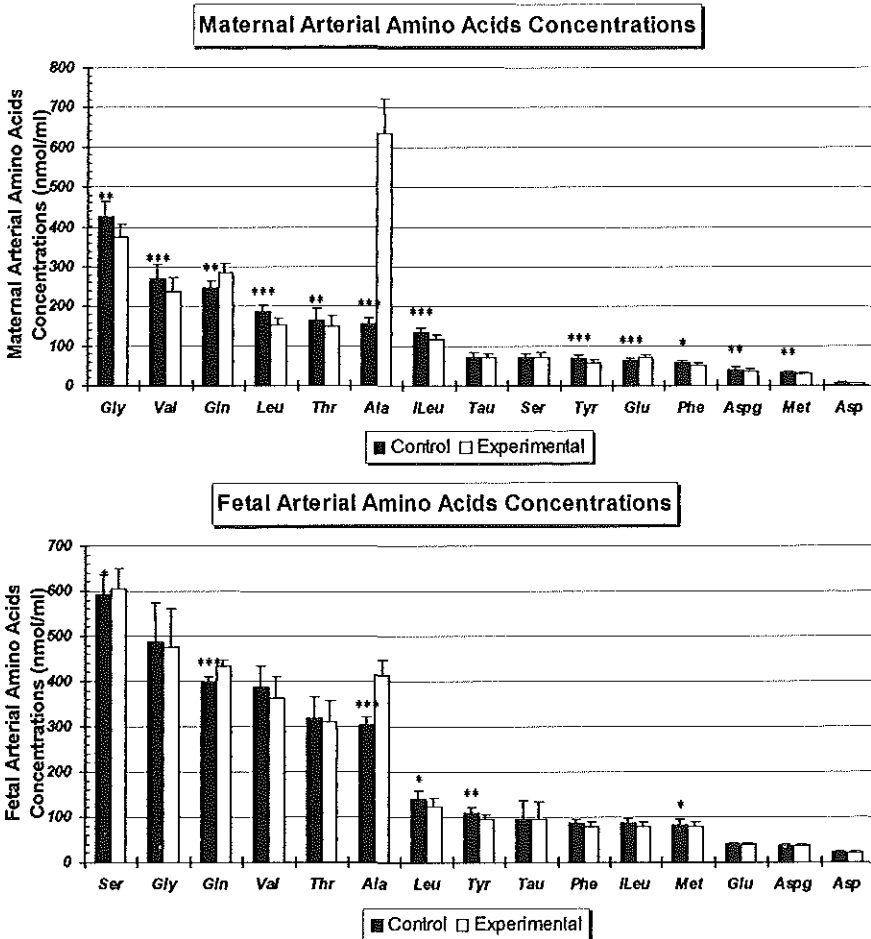
Figure 4.6 Comparison of maternal (top) and fetal (bottom) neutral and acidic amino acid concentrations between control (filled bars) and experimental (open bars) study periods. Values are means $\pm$ SE. Significant changes between 2 periods: * P < 0.05, ** P < 0.01, *** P < 0.001.

4.4 DISCUSSION

The present study is relevant to the question as to whether it is possible to increase fetal amino acid uptake by increasing the concentration of amino acids in maternal plasma. This question has both practical and theoretical interest. From the practical point of view, it is important to know whether the nutrition of a growth-restricted fetus could be improved by increasing the concentration of nutrients in the maternal circulation. Basic understanding of placental amino acid transport and metabolism requires experimental data about placental and fetal amino acid uptakes as a function of maternal amino acid concentration. Tracing the flux of maternal alanine into the fetal circulation yields a range of flux values, depending upon the type of tracers that are used. The infusion of deuterium labeled alanine into the maternal circulation results in an umbilical arterial enrichment that is about 1% of the maternal arterial enrichment. The normal transplacental maternal alanine flux estimated on the basis of this extremely low fetal enrichment is approximately 0.2 $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$ (Table 4.2, control data). The infusion of ^{13}C labeled alanine into the maternal circulation results in an umbilical arterial enrichment that is approximately 4% of the maternal arterial enrichment and an estimated normal maternal alanine flux into the fetal circulation of approximately 0.8 $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$ (Table 4.3). Before the methodological issue which is raised by this observation is addressed, it is important to note that the experiments with either tracer agree in showing that under normal physiological conditions the flux of maternal alanine into the fetal circulation ($R_{f,m}$) is a relatively small fraction of the alanine flux to the fetus from the placenta ($R_{f,P}$). The estimated value of the ($R_{f,m}$)/($R_{f,P}$) fraction ranged between 0.034 (Table 4.2, control data) and 0.15 (Table 4.3). This finding implies that most of the alanine flux from placenta to fetus represents alanine produced within the placenta.

If no other information were available, one might infer that there is virtually no alanine flux from the maternal plasma into the placental alanine pool supplying alanine to the fetus. However, during maternal alanine infusion, the increase in maternal plasma alanine concentration had the effect of doubling the fetal uptake of placental alanine. This observation shows that the placental alanine pool delivering alanine to the fetus has two inputs, i.e., alanine produced within the placenta and alanine entering the placenta

from the maternal circulation. Because the amount of maternal alanine escaping into the fetus is relatively small, the placental alanine pool must have a high turnover rate in comparison to the influx of maternal alanine. Maternal alanine transported from the maternal to the fetal surface of the placenta is diluted by mixing with a large flux of unlabeled alanine produced within the placenta.

The rate of placental alanine turnover is a function of placental protein turnover and transamination reactions. The turnover of placental proteins utilizes and releases amino acids at a rapid rate. This is demonstrated by the evidence that approximately one-half of the leucine flux to the fetus from the placenta represents leucine produced within the placenta (76). Because leucine is an essential amino acid, protein turnover is virtually the only source of placental leucine production. In addition to protein turnover, interconversion of alanine and pyruvate via transamination is likely to be the second most important mechanism for placental alanine turnover. Rapid alanine transamination within the placenta may explain why the estimate of maternal alanine flux into the fetus depends in part on the choice of the tracer used in the labeling of maternal alanine. The interconversion of alanine and pyruvate would cause the placental disposal rate of deuterium labeled alanine to be more rapid than the disposal rate of ^{13}C labeled alanine because the deuterium label is selectively lost in this process. Therefore, the labeling of maternal alanine with ^{13}C would yield a greater flux of maternal alanine into the fetus than the deuterium labeling, because the flux traced by ^{13}C labeled alanine includes maternal alanine molecules that underwent reversible transamination within the placenta. On the other hand, we cannot exclude that other mechanisms contributed to the discrepancy in the transplacental flux of the two tracers. For example, some of the deuterium may have been removed within the placenta via an exchange with water that did not involve enzymatic reactions.

The evidence presented in this study points to the conclusion that alanine transport across the placenta depends on the interaction between placental alanine metabolism and the activity of placental amino acid transporters. The flux of amino acids into the placenta from the maternal circulation is controlled by amino acid transporters located on the maternal surface of the organ (84). The relatively large increase in uterine uptake in response to an increase in maternal alanine into the placenta was not an

important factor in limiting the increase in fetal alanine uptake. Because maternal alanine infusion decreased the concentration of several neutral amino acids in maternal plasma, the increased uterine alanine uptake may have been the result, at least in part, of reduced competition in the sharing of transporters. Therefore, the observed increase in uterine uptake induced by maternal alanine infusion may not be predictive of uptake when alanine is infused with other amino acids. The flux of amino acids from placenta to fetus is controlled by exchange transporters located on the fetal surface of the placenta (39, 84). Placental sodium-independent (84) and sodium-dependent (41) exchange transporters have been described. An important role for these transporters in limiting fetal alanine uptake is suggested by the observation that, in response to the increase in maternal alanine concentration, the increase in umbilical alanine was approximately one-third of the increase in uterine uptake. The partitioning of alanine between placental utilization and transport to the fetus depends on the availability of both pathways for placental alanine metabolism and transport pathways that allow escape of alanine into the umbilical circulation.

In agreement with previous studies (64) there was a significant lactate output by the uteroplacental tissues in the control period. This output did not increase significantly in the experimental period, despite the increased uteroplacental alanine utilization. However, there was an increase in umbilical glucose uptake, which suggests that glucose was diverted from placental glucose utilization to fetal glucose uptake. The dephosphorylation of phosphoenolpyruvate to pyruvate is inhibited by high alanine concentrations (89). Thus, an increase of alanine concentration within the placenta during the experimental period may have decreased the entry rate of glucose into the glycolytic pathway and prevented a large increase in pyruvate and lactate concentrations via its inhibitory effect on pyruvate kinase. Figure 4.7 summarizes the changes in metabolic substrate fluxes associated with the maternal alanine infusion.

The increased placental utilization of alanine during the experimental period presents a problem of nitrogen excretion for the placenta. This problem could be solved in a variety of ways, e.g., by 1) increasing ammonia excretion, 2) increasing amidation of glutamate to glutamine and 3) decreasing deamination of branched chain amino acids. In the present study, there was a trend towards increased glutamine umbilical uptake and
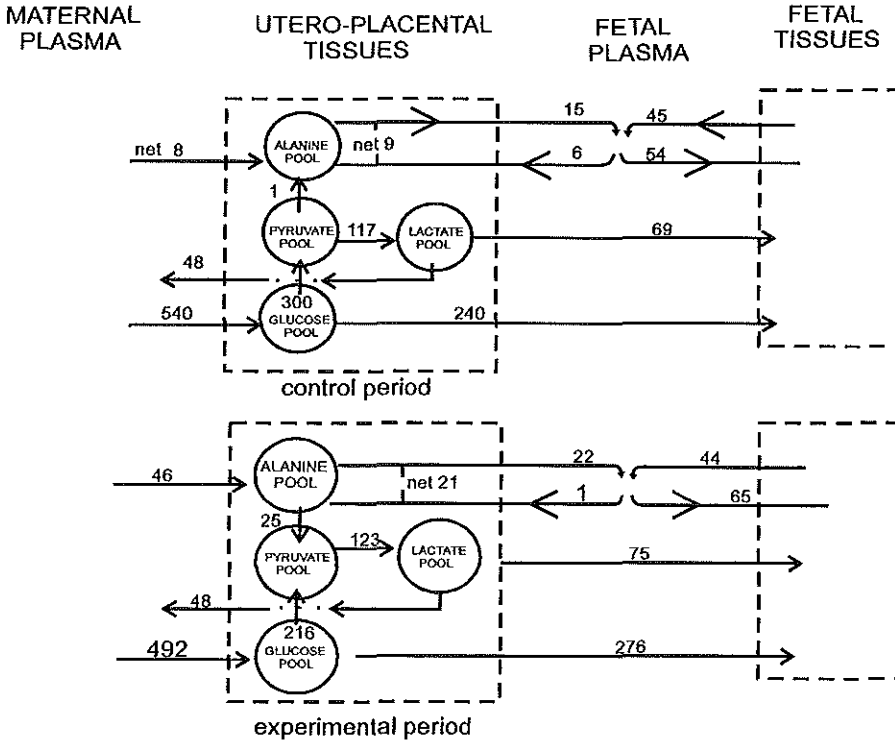
Figure 4.7 Summary balance of measured substrate fluxes between fetal, placental and maternal compartments during control and experimental periods. Mean fluxes are numbers representing μmol of carbon · min^{-1}·Kg$_{fetus}$$^{-1}$.

decreased placental utilization of branched chain amino acids. However, the only trend that attained statistical significance was a fall in placental leucine utilization ($p < 0.05$). Both maternal and fetal glutamine concentrations increased in the experimental period ($p =0.002$), suggesting raised maternal and fetal glutamine production. Ruderman et al. have shown that in the rat hind limb the rate of glutamine release can be stimulated by increasing the supply of alanine via the perfusate (77, 78).

In the control period, fetal plasma alanine disposal rate was much greater than the flux of alanine into the fetus from the placenta (19.6 vs. 5.0 μmol·min^{-1}·Kg$_{fetus}$$^{-1}$). In the experimental period, the alanine disposal rate increased by about 12% and by a value that

was virtually equal to the increased flux from the placenta. These observations add to previous evidence (30) showing that the ovine fetus has a high rate of alanine production in comparison to umbilical alanine uptake. Thus, a doubling of the umbilical uptake has a relatively small effect on fetal plasma alanine turnover.

It is likely that in all mammals placental alanine metabolism is one of the factors that limit the direct flux of alanine from mother to fetus. There may be, however, quantitative differences in the degree of limitation. Gilfillan et al. (26) have reported that the umbilical venous and arterial plasma enrichments of ^{13}C labeled tracer alanine infused at a constant rate into the maternal circulation were approximately 44 and 25%, respectively, of maternal peripheral venous enrichment in term human pregnancies studied at the time of cesarean section. These ratios are greater than the ones observed in the present study for the maternally infused ^{13}C alanine approximately 6 and 4%, respectively, and suggest a larger contribution of the maternal alanine flux across the human placenta to fetal alanine turnover. The larger contribution may be the expression of a difference in the rate of placental alanine metabolism as well as of differences in placental alanine transport and fetal alanine production rates.

Chapter 5

EFFECT OF DEXAMETHASONE ON FETAL HEPATIC GLUTAMINE-GLUTAMATE EXCHANGE

5.1 INTRODUCTION

Previous experiments have demonstrated that, under normal physiological conditions, the fetal liver takes up glutamine from the fetal circulation (59, 93) and that a fraction of the glutamine taken up is converted to glutamate (93). Furthermore, it has been shown that fetal dexamethasone infusion sufficient to induce parturition causes a decrease in net fetal hepatic glutamate output (4, 51). This fall in hepatic glutamate output under the influence of dexamethasone suggests a redirection of hepatic glutamine away from glutamate release towards entry of the glutamine carbon into the citric acid cycle and subsequent gluconeogenesis and glycogenesis. The present study was designed to measure the effect of dexamethasone on fetal hepatic glutamine uptake and its conversion to glutamate and CO_2 (Fig. 5.1).

5.2 MATERIALS AND METHODS

5.2.1 Surgery and animal care

Six late gestation Columbia-Rambouillet ewes, each pregnant with a single fetus were studied. Surgery was performed at 125-134 days of gestation. Preoperative preparation, surgery and animal care were performed as described in chapter 2.

5.2.2 Study design

On the day of study, 5 to 7 days after surgery, an infusate solution was prepared containing 100 mg 99% enriched L-[^{3}H$_5$+^{3}H$_4$] glutamate, 400 mg 99% enriched L-[1-^{13}C]
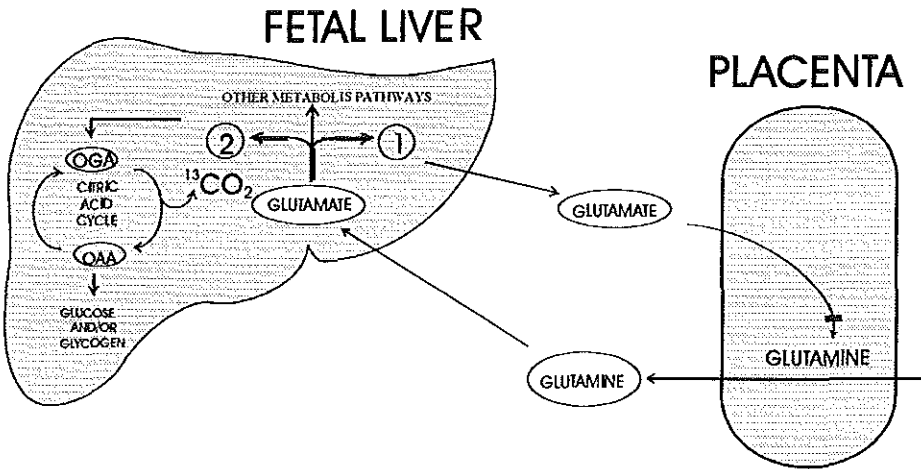
Figure 5.1. Possible metabolic pathways for glutamine carbon skeleton when converted to glutamate.

glutamine (CIL, Andover,MA) and 400 μCi tritiated water. The L-[1-^{13}C] glutamine was purified prior to the study to remove any L-[1-^{13}C] glutamate that might have been formed during storage. Two Biorad Econo-pac columns (1.5 by 14 cm) were packed to 10 cm height with 17 ml of Dowex 1.8 resin (CL-form, 200-400 mesh). The columns were washed with 20 ml of 10 mM imidazol buffer, pH 7.6, kept on ice. L-[1^{-13}C] glutamine, 400 mg, was dissolved in 10 ml water and the pH was adjusted to 7.6 with NaOH. Of the glutamine mixture 5 ml was loaded each column, the sample front was discarded. The glutamine was eluted three times with 5 ml imidazol buffer, pH 7.6 and the total of 15 ml of buffer fractions were collected on ice and filtered through a 0.2 μ filter.

The following experimental protocol was used to study six pregnant ewes (Fig. 5.2). The infusate was administered via the fetal venous infusion catheter. The first 4 ml were given as a bolus, the remaining infusate was administered at a rate of 0.085 ml/min. Beginning at 120 min and every 25 min thereafter, four sets of samples (S1-S4) were drawn simultaneously from the maternal arterial catheter, the uterine vein, the fetal aorta, the umbilical vein, and the left hepatic vein. The fetus was transfused with an equal volume of donor blood after each sampling. After the last sampling, the infusate was stopped and 500 mg ampicillin was administered through the amniotic catheter. The next

day a bolus of 0.2 mg dexamethasone was given, followed by a continuous fetal infusion of dexamethasone at a rate of 0.07 mg·hr^{-1}, a rate sufficient to induce labor (51). After approximately 26 hours of infusion the same study protocol was repeated. After completion of this second study period the ewe was killed. Fetal, placental, uterine and fetal liver weights were obtained and catheter positions were verified.
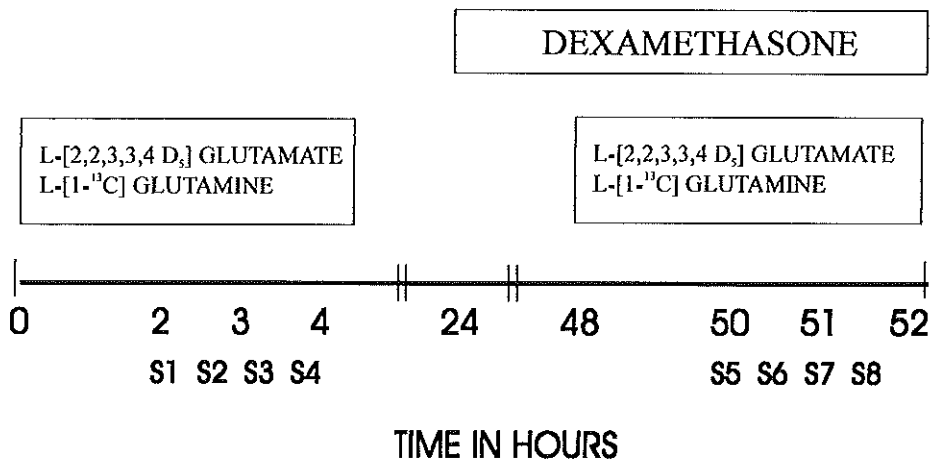
Fig. 5.1 Flow chart of study design

5.2.3 Analytical methods

Maternal and fetal samples were analyzed for hemoglobin, hematocrit, oxygen saturation, blood gases, tritiated water, glucose, lactate and amino acid concentrations. In addition, the maternal samples were analyzed for progesterone and the fetal samples were analyzed for $^{13}CO_2$, glutamine and glutamate enrichments. Except for glutamine and glutamate enrichment determinations the analytical methods were described in chapter 2.

For glutamine and glutamate determinations plasma (0.4 ml fetal arterial and umbilical venous; 0.25 ml hepatic venous) was mixed with 50% acetic acid and applied to the column. After washing the resin with destilled water, the amino acids were eluted twice with 500 μL NH$_4$OH and lyophilized. Tri-t-butyldimethylsilylether derivatives were formed as described above. Tandem GCMS was performed on a HP-5790 gas chromatograph mass spectrometer with a 30 m DB 1, 0.025 mm ID 0.25 μm film

thickness fused silica capillary column with helium as the carrier gas. The selected conditions for glutamate and glutamine were an injection port and T-line temperature of 280 °C, with an initial column temperature of 100 °C with a 10 °C/min ramp to 320 °C at a rate of 10 °C/min. This resulted in a glutamate M-57 peak at approximately 13 min and an M-57 peak for glutamine at approximately 14 min. The ion clusters monitored were M/Z 432,433, 434, 435, 436 and 437 for glutamate and 431,432,433, 434, 435 and 436 for glutamine. The enrichments were corrected for natural abundance as described in chapter 4.

5.2.4. Calculations

Blood flows and uptakes

Calculations of blood flows and net uterine and fetal uptakes of oxygen, glucose, lactate and amino acids were performed as described in chapters 3 and 4.

Hepatic uptakes

Hepatic uptakes of glucose, lactate and amino acids were normalized for hepatic O_2 uptake by calculating the substrate to O_2 uptake molar ratio. This required calculation of the ratio:

$$(h_i - h) \div (h_i - h)_{O2}$$

where h_i represents the concentration of substrate at the hepatic input and h represents the directly measured substrate concentration in the left hepatic vein.

The fetal left hepatic lobe is perfused by fetal arterial and umbilical venous blood, with the umbilical venous blood making a greater than 80% contribution (21). In each fetus, the fractional contribution of umbilical venous blood (F_γ) to total left hepatic blood flow was calculated as follows:

$$F_\gamma = (C_{T,h} - C_{T,\alpha}) \div (C_{T,\gamma} - C_{T,\alpha})$$

where $C_{T,h}$, $C_{T,\alpha}$ and $C_{T,\gamma}$ represent the tritiated water concentration (dpm/ml) in the fetal hepatic venous, fetal arterial and umbilical venous blood, respectively.

Hepatic input concentrations of metabolic substrates (C_{hi}) were then calculated using the equation:

$$C_{hi} = (F_\gamma \cdot C_\gamma) + (F_\alpha \cdot C_\alpha)$$

where F_α is the fractional arterial contribution to left hepatic flow ($F_\alpha = 1-F_\gamma$) and C_α and C_γ are the substrate concentrations in the umbilical arterial and venous blood.

The hepatic amino acid (AA) to oxygen uptake molar ratio was calculated as:

$$\text{AA uptake} \div O_2 \text{ uptake} = ((C_{hi} - C_h)_{AA} \cdot (1 - Ht)) \div (C_{hi} - C_h)_{O2}$$

Disposal rates

The fetal plasma glutamine and glutamate disposal rates (DR) were calculated as:

$$DR = [(100 \div MPE_\alpha)-1] \cdot C \cdot I$$

where MPE_α is the fetal arterial plasma enrichment of tracer at steady state, C is the concentration of the tracer in the infusate (μmol/ml) and I is the infusion rate (ml/min). For the deuterium-labeled glutamate, I represents the $^2H_4 + {}^2H_5$ infusion rate and MPE_α represents $^2H_4 + {}^2H_5$ MPE. This equation does not correct for any unlabeled glutamine or glutamate present in the infusate. However, both 1-^{13}C glutamine and $^2H_4 + {}^2H_5$ glutamate accounted for > 95% of the total glutamine and glutamate infused.

Tracer glutamine and glutamate concentrations

Plasma tracer glutamine and glutamate were calculated as total plasma concentrations times their respective MPE's divided by 100.

Hepatic conversion of plasma glutamine to hepatic glutamate and CO₂ output

The hepatic output of glutamate formed from glutamine ($R_{GLU,GLN}$), expressed as substrate output to O_2 uptake ratio, was calculated using the plasma concentration difference of L-[1^{13}C] glutamate across the hepatic circulation, $(C_h-C_{hi})_{13\text{-}GLU}$, and the percent enrichment of L-[1^{13}C] glutamine at the hepatic input, $(MPE_{hi})_{13\text{-}GLN}$, according to the equation:

$$R_{GLU,GLN} = 100 \cdot (C_h - C_{hi})_{13\text{-}GLU} \cdot (1 - Ht) \div (C_{hi} - C_h)_{O2} \div (MPE_{hi})_{13\text{-}GLN}$$

The output of CO_2 formed from C-1 of plasma glutamine, $(R_{CO2,GLN})$, was similarly calculated using the whole blood concentration difference of $^{13}CO_2$, $(C_h - C_{hi})_{13CO2}$:

$$R_{CO2,GLN} = 100 \cdot (C_h - C_{hi})_{13\text{-}CO2} \div (C_{hi} - C_h)_{O2} \div (MPE_{hi})_{13\text{-}GLN}$$

5.2.5 Statistical analysis

Statistical analysis was performed as described in chapter 2.

5.3 RESULTS

Gestational age was 127 ± 1 days. Fetal body, fetal liver and placental wet weights were 2851 ± 81, 102 ± 10 and 333 ± 1, respectively. Table 5.1 presents uterine and umbilical blood flows and oxygen uptakes for the six sheep. Fetal hematocrit and hemoglobin, and the blood oxygen content differences across the umbilical and left hepatic circulations increased after dexamethasone infusion. In addition, there was an increase in uterine blood flow accompanied by an increase in umbilical venous oxygen saturation. The fractional contribution of umbilical venous blood to left hepatic blood flow (F_γ) was 0.91 and 0.97, respectively, in two of the six fetuses and not significantly different from 1.0 in the other four.

Glucose and lactate concentrations and uptakes

During fetal dexamethasone infusion significant increases occurred in the concentrations of maternal plasma glucose, fetal plasma glucose and lactate. Concomitant with these changes, there was a significant rise in umbilical lactate uptake. Hepatic glucose uptake was virtually zero in both the control and experimental period, and hepatic lactate uptake did not increase significantly (Table 5.2).

TABLE 5.1 Blood flows and oxygen data in six sheep before (control) and during (experimental) fetal dexamethasone infusion. Values are means ± SE.

	Control	*Experimental*	*P-value*
Fetal hematocrit, %	32 ± 2	35 ± 2	0.02
Fetal hemoglobin, O_2 capacity, mM	5.5 ± 0.3	6.0 ± 0.3	< 0.001
Blood flows, ml·min^{-1}·Kg$_{fetus}^{-1}$			
Umbilical	250 ± 20	221 ± 25	NS
Uterine	479 ± 53	573 ± 48	0.03
Fetal blood O_2 saturations, %			
Umbilical artery	50.1 ± 3.0	54.4 ± 2.4	NS
Umbilical vein	78.2 ± 2.4	86.5 ± 1.5	0.01
Left hepatic vein	68.9 ± 2.5	73.0 ± 1.7	NS
Blood O_2 content differences, mM			
Across umbilical circulation	1.45 ± .09	1.75 ± 0.13	0.014
Across left hepatic lobe circulation	0.44 ± 0.05	0.60 ± 0.04	0.002
O_2 uptakes, µmol·min^{-1}·Kg$_{fetus}^{-1}$			
Umbilical	353 ± 13	373 ± 25	NS
Uterine	668 ± 47	613 ± 44	NS

Amino acid concentration, uptakes and fluxes

Dexamethasone infusion caused a large increase in fetal plasma glutamine concentrations (Table 5.3). In two of the six fetuses, the increase in glutamine concentration was so large that it could not be measured under the conditions that allowed chromatographic analysis of all the other amino acids. Concomitant with the rise in plasma glutamine concentration, there was a significant increase in the disposal rate of plasma glutamine, and a fall in hepatic glutamine uptake without significant change in umbilical glutamine uptake (Table 5.3).

In contrast to the rise in the fetal plasma concentration of glutamine, the fetal plasma concentration of glutamate decreased significantly. The fall was accompanied by a reduction in the plasma glutamate disposal rate and a decrease in both hepatic glutamate output and placental uptake of fetal glutamate (Table 5.3). The hepatic output and the placental uptake of glutamate were significantly correlated ($p < 0.01$).

TABLE 5.2 Glucose and lactate concentrations and uptakes in six sheep before (control) and during (experimental) fetal dexamethasone infusion. Values are means ± SE.

	Control	Experimental	P-value
Plasma glucose concentrations, mM			
Umbilical artery	1.25 ± 0.08	2.27 ± 0.19	< 0.001
Umbilical vein	1.43 ± 0.08	2.45 ± 0.17	< 0.001
Fetal left hepatic vein	1.42 ± 0.07	2.45 ± 0.18	< 0.001
Plasma lactate concentrations, mM			
Umbilical artery	2.18 ± 0.29	4.50 ± 0.34	< 0.005
Umbilical vein	2.31 ± 0.31	4.71 ± 0.34	< 0.005
Fetal left hepatic vein	2.15 ± 0.29	4.37 ± 0.32	< 0.005
Umbilical uptakes, $\mu mol \cdot min^{-1} \cdot Kg\, fetus^{-1}$			
Glucose	38.2 ± 3.0	31.4 ± 3.2	NS
Lactate	29.1 ± 2.3	44.3 ± 4.8	0.05
Hepatic uptake to O_2 uptake molar ratios			
Glucose	-0.01 ± 0.03	-0.01 ± 0.03	NS
Lactate	0.36 ± 0.07	0.47 ± 0.04	NS

The constant infusion of glutamine and glutamate tracers into the fetal circulation resulted in steady state plasma enrichments of the infused tracers and their metabolic products (Fig. 5.3). The infusion of L-[1-^{13}C] glutamine produced a relatively large L-[1-^{13}C] glutamate enrichment in the fetal circulation. The arterial glutamate/glutamine enrichment ratios were 0.30 ± 0.02 and 0.35 ± 0.05 in the control and the experimental period, respectively, and were not significantly different. The infusion of D$_5$ glutamate produced a small deuterium enrichment of plasma glutamine. The arterial glutamine/glutamate enrichment ratio was 0.02 ± 0.007 in the control period and decreased significantly to 0.007 ± 0.002 during dexamethasone infusion.

There was no detectable difference in plasma glutamine enrichment between left hepatic input and hepatic vein, both during the control and experimental period, thus indicating that at steady state the glutamine flux into the liver was virtually equal to hepatic glutamine uptake. The hepatic glutamine uptake was accompanied by hepatic output of glutamate formed from glutamine ($R_{GLU,GLN}$) and by hepatic output of CO_2

TABLE 5.3 Fetal glutamine and glutamate concentrations, uptakes and fluxes, before (control) and during (experimental) fetal dexamethasone infusion. Values are means ± SE.

	Control	Experimental	P-value
GLUTAMINE			
Plasma concentrations, μM			
Umbilical artery (α)	431 ± 22	*1151 ± 129	0.011
Umbilical vein (γ)	477 ± 25	*1203 ± 124	0.011
Left hepatic input (h_i)	476 ± 25	*1203 ± 124	0.011
Left hepatic vein (h)	381 ± 32	*1152 ± 132	0.010
Umbilical uptake, $\mu mol \cdot min^{-1} \cdot Kg\ fetus^{-1}$	7.4 ± 1.0	*7.7 ± 1.4	NS
Disposal rate, $\mu mol \cdot min^{-1} \cdot Kg\ fetus^{-1}$	21.4 ± 1.5	32.7 ± 1.8	< 0.001
Hepatic uptake to O_2 uptake ratio, $\mu mol/mmol$	150 ± 15	*53 ± 6	0.01
GLUTAMATE			
Plasma concentrations, μM			
Umbilical artery (α)	47.2 ± 8.2	17.0 ± 5.1	0.001
Umbilical vein (γ)	9.4 ± 1.9	1.3 ± 0.4	0.008
Left hepatic input (h_i)	9.9 ± 1.8	1.4 ± 0.4	0.008
Left hepatic vein (h)	108.8 ± 17.9	58.3 ± 17.4	0.008
Umbilical uptake, $\mu mol \cdot min^{-1} \cdot Kg\ fetus^{-1}$	-6.1 ± 0.6	-2.1 ± 0.7	< 0.001
Disposal rate, $\mu mol \cdot min^{-1} \cdot Kg\ fetus^{-1}$	9.6 ± 0.8	5.6 ± 0.5	< 0.001
Hepatic fluxes to O_2 uptake ratios, $\mu mol/mmol$			
Uptake	-150 ± 19	-58 ± 17	< 0.001
Output of glutamate formed from plasma glutamine ($R_{GLU,GLN}$)	53 ± 7	11 ± 2	< 0.001
Output of CO_2 formed from C_1 of plasma glutamine ($R_{CO2,GLN}$)	95 ± 14	50 ± 4	0.009
$R_{GLU,GLN} \div (R_{CO2,GLN} + R_{GLU,GLN})$	0.36 ± 0.02	0.18 ± 0.04	.001

. $n = 4$, $R_{GLU,GLN} + R_{CO2,GLN}$
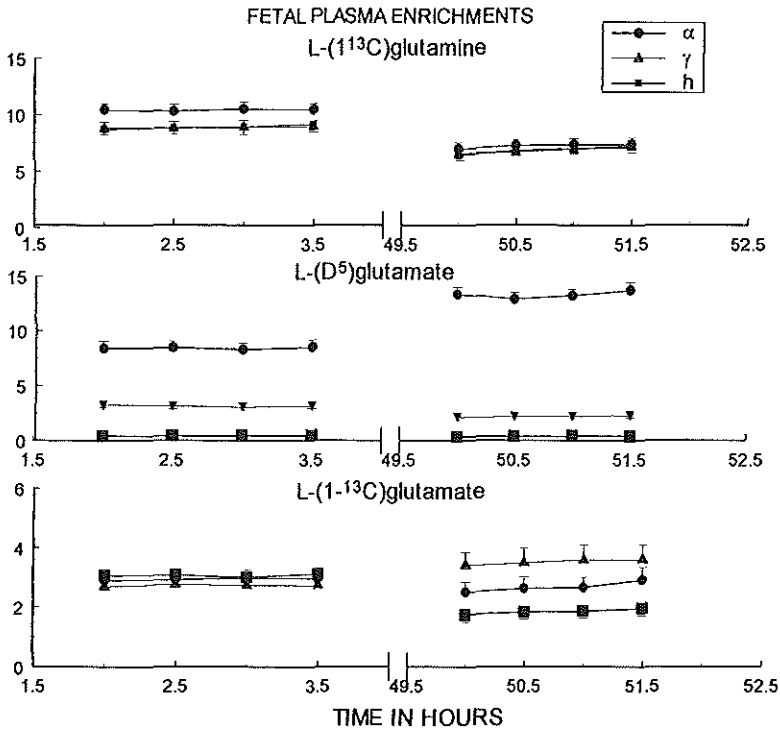
Fig. 5.3 Fetal plasma enrichments of $1\text{-}^{13}C$ glutamine, $1\text{-}^{13}C$ glutamate and $[^3H_4 + ^3H_5]$ glutamate during both study days are molar percent enrichments (MPE, means $\pm$ SE) plotted against time. α: fetal aorta, γ: umbilical vein, h: left hepatic vein.

derived from the C-1 carbon of glutamine ($R_{CO2,GLN}$). These two outputs accounted for virtually all of the glutamine uptake, i.e., the ($R_{GLU,GLN} + R_{CO2, GLN}$) sum was not significantly different from hepatic glutamine uptake. Both $R_{GLU,GLN}$ and $R_{CO2,GLN}$ decreased significantly in response to dexamethasone (Table 5.3). However, the fall in glutamate output was relatively greater than the decrease in CO_2 output. The contribution of $R_{GLU,GLN}$ to the combined ($R_{GLU,GLN} + R_{CO2,GLN}$) output was $36 \pm 2\%$ in the control period and decreased significantly to $18 \pm 4\%$ in the experimental period, ($p < 0.001$) (Table 5.3).

Several neutral amino acids shared the response of glutamine to dexamethasone infusion. Fetal plasma concentrations of eight amino acids (threonine, asparagine,

proline, glycine, alanine, isoleucine, tyrosine and phenylalanine) showed a significant
increase in plasma concentration and three of these (threonine, glycine and alanine) also
demonstrated a significant decrease in hepatic uptake (Table 5.4). By contrast, serine, the
amino acid which, in addition to glutamate, is released by the fetal liver into the fetal
circulation, showed no significant changes with regard to concentration and hepatic
output.

**TABLE 5.4 Fetal arterial concentrations, umbilical and hepatic uptakes of amino acids in the control
period (C) and during fetal dexamethasone infusion (E). Values are means ± SE.**

	Plasma concentration, mM		Umbilical uptake, μmol·min^{-1}·kg$_{fetus}$$^{-1}$		Hepatic uptake, μmol/mmol O_2	
	C	E	C	E	C	E
Asp	14 ± 4	9 ± 3	-0.2 ± 0.1	0.0 ± 0.1	-1 ± 1	-0.7 ± 0.8
Thr	284 ± 35	536 ± 51**	3.7 ± 0.3	2.4 ± 0.6	42 ± 2	26 ± 4**
Ser	641 ± 44	569 ± 45	-1.2 ± 0.6	-1.5 ±1.1	-47 ± 8	-51± 11
Asn	46 ± 9	108 ± 7***	1.6 ± 0.3	0.7 ± 0.5	19 ± 3	6 ± 5*
Pro	135 ± 18	346 ± 38***	2.1 ± 0.8	0.7 ± 0.8	11 ± 7	0 ± 11**
Gly	404 ± 26	757 ± 63**	4.1 ± 0.5	3.1 ± 0.8	48 ± 7	27 ± 6
Ala	280 ± 21	837 ± 75***	3.2 ± 0.3	1.3 ± 0.8*	72 ± 10	21 ± 6
Val	531 ± 59	587 ± 66	5.7 ± 0.4	5.9 ± 1.3	18 ± 4	14 ± 7
Met	70 ± 12	89 ± 12	1.0 ± 0.5	0.8 ± 0.2	13 ± 5	10 ± 3
Ile	122 ± 15	161 ± 17*	2.8 ± 0.3	3.6 ± 0.4	8 ± 1	8 ± 1
Leu	179 ± 22	219 ± 40	4.6 ± 0.4	4.7 ± 0.8	17 ± 1	17 ± 5
Tyr	107 ± 11	163 ± 14**	1.8 ± 0.3	1.7 ± 0.2	26 ± 3	30 ± 5
Phe	88 ± 6	183 ± 18**	1.7 ± 0.1	1.6 ± 0.2	22 ± 1	24 ± 4

*$P< 0.05$, **$P< 0.01$, ***$P< 0.001$

Progesterone output by the pregnant uterus

The output of progesterone into the uterine circulation, calculated as the uterine
plasma flow times plasma progesterone venous-arterial concentration difference across

the uterus, decreased significantly ($p < 0.01$) with dexamethasone infusion from 21 ± 3 to 3 ± 2 nmol·min^{-1+}·Kg$_{fetus}$$^{-1}$.

5.4 DISCUSSION

The present study provides the first demonstration that the flux of L-[1-^{13}C] glutamine into the fetal liver is virtually equal to the sum of L-[1-^{13}C] glutamate and ^{13}CO$_2$ hepatic output (Table 5.3). This finding implies that most of the plasma glutamine taken up by the fetal liver is converted to glutamate, which is then utilized in two different ways: part of it is excreted into the fetal circulation and part of it is routed into the citric acid cycle via deamination to α-ketoglutarate and subsequent oxidative decarboxylation to form succinyl CO-A. It also implies that the direct flux of plasma glutamine into hepatic protein synthesis is relatively small in comparison to the other routes of hepatic disposal.

Dexamethasone infusion was shown to cause a decrease in the fetal hepatic glutamate output/oxygen uptake ratio to 35% of control values (Table 5.3). Although some of this change could be due to an increase in hepatic oxygen uptake, the decrease in the ratio was accompanied by a proportionally comparable fall in fetal arterial glutamate concentration and in the placental uptake of fetal glutamate, as well as by a significant decrease in fetal plasma glutamate disposal rate from 9.6 ± 0.8 to 5.6 ± 0.5 μmol·min^{-1}·Kg$_{fetus}$$^{-1}$, (Table 5.3). Therefore, we may conclude that the fall in hepatic glutamate output/oxygen uptake ratio primarily represents a decrease in hepatic glutamate output.

Because it has been previously shown that glucocorticoids increase the activity of hepatic glucogenic enzymes in the fetal lamb (5), dexamethasone may decrease fetal hepatic glutamate output because the glutamate produced by the liver is diverted into the glucogenic pathway. This leads to the hypothesis that a larger fraction of the glutamate formed from the hepatic glutamine uptake enters the citric acid cycle under the influence of dexamethasone. The results of the present study support this hypothesis, because dexamethasone significantly reduced the $R_{GLU,GLN}/(R_{GLU,GLN}+R_{CO2,GLN})$ ratio from 0.36 ± 0.02 to 0.18 ± 0.04 (Table 5.3 and Fig 5.4).
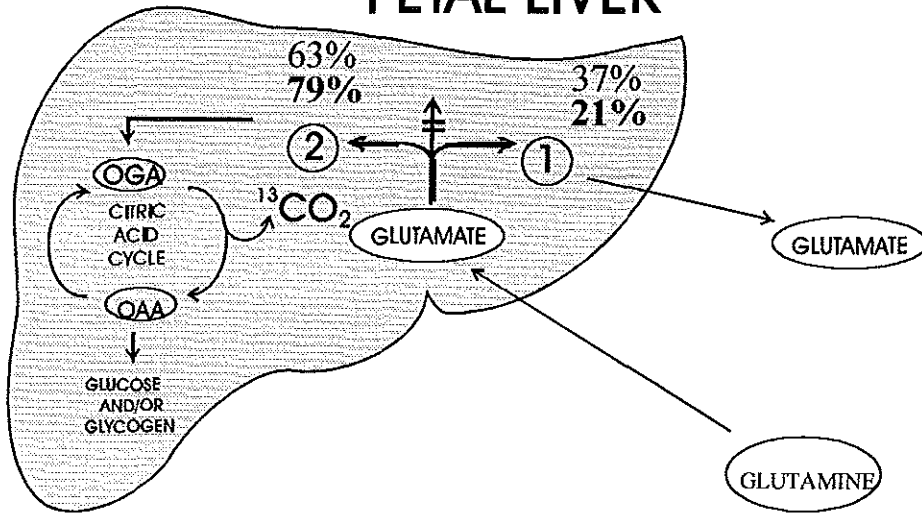
Fig. 5.4 Partitioning of glutamate within the fetal liver before (normal) and after (bold) dexamethasone administration.

The study also shows that a reduction in the hepatic uptakes of glutamine and alanine is an additional mechanism of the reduced hepatic glutamate output (Tables 5.3 and 5.4). Given the large increase in plasma glutamine and alanine concentrations during dexamethasone infusion, direct evidence for changes in glutamine and alanine uptake rests on the measurement of relatively small changes in the extraction of the two amino acids across the hepatic circulation. Hepatic glutamine extraction decreased from 20% to 4% instead of decreasing to the 11% predicted by a zero hepatic uptake change. Similarly, alanine extraction fell from 17% to 2% instead of decreasing to 8%. Since these small changes cannot be measured precisely, it is important to note that dexamethasone caused the hepatic output of CO_2 formed from plasma glutamine, ($R_{CO2, GLN}$), to fall significantly to 53% of control values, and that the change in the ($R_{GLU,GLN}+R_{CO2,GLN}$) sum was approximately equal to the estimated change in hepatic glutamine uptake (-87 vs. -97 μmol/mmol of oxygen). Therefore, a fall in fetal hepatic glutamine uptake is supported by the decrease in the combined output of its two major metabolic products.

The finding that dexamethasone reduces fetal hepatic glutamine and alanine uptake is contrary to evidence in adult mammals. In fasting dogs, dexamethasone has no detectable effect on glutamine uptake by the liver and increases hepatic alanine uptake (86). In adult rats, dexamethasone stimulates sodium dependent glutamine uptake via system N transporters by cultured hepatocytes (25) and by sinusoidal membrane vesicles isolated from liver homogenates (55). Developmental changes in the effect of dexamethasone on amino acid transport and metabolism have been previously demonstrated. In human newborns (71, 96) and in newborn rats (27) dexamethasone infusion causes a marked increase in the plasma concentrations of several amino acids, glutamine and alanine included, whereas in adult men (78) it causes no significant concentration changes, with the exception of a small increase in alanine. Fetal rat hepatocytes do not respond to dexamethasone with an increase in system A-mediated transport (32) in contrast to hepatocytes isolated from adult rats.

The data obtained in this study does not allow to decide if the inhibitory effect of dexamethasone on fetal hepatic glutamine and alanine uptakes represents inhibition of amino acid transport into the liver or downregulation of amino acid metabolism within the liver. Furthermore, the possibility cannot be excluded that the dexamethasone effect on hepatic amino acid uptake is indirect, e.g. via some other hormonal change caused by the dexamethasone infusion. At the time at which the measurements were performed, i.e. 26 hours after starting dexamethasone infusion, there was no evidence for the net increase in either hepatic lactate uptake or glucogenic amino acid carbon uptake, or both, that one would expect if the hormone stimulated rapid glycogen deposition. However, it remains possible that glycogen accumulated more rapidly at an earlier stage of dexamethasone stimulation, or that the dose and potency of the glucocorticoid determine whether glycogen accumulation or inhibition of glucogenic amino acid uptake predominates.

With dexamethasone infusion, the disposal rate of fetal plasma glutamine increased significantly. This indicates that, in contrast to the liver, elsewhere in the fetus the glutamine flux from plasma to tissues had increased. Part of the increased flux may represent elevated interorgan exchange. Indeed, in dogs dexamethasone increases glutamine release by skeletal muscle and glutamine uptake by gut and kidney (86).

The dose of dexamethasone used in the present study was sufficient to produce a decrease in placental progesterone output and would have been sufficient to induce labor about 50 hours after the start of the infusion (4). This raises the question whether spontaneous parturition could also be accompanied by changes in fetal and placental amino acid metabolism, similar to the changes that follow administration of dexamethasone.

Chapter 6

NET AMINO ACID FLUX ACROSS THE FETAL LIVER AND PLACENTA DURING SPONTANEOUS OVINE PARTURITION

6.1 INTRODUCTION

The fetal cortisol surge plays an important initiating role in ovine parturition. It was first shown by Liggins et al. that preterm delivery can be induced by fetal administration of glucocorticoids (49, 50, 51, 53). As shown in chapter 5 an infusion of dexamethasone into the fetal circulation, sufficient to induce parturition, alters the normal glutamine-glutamate exchange between the fetal liver and placenta that exists in late gestation ovine pregnancy. In chapter 5 it was reported that virtually all glutamine taken up by the fetal liver is converted to glutamate, and that under the influence of dexamethasone, net fetal hepatic uptake of glutamine decreases significantly. Furthermore, the glutamine-derived glutamate was redirected towards fetal hepatic oxidation in the citric acid cycle rather than released into the fetal circulation. In these studies, the changes in glutamine-glutamate flux coincided with a fall in placental progesterone production, a hormone that plays a pivotal role in the maintenance of pregnancy.

The present study was designed to examine whether spontaneous parturition and its associated rise in fetal cortisol concentration are associated with changes in fetal glutamine and glutamate exchange between the placenta and fetal liver that are similar to those demonstrated in dexamethasone-induced parturition.

6.2 MATERIALS AND METHODS

6.2.1 Surgery and animal care

Seven late gestation ewes, each pregnant with a single fetus were studied. Surgery was performed at 125-128 days of gestation. Preoperative preparation, surgery and animal care were performed as described in chapter 2.

6.2.2 Study design

From day 135 of gestation, samples were drawn from fetal and maternal catheters three times a day, at 7 am, 3 pm and 10 pm until spontaneous delivery occurred. Maternal blood samples were taken from the maternal common iliac artery and the uterine vein. Fetal samples were taken from the fetal aorta, common umbilical vein and left hepatic vein. The volume of fetal blood removed was replaced by fetal donor blood. Since the actual delivery date varied between 143 to 152 days from conception only the data from the last five days until eight hours prior to spontaneous delivery were analyzed. This study window was chosen since the fetal cortisol surge is known to occur 3 to 4 days before spontaneous delivery (53). The last eight hours prior to delivery were dismissed based on the fact that in that period uterine contractions commence accompanied by a rise in catecholamine concentrations (22), which induces large variability in substrate concentrations.

After parturition, the ewe and the lamb were killed for verification of the position of the fetal catheters.

6.2.3 Analytical methods

Fetal samples were analyzed for hemoglobin, hematocrit, oxygen saturation, glucose, lactate and amino acid concentrations as well as cortisol, and insuline levels. The maternal samples were analyzed for progesterone concentration. The analytical methods were as described in chapter 2.

6.2.4 Calculations

Uptakes

Because blood flows were not measured throughout the days preceding parturition, placental and hepatic uptakes of metabolic substrates were expressed per unit O_2 consumption, i.e. as the ratio of two arterio-venous differences, μmoles substrate per mmol O_2, as follows:

placental uptake = $(\gamma-\alpha)_{substrate} \div (\gamma-\alpha)_{O_2}$

hepatic uptake = $(\gamma-h)_{substrate} \div (\gamma-h)_{O_2}$

The fetal left hepatic lobe is perfused solely by fetal arterial and umbilical venous blood, the umbilical venous blood accounting for > 95% of the flow whereas the right hepatic lobe also receives portal venous blood (21). This was confirmed in the experiments reported in chapter 5. Therefore, blood samples taken from the left hepatic venous and umbilical venous catheters are considered representative of the amino acid concentration differences across the left lobe of the fetal liver (59).

6.2.5 Statistical analysis

Data are expressed as means ± SE. In order to compensate for differences in gestational age at the time of delivery, results are expressed in relation to the time from parturition, using the actual delivery date as the reference point. Regression lines were calculated for the pooled data from all studies taking into account the inter- and intra-animal variability using the mixed effect methodology described by Laird and Ware (45). A *p* value of < 0.05 was taken to represent significance.

6.3 RESULTS

The mean ± SE on the first day of study for the O_2 contents, glucose and lactate concentrations for the fetal artery, umbilical vein and fetal left hepatic vein are presented in Table 6.1. Umbilical arterio-venous oxygen content difference increased in the days preceding parturition. The regression for the umbilical arterio-venous difference was (α-

$\gamma) = (1.97 \pm 0.28) - (0.18 \pm 0.06) \cdot D \pm 0.08$ where the group mean intercept and slope are 1.97 and 0.18 mmol/day, respectively, and D is time in days prior to parturition. The mean slope was significantly less than zero ($p < 0.003$). By contrast, the oxygen content difference across the fetal liver showed no significant increase. There were no significant changes in fetal hemoglobin and hematocrit.

Table 6.1 Oxygen content, glucose and lactate concentrations on the first day of study. Values are means $\pm$ SE.

O_2 content, mM	
Fetal artery	2.7 ± 0.1
Umbilical vein	4.6 ± 0.1
Hepatic vein	3.6 ± 0.6
Glucose concentrations, mM	
Fetal artery	0.98 ± 0.02
Umbilical vein	1.16 ± 0.03
Hepatic vein	1.13 ± 0.02
Lactate concentrations, mM	
Fetal artery	2.41 ± 0.24
Umbilical vein	2.53 ± 0.23
Hepatic vein	2.33 ± 0.22

Net fluxes of glucose and lactate

Although both glucose and lactate were delivered to the fetal liver in large quantities by the umbilical vein, only lactate was taken up in significant amounts. The regression equations describing the changes in glucose and lactate concentrations and their placental and fetal hepatic uptakes for the days preceding parturition are presented in Table 6.2. Glucose concentrations in the fetal artery, umbilical vein and hepatic vein increased significantly without a significant change in umbilical glucose uptake. Net fetal hepatic glucose output was not significantly different from zero and did not change significantly in the days prior to delivery. Lactate concentrations and net umbilical lactate uptake showed no significant changes prior to parturition. By contrast, the net fetal hepatic lactate uptake increased significantly in the days preceding parturition, $p < 0.02$.

Net fluxus of amino acids

Table 6.3 presents the mean ± SE of the fetal amino acid concentrations on the first day of study and were in good agreement with data presented in chapter 4. Glycine, alanine, asparagine, proline and threonine concentrations showed no significant changes in the days preceding parturition. By contrast, the concentrations of leucine, isoleucine, valine, phenylalanine, tyrosine, serine and glutamate decreased significantly.

One metabolically related group with significant changes includes glutamate and glutamine. Table 6.4 presents the data demonstrating that glutamate concentrations fell significantly in fetal arterial and hepatic venous blood. Since umbilical venous concentration did not change, the placental uptake of glutamate and its hepatic release decreased significantly. Figure 6.1 presents the net glutamate fluxes across the fetal liver and placenta. Net placental glutamate uptake was dependent on net fetal hepatic glutamate release (Fig. 6.2). Glutamine net uptake across the fetal hepatic circulation decreased significantly as a function of the days preceding parturition without a detectable change in glutamine umbilical uptake (Table 6.4).

Table 6.2 Regression of glucose and lactate concentrations and uptakes.

GLUCOSE		p- value
Fetal arterial blood, mM	$(1.15 \pm 0.06) + (0.05 \pm 0.01) \cdot D \pm 0.1$	< 0.02
Umbilical venous blood, mM	$(1.37 \pm 0.06) + (0.06 \pm 0.01) \cdot D \pm 0.1$	< 0.01
Hepatic venous blood, mM	$(1.48 \pm 0.10) + (0.09 \pm 0.02) \cdot D \pm 1.4$	< 0.01
Net placental uptake, nmol/mmol O_2	$(94 \pm 8) - (0.5 \pm 4) \cdot D \pm 30$	NS
Net hepatic uptake, nmol/mmol O_2	$-(110 \pm 60) - (3 \pm 2) \cdot D \pm 120$	NS

LACTATE		
Fetal arterial blood, mM	$=(3.07 \pm 0.56) + (0.18 \pm 0.11) \cdot D \pm 0.4$	NS
Umbilical venous blood, mM	$=(3.30 \pm 0.55) + (0.20 \pm 0.11) \cdot D \pm 0.5$	NS
Hepatic venous blood, mM	$=(2.95 \pm 0.55) + (0.16 \pm 0.11) \cdot D \pm 0.4$	NS
Net placental uptake, μmol/mmol O_2	$=(96 \pm 14) + (4 \pm 4) \cdot D \pm 50$	NS
Net hepatic uptake, μmol/mmol O_2	$=(551 \pm 76) + (50 \pm 16) \times D \cdot 190$	< 0.02

Table 6.3 Fetal arterial plasma concentrations (μM) on the first day of the study period. Values are means ± SE.

Glutamate	40 ± 6	Tyrosine	175 ± 23
Glutamine	388 ± 21	Asparagine	52 ± 6
Serine	637 ± 111	Valine	599 ± 47
Glycine	613 ± 30	Leucine	210 ± 21
Alanine	421 ± 33	Isoleucine	112 ± 10
Proline	205 ± 42	Threonine	377 ± 39
Tyrosine	175 ± 23	Phenylalanine	120 ± 13

Serine concentration decreased significantly, ($p < 0.001$) in all vessels as a function of the days preceding parturition. The regression of arterial fetal serine concentration was: α (μM) = (346 ± 38)-(74 ± 15)·D ± 60. However, no significant changes in the fluxes of serine across the hepatic and umbilical circulations could be detected. There were no significant changes in glycine concentrations or net fluxes. The concentrations of the branched chain amino acids, leucine, isoleucine and valine, also fell significantly as a function of time prior to delivery. The fetal arterial regressions were: α (μM) = (160 ± 11)-(13 ± 4)·D ± 20, $p < 0.02$, α (μM) = (93 ± 9)-(8 ± 2))·D ± 13, $p < 0.01$, and α (μM) = (410 ± 36)-(48 ± 7)·D ± 54, $p < 0.001$, for leucine, isoleucine and valine, respectively. No decrease was found in their net placental and hepatic fluxes.

Endocrine changes

There were significant changes in the fetal arterial concentrations of cortisol in the days preceding parturition. Cortisol concentration (μg/dL) was described by the following: ln cortisol = (2.8 ± 0.1)+(0.4 ± 0.0)·D ± 0.3, $p < 0.001$. The net fetal hepatic glutamate release was significantly correlated to the fetal arterial cortisol concentration. The uterine arterio-venous concentration difference for progesterone (nmol/L) decreased significantly during parturition (A-V = (26 ± 5)- (8 ± 2)·D ± 12, $p < 0.003$). It was significantly correlated with placental glutamate uptake ($p < 0.01$). Fetal arterial
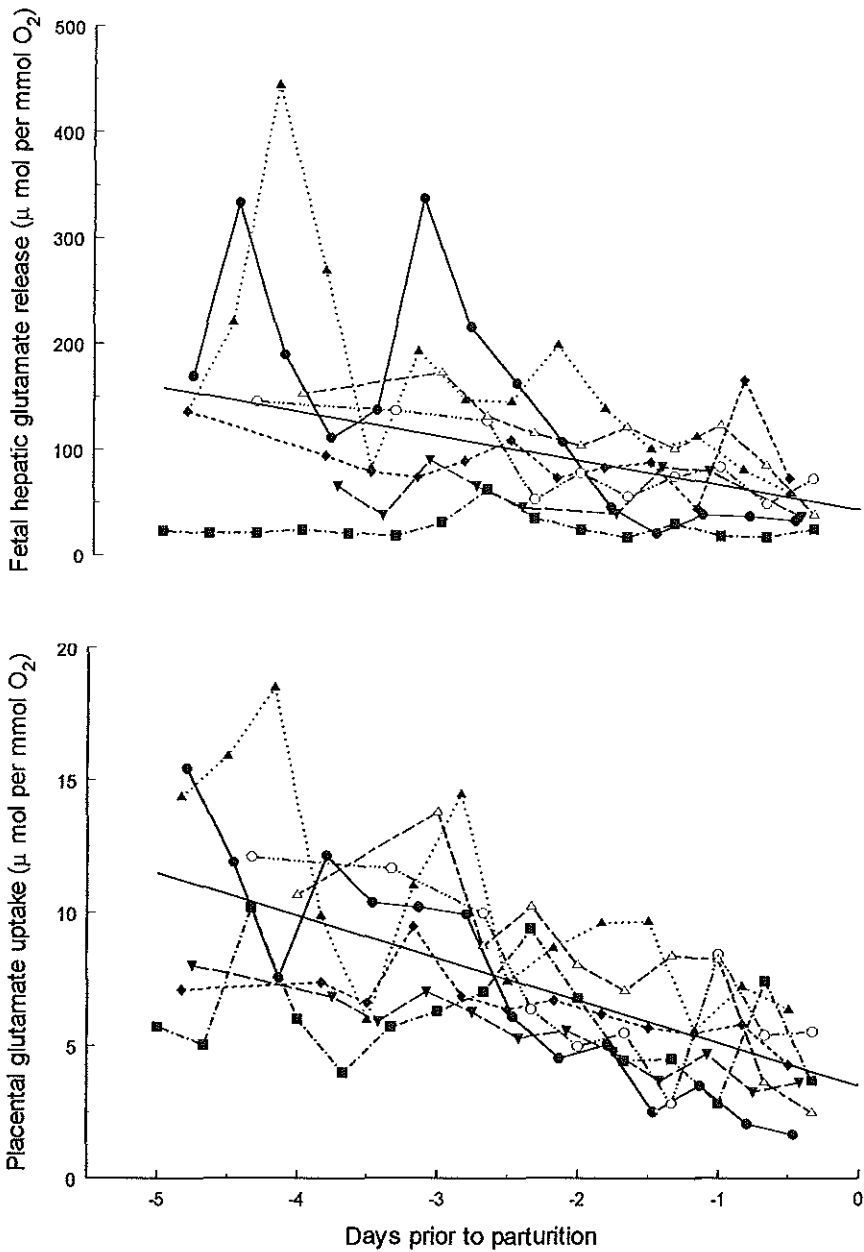
Fig 6.1 The course of net fetal hepatic glutamate release (top) and net placental glutamate uptake (bottom) in the days prior to parturition in the seven fetal lambs studied.

catecholamine concentrations were determined in two fetuses and did not change significantly in the period of study.

Table 6.4 Regression of glutamate and glutamine concentration and uptakes.

GLUTAMATE		p-value
Fetal arterial concentration, μM	$=(17.9 \pm 2.8) - (5.2 \pm 1.4) \times D \pm 5.5$	< 0.01
Umbilical venous concentration, μM	$=(4.0 \pm 1.6) - (0.9 \pm 0.5) \times D \pm 3.2$	NS
Hepatic venous concentration, μM	$=(55.1 \pm 6) - (11.7 \pm 3.7) \times D \pm 16.8$	< 0.02
Net placental uptake, $\mu mol/mmol\ O_2$	$=-(3.5 \pm 0.7) + (1.6 \pm 0.4) \times D \pm 1.9$	< 0.005
Net hepatic release, $\mu mol/mmol\ O_2$	$=-(43 \pm 12) + (23 \pm 8) \times D \pm 50$	< 0.03
GLUTAMINE		
Fetal arterial concentration, μM	$=(407 \pm 25) + (5 \pm 6) \times D \pm 38$	NS
Umbilical venous concentration, μM	$=(431 \pm 23) + (1 \pm 6) \times D \pm 44$	NS
Hepatic venous concentration, μM	$=(366 \pm 31) - (0 \pm 8) \times D \pm 39$	NS
Net placental uptake, $\mu mol/mmol\ O_2$	$=(5 \pm 2) - (2 \pm 1) \times D \pm 10$	NS
Net hepatic uptake, $\mu mol/mmol\ O_2$	$=(44 \pm 12.6) - (17 \pm 7) \times D \pm 54$	> 0.05

6.4 DISCUSSION

It is only recently that studies have demonstrated the importance of the interorgan exchange of amino acids between the fetal liver and the placenta and its changes with dexamethasone-induced parturition (4, 59, 93 and chapter 5). However, no data are available on placental and fetal hepatic amino acid metabolism in spontaneous parturition.

In the present study, the umbilical and fetal hepatic blood flows were not measured because of the technical difficulties in completing such measurements when the precise time of delivery of the fetus is unknown. For this reason, all substrate arterio-venous differences across the umbilical or fetal hepatic circulations were expressed per mmole O_2, i.e. substrate uptake has been expressed per unit O_2 consumption of the fetus or by the fetal liver. There was an increase in the umbilical arterio-venous difference in oxygen content as the pregnancy progressed towards the day of delivery. The most likely explanation for this is that there may be a progressive decrease in umbilical blood flow, although the possibility of an increase in fetal O_2 consumption cannot be excluded.
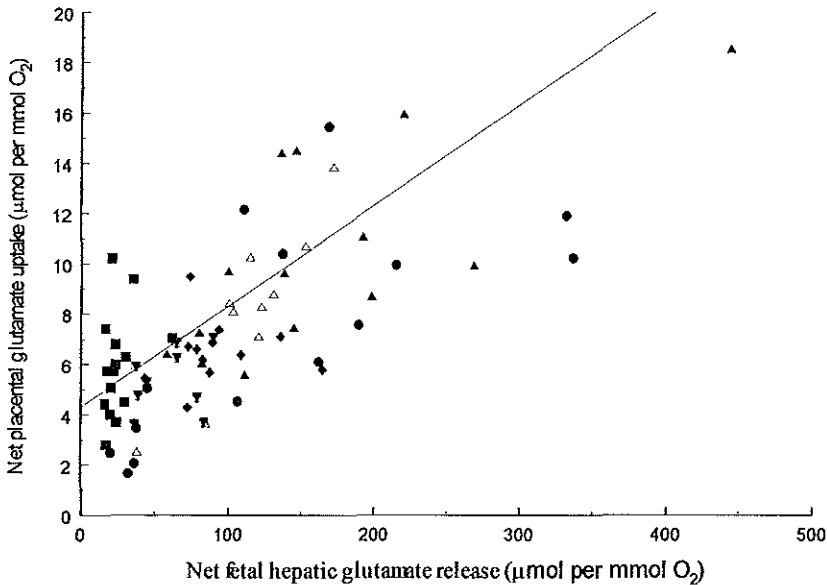
Fig. 6.2 Correlation between net placental glutamate uptake and net hepatic release. (Y = (4.3 ± 0.4) + (0.04 ± 0.00) ·x ± 2.3, p < 0.0002.

We found a significant increase in net fetal hepatic lactate uptake which presumably could be directed towards glucogenesis and glycogen accretion. However, there were no significant increases in the hepatic uptake of both gluconeogenic amino acids alanine and glutamine.

Previously, we hypothesized that the net fetal hepatic release of the amino acids serine and glutamate serves as a means of delivering nutrients to the fetus without net hepatic glucose release. Glucose release would increase fetal glucose concentration and curtail the delivery of exogenous glucose through the placenta. In chapter 5, we reported the effect of a fetal dexamethasone infusion, sufficient to induce parturition, on the fetal hepatic and placental fluxes of glutamine and glutamate. The results of that study are similar in some respects to those obtained in the present study of spontaneous parturition, but there are also marked differences. The similarity rests primarily with fetal hepatic glutamine and glutamate fluxes such as the decrease in the net fetal hepatic glutamate

release and decrease in net glutamine uptake by the fetal liver. In both conditions there was also a decreased placental glutamate uptake. The fall in net fetal hepatic glutamate release was accompanied by a significant rise in net fetal hepatic lactate uptake. This could account for the glycogen deposition known to occur prior to birth and mediated by cortisol (5). The net fetal hepatic uptake of other gluconeogenic substrates as alanine and glutamine did not increase notably.

Although we measured a significant fall in fetal plasma serine concentrations, we were not able to show a significant decrease in net umbilical and fetal hepatic serine uptake or release. Unfortunately, the high concentration of serine makes it difficult to detect its relatively small arterio-venous concentration differences across the fetal liver and placenta. The significant fall in serine concentration, however, suggests a decrease in net fetal hepatic serine production as well.

The difference between spontaneous and dexamethasone-induced parturition lies in the changes in fetal amino acid concentrations. In the present study, there was a significant decrease in concentration of many amino acids that had increased in concentration during dexamethasone infusion. The reasons for this difference are not clear but may reflect differences in skeletal muscle metabolism between spontaneous parturition versus dexamethasone-induced parturition (38, 40, 79). The fall in the concentrations of the branched chain amino acids during spontaneous parturition could be explained by the increased conversion of these substrates into their related keto acids under the influence of glucocorticoids (23, 95).

The redirection of glutamate within the fetal liver may be due to an upregulation of the enzyme glutamate dehydrogenase that converts glutamate into the citric acid intermediate α-keto glutarate. Although some studies indicate that glucocorticoids increase the activity of this enzyme in adult liver tissue, no studies have been performed in fetal life (72, 100). Also a change in number and/or affinity of the X^-_{AG} transporter responsible for glutamate transport out of the hepatocyte could play a role since glucocorticoids have been reported to influence amino acid transporters (84). Further studies will be needed to clarify the mechanisms within the fetal liver leading to the decrease in net fetal hepatic glutamate production.

Klimek et al. have suggested a direct relationship between placental glutamate metabolism and progesterone synthesis (42). Placental glutamate oxidation is an important source for NADPH required for placental steroidogenesis since the placenta lacks an active pentose phosphate pathway (11, 42). Placental progesterone synthesis is crucial in the maintenance of pregnancy; inhibition of placental progesterone synthesis leads to preterm delivery (90). If Klimek's hypothesis were true, it would provide a mechanism whereby fetal hepatic metabolism may alter placental steroidogenesis through its role as the major source of fetal glutamate production and thus, of placental glutamate supply. (42).

The present study demonstrates that the endocrine changes associated with parturition are accompanied by a marked reduction in net fetal hepatic output of glutamate and net fetal hepatic glutamine uptake, similar to the changes observed in dexamethasone-induced parturition. The change in the fetal liver metabolism also affects net placental glutamate uptake and is associated with a marked reduction in progesterone output by the pregnant uterus.

Chapter 7

INDUCTION OF GLUTAMATE DEHYDROGENASE IN THE OVINE FETAL LIVER BY DEXAMETHASONE INFUSION DURING LATE GESTATION

7.1 INTRODUCTION

The mitochondrial enzyme glutamate dehydrogenase (GDH, EC 2.1.14) catalyzes the reversible conversion of ammonium nitrogen into organic nitrogen, glutamate production, and oxidative deamination of glutamate, resulting in 2-oxoglutarate. The equilibrium of the enzyme is in favor of the formation of glutamate (24, 85). This reaction is the only pathway by which ammonia can become bound to the α-carbon of a α-carboxylic acid.

The present study was induced by a number of studies including those described in the chapters 5 and 6, directed at glutamine and glutamate exchange between the fetal liver and placenta. During ovine fetal life, glutamine derived from maternal plasma glutamine through placental transport, is taken up by the fetal liver in large quantities (chapters 3 and 5). The glutamine is utilized in part for net glutamate release from the liver. The glutamate is either released into the fetal circulation or further oxidized in the citric acid cycle. The hepatic glutamate released maintains the fetal glutamate concentration and is the key determinant of glutamate delivery to the placenta. As shown in chapters 5 and 6 fetal hepatic glutamate release falls markedly in dexamethasone-induced as well as in spontaneous parturition. In dexamethasone-induced parturition, using tracer methodology, we demonstrated that the fate of hepatic glutamate changes in favor of a further metabolism in the citric acid cycle rather than release into the fetal circulation (chapter 5). This suggests increased GDH activity under these conditions. The present study was designed to determine whether there is an increased expression of GDH mRNA and of GDH activity following fetal dexamethasone infusion.

7.2 MATERIALS AND METHODS

7.2.1 Surgery and animal care

Twelve Columbia-Rambouillet ewes pregnant with a single fetus were used. Surgery was performed on seven ewes to insert fetal venous catheters for dexamethasone infusion. Preoperative preparation, surgical techniques and animal care were as described in chapter 2

7.2.2 Study design

Studies were performed at 130-135 days of gestation. On the day of study, a bolus of 0.2 mg of dexamethasone was given via the fetal brachial vein followed by a continuous infusion (0.07 mg·hr^{-1}) for 26 hours. After infusion the fetus was delivered by cesarean section, the fetal liver was collected and weighed. The right lobe was removed and the left lobe sectioned into slices after trimming the peripheral tissues. The five control animals were killed and their livers collected at comparable gestational ages. Approximately 10 g of liver tissue was placed in mitochondria isolation medium buffer (IM buffer, mannitol, 220mmol/l; sucrose, 70 mmol/l; Hepes, 2 mmol/l; BSA 0,5 mg/ml; pH 7.4; 4°C) for immediate mitochrondrial fraction isolation. Mitochondrial pellets for GDH activity determination were obtained as described elsewhere (44). Pellets were resuspended in IM buffer and stored at –80°C. GDH activity was measured within 3 weeks of pellet preparation. The remaining liver slices were frozen in liquid N_2 and stored at -80°C until later RNA extraction.

7.2.3 Analytical methods

Enzymatic analysis

The catalytic activity of GDH was determined at room temperature by measuring the decrease in absorbence due to the oxidation of NADH (80). In preliminary trials, the oxidation rate was found to be too high to observe a linear response, when using reagent concentrations as reported in the original method. As a result, modifications were made and these are described below. Mitochondrial pellet samples were thawed on ice, diluted 1:1 with isolation medium, sonicated and centrifuged at 100 000 x g for 1 hour at 4°C.

The supernatant was further diluted 1:20 with IM prior to assay. Ten to 50 μl of diluted sample was added to a reaction mixture ((TEA, 64.5 mmol/l; EDTA, 3.22 mmol/l; $CH_3COO-NH_4$, 129 mmol/l pH 8.0)(NADH, 17.6 mmol/l; ADP, 96 mmol/l; LDH 160 kU/l)). Absorbence was read at 339 nm until all endogenous pyruvate had been consumed. Once a steady state had been achieved, 100 μl 2-oxoglutarate (7 mmol/l) was added and changes in absorbence recorded every 30 seconds for up to 5 minutes. Sample sizes that produced a linear decline in absorbence no greater than 0.05/minute over the 5 minute oxidation period were used. In addition, two further samples were run to confirm linearity of the activity. Samples were then assayed for citrate synthase (CS) activity by the method of Shepard and Garland (81). GDH activity was expressed as GDH units of activity/CS units of activity. Protein concentrations were determined with bicinchoninic acid (BCA) (Pierce, USA) with BSA as the standard.

RNA preparation, electrophoresis and transfer

Total cellular RNA was prepared using TRI REAGENT (Molecular Resource Center, Cincinnati, OH, USA). Total cellular RNA (20 μg) was denatured and subjected to electrophoresis in a 1% agarose gel containing glyoxal/DMSO in 0.01 M PO_4 buffer. The RNA was transferred in alkaline solution to an Amersham N+ Hybond membrane (Amersham, Amersham UK) using a pressure blotter PosiBlot® (Stragene, La Jolla CA, USA). After transfer, membranes were UV cross-linked and rinsed in 6 x SSC (0.72 M NaCl, 40 mM $Na_2HPO_4.7H_2O$, and 4 mM EDTA) before the integrity of RNA and transfer efficacy was confirmed with methylene blue staining. Membranes were washed in 5% acetic acid for 15 minutes before being further washed in a 0.04% methylene blue sodium acetate solution (0.5 M sodium acetate pH 5.2, 0.04 % methylene blue) for 10 minutes. The membranes were then rinsed in depc treated water until the membrane was clear of background stain. All procedures were conducted at room temperature. Stained membranes were photographed and samples not displaying distinct and clear 28S and 18S sub units were discarded.

Glutamate dehydrogenase reverse transcription-polymerase chain reaction

An ovine GDH cDNA probe was generated through the reverse transcription-polymerase chain reaction (RT-PCR). PCR primers were chosen, based on published sequences of the human glutamate dehydrogenase (X07769) and the rat protein (X14223) (5' - GCGAGGACGACCCCAACTT 3', 5' - TCAATGCCAGGACCAATAA 3'). The RT reaction was carried out using approximately 1µg of fetal liver total cellular RNA, using SuperScript™II (Gibco BRL). Thermal cycling using Taq DNA polymerase included 35 cycles at 94°C for 1 minute, annealing at 60°C for 1 minute and extension at 72°C for a further 1 minute. The PCR reaction was held at 72°C for a final extension for 10 minutes before being cooled to room temperature. An aliquot of the product was fractionated on a 1.5% agarose gel and stained with ethidium bromide. Liver total cellular RNA samples yielded a single product of approximately 481 base pairs. Following PCR the ligation was cloned into pCR® 2.1 (Invitrogen® Carlsbad, CA, USA), and then transformed using a INVαF' One Shot™ kit (Invitrogen® Carlsbad, CA, USA). The 481 bp oGDH insert placement was confirmed on a 1.5% agarose gel, following incubation with EcoR1.

Sequencing data

Sequencing of the DNA was conducted on several clones using an ABI 377 automated DNA sequencer with dye rhodamine terminator chemistry. For sequence confirmation, both strands of the DNA were sequenced. Sequences were then compared to other sequences by using National Center for Biotechnology Information basic BLAST search software and GeneStream alignment software.

Hybridization

Blots were prehybridized (6 x SSC, 50% v/v formamide, 10% w/v sulfate dextran, 7% w/v sodium dodecyl sulfate (SDS), 5µg/ml denatured salmon sperm DNA and 100 5µg/ml yeast tRNA (Gibco BRL, Gaithersburg, Md, USA) for 2-4 hours at 42°C. Blots were then hybridized with a 481 pb EcoRI-EcoRI ^{32}P-labeled oGDH cDNA fragment, at a concentration of 1-2 x 10^6 cpm/ml. Following a 16 hour hybridization period at 42°C, membranes were washed twice with 2 x SSC/0.1% SDS for 20 minutes at room

temperature and then twice with 0.1 x SSC/0.1% SDS for 15 minutes each at 65°C. The molecular size of the oGDH-specific band was estimated from a 0.4 to 9.5 kb RNA ladder (Gibco-BRL, Gaithersburg, MD, USA). Each gel was run with a sample of postnatal lamb and maternal liver, which served as a positive control for GDH expression. Saran-wrapped membranes were exposed to a phosphorus plate for at least 12 hours and scanned by a Storm™ system (Molecular Dynamics, Sunnyvale, CA, USA). Resulting images were analyzed through ImageQuant® (Molecular Dynamics, Sunnyvale CA, USA), generating data in the form of mRNA expression volumes. To correct for loading and transfer differences following oGDH quantification, the membranes were stripped (0.5% boiling SDS allowed to cool to room temperature) and reprobed with an 18s rRNA probe. The 18s rRNA levels were measured by hybridization with an oligonucleotide probe (ACGGTATCTGATCCGTCTTCGAACC) labeled with ^{32}P-dTCP using terminal deoxytransferase (Boehringer Mannheim Biochemicals, Indianapolis, IN, USA). After this, the membranes were subjected to the same washing procedures and image analysis as described.

7.2.4 Statistical analysis

Values from the enzymatic analysis are reported as means ± SE. Results from the phosphorus imaging scanning, a ratio of oGDH mRNA expression compared to 18s RNA expression, are also reported as means ± SE. Differences among groups were analyzed by Students t-test with significance set at $p < 0.05$.

7.3 RESULTS

Tissue was collected at approximately 130 days gestation from seven dexamethasone treated and five control fetal lambs. A 26 h dexamethasone infusion had significant effects on fetal body or fetal liver weights (Table 7.1).The nucleotide 2.8 kb, which is in agreement with other published GDH message data (19). The cDNA sequence for ovine GDH exhibits 95.2, 91.7 and 90.9 % homology with human (X07769), rat (X14223) and murine (X51081) sequences, respectively.

```
        10         20         30         40         50
GCGAGGACGA CCCCAACTTC TTCAAGATGG TGGAGGGCTT CTTTGACCGC
:::::::::: :::::::::: :::::::::: :::::::::: ::: :: :::
GCGAGGACGA CCCCAACTTC TTCAAGATGG TGGAGGGCTT CTTCGATCGC
         200        210        220        230        240


        60         70         80         90        100
GGTGCCAGCA TCGTGGAGGA CAAGCTGGTG GAGGACCTCA AGACCCGGGA
:: :::::::: :::::::::: :::::::::: :::::::: : ::::::::::
GGCGCCAGCA TCGTGGAGGA CAAGCTGGTG GAGGACCTGA GGACCCGGGA
         250        260        270        280        290


       110        120        130        140        150
GACCGAGGAG CAGAAGCGGA ACCGGGTGCG TGGCATCCTG CGGATCATCA
:: ::::::: :::::::::: :::::::::: :::::::::: ::::::::::
GAGCGAGGAG CAGAAGCGGA ACCGGGTGCG CGGCATCCTG CGGATCATCA
         300        310        320        330        340


       160        170        180        190        200
AGCCCTGCAA CCATGTGCTG AGCCTGTCCT TCCCCATCCG GCGCGACGAC
:::::::::: :::::::::: :: :: :::: :::::::::: ::::::::::
AGCCCTGCAA CCATGTGCTG AGTCTCTCCT TCCCCATCCG GCGCGACGAC
         350        360        370        380        390


       210        220        230        240        250
GGCTCCTGGG AAGTCATTGA GGGCTACCGG GCCCAGCACA GCCAGCACCG
:::::::::: : ::::: :: :::::::::: :::::::::: ::::::::::
GGCTCCTGGG AGGTCATCGA AGGCTACCGG GCCCAGCACA GCCAGCACCG
         400        410        420        430        440


       260        270        280        290        300
CACGCCCTGC AAGGGAGGTA TCCGTTACAG CACCGATGTG AGTGTAGACG
:::::::::: :::::::::: :::::::::: ::: :::::: :::::::: :
CACGCCCTGC AAGGGAGGTA TCCGTTACAG CACTGATGTG AGTGTAGATG
         450        460        470        480        490


       310        320        330        340        350
AAGTTAAAGC TCTGGCTTCT CTGATGACGT ATAAGTGTGC AGTGGTTGAT
:::: ::::: : :::::::: :::::::: : : :::::::: ::::::::::
AAGTAAAAGC TTTGGCTTCT CTGATGACAT ACAAGTGTGC AGTGGTTGAT
         500        510        520        530        540


       360        370        380        390        400
GTGCCATTTG GGGGTGCCAA AGCTGGTGTT AAGATCAATC CCAAGAACTA
::::: :::: :::::::: :: :::::::::: :::::::::: ::::::::::
GTGCCGTTTG GGGGTGCTAA AGCTGGTGTT AAGATCAATC CCAAGAACTA
         550        560        570        580        590


       410        420        430        440        450
TACTGATAAC GAATTGGAAA AGATCACAAG GAGGTTCACC ATGGAGCTGG
:::::::::: :::::::::: :::::::::: :::::::::: :::::::: :
TACTGATAAT GAATTGGAAA AGATCACAAG GAGGTTCACC ATGGAGCTAG
         600        610        620        630        640


       460        470        480
CAAAGAAGGG CTTTATTGGT CCTGGCATTG A
:::: ::::: :::::::::: :::::::::: :
```

Fig. 7.1 The sequence for sheep cDNA for glutamate dehydrogenase (GDH) aligned with the predicated sequence for human (M20867).

Table 7.1 Fetal parameters in control (n=5) and dexamethasone (n=7) infused fetuses at delivery. Values are means ± SE.

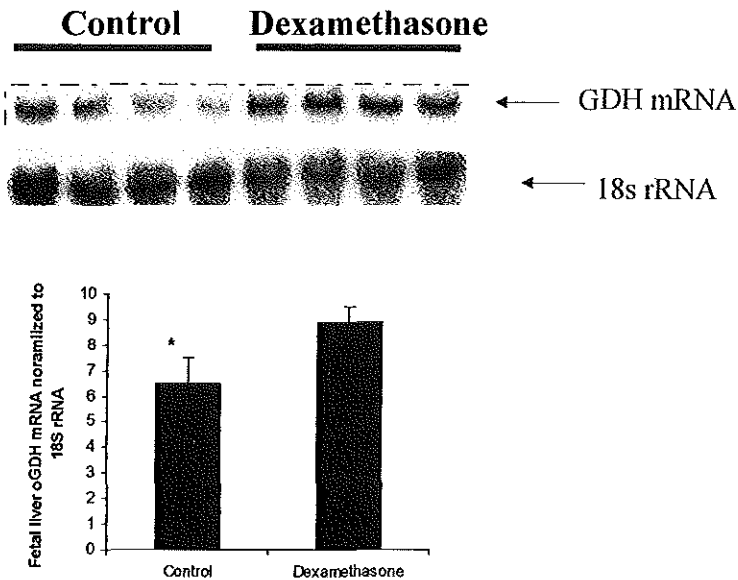|	Control	Dexamethasone
Gestational age, days	130 ± 2	130 ± 2
Fetal weight, g	2938 ± 322	3248 ± 207
Fetal liver weight, g	117 ± 11	103 ± 5

Fig. 7.2 Northern blot analysis of ovine GDH mRNA levels of control and dexamethasone treated fetal liver. Quantification of the oGDH mRNA levels normalized to the 18s mRNA levels is shown above. Values are means ± SE.

Dexamethasone infusion for 26 hours significantly increased GDH mRNA expression by approximately 44% (p< 0.02, Fig. 7.2). This increased oGDH mRNA expression was accompanied by an increase in mitochondrial GDH activity, from 26.5 ± 9.6 to 66.1 ± 10.9 U GDH/U CS (p<0.03, Figure 7.3). Moreover, there was a significant correlation between GDH mRNA expression and activity (p <0.001, Fig. 7.4).
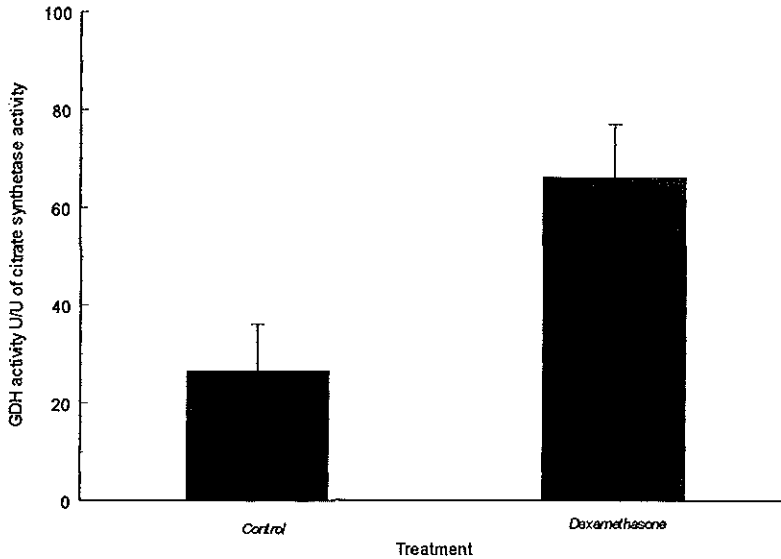
Fig. 7.3 Hepatic GDH activity per citrate synthetase activity for control and dexamethasone treated liver. Values are means ±SE.

7.4 DISCUSSION

These results demonstrate that, under the influence of a fetal dexamethasone infusion near term sufficient to induce parturition, the expression and activity of glutamate dehydrogenase in the fetal liver is increased. Glutamate dehydrogenase is exclusively located in the mitochondrial matrix and catalyzes the reaction: glutamate + NAD^+ + H_2O $\leftrightarrow$ α-ketoglutarate + NH_4^+ + NADH + H^+. The equilibrium coefficient of this reaction is $1{\sim}10 \times 10^{-14}$, which strongly favors glutamate production, or what is termed the reverse reaction (24). In chapter 5 we reported that fetal dexamethasone
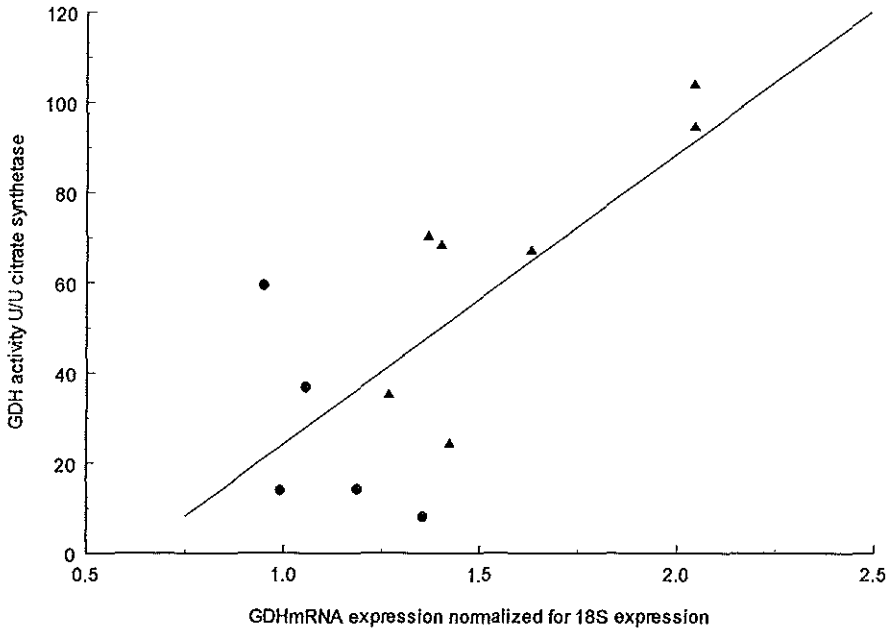
Fig. 7.4 Relationship between fetal hepatic GDH mRNA expression and activity in control (●)and dexamethasone (▲) treated livers. Y= 0.0081X+0.989; R^2=0.52.

infusion decreases fetal glutamate output in favor of further oxidation in the citric acid cycle. The results demonstrated here suggest that the activity of GDH is increased to favor the forward reaction, that is the production of α-ketoglutarate and associated reductive units. This change in GDH activity under the influence of dexamethasone may be ascribed to the increased need for citric acid cycle intermediates for gluconeogenesis and glycogen synthesis. These changes occur naturally as parturition approaches, and both metabolic pathways are reported to be stimulated by glucocorticoids (5).

The observation of increased GDH mRNA expression following fetal infusion of dexamethasone is the first report of *in utero* alterations in hepatic enzymatic activity in the fetal sheep. While GDH activity has been documented in other species for different physiological states (45, 72, 100), gestational changes in GDH expression *in utero* have

only been been examined in the rat (19). In this species a relatively low activity of hepatic GDH has been described *in utero*, in comparison with GDH activity in adult liver (19, 43). In rats GDH mRNA expression varies significantly during development, reaching maximal levels two days prior to birth, decreasing at birth and then rising again to peak levels in the 3rd-4th postnatal week (19). GDH mRNA expression following birth is thought to be regulated predominantly at the pretranslational level, whereas during the prenatal period, GDH expression is regulated at the translational as well as at the pretranslational level (19). The present study clearly demonstrates that, prior to term, following glucocorticoid administration ovine fetal hepatic GDH mRNA expression is upregulated, a result that mimics the developmental profile observed prior to term in the rat fetus. In the fetal lamb, cortisol infusions have been demonstrated to increase fetal liver glycogen concentrations (5), presumably through glucogenic enzymes such as GDH and cytosolic phosphoenolpyruvate carboxykinase (PEPCK). The direct effects of dexamethasone on the translation of mRNA are still unknown. Studies in adult rats have shown that T_3 treatment and cortisol increase the level of GDH, suggesting hormonal regulation of this gene (100). Furthermore, the induction of GDH activity by thyroid hormones is partly inhibited by blocking *de novo* protein synthesis, suggesting that T_3 is involved in altering gene transcription as well as specific enzyme characteristics (100).

Glucocorticoid induced increases in GDH activity were previously reported in adult rat liver (72). GDH activity nearly doubled in our study following dexamethasone administration. This increase in activity was correlated with the increase in GDH message expression (Fig. 7.4). Through the actions of this enzyme the transfer of nitrogen between fetus and placenta occurs as well as the proposed supply of an oxidative fuel source in the form of NADPH from glutamate formation for placental steroidogenesis (69).

In conclusion, the study reported here demonstrates that infusion of dexamethasone induces significant increases in GDH message expression and GDH activity. These data are consistent with the *in vivo* studies reported in chapter 5, which showed a redirection of glutamine carbon away from net hepatic glutamate release and into the citric acid cycle through the forward reaction catalyzed by GDH, i.e. glutamate to oxoglutarate.

Chapter 8

GENERAL DISCUSSION

One of the difficulties encountered in the study of amino acid metabolism is the relatively high plasma concentration of amino acids as compared to the small arteriovenous whole blood concentration difference across a selected organ. One study reported that amino acids carried within the erythrocyte made no significant contribution to the rapid amino acid exchange between circulation and body organs in the adult sheep (54). However, several amino acid transporters have been found on the erythrocyte membrane and there are marked amino acid concentration differences between erythrocytes and plasma (31, 101). We investigated whether amino acid exchange between plasma and erythrocytes in fetal ovine blood plays a significant role in amino acid uptake between the circulation and tissue. Using plasma amino acid concentrations rather than whole blood amino acid concentrations would result in a more accurate assessment of amino acid metabolism. In the study reported in chapter 3 we found no detectable efflux from erythrocytes into the plasma compartment during a period of time of up to 5 minutes, with the exception of a slow efflux of alanine that bears no significance under the study conditions in our laboratory. Although amino acid transporters have been found on the erythrocyte membrane, their transport velocity appears to be too slow to play a role in amino acid exchange between tissues and blood.

The results of the simultaneous measurements of uterine and umbilical amino acid uptake presented in chapter 3.3.2. demonstrate that in normal ovine pregnancy the supply of non-essential as well as essential amino acids to the fetus exceeds the accretion rate for normal fetal growth. We found no net significant placental utilization of amino acids with the exception of the branched chain amino acids. This is in agreement with data from previous studies and the finding of branched chain amino acid aminotransferase in placental tissue (29). In contrast, there was a net placental production of glutamine. Serine and glutamate were taken up by the placenta from the umbilical circulation. No

net uterine uptake of glutamate was detected, which agrees with the absence of the mother-to-fetus glutamate transfer observed in primate pregnancy (88).

The study presented in chapter 4 was designed to assess the relationship between maternal plasma concentration and fetal uptake of alanine, because no data could be found in the accessible literature on this physiologically important issue. We showed that by increasing maternal alanine concentration, net uterine alanine uptake increased six times resulting in a twofold net umbilical alanine uptake. This means that most of the increased uterine alanine uptake is utilized by the placenta. The study showed that the alanine transported from the placenta to the fetus was predominantly formed de novo within the placenta and not derived from the maternal circulation. This implies a major obstacle in putative treatment of fetal growth restriction by means of artificially increasing maternal amino acid concentration. However, alanine is a non-essential amino acid that is readily interchangeable with lactate and pyruvate, and for that reason it may be an extreme amino acid in that regard since previous studies using the essential amino acids leucine and threonine showed a contribution of direct maternal to fetal flux of approximately 75 and 90%, respectively. The reduced net placental glucose utilization during the raised maternal plasma alanine concentration also demonstrates the adaptive character of placental metabolism.

Amino acid concentrations in growth restricted fetuses are significantly lower compared to those in normally grown fetuses (13, 16, 18). A shortage of one essential amino acid with adequate energy and protein equivalent intake leads to fetal growth restriction in pregnant rats (66). However, in light of possible future treatment of growth restriction, the increase in net uterine alanine uptake under conditions of a markedly elevated maternal alanine plasma concentration may be markedly less in growth restricted pregnancies because placental amino acid transport on both the fetal and maternal side, is mediated through amino acid transporters that have been shown to be markedly reduced on the placental surfaces of growth restricted fetuses (20, 58). Furthermore, amino acid transporters are semi-specific, which may lead to competitive inhibition within groups of amino acids when the plasma concentration of more than one amino acid concentration is artificially elevated.

A host of data has been collected regarding the hormonal changes leading up to ovine parturition. There is evidence that these hormonal changes are accompanied by changes in amino acid metabolism, especially with respect to glutamine and glutamate (4).

In late ovine gestation an interorgan cycling of glutamine and glutamate by the fetal liver and placenta has been demonstrated (93). Glutamine is delivered to the fetal liver by the umbilical vein. Within the fetal liver approximately half of the glutamine taken up is converted to glutamate (69). This is in agreement with the findings described in chapter 5. A fraction of the glutamate is released by the fetal liver into the fetal circulation from which most of the glutamate is taken up and oxidized by the placenta. For obvious reasons, no data is available on fetal hepatic metabolism of glutamine and glutamate in humans. However, placental uptake of glutamate from the fetal circulation has been demonstrated (34, 74).

Ovine parturition is initiated by a fetal cortisol surge (6, 49, 50). It is possible to induce ovine parturition by fetal administration of glucocorticoids (51). The mechanism by which normal human pregnancy is terminated is far less clear, due to the obvious limitations of human experimental studies. However, fetal cortisol is not thought to be the initiating factor in human parturition because no differences in mean gestational length were demonstrated between pregnancies with normal and those with anencephalic fetuses, as opposed to observations in sheep (37).

In dexamethasone-induced parturition a significant fall in net fetal hepatic release of glutamate was demonstrated (4). We suggested that the decrease in net fetal hepatic glutamate output under the influence of dexamethasone could be mediated by a redirection of glutamine carbon into the citric acid cycle and subsequent use for glycogenesis since glucocorticoids are known to stimulate glycogen deposition (5). In the experiments in late ovine gestation reported in chapter 5 using tracer methodology, we found that, first, nearly all of the glutamine taken up by the fetal liver is converted to glutamate. Second, that the fate of the glutamate is either release into the fetal circulation or further oxidation in the citric acid cycle. Third, that under the influence of dexamethasone net fetal hepatic glutamine uptake falls markedly, although net fetal glutamine uptake does not change significantly. Finally, the partitioning of the glutamate

within the fetal liver changes significantly in favor of oxidation in the citric acid cycle under the influence of dexamethasone.

It was not known if the hormonal changes in spontaneous parturition were accompanied by similar changes in fetal hepatic and placental amino acid metabolism. In the study presented in chapter 6 we found that also in spontaneous parturition net fetal hepatic glutamate release falls significantly. In dexamethasone-induced parturition this was accompanied by a marked increase in fetal plasma glutamine concentrations and a fall in net fetal hepatic glutamine uptake. In contrast, although there was a similar fall in net fetal hepatic glutamine uptake in spontaneous parturition, no changes in glutamine concentrations were detected. This difference may be explained by the fact that dexamethasone stimulates the release of glutamine and alanine from muscle tissue (77, 79). The reduction in net fetal hepatic uptake of glutamine was more unexpected, since earlier studies reported an increased uptake of glutamine by adult rat liver under the influence of dexamethasone, mediated through the system N transporter (25, 55). Different regulatory mechanisms of amino acid transporters in fetal and adult life have been described (32). The decrease in net fetal glutamine uptake may be a mechanism to reduce net fetal hepatic glutamate output.

Another difference between dexamethasone-induced and spontaneous parturition that became apparent from the studies presented in chapters 5 and 6 is the net fetal hepatic uptake of lactate. Lactate is a gluconeogenic precursor and it was expected that its net fetal hepatic uptake would increase under the influence of glucocorticoids. Indeed, net fetal hepatic lactate increased significantly in spontaneous parturition, but in dexamethasone-induced parturition no change in uptake could be demonstrated. One explanation could be that glycogen deposition took place at an earlier stage than our sampling time of 26 hours after the start of dexamethasone infusion, or that the dose and potency of the glucocorticoid administered determines whether stimulation or inhibition of uptake of gluconeogenic substrates occurs.

Further support for the hypothesis of a redirection of glutamine carbon into the citric acid cycle is the finding reported in chapter 7 of an increased activity of glutamate dehydrogenase, the enzyme converting glutamate to the citric acid cycle intermediate α ketoglutarate, found in ovine fetal liver tissue after 26 hours of continuous

dexamethasone administration. The increased activity of the enzyme was significantly correlated with its mRNA expression. However, the proportionally small increase as well as the wide range in enzyme activity in control and dexamethasone exposed fetal liver tissue, suggest that this is not a key enzyme in directing carbon into the citric acid cycle for gluconeogenesis.

In dexamethasone-induced as well as in spontaneous parturition net placental glutamate uptake was markedly reduced and significantly dependent on net fetal hepatic glutamate release. This was accompanied in spontaneous as well as in dexamethasone-induced parturition by a marked fall in placental progesterone output. Because the term placenta lacks an active pentosephosphate pathway, it has been postulated in the literature that glutamate oxidation is its only source for NADPH necessary for steroidogenesis (11, 42, 67). Whether the decrease in placental progesterone production is not solely mediated through induction of enzymes involved in steroidogenesis but also through placental glutamate availability needs to be clarified by future research.

SUMMARY

CHAPTER ONE presents a general introduction to the thesis. Placental amino acid metabolism has been shown to be important in determining fetal nutrient supply. In vivo experiments have reported altered placental amino acid metabolism and fetal amino acid concentrations in late gestation ovine pregnancy complicated by fetal growth-restriction. Therefore, understanding placental metabolism and its adaptive changes under different circumstances is important. This leads to the objectives of the studies reported in chapters three and four. The available data on fetal hepatic and placental glutamine and glutamate metabolism in late ovine gestation are discussed, with special emphasis on the changes known to occur with dexamethasone-induced parturition. This leads to the objectives dealt with in the chapters five, six and seven.

CHAPTER TWO describes the animal care and surgical techniques used to establish the chronically instrumented sheep preparation with fetal and maternal indwelling catheters, used for the experiments reported in the thesis.

CHAPTER THREE reports the uterine and umbilical uptakes of plasma amino acids measured simultaneously in eighteen singleton pregnant ewes at late gestation, for the purpose of establishing which amino acids are produced or utilized by the uteroplacental unit under normal physiological conditions, and at what rates. The branched chain amino acids (BCAA) had significantly greater uterine than umbilical uptakes. Net uteroplacental BCAA utilization was 8.0 ± 2.5 $\mu mol \cdot Kg_{fetus}^{-1} \cdot min^{-1}$, and represented 42% of the total BCAA utilization by fetus plus uteroplacental tissues. There was placental uptake of fetal glutamate (4.2 ± 0.3 $\mu mol \cdot Kg_{fetus}^{-1} \cdot min^{-1}$) and no uterine uptake of maternal glutamate. Umbilical uptake of glutamine was approximately 61% greater than uterine uptake, thus demonstrating net uteroplacental glutamine production of 2.2 ± 0.9 $\mu mol \cdot Kg_{fetus}^{-1} \cdot min^{-}$. In conjunction with other evidence, these data indicate rapid placental metabolism of glutamate, which is supplied in part by the fetus and in part produced locally via BCAA transamination. Most of the glutamate is oxidized and some is used to synthesize glutamine that is delivered to the fetus. There appeared to be net uteroplacental utilization of maternal serine, and umbilical uptake of glycine produced by

the placenta. Maternal serine utilization and glycine umbilical uptake were virtually equal $(3.14 \pm 0.50$ vs. 3.10 ± 0.46 $\mu mol \cdot Kg_{fetus}^{-1} \cdot min^{-1})$. This evidence supports the conclusion that the ovine placenta converts large quantities of maternal serine into fetal glycine.

CHAPTER FOUR presents the uterine and umbilical uptakes of alanine measured in 10 ewes in late gestation, before (control) and during (experimental) intravenous infusion of alanine, which significantly increased maternal arterial alanine concentration from 155 ± 14 to 629 ± 78 μM. In eight of these ewes, placental alanine fluxes were traced by constant intravenous infusion of $L-[3,3,3-D_3]$ alanine in the mother and $L-[1-^{13}C]$ alanine in the fetus. Rates are reported as $\mu mol \cdot min^{-1} \cdot Kg_{fetus}^{-1}$. Alanine infusion significantly increased uterine uptake $(2.5 \pm 0.6$ to $15.5 \pm 3.1)$, umbilical uptake $(3.1 \pm 0.5$ to 6.9 ± 0.8, $P < 0.001)$ and net uteroplacental utilization $(-0.6 \pm 0.8$ to $8.6 \pm 2.7)$ of alanine. Control alanine flux to fetus from mother $(R_{f,m})$ was much less than the alanine flux to fetus from placenta $(R_{f,p})$, $(0.17 \pm 0.04$ vs $5.0 \pm 0.6)$. Two additional studies utilizing $L-[U-^{13}C]$ alanine as the maternal tracer confirmed the small contribution of $R_{f,m}$ to $R_{f,p}$. During maternal alanine infusion, $R_{f,m}$ increased significantly but remained a small fraction of $R_{f,p}$ $(0.71 \pm 0.2$ vs $7.3 \pm 1.3)$. It is concluded that maternal alanine entering the placenta is metabolized and exchanged for placental alanine, so that most of the alanine delivered to the fetus is produced within the placenta. An increase in maternal alanine concentration increases placental alanine utilization as well as fetal uptake of both maternal and placental alanine.

CHAPTER FIVE explores the underlying mechanisms of the marked decrease in net fetal hepatic glutamate release and plasma glutamate concentration and the two to three fold increase in plasma glutamine and other glucogenic amino acids with dexamethasone- induced parturition in the fetal lamb. Hepatic amino acid uptake and conversion of $L-[1-^{13}C]$ glutamine to $L-[1-^{13}C]$ glutamate and $^{13}CO_2$ were measured in six term sheep fetuses, before and in the last 2 hr of a 26 hr fetal dexamethasone infusion. Dexamethasone significantly reduced net hepatic glutamine and alanine uptakes and hepatic glutamate output. Hepatic outputs of the glutamate $(R_{GLU, GLN})$ and CO_2 formed from plasma glutamine decreased significantly to 21% and 53% of control, respectively. $R_{GLU,GLN}$, expressed as a fraction of both outputs, decreased from 0.36 ± 0.02 to $0.18 \pm$

0.04. Hepatic glucose output remained virtually zero throughout the experiment. It is concluded that dexamethasone reduces net fetal hepatic glutamate output by increasing the routing of glutamate carbon into the citric acid cycle and by decreasing the net hepatic uptake of glucogenic amino acids.

CHAPTER SIX describes the changes in net fetal hepatic and placental glutamine-glutamate exchange accompanying the endocrine changes in spontaneous ovine parturition. During the five days prior to parturition net fetal hepatic glutamate as well as net placental glutamate uptake fell significantly, and the two were significantly correlated. Also net fetal hepatic glutamine uptake decreased markedly. Those changes in glutamine and glutamate metabolism are similar to those occurring in dexamethasone-induced parturition.

CHAPTER SEVEN explores the effect of dexamethasone on hepatic glutamate dehydrogenase in the fetal lamb. During dexamethasone-induced parturition in late gestation ewes, a significant change was observed in the partitioning of the glutamate within the fetal liver between release into the fetal circulation and oxidation in the citric acid cycle in favor of the latter. Glutamate dehydrogenase (GDH) is the enzyme converting glutamate into α ketoglutarate, a citric acid intermediate. The results of the study show that GDH activity doubles during dexamethasone infusion, accompanied by a marked increase in GDH mRNA expression. Therefore, the data is in agreement with the results in the in vivo studies.

CHAPTER EIGHT presents a general discussion of the results of the studies described in the thesis. There is no net placental amino acid utilization under physiological conditions, with the exception of the branched chain amino acids. However, the placenta has a high amino acid turnover rate, which becomes apparent when investigating the direct maternal-fetal flux for the non-essential amino acid alanine that accounts for only a small fraction of the alanine flux from placenta to fetus. Most of the alanine delivered to the fetus is formed within the placenta. Raising maternal plasma alanine concentration does increase fetal alanine delivery. However, most of the increase in uterine alanine uptake is utilized within the placenta itself, affecting the oxidation rate of other nutrients. This demonstrates the complexity of placental metabolism and

indicated one of the difficulties that may be encountered when attempting treatment of fetal growth restriction by altering maternal plasma amino acid concentration.

In dexamethasone-induced as well as in spontaneous parturition net fetal hepatic glutamine uptake and glutamate release fall markedly. This is accompanied by a significant decrease in placental glutamate availability and placental progesterone output. Under the influence of glucocorticoids glutamate is redirected in the fetal liver towards oxidation in the citric acid cycle rather than towards release into the fetal circulation. This observation supports the hypothesis that glutamate carbon is used for glycogen deposition. The redirection of glutamate carbon can be explained, at least in part, by an increase in mRNA expression and activity of the enzyme glutamate dehydrogenase.

SAMENVATTING

HOOFDSTUK EEN geeft een algemene inleiding tot het proefschrift. Het is gebleken dat placentair aminozuur metabolisme een belangrijke rol te speelt bij het reguleren van de aanvoer van voedingsstoffen naar de foetus. In vivo experimenten bij het laat-drachtige schaap hebben veranderingen aangetoond in het placentair aminozuur metabolisme en de foetale aminozuur concentraties in zwangerschappen gecompliceerd door foetale groei vertraging. Het is daarom van belang inzicht te krijgen in placentair aminozuur metabolisme en de adaptieve veranderingen die daarin plaatsvinden onder verschillende omstandigheden. Deze overwegingen leiden tot de doelstellingen beschreven in de hoofdstukken drie en vier. De beschikbare literatuur betreffende het foetale hepatische en placentaire glutamine en glutamaat metabolisme in het laat-drachtige schaap wordt beschreven, met speciale nadruk op de veranderingen die optreden bij de door middel van dexamethason-geinduceerde baring. Hieruit worden de doelstellingen afgeleid van het onderzoek beschreven in de hoofdstukken vijf, zes en zeven.

HOOFDSTUK TWEE beschrijft de verzorging en de chirurgische technieken toegepast voor het tot stand brengen van het chronisch geinstrumenteerde onderzoeks bij het drachtige schaapmodel met foetale en maternale vasculaire catheters. Dit model is gebruikt voor de experimenten beschreven in het proefschrift.

HOOFDSTUK DRIE presenteert de gelijktijdig bepaalde uteriene en umbilicale opname van plasma aminozuren in achttien laat-drachtige schapen, met het doel vast te stellen welke aminozuren worden geproduceerd of verbruikt door de placenta onder normale fysiologische omstandigheden, en met welke snelheid. De aminozuren valine, leucine en isoleucine hadden een significant grotere uteriene dan umbilicale opname. Het netto placentaire verbruik van deze aminozuren was $8.0 \pm 2.5 \ \mu mol \cdot Kg_{foetus}^{-1} \cdot min^{-1}$ en vertegenwoordigde 42% van het totale verbruik van deze aminozuren door de placenta en de foetus samen. Er was netto opname van foetaal glutamaat door de placenta zonder opname van maternaal glutamaat. De umbilicale opname van glutamine was 61% groter dan de uteriene glutamine opname. Dit betekent een placentaireglutamine productie van $2.2 \pm 0.9 \ \mu mol \cdot Kg_{foetus}^{-1} \cdot min^{-1}$. In samenhang met andere gegevens, wijzen deze resulten

op een snel placentair metabolisme van glutamaat wat deels wordt geleverd door de foetus en deels wordt verkregen door lokale productie van glutamaat via de transaminatie van valine, leucine en isoleucine. Het grootste deel van het glutamaat wordt geoxideerd en een deel wordt gebruikt voor de synthsese van glutamine dat wordt afgegeven aan de foetus. Er lijkt een netto verbruik te zijn van maternaal serine door de placenta en een umbilicale opname van, door de placenta geproduceerd, glycine. Het placentaire verbruik van maternaal serine en de umbilicale opname van glycine waren nagenoeg gelijk namelijk, 3.14 ± 0.50 versus 3.10 ± 0.46 $\mu mol \cdot Kg_{foetus}^{-1} \cdot min^{-1}$. Deze resultaten steunen de hypothese dat de placenta van het schaap grote hoeveelheden maternaal serine omzet in foetaal glycine.

HOOFDSTUK VIER beschrijft de uteriene en umbilicale opname van alanine bepaald in tien laat-drachtige schapen, voor (controle periode) en na (experimentele periode) een maternale infusie met alanine. De alanine infusie veroorzaakte een stijging van de maternale alamime van 155 ± 14 naar 629 ± 78 μM. In acht van deze ooien werden de placentaire fluxen bepaald door middel van een constante intraveneuse infusie van L-$[3,3,3$-D$_3]$ alanine in de moeder en L-$[1$-^{13} C] alanine in de foetus. Snelheden worden gegeven als $\mu mol \cdot Kg_{foetus}^{-1} \cdot min^{-1}$. Maternale alanine infusie ging gepaard met een significante verhoging van de uteriene alanine opname van 2.5 ± 0.6 naar 15.5 ± 3.1, de umbilicale alanine opname van 3.1 ± 0.5 naar 6.9 ± 0.8, en het netto placentaire verbruik van alanine van -0.6 ± 0.8 naar 8.6 ± 2.7. De alanine flux van de moeder naar de foetus($R_{f,m}$) in de controle periode was veel kleiner dan die van de placenta naar de foetus ($R_{f,p}$), (0.17 ± 0.04 vs 5.0 ± 0.6). Twee additionele experimenten waarbij L-[U-^{13}C] alanine werd gebruikt als maternale isotoop bevestigden het geringe aandeel van de $R_{f,m}$ in de $R_{f,p}$. Gedurende de maternale infusie van alanine nam de $R_{f,m}$ significant toe maar het bleef een klein deel van de $R_{f,p}$ (0.71 ± 0.2 vs 7.3 ± 1.3). Geconcludeerd moet worden dat het maternale alanine dat door de placenta wordt opgenomen, gemetaboliseerd en uitgewisseld wordt met placentair alanine, zodat het grootste deel van de alanine dat ter beschikking komt van de foetus geproduceerd is door de placenta. Een stijging van de maternale alanine concentratie doet het placentaire alanine verbruik toenemen samen, met de foetale opname van zowel maternaal als placentair alanine.

HOOFDSTUK VIJF beschrijft een onderzoek naar de mechanismen die ten grondslag liggen aan de afname van de glutamaat afgifte door de foetale lever en de twee- tot drievoudige stijging van de glutamine concentratie samen met een toename van de overige glucogene aminozuren in door middel van dexamethason geinduceerde baringen. De aminozuur opname door de foetale lever en de omzetting van L-[1-^{13}C] glutamine naar L-[1-^{13}C] glutamaat en $^{13}CO_2$ werd bepaald in zes foetale schapen, voor afgaand aan en na een 26 uur durende foetale dexamethason. Dexamethason verminderde de opname van glutamine en alanine en de glutamaat afgifte door de lever significant. De afgifte door de lever van glutamaat ($R_{GLU, GLN}$) en $^{13}CO_2$ afkomstig van plasma glutamine daalde significant tot respectievelijk 21% en 53% in vergelijking met de controle periode. $R_{GLU,GLN}$ als fractie van de beide afgiften verminderde van 0.36 ± 0.02 tot 0.18 ± 0.04. Gedurende het gehele experiment werd geen glucose afgifte door de lever aangetoond. Geconcludeerd wordt dat dexamethason de glutamaat afgifte door de foetale lever reduceert door de instroom van glutamine koolstof in de citroenzuur cyclus te verhogen en door de opname van glucogene aminozuren door de lever te verminderen.

HOOFDSTUK ZES presentteerde resultaten van onderzoek naar de veranderingen die optreden in de uitwisseling van glutamine en glutamaat tussen de foetale lever en de placenta in samenhang met de endocriene veranderingen bij de spontane baring bij het schaap. In de dagen voorafgaand aan de spontane baring nam de afgifte van glutamaat door de foetale lever significant af evenals de glutamaat opname door de placenta. De netto glutamaat opname door de placenta was afhankelijk van de afgifte van glutamaat door de foetale lever. De netto opname van glutamine door de foetale lever nam eveneens significant af. Deze veranderingen in glutamine en glutamaat metabolisme zijn identiek aan die welke optreden bij de met behulp van dexamethason geinduceerde baring.

HOOFDSTUK ZEVEN beschrijft het effect van dexamethason op glutamaat dehydrogenase (GDH) in de lever van het foetale lam. Tijdens de met behulp van dexamethason geinduceerde bevalling, werd een significante verschuiving waargenomen in de verdeling van glutamaat in de foetale lever, met toegenomen oxidatie in de citroenzuur cyclus ten koste van afgifte aan de foetale circulatie. Glutamaat dehydrogenase is het enzym dat glutamaat omzet in α- ketoglutaraat, een intermediair in de citroenzuur cyclus. De resultaten laten zien dat de activiteit van het enzym GDH

verdubbelt tijdens infusie met dexamethason met stijging van de expressie van GDH mRNA. Dit is in overeenstemming met de resultaten van de in vivo experimenten.

HOOFDSTUK ACHT geeft een algehele bespreking van de resultaten van de onderzoeken, die worden beschreven in het proefschrift. Onder normale fysiologische omstandigheden is er geen netto placentair verbruik van aminozuren, met als uitzondering de aminozuren valine, leucine en isoleucine. De placenta heeft echter een hoge turnover van aminozuren, wat duidelijk wordt wanneer men de directe moeder-foetus flux van het niet-essentiele aminozuur alanine, beschouwt. Hieruit blijkt namelijk dat deze een zeer klein aandeel levert aan de totale alanine flux van placenta naar foetus. Het significant verhogen van de maternale plasma concentratie van het niet-essentiele aminozuur alanine doet zowel de uteriene opname als de foetale toevoer van alanine toenemen. Het grootste deel van de alanine wordt echter door de placenta verbruikt, wat ook een duidelijk effect heeft op het placentaire verbruik van andere substraten. Hieruit komt het complexe karakter van het placentaire aminozuur metabolisme tot uiting en dit vormt een van de problemen bij eventuele behandeling van foetale groeivertraging door middel van het verhogen van de maternale aminozuurconcentratie.

Bij het schaap neemt zowel bij de door middel van dexamethasonge induceerde als bij de spontane baring de netto opname van glutamine en de afgifte van glutamaat door de foetale lever af. Dit gaat samen met een significante vermindering van de placentaire beschikbaarheid van glutamaat en van de placentaire progesteron productie. Onder invloed van glucocorticosteroiden wordt glutamaat in de foetale lever bij voorkeur geoxideerd in de citroenzuur cyclus ten koste van afgifte aan de foetale circulatie. Dit steunt de hypothese dat glutamaat koolstof wordt gebruikt voor glycogeen depositie. Deze herverdeling van glutamaat koolstof kan ten dele worden toegeschreven aan een toegenomen activiteit en mRNA expressie van het enzym glutamaat dehydrogenase.

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CURRICULUM VITAE AUCTORIS

1969	Born in Rotterdam
1982-1988	Atheneum, O.S.G. Walburg at Zwijndrecht
1988-1995	Medical School and Internships, Erasmus University Rotterdam
1995-1997	Research Fellow, Department of Perinatal Medicine, University of Colorado Health Sciences Center (Prof. F.C. Battaglia and Prof. G. Meschia)
1997-present	Resident in Obstetrics and Gynecology, Sint Franciscus Gasthuis te Rotterdam (Dr. A.T. Alberda), and University Hospital Rotterdam (Prof.dr. H.C.S. Wallenburg, Prof.dr. Th.J.M. Helmerhorst).