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Timmerman, Michelle, Misoo Chung, Randall B. Wilkening, Paul V. Fennessey, Frederick C. Battaglia, and Giacomo Meschia. Relationship of fetal alanine uptake and placental alanine metabolism to maternal plasma alanine concentration. *Am. J. Physiol.* 275 (*Endocrinol. Metab.* 38): E942–E950, 1998.—Uterine and umbilical uptakes of alanine (Ala) were measured in 10 ewes before (control) and during intravenous infusion of Ala, which increased maternal arterial Ala concentration from 115 ± 14 to 629 ± 78 μM ($P < 0.001$). In 8 of these ewes, placental Ala fluxes were traced by constant intravenous infusion of L-[3,3,3-$^2H_3$]Ala in the mother and L-[1-^{13}C]Ala in the fetus. Rates are reported as micromoles per minute per kilogram fetus. Ala infusion increased uterine uptake (2.5 ± 0.6 to 15.6 ± 3.1, $P < 0.001$), umbilical uptake (3.1 ± 0.5 to 6.9 ± 0.8, $P < 0.001$), and net uteroplacental utilization (−0.7 ± 0.8 to 8.6 ± 2.7, $P < 0.01$) of Ala. Control Ala flux to fetus from mother ($R_{f,m}$) was much less than the Ala flux to fetus from placenta ($R_{f,p}$) (0.17 ± 0.04 vs. 5.0 ± 0.6). Two additional studies utilizing L-[U-^{13}C]Ala as the maternal tracer confirmed the small relative contribution of $R_{f,m}$ to $R_{f,p}$. During maternal Ala infusion, $R_{f,m}$ increased significantly ($P < 0.02$) but remained a small fraction of $R_{f,p}$ (0.71 ± 0.2 vs. 7.3 ± 1.3). We conclude that maternal Ala entering the placenta is metabolized and exchanged for placental Ala, so that most of the Ala delivered to the fetus is produced within the placenta. An increase in maternal Ala concentration increases placental Ala utilization and the fetal uptake of both maternal and placental Ala.

alanine turnover; amino acids; umbilical uptake; alanine fluxes

PLACENTAL TRANSPORT of amino acids is a complex process in which specificity, activity, and location of amino acid transporters within the placenta (17) 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 (12).

There are no studies on the role of placental metabolism in determining the supply of alanine to the fetus. In sheep, alanine is transported to the pregnant uterus (3, 7) and from the placenta to the fetus (2, 6, 10). 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 (9). However, the increase in fetal concentration was only 12% of the increase in maternal concentration, and there was no attempt 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 on placental and fetal alanine uptake and utilization?

METHODS

Biological Preparation

Twelve Columbia-Rambouillet ewes pregnant with a singleton fetus were studied. Surgery was performed at 120–128 days of gestation after a 48-h fast with free access to water. Anesthesia and surgery were performed as previously described (14). Polyvinyl 20-gauge catheters were inserted in the maternal femoral artery and vein and in the uterine veins draining each uterine horn, fetal pedal artery and vein, and the common umbilical vein. An amniotic catheter was also placed for the injection of antibiotics. All catheters were tunneled subcutaneously to a pouch on the ewe's flank.

Antibiotics were given pre- and postoperatively, and analgesics were given during the first postoperative day. All catheters were flushed daily with a solution of heparinized saline.

At least five days were allowed for full recovery, as assessed by normal O_2 content and glucose concentration in the fetal circulation and normal food intake. The ewes were provided with water, alfalfa pellets, and salt ad libitum.

Experimental Design

The following experimental protocol was used to study 8 sheep at 127–134 days of gestation. Maternal and fetal blood samples were drawn for tritium and alanine enrichment blanks. Then, a fetal infusion of L-[1-^{13}C]alanine (0.83 ± 0.09 μmol·min^{-1}·kg fetus^{-1}) and tritiated water (0.22 μCi·min^{-1}·kg fetus^{-1}) was started via the pedal vein. Simultaneously, a maternal infusion of L-[3,3,3-$^2H_3$]alanine (CIL,

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Andover, MA; 0.17 ± 0.02 μmol·min^{-1}·kg mother^{-1}) was started.

At steady state, four sets of samples were collected simultaneously from the maternal artery, uterine veins, fetal artery, and the common umbilical vein at ~120, 150, 180, and 210 min from the start of the infusions. Each sampling represented the loss of ~10 ml of fetal blood. Fetal blood loss was corrected by transfusing the fetus, between sampling sets, 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-$^2H_3$]alanine and reagent-grade L-alanine (1,000 μmol/ml) to raise maternal alanine concentration four- to fivefold. At steady state, 2 h after the start of the new maternal infusate, four sets of blood samples were collected at ~330, 360, 390, and 420 min from the beginning of the study by following the same procedure used in collecting the control samples. This part of the experiment is referred to as the experimental period. All samples were analyzed for hemoglobin, hematocrit, O_2 saturation, glucose, lactate, tritiated water, amino acid concentrations, and plasma alanine enrichments. Maternal and fetal arterial samples were analyzed for plasma insulin.

After collection of the last set of samples, the ewe and fetus were euthanized by intravenous injection (Sleepaway, Fort Dodge, IA). Necropsy was performed to obtain fetal, placental, and uterine weights. To help in the interpretation of the tracer data generated by these experiments, four additional animals were studied. In two of these, an identical protocol was followed, with the exception that L-[3,3,3-$^2H_3$]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 mother.

Analytic Methods

Hemoglobin concentration and O_2 saturation were measured spectrophotometrically (OSM-3, Radiometer, Copenhagen, Denmark). The blood O_2 content was calculated from the hemoglobin concentration expressed as O_2 capacity and multiplied by 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 ^3H_2O was measured on triplicate aliquots in a scintillation counter and converted to blood ^3H_2O on the basis of the hematocrit measurement (19). Plasma insulin concentrations were determined using the RAT insulin RIA kit (Linco Research, St. Charles, MO). 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 O.3 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 had a mean value of ±2%. Samples from all vessels drawn simultaneously were loaded to run within 12 h. Amino acid absorbances were measured after reaction with ninhydrin at 570 nm except glutamate, which was measured at a wavelength of 440 nm.

For mass spectrometry, amino acids were first separated on 0.2-ml AG50 cation exchange resin (Bio-Rad Mesch 100–200). Plasma (0.2 ml) was mixed with 300 μl 30% acetic acid (Fisher Scientific, Pittsburgh, PA) to form zwitter ions and was applied to the column. After the resin was washed with 2 ml of distilled water, the amino acids were eluted with 750 μl NH_4OH and lyophilized. Tri-*t*-butyldimethylsilyl derivatives were formed with 200 μl of 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 was performed on a Hewlett-Packard HP-5790 gas chromatographer with a 30-m DB-17 0.025-mm-ID 0.25 μm film thickness 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 mass-to-charge ratios 260, 261, 262, and 263.

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 with tritiated water (19). The uterine and umbilical uptakes of O_2, glucose, and lactate were calculated by application of the Fick principle

$$\text{uterine uptake} = Q_m(\text{a-v})_{\text{blood}} \quad (1)$$

$$\text{umbilical uptake} = Q_f(\gamma\text{-}\alpha)_{\text{blood}} \quad (2)$$

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

$$\text{uterine alanine uptake} = Q_m(1 - H_m)(\text{a-v})_{\text{plasma}} \quad (3)$$

$$\text{umbilical alanine uptake} = Q_f(1 - H_f)(\gamma\text{-}\alpha)_{\text{plasma}} \quad (4)$$

where H_m and H_f represent the maternal and fetal fractional hematocrits, respectively. *Equations 3* and *4* 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. This observation is supported by observations in vivo (8) and by measurements of amino acid fluxes between red cells and plasma in vitro (21).

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

$$\text{MPE} = [100(r_s - r_o)] \div [1 + (r_s - r_o)] \quad (5)$$

Disposal rates. The maternal plasma alanine disposal rate (DR_m) was calculated as follows

$$DR_m = \left(\frac{100}{{}^m\text{MPE}_a} \cdot {}^mC \cdot {}^mI\right) - {}^mC \cdot {}^mI \quad (6)$$

where ${}^m\text{MPE}_a$ is the maternal arterial plasma enrichment of the maternally infused tracer at steady state, ${}^mC$ is the concentration of the tracer in the maternal infusate (μmol/ml), and ${}^mI$ is the infusion rate of the maternal infusate (ml/min).

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

$$DR_f = \left(\frac{100}{{}^f\text{MPE}_\alpha} \cdot {}^fC \cdot {}^fI\right) - {}^fC \cdot {}^fI \quad (7)$$

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 (μmol/ml), and ^{f}I is the infusion rate of the fetal infusate (ml/min). We note that *Eqs. 6* and *7* do not include the disposal rate of the naturally occurring isotopes (13).

Because the DR_m and DR_f calculations are based on steady-state measurements, they also represent estimates of the entry rate (i.e., 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 maternal tracer concentration differences across the uterine circulation [$(a\text{-}v)_{mat.\ tracer}$] 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 uteroplacenta from the maternal circulation ($R_{UP,m}$)

$$R_{UP,m} = [(a\text{-}v)_{mat\cdot tracer} \cdot \text{uterine plasma flow}] \div 0.01\ {}^{m}MPE_a \quad (8)$$

The fetal tracer concentration differences across the umbilical circulation, $[(\alpha\text{-}\gamma)_{fetal\ tracer}]$ 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 ($R_{P,f}$)

$$R_{P,f} = [(\alpha\text{-}\gamma)_{fetal\ tracer} \cdot \text{umbilical plasma flow}] \div 0.01\ {}^{f}MPE_\alpha \quad (9)$$

Fetal alanine disposal rate and the fetal-to-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 ($R_{f,m}$)

$$R_{f,m} = DR_f \cdot ({}^{m}MPE_\alpha \div {}^{m}MPE_a) \quad (10)$$

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

$$R_{f,P} = R_{P,f} + \text{umbilical alanine uptake} \quad (11)$$

Statistics

The data were analyzed using the Statistical Analysis System program (SAS Institute, Cary, NC). Each sheep provided two averaged data points, control and experimental, for each variable. All data presented in the tables are expressed as sample means ± SE for both study periods. 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 (22). This program gave the same average line as the line that was calculated by averaging each slope and intercept for all paired lines.

RESULTS

Table 1 presents mean gestational age, fetal and placental weights, 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.

Table 1. *Maternal and fetal hematocrits, O_2 capacities, arterial O_2 saturations, glucose and lactate concentrations, uterine and umbilical blood flows, and O_2, glucose, and lactate uptakes in 10 ewes before (control) and during (experimental) maternal alanine infusion*

	Control	Experimental	*P*
Hematocrit, %			
Maternal	32 ± 1	32 ± 1	NS
Fetal	37 ± 1	37 ± 1	NS
Hemoglobin (O_2 capacity), mM			
Maternal	6.1 ± 0.2	6.0 ± 0.2	NS
Fetal	6.5 ± 0.2	6.4 ± 0.2	NS
Arterial O_2 saturation, %			
Maternal	94.8 ± 0.6	94.5 ± 0.7	NS
Umbilical	54.3 ± 2.9	48.1 ± 3.4	<0.01
Arterial plasma glucose, mM			
Maternal	3.71 ± 0.06	4.01 ± 0.07	0.001
Fetal	0.99 ± 0.07	1.06 ± 0.07	<0.01
Arterial plasma lactate, mM			
Maternal	0.59 ± 0.06	0.63 ± 0.05	NS
Fetal	1.76 ± 0.06	1.80 ± 0.09	NS
Blood flow, ml·min^{-1}·kg fetus^{-1}			
Uterine	595 ± 52	551 ± 46	NS
Umbilical	212 ± 12	216 ± 16	NS
O_2 uptake, μmol·min^{-1}·kg fetus^{-1}			
Uterine	596 ± 28	540 ± 30	NS
Umbilical	360 ± 11	375 ± 14	NS
Glucose uptake, μmol·min^{-1}·kg fetus^{-1}			
Uterine	90 ± 8	82 ± 10	NS
Umbilical	40 ± 3	46 ± 3	<0.01
Lactate uptake, μmol·min^{-1}·kg fetus^{-1}			
Uterine	−16 ± 3	−19 ± 2	NS
Umbilical	23 ± 2	25 ± 2	NS

Values are means ± SE. Mean (±SE) fetal age was 131 (±1) days; fetal weight was 3,064 (±156) g; placental weight was 321 (±19) g. NS, nonsignificant.

Alanine Concentrations and Uptakes

The infusion of alanine into the maternal circulation elevated maternal plasma alanine concentration approximately fourfold and increased fetal plasma alanine concentration 36% (Table 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 concentration (Fig. 1). Uterine alanine uptake was similar to umbilical uptake in the control period (2.5 vs. 3.1 μmol·min^{-1}·kg fetus^{-1}) but became significantly greater than umbilical uptake during the alanine infusion (15.5 vs. 6.9 μmol·min^{-1}·kg fetus^{-1}, $P < 0.01$). Therefore, net utilization of alanine by the uteroplacenta increased markedly in response to

Table 2. *Alanine concentrations, uptakes, and fluxes*

	n	Control	Experimental	*P*
Arterial plasma alanine concn, μM				
Maternal	10	155 ± 14	629 ± 78	<0.001
Fetal	10	303 ± 17	413 ± 30	<0.001
Alanine uptake, μmol·min^{-1}·kg fetus^{-1}				
Uterine	10	2.5 ± 0.6	15.5 ± 3.1	<0.001
Umbilical	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	<0.01
Plasma alanine disposal rate, μmol·min^{-1}·kg fetus^{-1}				
Maternal	8	6.1 ± 0.9	16.0 ± 1.6	<0.001
Fetal	8	19.6 ± 1.3	22.0 ± 1.2	<0.01
MPE ratios of maternal tracer × 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, μmol·min^{-1}·kg fetus^{-1}				
To uteroplacenta from mother ($R_{UP,m}$)	8	8.2 ± 2.1	32.8 ± 7.1	<0.01
Direct to fetus from mother ($R_{f,m}$)	8	0.17 ± 0.04	0.71 ± 0.2	<0.02
To fetus from placenta ($R_{f,p}$)	8	5.0 ± 0.6	7.3 ± 1.3	0.06
To placenta from fetus ($R_{p,f}$)	8	1.9 ± 0.6	0.3 ± 1.3	NS

Values are means ± SE; *n*, no. of ewes. Fluxes were estimated by iv infusion of L-[3,3,3-D_3]alanine in mother and L-[1-^{13}C]alanine in fetus. MPE, molar percent enrichment.

maternal alanine infusion, from −0.6 to 8.6 μmol·min^{-1}·kg fetus^{-1} ($P < 0.01$).

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 isotopomer (Fig. 2). Maternal alanine disposal rate increased significantly in response to the alanine infusion (Table 2). The increase in maternal disposal rate was approximately equal to the alanine infusion rate (9.9 ± 1.1 vs. 8.4 ± 1.0 μmol·min^{-1}·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. 2). This observation, coupled with the uterine uptake data, demonstrated bidirectional alanine fluxes between the maternal circulation and the pregnant uterus. Similarly, the fetal alanine tracer data demonstrated bidirectional alanine exchange between placenta and fetus (Table 2 and Fig. 2). 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 ~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 compared with the other alanine fluxes, ~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 (Ta-

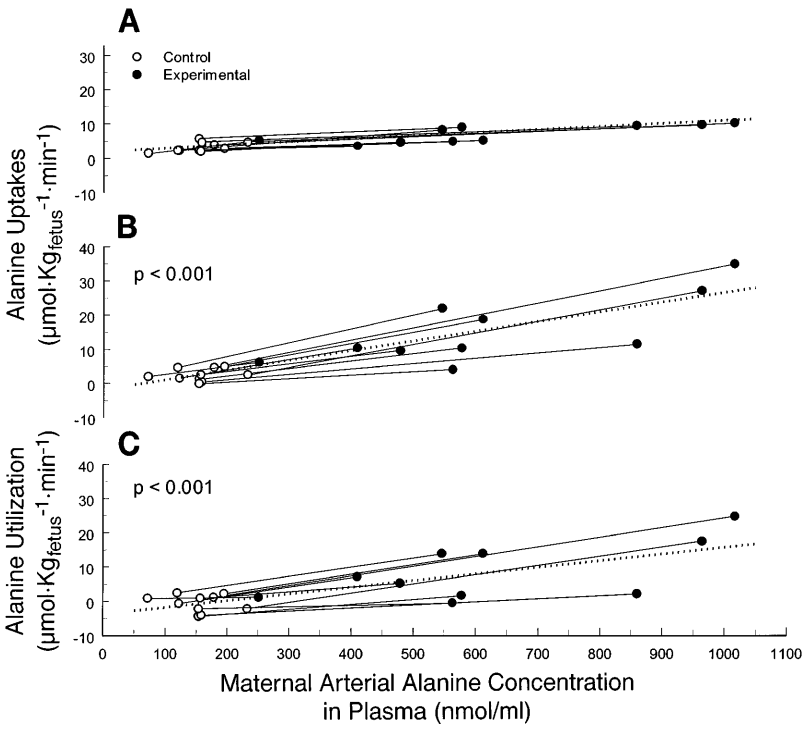

Fig. 1. Umbilical and uterine alanine uptakes (*A* and *B*) and uteroplacental alanine utilization (*C*) vs. maternal alanine concentration.

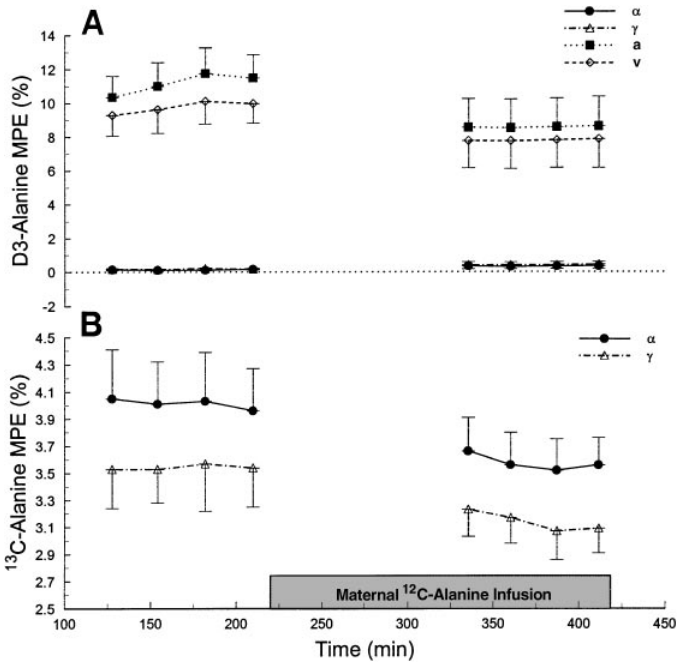

Fig. 2. Plasma enrichments for both L-[3,3,3-$^2H_3$]alanine (maternal tracer, *A*) and L-[1-^{13}C]alanine (fetal tracer, *B*) in control and experimental periods are molar percent enrichments (MPE, means ± SE) plotted against time. α, umbilical artery; γ, umbilical vein; a, maternal artery; v, uterine vein.

ble 2) and by a value that 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 ($R_{up,m}$) ($P < 0.01$). However, the slope of the regression line relating $R_{f,m}$ to $R_{up,m}$ was about one-tenth of the slope relating umbilical and uterine uptakes (Fig. 3). The increase in umbilical uptake was ~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.

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 (20). 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-$^2H_3$]alanine (*Eq. 10* in *Calculations*). Two fetal sheep infused with L-[3,3,3-$^2H_3$]alanine yielded fetal plasma alanine disposal rates equal to 19.5 and 31.3 μmol·min^{-1}·kg fetus^{-1}, respectively. Compared with the fetal disposal rates measured with L-[1-^{13}C]alanine (mean 19.6, range 14.6–23.6), 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 by use of maternal infusion of L-[U-^{13}C]alanine. The relevant results are summarized in Table 3. In this table, the fetal-to-maternal MPE ratios of maternal tracer and the $R_{f,m}$ flux are all greater than the corresponding mean values in Table 2 by more than four standard deviations, thus indicating a greater transplacental

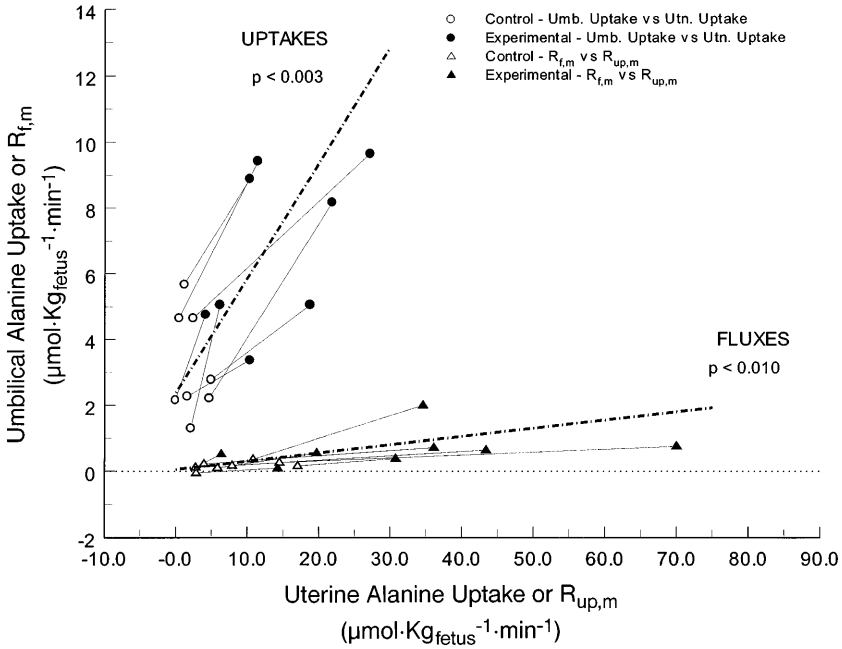

Fig. 3. Relation of umbilical (Umb) alanine uptake to uterine (Utn) alanine uptake and flux of maternal alanine into the fetus ($R_{f,m}$) to flux of maternal alanine into the uteroplacenta ($R_{UP,m}$).

Table 3. *Summary of results in 2 sheep infused iv with L-[U-^{13}C]alanine in the mother and L-[1-^{13}C]alanine in the fetus*

	Sheep No. 1	*Sheep No. 2*
Fetal age, days	128	134
Fetal weight, g	2897	3946
Placental weight, g	393	434
Arterial plasma alanine concn, μM		
Maternal	158	150
Fetal	270	295
Umbilical alanine uptake, μmol·min^{-1}·kg fetus^{-1}	2.9	3.7
Fetal plasma alanine disposal rate, μmol·min^{-1}·kg fetus^{-1}	19.4	21.0
MPE ratio, maternal tracer × 100		
Fetal arterial/maternal arterial	3.8	4.3
Umbilical venous/maternal arterial	5.9	5.5
Alanine flux to fetus, μmol·min^{-1}·kg fetus^{-1}		
Direct from mother ($R_{f,m}$)	0.7	0.9
From placenta ($R_{f,p}$)	8.1	6.1

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 3, the direct flux of maternal alanine across the placenta was only 9 and 15%, respectively, of the alanine flux from placenta to fetus.

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). 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}, $P < 0.05$).

DISCUSSION

The present study is relevant to the question of 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

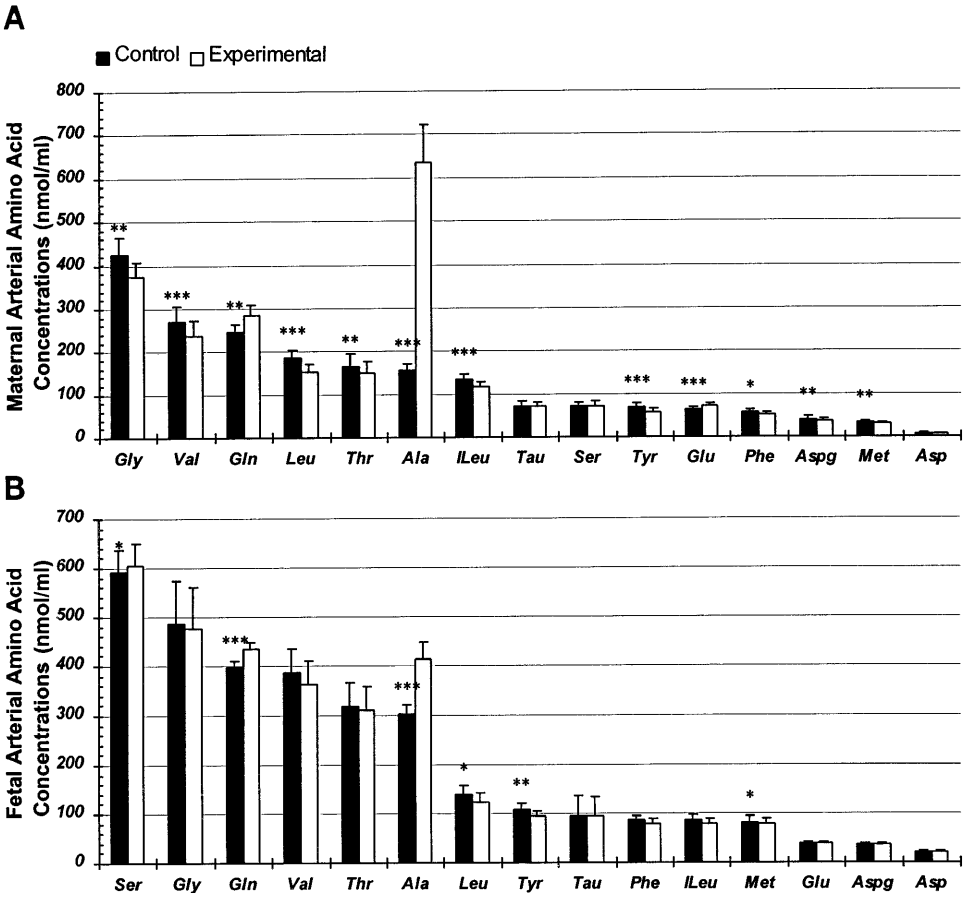

Fig. 4. Comparison of maternal (*A*) and fetal (*B*) neutral and acidic amino acid concentrations between control (filled bars) and experimental (open bars) study periods. Values are means ± SE. Significant changes between 2 periods: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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 uptake 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 on 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 ~1% maternal arterial enrichment. The normal transplacental maternal alanine flux estimated on the basis of this extremely low fetal enrichment is ~0.2 $\mu mol \cdot min^{-1} \cdot kg\ fetus^{-1}$ (Table 2, control data). The infusion of ^{13}C-labeled alanine into the maternal circulation results in an umbilical arterial enrichment that is ~4% of the maternal arterial enrichment and an estimated normal maternal alanine flux into the fetal circulation of ~0.8 $\mu mol \cdot min^{-1} \cdot kg\ fetus^{-1}$ (Table 3). Before the methodological issue that 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 $R_{f,m}$-to-$R_{f,p}$ fraction ranged between 0.034 (Table 2, control data) and 0.15 (Table 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 compared with 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 has been demonstrated by the evidence that approximately one-half of the leucine flux to the fetus from the placenta represents leucine produced within the placenta (14). 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 tracer that is 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 (17). The relatively large increase in uterine uptake in response to an increase in maternal alanine concentration suggests that transport of 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 together 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 (4, 17). Placental sodium-independent (17) and sodium-dependent (5) 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 uptake was only 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 the escape of alanine into the umbilical circulation.

In agreement with previous studies (11), 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

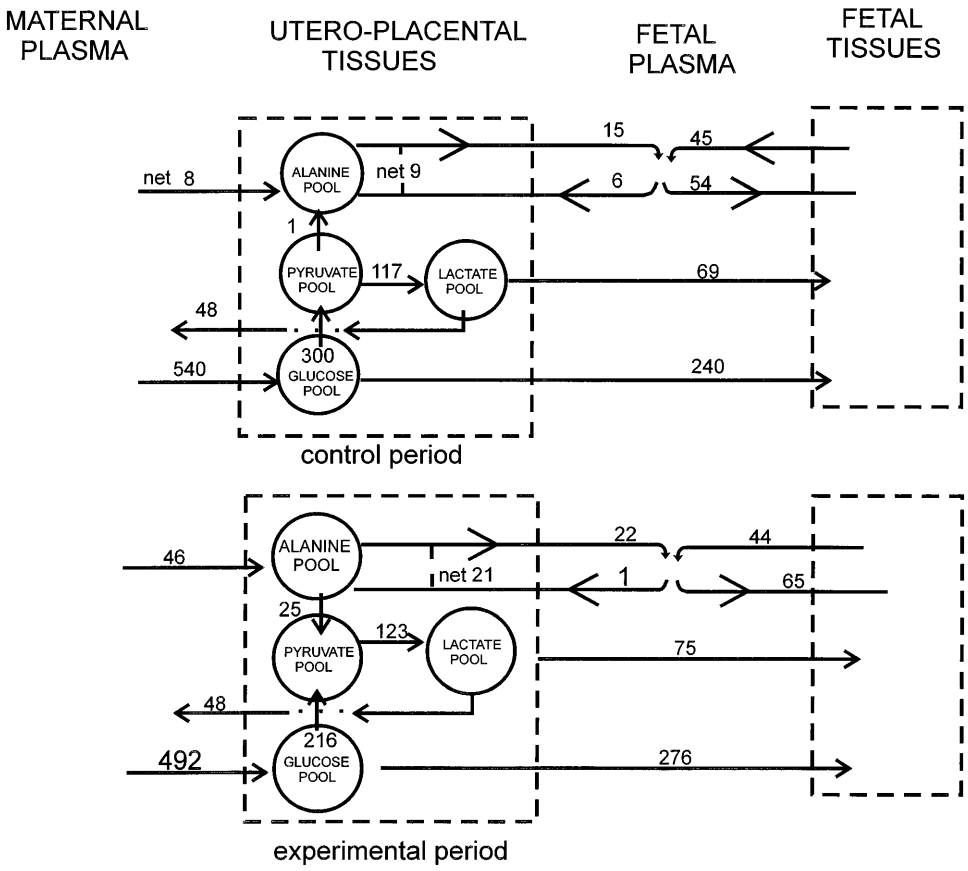

Fig. 5. Summary balance of measured substrate fluxes between fetal, placental, and maternal compartments during control and experimental periods. Mean fluxes are nos. representing μmol of carbon·min^{-1}·kg fetus^{-1}.

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 phospho*enol*pyruvate to pyruvate is inhibited by high alanine concentrations (18). 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 5 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 toward increased glutamine umbilical uptake and decreased placental utilization of branched-chain amino acids. However, the only trend that attained significance was a decrease in placental leucine utilization ($P = 0.04$). Both maternal and fetal glutamine concentrations increased in the experimental period ($P = 0.002$), suggesting increased maternal and fetal glutamine production. Ruderman and co-workers (15, 16) have shown that, in the rat hindlimb, the rate of glutamine release can be increased by increasing the supply of alanine via the perfusate.

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 ~12% and by a value that was virtually equal to the increased flux from the placenta. These observations add to previous evidence (2) showing that the ovine fetus has a high rate of alanine production compared with 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 limits the direct flux of alanine from mother to fetus. There may be, however, quantitative differences in the degree of limitation. Gilfillan et al. (1) 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 ~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 one observed in the present study for the maternally infused [^{13}C]alanine (~6 and 4%) 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 differences in placental alanine transport and fetal alanine production rates.

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