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Effect of dexamethasone on fetal hepatic glutamine-glutamate exchange

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[1]*Department of Obstetrics and Gynecology, Erasmus University, 3000 DR Rotterdam, The Netherlands; and* [2]*Division of Perinatal Medicine, Departments of Pediatrics, Pharmacology, and Physiology, University of Colorado Health Sciences Center, Denver, Colorado 80262*

Timmerman, Michelle, Cecilia Teng, Randall B. Wilkening, Paul Fennessey, Frederick C. Battaglia, and Giacomo Meschia. Effect of dexamethasone on fetal hepatic glutamine-glutamate exchange. *Am J Physiol Endocrinol Metab* 278: E839–E845, 2000.—Intravenous infusion of dexamethasone (Dex) in the fetal lamb causes a two- to threefold increase in plasma glutamine and other glucogenic amino acids and a decrease of plasma glutamate to approximately one-third of normal. To explore the underlying mechanisms, 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 sheep fetuses before and in the last 2 h of a 26-h Dex infusion. Dex decreased hepatic glutamine and alanine uptakes ($P < 0.01$) and hepatic glutamate output ($P < 0.001$). Hepatic outputs of the glutamate ($R_{Glu,Gln}$) and CO_2 formed from plasma glutamine decreased to 21 ($P < 0.001$) and 53% ($P = 0.009$) of control, respectively. $R_{Glu,Gln}$, expressed as a fraction of both outputs, decreased ($P < 0.001$) from 0.36 ± 0.02 to 0.18 ± 0.04. Hepatic glucose output remained virtually zero throughout the experiment. We conclude that Dex decreases fetal hepatic glutamate output by increasing the routing of glutamate carbon into the citric acid cycle and by decreasing the hepatic uptake of glucogenic amino acids.

fetus; amino acids; fetal liver; placenta

IN THE SHEEP FETUS, THE CONCENTRATION of plasma cortisol increases before parturition (2), and premature labor can be induced by infusing glucocorticoids into the fetal circulation (11). Glucocorticoids also induce several metabolic changes that are important for survival after birth, e.g., increased production of lung surfactant (5) and liver glycogen accumulation (2). A recent study has shown that the infusion of dexamethasone into the circulation of fetal lambs has a remarkable effect on fetal plasma amino acid concentrations (1). Several amino acids, e.g., glutamine and alanine, show a two- to threefold increase in concentration, whereas plasma glutamate decreases to approximately one-third of normal. By contrast, plasma serine concentration is virtually unchanged. Analysis of the mechanisms that underlie this complex response may enhance our understanding of the hormonal regulation of fetal amino acid metabolism in the perinatal period.

Previous experiments have demonstrated that, under normal physiological conditions, the fetal liver takes up glutamine from the fetal circulation (13, 19), that some of the glutamine taken up is converted to hepatic glutamate output (19), and that a large fraction of the glutamate delivered by the liver into the fetal circulation is taken up and oxidized by the placenta (14). These observations have led to the hypothesis that fetal hepatic glutamate output serves two important functions. The first is to dissociate glucogenic amino acid oxidation from glucogenesis, thus allowing the fetus to maintain simultaneously a low rate of glucose production and a high rate of amino acid oxidation (14). The second is to contribute to placental glutamate oxidation, which has been linked to placental NADPH production and progesterone synthesis (10). At parturition, these functions are no longer required; therefore, the decrease in fetal plasma glutamate caused by dexamethasone (Dex) infusion may be another example of glucocorticoids coordinating the metabolic changes that precede birth. The present study was designed to measure the effect of Dex on fetal plasma glutamine and glutamate disposal rates and on fetal hepatic glutamine uptake, as well as its conversion to glutamate and CO_2.

MATERIALS AND METHODS

Experimental Design

The studies were approved by the University of Colorado Health Sciences Center Animal Care and Use Committee. Six late-gestation mixed-breed Columbia-Rambouillet ewes, each pregnant with a single fetus, were studied. Surgery was performed under a combination of general pentobarbital (65 mg/ml) and spinal anesthesia (2 ml 1% pontacaine) after a 48-h fast with free access to water. Preoperatively, each ewe was given 500 mg of ampicillin and 500 mg of gentamycin intramuscularly. Polyvinyl catheters were inserted into the maternal femoral artery and into the uterine vein draining the pregnant horn, in the fetal aorta via the fetal pedal artery, the common umbilical vein, and one fetal brachial vein. A left hepatic venous catheter was placed through a right thoracotomy. Fetal muscular tone was reduced by an intravenous injection of pancuronium (0.3 mg). The catheter was guided into the left hepatic vein and anchored to its insertion site in the vena cava. An amniotic catheter was also placed for the

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injection of antibiotics. All catheters were tunneled subcutaneously to a pouch on the ewe's flank.

After surgery, the animals were allowed free access to water and alfalfa pellets. On the day of study (5–7 days after surgery), an infusate solution was prepared containing 100 mg of 99% enriched L-[2,3,3,4,4-^{2}H]glutamate or L-[^{5}D]glutamate, 400 mg of 99% enriched L-[1-^{13}C]glutamine (Cambridge Isotope Laboratories, Andover, MA), and 400 μCi of tritiated water (Amersham, Arlington Heights, IL). The L-[1-^{13}C]glutamine was purified before the study to remove any L-[1-^{13}C]-glutamate that might have been formed during storage. Two Bio-Rad econo-pac columns (1.5 × 14 cm) were packed 10 cm in height with 17 ml of Dowex 1 × 8 resin (CL-form, 200–400 mesh). The columns were washed with 20 ml of 10 mM imidazole buffer pH 7.6 kept on ice. L-[1-^{13}C]glutamine (400 mg) was dissolved in 10 ml of water, and the pH was adjusted to 7.6 with 1 N NaOH. The glutamine mixture was loaded onto the columns (5 ml each). The sample front was discarded. The glutamine was eluted three times with 5 ml of imidazole buffer, pH 7.6, and the total of 15 ml of buffer fractions was collected on ice and filtered through a 0.2-μm filter.

Study Protocol

The infusate was given via the fetal brachial vein. 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 were drawn simultaneously from the maternal femoral artery, uterine vein, fetal abdominal aorta, umbilical vein, and left hepatic vein. 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. The fetus was transfused with an equal volume of donor blood after each draw. After the last draw, the infusate was stopped, and 500 mg of ampicillin were administered via the amniotic catheter. The next day, water-soluble cyclodextrin-encapsulated dexamethasone (Sigma Chemical, St. Louis, MO) was dissolved in isotonic saline (1.4 mg Dex/100 ml); then, a bolus of 0.2 mg of Dex was given to the fetus, followed by a continuous infusion at 0.07 mg/h. After ~26 h of infusion, the same study protocol was repeated. After completion of this second study period, the ewe was euthanized. Fetal, placental, uterine, and fetal liver weights were obtained, and catheter positions were verified.

Analytical Measurements

Hemoglobin and O_2 saturation were measured spectrophotometrically (OSM-3, Radiometer, Copenhagen, Denmark). The blood O_2 content was calculated as the product of hemoglobin content, expressed as O_2 capacity times the O_2 saturation. Glucose and lactate concentrations were measured in duplicate with a glucose-lactate analyzer (YSI model 2700 Select and Dual Standard). Plasma $^{3}H_2O$ was measured on triplicate aliquots in a scintillation counter and converted to blood $^{3}H_2O$ on the basis of the hematocrit measurement (18). Respiratory gas tensions and pH were measured with an ABL330 Radiometer. Total CO_2 concentration in blood was calculated by using the Henderson-Hasselbach equation and a nomogram from Van Slyke and Sendroy (17). $^{13}CO_2$ enrichment was measured in samples of 0.5 ml of whole blood, which were collected in exutainers (Metabolic Solutions) and analyzed by means of tandem gas chromatography-isotope radio mass spectrometry.

Progesterone was measured using a validated (3) Coat-a-count progesterone RIA kit (Diagnostic Product, Los Angeles, CA). Inter- and intra-assay coefficients of variation (CV, %) for the low-quality control were 9.23 and 6.02%, respectively, and for the high-quality control were 6.69 and 3.44%, respectively.

Plasma samples for amino acid concentrations were stored at −70°C until the day of analysis. At that time, the samples were thawed quickly and deproteinized with 15% sulfosalicylic acid containing 300 μM norleucine as internal standard. The pH was adjusted to 2.2 with 1.5 N LiOH. After centrifugation, the supernatant was analyzed with a Dionex HPLC amino acid analyzer (Dionex, Sunnyvale, CA). The same column was used for all samples from an individual animal. Reproducibility within the same column was ±2%. Samples from all vessels drawn simultaneously were loaded to run within 12 h. Amino acid absorbencies were measured after reaction with ninhydrin at 570 nm, except proline, which was measured at 440 nm wavelength.

For mass spectrometry, amino acids were first separated on ~0.2 ml of AG-50 × 8 (BioRA mesh 100–200) cation exchange resin. 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 the resin was washed, the amino acids were eluted with 2 × 500 μl of NH_4OH and lyophilized. *Tert*-butyldimethylsilyl ether derivatives were formed by adding 200 μl acetonitrile containing 15% *N*-methyl-*N*(*t*-butyldimethylsilyl)trifluoroacetamide and 1.5% *t*-butyldimethylchlorosilane and heating at 100°C for 30 min. Tandem gas chromatography-mass spectrometry 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 (velocity, 40 cm/s). The selected conditions for glutamate and glutamine were an injection port and T-line temperature of 280°C, and oven temperature programmed from 100 to 320°C at a rate of 10°C/min. This resulted in a glutamate *m*-57 peak at ~13 min and a glutamine *m*-57 peak at ~14 min. The ion clusters monitored were mass-to-charge ratios (*m*/*z*) 432, 433, 434, 435, 436, and 437 for glutamate and 431, 432, 433, 434, 435, and 436 for glutamine. The enrichments were calculated by using the increase in the isotope peaks after correction for the natural abundance.

Calculations

A schematic drawing of the fetoplacental circulation showing the position of the fetal catheters has been published (19). Reference to this drawing clarifies the rationale of the following calculations.

Blood flows and uptakes. Umbilical blood flow (Q_f) was measured by application of the steady-state transplacental diffusion method, using tritiated water as the flow indicator (18). The umbilical uptakes of oxygen, glucose, and lactate were calculated by application of the Fick principle

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

where γ and α refer to umbilical venous and umbilical arterial blood concentrations, respectively.

Umbilical uptakes of amino acids were calculated using plasma flows and plasma concentrations as follows

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

where Ht is the fractional hematocrit. The use of plasma flows is based on evidence 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 (4).

Hepatic uptake of metabolic substrates was normalized for hepatic O_2 uptake by calculating the substrate to O_2 uptake molar ratio. This required calculation of the ratio

$$(h_i - h) \div (h_i - h)_{O_2}$$

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 >80% contribution (6). In each fetus, the fractional contribution of umbilical venous blood (F_V) to total left hepatic blood flow was calculated (19) as

$$F_v = (C_{T,H} - C_{T,\alpha}) \div (C_{T,\gamma} - C_{T,\alpha})$$

where $C_{T,h}$, $C_{T,\alpha}$, and $C_{T,\gamma}$ represent the tritiated water concentrations (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

$$C_{hi} = (F_v \times C_\gamma) + (F_a \times C_\alpha)$$

where F_a is the fractional arterial contribution to left hepatic flow ($F_a = 1 - F_v$), 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

$$\text{AA uptake} \div O_2 \text{ uptake} = \{[(C_{hi} - C_h)_{AA}] \times (1 - Ht)]\} \div (C_{hi} - C_h)_{O_2}$$

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

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

where MPE_α is the fetal arterial plasma molar percent 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 concentrations were calculated as total plasma concentrations times their respective MPEs divided by 100.

Hepatic conversion of plasma glutamine to hepatic glutamate and CO_2 output. The hepatic output of glutamate formed from plasma glutamine ($R_{Glu,Gln}$), expressed as a substrate output-to-O_2 uptake ratio, was calculated by 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

$$R_{Glu,Gln} = 100 \times (C_h - C_{hi})_{13\text{-}Glu} \times (1 - Ht) \div (C_{hi} - C_h)_{O_2} \div (MPE_{hi})_{13\text{-}Gln}$$

The output of CO_2 formed from C-1 of plasma glutamine ($R_{CO_{2,Gln}}$), was similarly calculated, using the whole blood concentration difference of $^{13}CO_2$, $(C_h - C_{hi})_{^{13}CO_2}$

$$R_{CO_2,Gln} = 100 \times (C_h - C_{hi})_{13\text{-}CO_2} \div (C_{hi} - C_h)_{O_2} \div (MPE_{hi})_{13\text{-}Gln}$$

Statistics

Differences between study periods were tested using Student's *t*-test for paired samples. Two-sided *P* values were considered significant at $P < 0.05$. Linear regressions were calculated by the least squares method.

RESULTS

Table 1 presents mean gestational age, fetal, placental, and fetal liver weights, 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 Dex infusion. In addition, there was an increase in uterine blood flow that was accompanied by an increase in umbilical venous oxygen saturation. The fractional contribution of umbilical venous blood to left hepatic blood flow (F_v) 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 Dex infusion, there were significant increases in the concentrations of maternal plasma glucose, fetal plasma glucose, and lactate. Concomitant with these changes, there was a significant increase in umbilical lactate uptake. Hepatic glucose uptake was virtually zero in both the control and the experimental period, and hepatic lactate uptake did not increase significantly (Table 2).

Amino Acid Concentration, Uptakes, and Fluxes

Dex infusion caused a large increase in fetal plasma glutamine (Table 3). In two of the six fetuses, the

Table 1. *Gestational age, fetal, fetal hepatic and placental weights, umbilical and uterine blood flows, fetal hematocrits, blood O_2 capacities and saturations, O_2 content differences across umbilical and left hepatic circulations, and umbilical and uterine O_2 uptakes in 6 sheep before and during fetal dexamethasone infusion*

Gestational age, days	127 ± 1		
Fetal weight, g	2,851 ± 81		
Fetal liver weight, g	102 ± 10		
Placental cotyledons weight, g	333 ± 31		
	Control	Dexamethasone	*P*
Blood flows, ml·min^{-1}·kg fetus^{-1}			
Umbilical	250 ± 20	221 ± 25	NS
Uterine	479 ± 53	573 ± 48	0.03
Blood hematocrit, %	32 ± 2	35 ± 2	0.02
Blood O_2 capacity, mM	5.46 ± 0.3	5.97 ± 0.3	<0.001
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 ± .13	0.014
Across left hepatic lobe circulation	0.44 ± .05	0.60 ± .04	0.002
O_2 uptakes, μmol·min^{-1}·kg fetus^{-1}			
Umbilical	353 ± 13	373 ± 25	NS
Uterine	668 ± 47	613 ± 44	NS

Values are means ± SE. NS, not significant.

Table 2. *Glucose and lactate concentrations and uptakes in six sheep before and during fetal dexamethasone infusion*

	Control	Dexamethasone	*P*
Plasma glucose concentrations, mM			
Maternal artery	4.26 ± .11	5.24 ± .05	<0.001
Umbilical artery	1.25 ± .08	2.27 ± .19	<0.001
Umbilical vein	1.43 ± 0.8	2.45 ± .17	<0.001
Fetal left hepatic vein	1.42 ± .07	2.45 ± .18	<0.001
Plasma lactate concentrations, mM			
Umbilical artery	2.22 ± .29	4.50 ± .34	<0.005
Umbilical vein	2.31 ± .31	4.71 ± .34	<0.005
Fetal left hepatic vein	2.15 ± .29	4.37 ± .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 ± .03	−0.01 ± .03	NS
Lactate	0.36 ± .07	0.47 ± .04	NS

Values are means ± SE.

glutamine increase was very large and could not be measured under the conditions that allowed chromatographic analysis of all the other amino acids. Concomitant with the increase in plasma glutamine, there was a significant increase in the disposal rate of plasma glutamine, a decrease in hepatic glutamine uptake, and no significant change in umbilical glutamine uptake (Table 3).

In contrast to glutamine, the fetal plasma concentration of glutamate decreased significantly. This decrease was accompanied by a decrease in the plasma glutamate disposal rate and a decrease in both hepatic glutamate output and placental uptake of fetal glutamate (Table 3). The hepatic output and the placental uptake of glutamate were significantly correlated (r = 0.87).

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. 1). 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-to-glutamine enrichment ratios were 0.30 ± 0.02 and 0.35 ± 0.05 in the control and the experimental periods, respectively. These two ratios were not significantly different ($P > 0.1$). The infusion of [^{5}D]glutamate produced a very small deuterium enrichment of plasma glutamine. The arterial glutamine-to-glutamate enrichment ratio was 0.02 ± 0.007 in the control period and decreased significantly ($P < 0.05$) to 0.007 ± 0.002 during Dex infusion.

There was no detectable difference in plasma glutamine enrichment between left hepatic input and hepatic vein, during both the control and experimental periods, 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 derived from the C-1 carbon of glutamine ($R_{CO_2,Gln}$). These two outputs accounted for virtually all of the glutamine uptake, i.e., the ($R_{Glu,Gln} + R_{CO_2,Gln}$) sum was not significantly different from hepatic glutamine uptake. Both $R_{Glu,Gln}$ and $R_{CO_2,Gln}$ decreased significantly in response to Dex (Table 3). However, the decrease in glutamate output was relatively greater than the decrease in CO_2 output. The contribution of $R_{Glu,Gln}$ to the combined ($R_{Glu,Gln} + R_{CO_2,Gln}$) output was 36 ± 2% in the control period and decreased significantly to 18 ± 4% in the experimental period, (P 0.001) (Table 3).

Several neutral amino acids shared the response of glutamine to Dex infusion. Eight amino acids (threonine, asparagine, proline, glycine, alanine, isoleucine,

Table 3. *Fetal glutamine and glutamate concentrations, uptakes, and fluxes before and during fetal dexamethasone infusion*

	Control	Dexamethasone	*P*
Glutamine			
Plasma concentrations (μgM)			
Umbilical artery (α)	431 ± 22	1,151 ± 129*	0.011
Umbilical vein (γ)	477 ± 25	1,203 ± 124*	0.011
Left hepatic input (h_i)	476 ± 25	1,203 ± 124*	0.011
Left hepatic vein (h)	381 ± 32	1,152 ± 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, μmol/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, μmol/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 ($R_{CO_2,Gln}$)	95 ± 14	50 ± 4	0.009
$R_{Glu,Gln} \div (R_{CO_2,Gln} + R_{Glu,Gln})$	0.36 ± 0.02	0.18 ± 0.04	0.001

Values are means ± SE; *n = 4. $R_{Glu,Gln}$, hepatic output of glutamate (Glu) formed from glutamine (Gln); $R_{CO_2,Gln}$, hepatic output of CO_2 derived from C-1 carbon of glutamine.

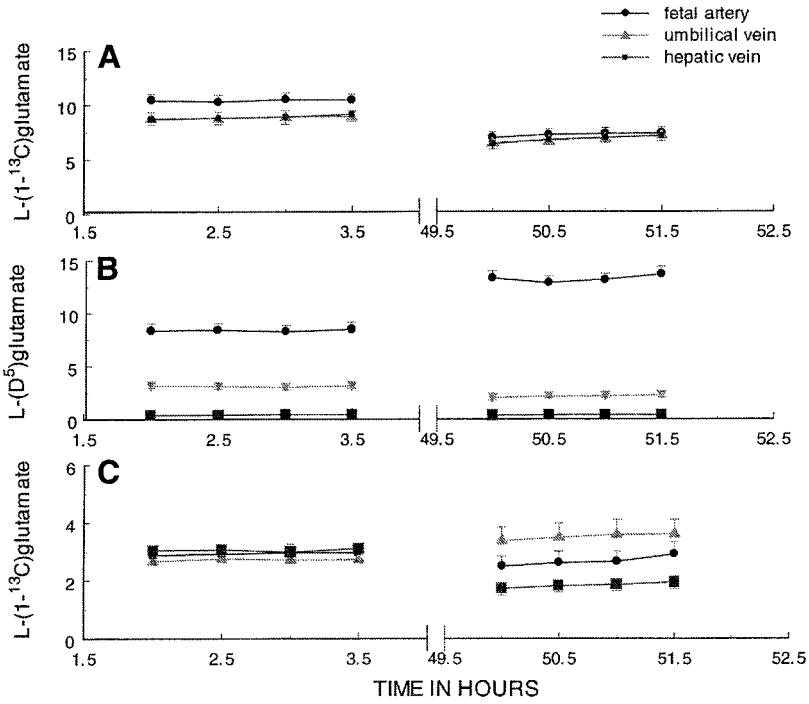

Fig. 1. Fetal plasma enrichments of L-[1-^{13}C]glutamine (*A*), L-[^{5}D]glutamate (*B*), and L-[1-^{13}C]glutamate (*C*) during both study days. Enrichments on the *y*-axis are in units of molar percent enrichment.

tyrosine, and phenylalanine) demonstrated a significant increase in plasma concentration, and three of these (threonine, glycine, and alanine) also demonstrated a significant decrease in hepatic uptake (Table 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 in concentration and hepatic output (Table 4).

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 Dex infusion from 21 ± 3 to 3 ± 2 nmol·min^{-1}·kg fetus^{-1}.

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 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: some is excreted into the fetal circulation, and some is routed into the citric acid cycle via deamination to α-ketoglutarate and subsequent oxidative decarboxylation to form succinyl-CoA. It also

Table 4. *Fetal arterial concentrations and umbilical and hepatic uptakes of amino acids in the control period (C) and during fetal dexamethasone infusion (D)*

	Plasma Concentration, μM		Umbilical Uptake, μmol·min^{-1}·kg fetus^{-1}		Hepatic Uptake, μmol/mmol O_2	
	C	D	C	D	C	D
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

Values are means ± SE. *$P < .05$; †$P < .01$; ‡$P < .001$.

implies that the direct flux of plasma glutamine into hepatic protein synthesis is relatively small in comparison with the other routes of hepatic disposal.

Dexamethasone infusion caused a decrease in the fetal hepatic glutamate output-to-oxygen uptake ratio to 35% of control (Table 3). Although some of this change could be due to an increase in hepatic oxygen uptake, it is important to note that the decrease in the ratio was accompanied by comparable percent decreases in fetal arterial glutamate concentration and in the placental uptake of fetal glutamate, as well as a significant ($P < 0.001$) decrease in the fetal plasma glutamate disposal rate from 9.6 ± 0.8 to 5.6 ± 0.5 $\mu mol \cdot min^{-1} \cdot kg\ fetus^{-1}$ (Table 3). Therefore, we can conclude that the decrease in the hepatic glutamate output-to-oxygen uptake ratio represents primarily a decrease in hepatic glutamate output.

Knowledge that glucocorticoids increase the activity of hepatic glucogenic enzymes in the fetal lamb (2) leads to the hypothesis that dexamethasone decreases fetal hepatic glutamate output because the glutamate produced by the liver is diverted into the glucogenic pathway. More specifically, it leads to the hypothesis that a larger fraction of the glutamate formed from the hepatic glutamine uptake enters the citric acid cycle. The present study agrees with this hypothesis by showing that dexamethasone decreased significantly the $R_{Glu,Gln}$-to-($R_{Glu,Gln} + R_{CO_{2,Gln}}$) ratio from 0.36 ± 0.02 to 0.18 ± 0.04 (Table 3).

The study also establishes that a decrease in the hepatic uptakes of glutamine and alanine was an additional mechanism for the decreased hepatic glutamate output (Tables 3 and 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 declined from 20 to 4% instead of decreasing to the 11% predicted by a constant hepatic glutamine-to-oxygen uptake ratio. Similarly, alanine extraction declined from 17 to 2% instead of decreasing to 8%. Because 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, ($R_{CO_{2,Gln}}$), to decrease significantly to 53% of control and that the change in the ($R_{Glu,Gln} + R_{CO_{2,Gln}}$) sum was approximately equal to the estimated change in hepatic glutamine uptake (−87 vs. −97 μmol/mmol oxygen). Therefore, a decrease in fetal hepatic glutamine uptake is confirmed by the decrease in the combined output of its two major metabolic products.

The finding that dexamethasone decreases 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 (16). In rats, dexamethasone stimulates sodium-dependent glutamine uptake via system N transporters by cultured hepatocytes and by sinusoidal membrane vesicles isolated from liver homogenates (7). Developmental changes in the effect of dexamethasone on amino acid transport and metabolism have been previously noted. In newborn babies (15, 20) and in newborn rats (8), dexamethasone infusion causes a marked increase in the plasma concentration of several amino acids, glutamine and alanine included, whereas in adult men (21), it causes no significant concentration changes, with the exception of a small increase in alanine. In contrast to hepatocytes isolated from adult rats, fetal rat hepatocytes do not respond to dexamethasone with an increase in system A-mediated transport (9). In vivo data do not provide the detailed information that is needed to establish whether 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, we cannot exclude the possibility that the dexamethasone effect on hepatic amino acid uptake was indirect via some other hormonal or metabolic change caused by the dexamethasone infusion. At the time the measurements were made, i.e., 26 h after starting dexamethasone infusion, there was no evidence for the net increase in either hepatic lactate uptake and/or glucogenic amino acid carbon uptake that one would expect if the hormone stimulated rapid glycogen deposition (2). We cannot exclude the possibility that glycogen had been accumulating 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. The rationale for studying the effect of dexamethasone at 26 h was based on a previous study (1) showing that 3 h after starting the dexamethasone infusion (0.2 mg bolus followed by 0.07 mg/h), there were no significant changes in fetal plasma glutamine, glutamate, glucose, and lactate concentrations, whereas marked changes in the concentrations of these metabolites were present at 25 h. The dose of dexamethasone used in the present and previous studies was selected because it may produce some of the metabolic changes associated with parturition; it decreases progesterone output by the pregnant uterus and it has been shown to induce premature labor ~56 h after the start of the infusion.

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 increased interorgan exchange. In dogs, dexamethasone increases glutamine release by skeletal muscle and glutamine uptake by gut and kidney (16).

In sheep, glucocorticoids play an important role in stimulating the metabolic changes that initiate parturition and adapt the lamb to postnatal life (2, 5, 11). Decreasing the glutamate supply to the placenta and diverting glutamate carbon into glucogenesis may represent additional aspects of this role. During parturition, glucocorticoids cause a decrease in progesterone synthesis by the placenta. This change may entail a

decrease in glutamate oxidation by placental mitochondria (10) and a decrease in placental glutamate requirements. It would appear that glucocorticoids serve the dual function of reducing placental glutamate requirements and the supply of glutamate to the placenta by the fetal liver.

Stimulation of fetal hepatic glucogenesis by glucocorticoids is indicated by the findings that cortisol increases the activity of hepatic glucogenic enzymes and hepatic glycogen storage (2) and that dexamethasone diverts glutamate carbon from hepatic output to flux into the citric acid cycle. However, dexamethasone infusion into the fetus decreases the hepatic uptake of glucogenic amino acids and does not induce hepatic glucose output. The functional meaning of these seemingly contradictory effects is obscure. Studies of fetal amino acids and carbohydrate metabolism during spontaneous parturition are needed for a better understanding of the relevance of fetal glucocorticoid infusion experiments to normal physiology. It should be noted that a glucocorticoid, betamethasone, is used in pregnant patients to stimulate pulmonary maturation in the human fetus. Although the original usage had been confined to a single dose given shortly before anticipated delivery, currently it may be given in repeated doses some weeks before delivery. We hypothesize that the changes in fetal glucogenic amino acid concentration and metabolism found in the present study might also occur in this clinical situation.

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