Arginine and Mixed Amino Acids Increase Protein Accretion in the Growth-Restricted and Normal Ovine Fetus by Different Mechanisms

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ABSTRACT

Protein metabolism may be perturbed in intrauterine growth restriction (IUGR). Arginine is indispensable for growth and nitrogen balance in young mammals. Fetuses with IUGR therefore may benefit from arginine supplementation. The purpose of this study was to determine *1*) the effects of IUGR on protein metabolism in the ovine fetus and *2*) the effects of arginine or mixed amino acid (AA) infusion on protein metabolism in these fetuses. Pregnant ewes and their fetuses were catheterized at 110 d gestation and randomly assigned to control or IUGR groups. IUGR was induced by repetitive placental embolization. Parameters of fetal protein metabolism were determined from [*ring*-²H₅]phenylalanine kinetics at baseline and in response to a 4-h infusion of either arginine or an isonitrogenous AA mixture. There were no differences in protein metabolism between control and IUGR groups either at baseline or in response to arginine or AA treatment. Both arginine and AA infusion increased fetal protein accretion in both groups. Arginine did this by decreasing protein turnover, synthesis, and breakdown. AAs increased protein turnover and synthesis while decreasing protein breakdown. AA infusion resulted in a significantly higher increase in protein accretion than arginine infusion. Thus, in the ovine fetus, placental embolization has no clear effect on protein metabolism. Arginine and AAs both stimulate protein accretion but do so in distinctly different ways. Mixed AA infusion has a greater effect on protein accretion than arginine alone and therefore may be a better strategy for stimulating fetal growth. (*Pediatr Res* **58: 270–277, 2005**)

Abbreviations
AA, amino acid
FA, fetal artery
FV, fetal vein
HlBF, hind limb blood flow
IUGR, intrauterine growth restriction
NB, net tracer balance
NO, nitric oxide
TTR, tracer/tracee ratio
UA, uterine artery
UOV, utero-ovarian vein
UV, umbilical vein

Intrauterine growth restriction (IUGR) is a major cause of perinatal mortality and morbidity (1,2). Although there are many possible causes, the key feature of IUGR is impaired fetal growth and, hence, by inference, impaired protein accretion. However, there are few data describing the alterations in protein metabolism that must underlie IUGR.

In the IUGR human neonate, the few studies available are conflicting (3–5). Protein accretion rates are reported to be normal, although both increased (3,4) and reduced (5) protein synthesis and turnover have been reported. A previous study on protein kinetics in the ovine fetus in which growth was restricted by heat stress showed no significant changes in protein synthesis, breakdown, or accretion (6). However, a number of reports provide indirect evidence of altered protein metabolism in human fetuses with IUGR. In human pregnancies that are complicated with IUGR, fetal plasma AA concentrations are reduced (7,8), and this persists after birth (9). Concentrations of most essential AAs are lower, particularly those of the branched-chain AAs valine, isoleucine, and leucine (7,9). The maternal plasma AA concentrations are higher than in uncomplicated pregnancies and often not significantly different from nonpregnant controls (8).

There is also indirect evidence of impaired urea production in association with IUGR. Plasma and urinary urea levels are

reduced and ammonia levels are elevated in both human neonates and guinea pig fetuses with (10,11). In the neonate with IUGR, ammonia levels increased with increasing protein intake (12). In the guinea pig fetuses with IUGR, urea cycle enzyme activities in the liver were markedly reduced. This resulted in significantly lower urea production rates, whereas the ammonia concentration in liver slices were 16-fold higher than in the non–growth restricted controls (11). As urea production is the final common pathway of protein metabolism, altered urea production suggests a limited capacity to process excess nitrogen.

To try to establish optimal postnatal growth, neonates with IUGR are usually fed the same high-protein, high-energy diet as their normally grown preterm peers. However, because of the suspected immaturity of the urea cycle in these infants, high-protein intakes may lead to hyperammonemia. Ammonia is a known neurotoxin, and hyperammonemia is associated with lethargy, coma, and irreversible neuronal damage (13). Thus, neonatal treatment of the infant with IUGR may require a trade-off between keeping protein intake low enough to prevent ammonia intoxication but high enough to facilitate adequate growth. Relieving the hyperammonemia by stimulating the urea cycle therefore may be a possible strategy in the nutritional treatment of the neonate with IUGR.

Arginine is a high nitrogen–containing AA with several anabolic functions. It is part of the urea cycle and as such is a potent stimulator of urea production (14). Under normal conditions, arginine is not essential for protein metabolism, but in certain stressful situations, it may become essential (15). In traumatized or burned rats, as well as in patients who have undergone surgery, arginine supplementation has an anticatabolic effect and improves nitrogen balance (16–18). It is also known to enhance wound healing (19). Furthermore, arginine may be an essential nutrient for mother and fetus during pregnancy (20). Arginine stimulates secretion of insulin in human and ovine fetuses (21,22) and also stimulates secretion of growth hormone (23) and glucagon (24). It therefore potentially could improve fetal growth and protein metabolism indirectly *via* these anabolic hormones. Arginine also serves as a precursor for polyamines involved in cell growth and DNA replication (25). The aims of our study, therefore, were to investigate the effect of IUGR on protein metabolism in the late-gestation ovine fetus and to determine the effects of arginine supplementation on protein metabolism in these animals.

METHODS

Animal preparation. Twenty-two pregnant Romney ewes that were carrying Dorset cross-breed singleton fetuses were housed in individual cages with free access to water and pelleted food. All experiments were approved by the University of Auckland Animal Ethics Committee.

The ewes underwent surgery under halothane anesthesia at 111 ± 0.6 d gestation (term = 145 d). Catheters were placed in both fetal femoral arteries and veins (FA and FV), the fetal common umbilical vein (UV), the main arteries that supply the maternal uterine horns (UA), the maternal utero-ovarian vein that drains the pregnant horn (UOV), and the maternal carotid artery (MA) and jugular vein (MV). Growth catheters were inserted s.c. around either side of the fetal chest and were attached to the spine and sternum (26). Fetal girth and hock-hoof length were measured. Ewes were allowed to recover for at least 3 d after surgery. All catheters were flushed with sterile saline that contained 10 U/mL heparin daily for the first 3 d postoperatively and subsequently every

other day until post mortem. Fetal chest girth increments were measured twice daily.

Embolization. Animals were assigned at random to control and IUGR groups. In the IUGR group, placental embolization was begun at 114 ± 0.9 d gestation. Microspheres (Superose 12; Pharmacia Biotech, Uppsala, Sweden; diluted 1:100, 1 mL per injection) were injected twice daily into the uterine arteries. Embolization was stopped 2 d before the first tracer study or if the fetal arterial Po_2 was <14 mm Hg, fetal blood lactate concentration was >2 mM, or chest girth did not increase for 2 consecutive days.

Experimental protocol. Tracer infusion studies were performed on each animal at 120 (study 1) and 126 d gestation (study 2). In one of the studies, fetal protein metabolism was assessed before and after the infusion of arginine and in the other before and after infusion of an isonitrogenous solution of mixed AAs. The order of the studies was randomly determined for each animal.

After baseline blood samples were taken, L-[*ring*-$^2H_5$]phenylalanine (D$_5$-phenylalanine, 0.8 μmol/min), L-[*ring*-$^2H_2$]tyrosine (D$_2$-tyrosine, 0.15 μmol/min), and antipyrine (0.4 mg/min) were infused into the FV catheter, and L-[1-^{13}C]phenylalanine (^{13}C-phenylalanine, 2.65 μmol/min) was infused into the MV catheter for 8 h (Fig. 1). All tracers were obtained from Cambridge Isotopes (ARC Laboratories, Amsterdam, The Netherlands). Priming doses equivalent to 100 min of infusate were given as a bolus at the start of infusion. An additional infusion of 0.9% NaCl at 3 mL/h was given *via* the FV catheter. Steady state was reached for all fetal tracers at 180 min, after which five sets of blood samples were taken simultaneously from FA, FV, UV, MA, and UOV catheters at 15-min intervals. The FA and FV samples were taken from the hind limb that was not used for infusion, and care was taken to draw the sample slowly and gently to avoid drawing blood back from the vena cava that may contain the infusate from the other FV catheter. Immediately after the fifth set of samples, the saline infusion was changed to L-arginine (12.2 μmol/min) or a mixed AA solution (10% FreAmine III; Braun Medical, Irvine, CA; 13.4 mL made up to 15 mL with saline, run at 3 mL/h; specific composition in Table 1). Both infusates contained 0.98 mM nitrogen. A new steady state was reached after another 180 min, after which an additional set of five blood samples were taken.

Whole blood was aliquotted into tubes in frozen gel blocks and stored at −80°C until further analysis. An additional aliquot was kept on ice for measurement of blood gases on a Chiron M845 blood gas analyzer (Chiron Corp., Emeryville, CA) within 15 min of sampling. Additional aliquots were centrifuged, and the plasma was frozen at −20°C.

After study 1, the fetus was transfused with 20 mL of maternal blood immediately after the last sample was taken to replace blood lost in sampling. On the morning after study 2 (127 ± 1.0 d gestation), ewes were killed with an overdose of pentobarbitone. The fetus was removed, and all catheter positions were checked. The fetus was weighed and measured, and placentomes were dissected, weighed, and individually classified according to Vatnick *et al.* (27).

Assays. Antipyrine concentrations were analyzed in whole blood by HPLC (28). Plasma IGF-1 and insulin concentrations were measured by double-antibody RIAs validated for sheep plasma (29,30).

Isotopic enrichments of phenylalanine and tyrosine were analyzed in plasma by gas chromatography–mass spectrometry as described previously (31). In short, phenylalanine and tyrosine were converted to their methylformated pentafluorobenzyl derivatives, with addition of $^2H_8$-phenylalanine and $^{13}C_9$-tyrosine as internal standards. Quality control standards were derivatized in triplicate with each set of samples. Isotopic enrichments were measured on an HP Engine (Hewlett-Packard, Avondale, PA) that consisted of a 5890-series II gas chromatograph and a 5989B quadrupole mass spectrometer. The analytes were monitored for their [M-H]-ions at 190.2, 191.2, 195.2, and 198.2 m/z for phenylalanine and at 264.2, 266.2, 268.2, and 273.2 m/z for tyrosine. Negative ion chemical ionization was used with ammonia as reaction gas at optimized pressure. Samples were injected using a heated split/splitless injec-
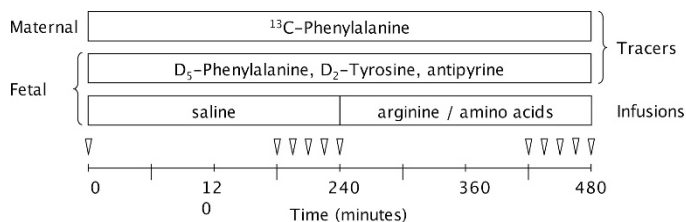

Figure 1. Experimental design of the protein metabolism studies. The arrows indicate times of blood sampling. Priming doses of all tracers were given at time 0 after baseline blood samples were taken.

Table 1. *Composition of the amino acid infusate*

	g/L
Alanine	6.34
Arginine	8.49
Cysteine	<0.14
Glycine	12.51
Histidine	2.50
Isoleucine	6.16
Leucine	8.13
Lysine	6.52
Methionine	4.73
Phenylalanine	5.00
Proline	1.00
Serine	5.27
Threonine	3.57
Tryptophan	1.34
Valine	5.90

Values are those stated by the manufacturer (10% FreAmine III, Braun Medical Inc., Irvine, USA) made to an 89.3% solution with sterile saline.

tor (T = 270 C). A CP-Sil-19CB 25 m × 0.25 mm (I.D.) × 0.2 μm (film thickness) column (Chrompak International, Middelburg, The Netherlands) was used with helium as carrier gas. Inter- and intra-assay variabilities were <8 and <4%, respectively, for each isotope.

Calculations. Umbilical and uterine blood flows (ml/min) were calculated from antipyrine concentrations according to the Fick principle, as the quotient of the infusion rate and the arteriovenous concentration difference across the placenta (32):

Umbilical blood flow = I/[Δantipyrine]$_{FA-UV}$

Uterine blood flow = I/[Δantipyrine]$_{UOV-MA}$

where I is the antipyrine infusion rate in μg/min, and the blood antipyrine concentrations are in μg/mL.

Hind limb blood flow (HlBF) was assumed to be a constant fraction of umbilical blood flow, based on a previous study in late-gestation sheep in which hind limb and umbilical blood flows were determined using radiolabeled microspheres (33). Blood flows were converted to plasma flows as follows:

PF = BF * (1 − fractional hematocrit)

Whole-body phenylalanine kinetics. Whole-body phenylalanine kinetics were estimated by the method of Clarke and Bier (34), adapted for the ovine fetus by Liechty *et al.* (35). Turnovers (Q; μmol/min) of phenylalanine and tyrosine were estimated from the plasma dilution of the infused tracers with the following equation:

Q = I * [(Ri/Rp) − 1]

where I is the tracer infusion rate (μmol/min) and Ri and Rp are the tracer/tracee ratios (TTR) in the infusate and plasma, respectively.

Phenylalanine hydroxylation to tyrosine (OH, μmol/min) was calculated as follows:

OH = Q_{D2-tyr} * (R_{D4-tyr}/R_{phe}) * [$Q_{phe}/(I_{phe} + Q_{phe})$]

where Q_{D2-tyr} is the turnover of tyrosine, derived from plasma enrichments of D_2-tyrosine; Q_{phe} is the turnover of phenylalanine; I_{phe} is infusion rate of D_5-phenylalanine; R_{D4-tyr} is the TTR of D_4-tyrosine in plasma, which is derived from hydroxylation of the D_5-phenylalanine tracer; and R_{phe} is the TTR of D_5-phenylalanine in plasma.

The influx of maternal phenylalanine to the fetal circulation ($Q_m \rightarrow$ f; μmol/min) was estimated by dilution of the maternal tracer in fetal plasma:

$Q_m \rightarrow$ f = [(R^m_f * Q_f)/I_m] * Q_m

where R^m_f is the TTR of the maternal tracer (^{13}C-phenylalanine) in the fetal plasma, I_m is the infusion rate of the maternal tracer, Q_f is the turnover of phenylalanine in the fetal plasma, and Q_m is the turnover of phenylalanine in maternal plasma. This estimate assumes that there is no utilization of maternally derived phenylalanine by the placenta. Therefore, it will overestimate $Q_m \rightarrow$ f to the extent that maternal tracer is used by the placenta (35).

The unidirectional flux of phenylalanine from the fetal plasma to the placenta ($Q_f \rightarrow$ m; μmol/min) was estimated by the Fick principle as the product of the umbilical plasma flow and the loss of tracer across the placenta:

$Q_f \rightarrow$ m = UmPF * [(R_{FA} * [FA]) − (R_{UV} * [UV])]

where R_{FA} and R_{UV} are the TTRs of D5-phenylalanine, and [FA] and [UV] are the concentrations of phenylalanine in FA and UV plasma, respectively (μmol/mL).

Because phenylalanine cannot be newly synthesized in the body, release of phenylalanine from body protein breakdown (B; μmol/min) is the difference between the total phenylalanine turnover and phenylalanine derived from the mother and tracer and AA infusions:

B = Q − (I + $Q_m \rightarrow$ f)

where I is the combined infusion rate of phenylalanine as tracer and in the AA mixture (μmol/min).

Phenylalanine used for protein synthesis (S; μmol/min) is the difference between the turnover and the disappearance of phenylalanine toward the mother and to tyrosine:

S = Q − (OH + $Q_f \rightarrow$ m)

Phenylalanine used for protein accretion is the net balance between synthesis and breakdown.

Hind Limb Phenylalanine kinetics. Phenylalanine kinetics across the hind limb were calculated as described by Wolfe (36). The net tracer balance (NB) across the hind limb (nmol/min) is a measure for hind limb protein accretion and is calculated as shown below:

NB = HlPF * [([FA] * R_{FA}) − ([FV] * R_{FV})]

where HlPF is the plasma flow across the hind limb (ml/min), [FA] and [FV] are the phenylalanine concentrations in FA and FV plasma, respectively (nmol/mL), and R_{FA} and R_{FV} are the TTRs in FA and FV plasma.

As there is relatively low phenylalanine 4-monooxygenase activity (37) and, therefore, phenylalanine hydroxylation, in muscle, the hind-limb disposal rate of phenylalanine (Rd; nmol/min) is a reflection of protein synthesis. This was calculated as follows:

Rd = NB/R_{FV}

The R_{FV} is thought to best represent the intracellular enrichment of the organ (38).

The production rate (Ra; nmol/min) of phenylalanine in muscle represents the rate of protein breakdown. The NB of an AA across an organ is the net difference between production and disposal; therefore, the hind limb phenylalanine production rate is represented as:

Ra = Rd − NB

Statistics. The results are presented as mean ± SD. For each animal, the means of the five measurements during saline infusion and the five during arginine or AA infusion were calculated. Baseline differences between control and embolized groups were analyzed using *t* test for unpaired measurements. The effects of arginine or AA infusion were analyzed using repeated measures ANOVA taking into account infusion (before *versus* after), group (embolized *versus* control), and infusion × group interaction. Because of the relatively small sample size, baseline differences between the two groups in hind limb protein kinetics were analyzed with Mann-Whitney tests. Differences within groups in hind limb protein kinetics were analyzed with Wilcoxon signed rank tests. The results of these are expressed as median (range). There were no significant differences between the two study days in baseline values of any of the major outcomes; therefore, the results for both days were pooled for statistical analysis.

RESULTS

The gestational ages at the two protein turnover studies were the same in both groups (120.3 ± 0.5 and 126.1 ± 1.1 d in the control animals and at 120.5 ± 0.5 and 126.4 ± 0.5 d in the embolized group). The gestational ages at post mortem were also similar in the two groups (control 127.1 ± 1.1 d; embolized 127.0 ± 0.9 d).

Embolization did not significantly reduce fetal weight at post mortem (Table 2). However, the fetal growth rate was reduced in the embolized group, as indicated by a decrease in the daily increment in hock-hoof length and chest girth between surgery and post mortem. Placental weight was also reduced by 17% in the embolized group, although this did not reach statistical significance ($p = 0.06$). The distribution of placentomes was shifted toward the more mature stage in the embolized group, with lower median weight of total type-A placentomes [median (range): control 329 (152–489) g *versus* embolized 194 (20–379) g; $p = 0.03$] and higher median weight of total type C placentomes [control 30 (15–46) g *versus* embolized 123 (15–216) g; $p = 0.05$].

Table 2. *Effect of embolization on fetal and placental size, and on blood gases, plasma hormone and metabolite concentrations and plasma flows at baseline*

	Control	n	Embolized	n
Measurements				
Fetal weight (g)	3585 ± 456	(10)	3347 ± 668	(11)
Hock-hoof increment (mm/d)	2.6 ± 0.5	(10)	2.1 ± 0.4*	(10)
Chest girth increment (mm/d)	5.1 ± 0.6	(11)	4.3 ± 0.7†	(11)
Placental weight (g)	432 ± 83	(10)	358 ± 73	(10)
Fetal arterial blood gases				
pO_2 (mmHg)	19 ± 3	(20)	16 ± 3†	(19)
pCO_2 (mmHg)	55 ± 3	(20)	54 ± 3	(19)
Hematocrit (%)	36 ± 6	(17)	42 ± 7*	(19)
pH	7.35 ± 0.02	(20)	7.35 ± 0.04	(19)
Plasma hormones (ng/ml)				
Fetal IGF-1	68 ± 20	(19)	51 ± 19*	(18)
Maternal IGF-1	115 ± 27	(19)	133 ± 28*	(19)
Fetal insulin	0.24 ± 0.16	(16)	0.17 ± 0.16	(17)
Maternal insulin	0.29 ± 0.10	(14)	0.51 ± 0.33*	(17)
Plasma Metabolites (mmol/L)				
Fetal glucose	0.69 ± 0.11	(20)	0.64 ± 0.14	(19)
Maternal glucose	2.54 ± 0.39	(20)	2.43 ± 0.35	(19)
Fetal lactate	1.35 ± 0.21	(20)	1.42 ± 0.47	(19)
Maternal lactate	0.72 ± 0.15	(18)	0.77 ± 0.28	(19)
Plasma flows (ml/min)				
Uterine plasma flow	1070 ± 195	(13)	758 ± 250‡	(15)
Umbilical plasma flow	370 ± 102	(18)	386 ± 111	(18)

Values are mean ± SD (n). Values for blood gases, hormone and metabolite concentrations and plasma flows are the baseline results on both study days.
* $p = 0.05$; † $p = 0.01$; ‡ $p = 0.001$ for comparison with control animals.

Blood Gases, Plasma Hormone and Metabolite Concentrations, and Plasma Flows

Effects of embolization. Placental embolization significantly reduced fetal arterial Po_2 and increased fetal hematocrit but did not result in hypercapnia or acidemia at the time of study (Table 2). Embolization reduced fetal and increased maternal IGF-1 concentrations. Fetal insulin concentrations were not different between embolized and control groups, but maternal concentrations were elevated in the embolized group. There were no differences in baseline fetal and maternal glucose or lactate concentrations between the two groups. Embolization reduced baseline uterine plasma flow by ~30%, but umbilical plasma flow was not changed (Table 2).

Effects of mixed AA or arginine supplementation. Fetal IGF-1 concentrations were not altered by either AA or arginine infusion in either group (AA infusion: controls 70 ± 20 to 66 ± 22 ng/mL, embolized 65 ± 11 to 60 ± 10 ng/mL; arginine infusion: controls 66 ± 21 to 64 ± 21 ng/mL, embolized 49 ± 24 to 51 ± 25 ng/mL). AA infusion increased fetal insulin concentrations in both groups (controls 0.30 ± 0.22 to 0.37 ± 0.23 ng/mL, embolized 0.21 ± 0.21 to 0.42 ± 0.52 ng/mL; infusion effect $p \leq 0.05$, group × infusion interaction $p = 0.3$). Arginine infusion also increased fetal plasma insulin concentrations to a similar extent in both groups (control 0.20 ± 0.09 to 0.29 ± 0.10 ng/mL, embolized 0.13 ± 0.14 to 0.22 ± 0.20 ng/mL; infusion effect $p \leq 0.001$, group × infusion interaction $p = 1.0$). Maternal hormone concentrations were not changed by either AA or arginine infusion (data not shown).

AA infusion did not change uterine or umbilical plasma flows in either group (data not shown). Umbilical plasma flows were reduced by arginine infusion to a similar extent in both groups (controls 374 ± 83 to 334 ± 72.6 mL/min, embolized 380 ± 106 to 344 ± 130 mL/min; $p \leq 0.01$, group × infusion interaction $p = 0.5$). Uterine plasma flows were not changed by arginine infusion in either group.

Phenylalanine Kinetics

Arginine concentrations before arginine or AA infusion were lower in the embolized animals when results from all studies were pooled (controls 108 ± 30 μM, embolized 85 ± 31 μM; $p \leq 0.05$) but not when separated for AA or arginine infusion studies (Table 3). There were no differences in phenylalanine or tyrosine concentrations between control and embolized animals. Mixed AA infusion increased fetal plasma phenylalanine and tyrosine concentrations in both control and embolized animals. Arginine infusion approximately doubled fetal plasma arginine concentrations in both groups (Table 3).

There were no differences in any of the phenylalanine or tyrosine kinetic data between control and embolized animals (Fig. 2). The effects of AA infusion were also not different between control and embolized animals. AA infusion increased whole-body phenylalanine turnover and phenylalanine hydroxylation rates (Fig. 2A). Tyrosine turnover did not change. Phenylalanine used for protein synthesis was increased, whereas phenylalanine released from protein breakdown was reduced. The net effect of mixed AA infusion in both groups was an increase in protein accretion.

The effects of arginine infusion were not different between the control and embolized animals. Arginine infusion decreased whole-body phenylalanine and tyrosine turnovers (Fig. 2B). Phenylalanine hydroxylation rates were not affected by arginine infusion. Arginine infusion reduced protein synthesis and breakdown, resulting in a net increase in protein accretion.

Table 3. *Effects of mixed amino acid or arginine infusion on plasma amino acid concentrations*

	Controls		Embolized	
	Before	After	Before	After
Amino acid infusion				
Phenylalanine	120 ± 19	156 ± 27	115 ± 12	161 ± 35§
Tyrosine	106 ± 21	116 ± 24	106 ± 24	117 ± 19‡
Arginine	109 ± 18	131 ± 21	94 ± 26	117 ± 45‡
Arginine infusion				
Phenylalanine	131 ± 19	133 ± 22	131 ± 46	128 ± 37
Tyrosine	99 ± 17	105 ± 16	108 ± 29	108 ± 29*
Arginine	108 ± 36	198 ± 74	87 ± 40	182 ± 60§

Values are mean ± SD in μmol/L for nine control and nine embolized animals receiving amino acid infusion and 10 control and 10 embolized animals receiving arginine infusion.

* $p = 0.05$; ‡ $p = 0.001$; § $p = 0.0001$ for infusion effect. There were no significant group effects or group x infusion interactions.
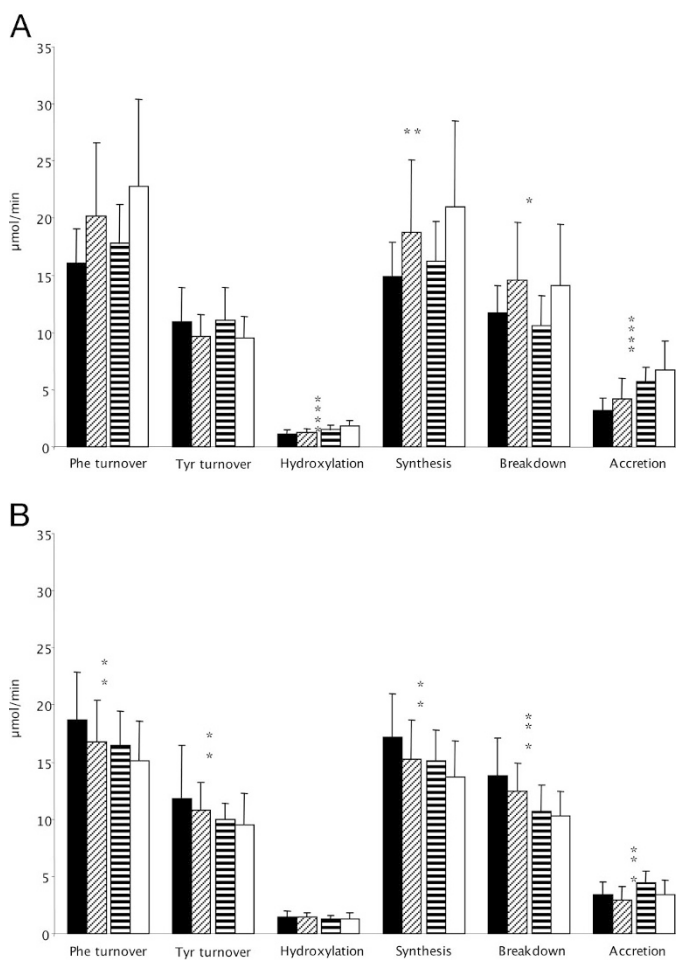

Figure 2. The effects of AA (*A*) and arginine infusion (*B*) on phenylalanine and tyrosine kinetics. Values are mean ± SD for nine control and eight embolized animals after AA infusion and 10 control and 10 embolized animals after arginine infusion. Synthesis, breakdown, and accretion indicate the amount of phenylalanine used for whole-body protein synthesis, breakdown, and accretion. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$ for infusion effect (before *vs* after, repeated measures ANOVA). There were no significant group effects or group × infusion interactions. ■ Controls before infusion; ▤ Controls after infusion; ▨ Embolized before infusion; □ Embolised after infusion.

Hind limb phenylalanine synthesis, breakdown, and accretion rates were not different between control and embolized animals (Table 4). Hind limb protein kinetics did not change in response to AA or arginine infusion.

When expressed as change from baseline values, AA and arginine supplementations had significantly different effects on protein turnover and synthesis in both groups (Table 5). AAs increased, whereas arginine reduced protein synthesis and turnover. The effects of treatments on protein breakdown were not different from each other. AA supplementation increased protein accretion more than arginine supplementation. Arginine and AA supplementation had similar effects on hind limb protein kinetics.

DISCUSSION

This study was undertaken to determine the effects of IUGR on protein metabolism in the ovine fetus and the effects of arginine or mixed AA infusion on protein metabolism in these animals. Our study showed no effect of placental embolization on any of the measured parameters of protein metabolism. This might be because the growth restriction in our animals was relatively mild. Fetal weight at post mortem was not significantly reduced in the embolized group, as a result of the large biologic variance. However, embolized fetuses did have significantly reduced growth as measured by chest girth increment and hind limb growth, measurements with much smaller variance in this study. In addition, embolized animals had reduced placental weight, uterine plasma flow, fetal P_{O_2}, and fetal IGF-1 concentrations. Nevertheless, our failure to detect significant changes in protein metabolism in these fetuses with mild growth restriction does not exclude the possibility that altered protein metabolism may be demonstrable after more severe or prolonged IUGR.

We found that arginine and mixed AA infusions both increased fetal protein accretion but did so by distinctly different mechanisms. Whereas mixed AA infusion increased protein turnover and synthesis and reduced protein breakdown, arginine infusion decreased turnover, synthesis, and breakdown. The increase in protein synthesis after AA infusion was to be expected as AAs are the prime substrate for protein synthesis. AAs are also known to have an inhibitory effect on protein breakdown (39), which is at least partly caused by reduction of proteasome activity (40).

The inhibitory effect of arginine on whole-body protein turnover has been observed before in a study of sepsis in pigs (41). This study showed that the decrease in whole-body protein turnover was most likely to be caused by a reduction in

Table 4. *Effects of mixed amino acid or arginine infusion on hind limb phenylalanine kinetics*

	Control		Embolized	
	Before	After	Before	After
Amino acid infusion				
Synthesis	673 (370–1602)	742 (346–1865)	642 (280–960)	505 (456–1146)
Breakdown	642 (353–1525)	713 (330–1783)	615 (267–916)	484 (431–1101)
Accretion	31 (18–77)	29 (16–82)	27 (13–44)	25 (16–45)
Arginine infusion				
Synthesis	540 (455–746)	663 (333–745)	575 (489–671)	629 (572–671)
Breakdown	516 (435–705)	620 (318–711)	549 (466–637)	601 (545–635)
Accretion	24 (20–40)	34 (15–42)	26 (23–34)	28 (27–36)

Values are median (range) for six control and four embolized animals receiving amino acid infusion and three control and three embolized animals receiving arginine infusion. Synthesis, breakdown and accretion values represent the amount of phenylalanine used for protein synthesis, breakdown and accretion. There were no significant effects of group or infusion.

Table 5. *Changes from baseline in phenylalanine/tyrosine kinetics after mixed amino acid or arginine infusion*

	Control		Embolized	
	Amino acids	Arginine	Amino acids	Arginine
Whole-body kinetics (μmol/min)				
$^F Q_{Phe}$	1.7 ± 2.0	-2.5 ± 3.7	2.7 ± 2.4	-1.5 ± 1.5†
$^F Q_{Tyr}$	0.4 ± 1.2	-2.7 ± 4.4	-0.1 ± 0.7	-0.9 ± 1.3
Hydroxylation	0.5 ± 0.3	-0.2 ± 0.5	0.5 ± 0.3	-0.1 ± 0.2‡
Synthesis	1.3 ± 2.0	-2.4 ± 3.2	2.2 ± 2.2	-1.4 ± 1.4†
Breakdown	-1.1 ± 1.7	-3.3 ± 3.6	-0.6 ± 1.7	-2.0 ± 1.1
Accretion	2.3 ± 0.7	0.8 ± 1.0	2.8 ± 1.1	0.6 ± 0.7§
Hind limb kinetics (nmol/min)				
Synthesis	−31	−83	32	0
	(−134–264)	(−122–205)	(−161–186)	(−3–140)
Breakdown	−27	−85	28	−2
	(−130–258)	(−117–195)	(155–185)	(−3–135)
Accretion	−2	2	−1.5	2
	(−5–6)	(−4–10)	(−7–12)	(0–5)

Values are mean $\pm$ SD in μmol/min for eight control and seven embolized animals for whole-body kinetics and median (range) in nmol/min for three control and three embolized animals for hind limb kinetics. There were no significant group effects or group x infusion interactions.

$^F Q_{Phe}$ and $^F Q_{Tyr}$ are whole-body phenylalanine and tyrosine turnover, respectively.

† $p \leq 0.01$; ‡ $p \leq 0.001$; § $p \leq 0.0001$ for comparison between effects of amino acid and arginine infusion.

liver protein synthesis and breakdown, despite an increase in hindquarter protein turnover. In our study, there was no change in hind limb protein metabolism in response to arginine infusion, and the cause of the decrease in whole-body protein turnover therefore must be located elsewhere. The liver may be a possible candidate. The biologic meaning of the inhibitory effect of arginine on whole-body protein turnover is as yet unexplained.

Arginine suppressed protein synthesis despite increased insulin concentrations, which would be expected to stimulate protein synthesis (42). A possible explanation may lie in the organ-specific actions of arginine. Skeletal muscle is the main site of insulin action (43). Although our study failed to show a change in hind limb protein metabolism after arginine infusion, previous studies in other animal models have shown an increase in muscle protein turnover and synthesis (17,41). Thus, arginine infusion may have an insulin-dependent action on protein metabolism in skeletal muscle while affecting other organs in different ways.

There are several possible reasons why our study failed to show an effect of arginine infusion on hind limb protein metabolism. First, the animals in the other studies were in a state of catabolism at the start of arginine supplementation, whereas our animals were not. Increased protein turnover is

thought to facilitate rapid elimination of damaged proteins (41). This may be an essential healing strategy during trauma such as burn injury or sepsis. Second, we studied the lambs *in utero*, whereas the other studies were done in adult or adolescent animals. Thus, the effects of arginine infusion on protein metabolism may be different in the rapidly growing and maturing organism. Finally, because of practical problems with sampling of the venous catheters, the number of measurements of hind limb metabolism is small in our study and the variance is large. It therefore is possible that the statistical power of our study was not large enough to detect anything but large differences in hind limb protein kinetics after arginine infusion.

Mixed AA infusion had a greater effect on protein accretion rates than arginine infusion and therefore seems to be a potentially better strategy for improving fetal growth. Charlton and Johengen (44,45) showed previously that both fetal intragastric and i.v. mixed AA and glucose supplementation prevented growth restriction in the ovine fetus induced by undernutrition or embolization, but they did not study fetal protein metabolism. We have shown that stimulating protein accretion may be one of the underlying mechanisms for this effect.

Arginine supplementation reduced umbilical plasma flows in both control and embolized groups, although this was not statistically significant in the embolized group. In many cell

types, arginine is a precursor for the production of nitric oxide (NO), a vasodilator (46,47). The increased arginine levels in our study may have led to increased fetal peripheral vasodilation by means of increased NO production, redistributing the blood flow away from the placenta toward the fetus. This would give rise to systematic errors in our estimation of hind limb protein metabolism, as in the absence of direct measures of HlBF, we were forced to assume that it remained a fixed percentage of the umbilical blood flow. This assumption would not have been valid if the observed fall in umbilical blood flow were accompanied by no change or even an increase in HlBF.

The only aspect of protein metabolism that we measured that is dependent on umbilical plasma flow is the phenylalanine flux from the fetal circulation back across the placenta. Although umbilical plasma flows were reduced after arginine infusion, this had no measurable effect on the fetal to maternal phenylalanine flux. Furthermore, if arginine led to increased fetal peripheral vasodilation, then it would be expected to lead to increased nutrient supply to fetal tissues, at least in the short term, and consequently to higher fetal protein turnover rates. However, arginine infusion decreased rather than increased fetal protein turnover in this study. Thus, it seems unlikely that arginine-induced increase in NO production has confounded our measurements of protein kinetics.

CONCLUSION

In conclusion, this study showed no effect of mild growth restriction on protein metabolism in the ovine fetus. Intravenous infusion of arginine or mixed AAs to the fetus increased protein accretion rates in both embolized and control fetuses. However, AA infusion increased protein turnover and synthesis rates, whereas arginine inhibited whole-body protein metabolism. AA infusion was more effective in stimulating protein accretion and therefore may be a better possible strategy for improving growth *in utero*. The biologic significance of the inhibitory effect of arginine on protein metabolism requires further investigation.

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