The Journal of Nutrition
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

Intravenous Administration of L-Citrulline to Pregnant Ewes Is More Effective Than L-Arginine for Increasing Arginine Availability in the Fetus[1–3]

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Abstract

L-Arginine administration may be useful for the treatment of intrauterine growth restriction, but concerns remain about effective precursors for administration into pregnant dams. Therefore, we used an ovine model to test the hypothesis that infusion of L-citrulline into the maternal circulation increases L-arginine availability to the fetus. On d 135 ± 1 of gestation, ewes received an i.v. bolus dose of L-citrulline (155 μmol/kg body weight) or the same dose of L-arginine-HCl. Maternal and fetal arterial blood samples were obtained simultaneously at −120, −60, 0, 5, 15, 30, 60, 120, 180, and 240 min relative to the time of amino acid administration. Concentrations of arginine in maternal plasma increased to peak values within 5 min after its injection in ewes and declined rapidly thereafter, whereas concentrations of arginine in fetal plasma increased between 15 and 30 min and returned to baseline values by 60 min. In contrast, administration of citrulline increased concentrations of citrulline and arginine in maternal and fetal plasma between 5 and 60 min and values remained elevated thereafter. The differential pharmacokinetics for arginine compared with citrulline infusion was consistent with the observation that the half-life of citrulline was twice that of arginine in ewes. We conclude that i.v. administration of citrulline is more effective than arginine in sustaining high concentrations of arginine in the maternal and fetal circulations of pregnant ewes. These novel findings provide support for studies of the clinical use of arginine and citrulline as therapeutic means to prevent or ameliorate fetal growth retardation in mammals. J. Nutr. 139: 660–665, 2009.

Introduction

Physiological levels of nitric oxide (NO)[6] and polyamines, which are products of L-arginine catabolism, play important roles in implantation, embryogenesis, and uterine quiescence throughout gestation (1–5). NO also regulates placental angiogenesis and uteroplacental blood flow, therefore affecting the transfer of nutrients from mother to fetus and fetal growth (6–12). Notably, administration of L-arginine ameliorated intrauterine growth restriction in women (13,14), whereas dietary L-arginine supplementation enhanced fetal survival and growth in rats (12) and gilts (15).

Arginine is alkaline in physiological solutions and, therefore, to prevent an acid-base imbalance, its HCl salt is generally used for i.v. administration into animals and humans (16). However, there are concerns about the effects of chronic provision of chloride on human health (17). Furthermore, the biological half-life ($T_{1/2}$) of L-arginine in mammals is relatively short (e.g. 45 min in ewes on d 105 of gestation) (18) due to a high arginase activity that rapidly degrades arginine in cells and tissues, including the ovine placenta (6,19,20). An alternative approach is to use L-citrulline (16), a neutral amino acid that can be utilized for arginine synthesis in cells (19). In addition, limited degradation of citrulline in the placenta allows maximum transfer from mother to fetus (21,22).

We hypothesized that i.v. administration of L-citrulline to pregnant ewes would be more effective than L-arginine for increasing arginine availability in the fetus. This hypothesis was tested in gestating ewes, an established and valuable animal model for studying human fetal growth (7,23), by determining the pharmacokinetics of arginine and citrulline in maternal and fetal plasma, as well as arginine availability to the fetus, in response to i.v. administration of either amino acid to the maternal circulation.

[1] Supported by grants from the NIH (1R21 HD049449) and the National Research Initiative Competitive Grant Program of the USDA Cooperative State Research, Education, and Extension Service (2006-35203-17283, 2008-35203-19120, and 2008-35206-18764).
[2] Author disclosures: A. Lassala, F. W. Bazer, T. A. Cudd, P. Li, X. L. Li, M. C. Satterfield, T. E. Spencer, and G. Wu, no conflicts of interest.
[3] Supplemental Tables 1–4 are available with the online posting of this paper at jn.nutrition.org.
[6] Abbreviations used: AUC, area under the concentration-time curve; CL, total clearance; C_{max}, maximum concentration; NO, nitric oxide; $T_{1/2}$, half-life.
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Manuscript received November 5, 2008. Initial review completed January 14, 2009. Revision accepted January 23, 2009.
First published online February 18, 2009; doi:10.3945/jn.108.102020.

Materials and Methods

Ewes. Six multiparous Suffolk crossbred ewes weighing 62.4 ± 3.2 kg (mean ± SEM) were used for catheterization of maternal and fetal blood vessels. Animals were sheared, washed, and confined in individual pens indoors with free access to drinking water and fed at 0700 and 1800 to meet 100% of the NRC 1985 nutrient requirements for pregnant sheep (22). A complete pelleted diet (**Table 1**) was purchased from Producers Cooperative Association. Ewes consumed all of the feed (22 g/kg body weight) provided daily. This study was approved by the Texas A&M University Institutional Animal Care and Use Committee.

Surgical instrumentation and experimental design. After a 7-d period of acclimation to their confinement conditions and coinciding with d 130 ± 1 (mean ± SEM) of gestation, ewes were instrumented with catheters to access maternal and fetal vessels as previously described (24). Briefly, anesthesia was induced by i.v. administration of diazepam (0.2 mg/kg body weight; Abbott Laboratories) and ketamine (4 mg/kg body weight; Fort Dodge). A surgical plane of anesthesia was maintained using isofluorane (0.5–2.5%, Abbott Laboratories) after endotracheal intubation. The ewes were positioned in dorsal recumbency and a ventral midline laparotomy was performed to expose uterus and fetal membranes, which were incised. After exteriorizing a hind leg of the fetus, a polyvinyl chloride catheter (0.08-cm i.d., 0.13-cm o.d.) was passed from the cranial tibial artery into the abdominal aorta. The procedure was repeated for the alternate leg, after which the fetus was returned to the amniotic sac and the uterus was closed with sutures prior to closing the maternal midline incision. The ewes were then instrumented to access arterial and venous blood vessels by advancing polyvinyl chloride catheters (0.13-cm i.d., 0.23-cm o.d.) into the maternal aorta and vena cava via the femoral artery and vein, respectively. Fetal and maternal catheters were passed through the abdominal wall in the flank of the ewe and secured in a pouch attached to the skin.

Following postsurgical recovery for 5 d, all ewes received a sterile i.v. bolus dose of L-arginine-HCl (Sigma Chemicals) equivalent to 155 μmol L-arginine/kg body weight on the first day of sampling (d 1) and the same dose of L-citrulline (Sigma Chemicals) on the subsequent day (d 2). The amount of infused arginine or citrulline was determined on the basis of our previous study with ewes at d 105 of gestation (18). Additionally, results from a previous study in our laboratory indicated that arginine was completely cleared from the circulation at 5 h after its i.v. administration to pregnant ewes (18). Infused solutions were prepared at concentrations of 1.5 g/5 mL for arginine and 1.5 g/20 mL for citrulline using sterile physiological saline (0.9% sodium chloride, Hospira). After adjusting the pH to 7.0 with 1 mol/L NaOH, solutions were filtered through a 0.22-μm cellulose acetate filter (Corning) into sterile glass containers fitted with sterile rubber caps. The total volumes of arginine-HCl and citrulline solutions administered to each ewe were ~6.0 and 22.5 mL, respectively. Infused volumes of the 2 solutions differed, because arginine is more soluble in water than citrulline. To facilitate i.v. administration of an amino acid into maternal circulation, we minimized the volume of the infused solution. Given a large volume of water (~44 L, or 70% of body weight) in a 62.4-kg pregnant ewe, the volume of the infused solution in the amounts of 6–23 mL has little impact on the circulating levels of metabolites.

TABLE 1 Composition of the diet (as-fed basis)[1]

Ingredients	*g/100 g*
Wheat midds	37.79
Corn	20.0
Dehydrated alfalfa	15.0
Soybean hulls	12.0
Soybean meal	5.25
Rice bran	5.0
Liquid binder	2.5
Ground limestone	1.42
Ammonium chloride	0.50
Mineral mixture[2]	0.30
Vitamin mixture[3]	0.05

[1] Provided the following (% of diet): crude protein, 15; crude fat, 4.09; crude fiber, 11.81; calcium, 1.0; phosphorus, 0.55; chlorine, 0.60; sodium, 0.20; potassium, 1.13; sulfur, 0.18; and magnesium, 0.27.
[2] Provided the following (mg/kg of the complete diet): manganese, 174; iron, 187; copper, 13.3; cobalt, 0.31; zinc, 150; iodine, 1.01; selenium, 0.56; and molybdenum, 1.00.
[3] Provided the following (mg/kg of the complete diet): retinyl acetate, 7.4; D-α-tocopherol,15.6; thiamin, 1.76; and menadione sodium bisulfate, 0.27.

On both treatment days, maternal and fetal arterial blood samples (1 mL) were obtained simultaneously at −120, −60, 0, 5, 15, 30, 60, 120, 180, and 240 min from the time of delivery of the amino acid solution and placed into vials containing 2 μL of 0.3 mol/L EDTA. Blood samples were carefully inverted to allow mixing with the anticoagulant and immediately centrifuged at 10,000 × *g*; 1 min. Plasma was separated and stored at −80°C until assayed for amino acids.

Determination of amino acids. Amino acids were determined using fluorometric HPLC methods involving precolumn derivatization with *o*-phthaldialdehyde as previously described (25). HPLC-grade water and methanol used for the analysis were obtained from Fisher Scientific. All other chemicals were purchased from Sigma-Aldrich. Plasma (40 μL) was deproteinated with 40 μL of 1.5 mol/L $HClO_4$, followed by the addition of 20 μL of 2 mol/L K_2CO_3 and 900 μL of HPLC water. Amino acids in samples were quantified on the basis of authentic standards (Sigma-Aldrich) using Millenium-32 Software (Waters) (26).

Calculations and statistical analyses. Pharmacokinetics of arginine or citrulline following administration of an i.v. bolus were analyzed after subtraction of baseline concentrations of plasma arginine and citrulline, using the single exponential model: plasma amino acid = $a \times e^{(-b \times t)}$, where a is maximum concentration (C_{max}) in plasma and b is the elimination rate, as described previously (18). The internal exposure to exogenous arginine or citrulline was estimated by calculating the area under the concentration-time curve (AUC; a/b), with total clearance (CL) = amino acid dose/AUC. The C_{max} of arginine and citrulline in plasma were calculated by back-extrapolation of the elimination curve to time zero. The $T_{1/2}$ of the infused amino acid was determined from the elimination curve (18).

Differences in pharmacokinetic parameters between arginine and citrulline following i.v. administration to ewes were determined by 1-way ANOVA. Data on concentrations of amino acids in plasma among different time points after arginine or citrulline infusion were analyzed by ANOVA for repeated measures, using the mixed model procedure of SAS (version 9.1, SAS Institute). Differences among treatment means were determined by the Student-Newman-Keuls multiple comparison test. In addition, the linear regression procedure was used to compare slopes of change in concentrations of each amino acid throughout the sampling periods. A P-value ≤ 0.05 was considered significant.

Results

Concentrations of amino acids in maternal or fetal plasma obtained at −120, −60, and 0 min did not differ on either sampling day. Hence, means for the −120, −60, and 0 min time points are provided as baseline values as time 0 min. In addition, baseline concentrations of all amino acids in maternal and fetal plasma did not differ among sampling days, indicating a lack of carryover effects of arginine administration on subsequent days of sampling.

Amino acids in maternal plasma. A rapid increase in the concentrations of arginine and citrulline in maternal plasma occurred within 30 min after i.v. bolus administration of these 2 amino acids to ewes (**Tables 2** and **3**). The concentration of arginine in maternal plasma peaked at 5 min after arginine infusion, with circulating levels remaining above ($P < 0.01$)

TABLE 2 Concentrations of arginine, citrulline, and ornithine in maternal and fetal plasma after a single i.v. bolus injection of L-arginine-HCl in late pregnant ewes[1]

	Time after bolus injection, *min*									
Amino acid	0	5	15	30	60	120	180	240	SEM	*P*-value
Maternal plasma					*μmol/L*					
Arginine	159^{e}	668^{a}	441^{b}	377^{b}	281^{c}	234^{cd}	181de	174de	55	0.001
Citrulline	119	140	136	157	144	145	128	127	11	0.086
Ornithine	56^{d}	88^{bc}	96^{ab}	108^{a}	93^{bc}	82^{c}	64^{d}	58^{d}	10	0.001
Fetal plasma										
Arginine	125^{c}	128^{c}	161^{b}	190^{a}	141^{bc}	135^{c}	128^{c}	134^{c}	12	0.001
Citrulline	119	114	119	118	114	115	115	126	6	0.335
Ornithine	35^{c}	39^{bc}	46^{ab}	49^{a}	47^{ab}	41^{bc}	38^{c}	40^{bc}	4	0.007

[1] Values are means with pooled SEM, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$.

baseline values for up to 120 min postinjection (Table 2). Similar results were obtained for concentrations of citrulline after its administration, except that values at 240 min remained greater ($P < 0.01$) than those at time 0 (Table 3).

Administration of arginine to the ewes did not increase concentrations of citrulline in maternal plasma at any time postinjection (Table 2). However, concentrations of arginine in maternal plasma increased ($P < 0.05$) between 5 and 240 min after citrulline infusion (Table 3). Compared with baseline values, concentrations of ornithine in maternal plasma increased ($P < 0.01$) 94% between 15 and 30 min after exogenous arginine infusion and remained elevated ($P < 0.05$) up to 120 min postinjection (Table 2). In contrast, there were no changes in concentrations of ornithine in maternal plasma at any time point after citrulline administration (Table 3).

When compared with time 0, concentrations of aspartate, glutamate, asparagine, threonine, and leucine in maternal plasma decreased ($P < 0.05$) and concentrations of glutamine, taurine, methionine, and lysine increased ($P < 0.05$) at various time points after i.v. administration of arginine to ewes (**Supplemental Table 1**). Particularly, the concentration of aspartate in plasma decreased ($P < 0.01$) by 50% at 180 min, whereas the concentration of lysine increased ($P < 0.01$) by 41% at 5 min (Supplemental Table 1). In addition, concentrations of proline in maternal plasma were higher than baseline values between 5 and 180 min after arginine injection (Supplemental Table 1). Arginine treatment did not affect the concentrations of other amino acids.

Concentrations of aspartate in maternal plasma decreased ($P < 0.01$) between 30 and 240 min, whereas concentrations of proline increased between 30 and 120 min after citrulline infusion. However, concentrations of other amino acids did not change at any time point after citrulline injection to ewes (**Supplemental Table 2**).

Amino acids in fetal plasma. Compared with baseline values, concentrations of arginine in fetal plasma were 50 and 29% greater ($P < 0.01$) at 30 min and 60 min, respectively, after i.v. administration of arginine (Table 2) and citrulline (Table 3) to ewes. Circulating levels of arginine in the fetus remained higher ($P < 0.05$) than baseline values between 30 and 240 min after citrulline administration (Table 3), but only between 15 and 30 min after arginine administration (Table 2). In contrast, concentrations of citrulline in fetal plasma increased ($P < 0.01$) between 15 and 240 min after i.v. infusion of citrulline to ewes (Table 3) but were not altered at any time point after administration of arginine (Table 2).

Concentrations of ornithine in fetal plasma increased ($P < 0.01$) between 15 and 30 min after bolus i.v. administration of arginine to ewes but did not change ($P > 0.10$) at other time points postinjection compared with the baseline value (Table 2). Interestingly, in response to citrulline infusion, concentrations of ornithine in fetal plasma increased substantially ($P < 0.05$) at 30 min and remained elevated throughout the sampling period for up to 240 min ($P < 0.05$) (Table 2).

Arginine infusion decreased ($P < 0.05$) concentrations of leucine in fetal plasma between 15 and 120 min and increased concentrations of proline between 15 and 180 min postadministration but did not affect other amino acids (**Supplemental Table 3**). In contrast, citrulline administration increased ($P < 0.05$) concentrations of lysine in fetal plasma between 120 and 240

TABLE 3 Concentrations of arginine, citrulline, and ornithine in maternal and fetal plasma after a single i.v. bolus injection of L-citrulline to late pregnant ewes[1]

	Time after bolus injection, *min*									
Amino acid	0	5	15	30	60	120	180	240	SEM	*P*-value
Maternal plasma					*μmol/L*					
Arginine	156^{b}	187^{a}	202^{a}	195^{a}	192^{a}	194^{a}	198^{a}	196^{a}	28	0.014
Citrulline	143^{f}	765^{a}	610^{b}	459^{c}	362^{d}	306de	285^{e}	256^{e}	43	0.001
Ornithine	59	61	67	65	66	69	69	67	7	0.134
Fetal plasma										
Arginine	131^{c}	134^{bc}	138^{bc}	148^{b}	169^{a}	166^{a}	172^{a}	164^{a}	9	0.001
Citrulline	112^{d}	121^{d}	131^{c}	145^{b}	162^{a}	168^{a}	170^{a}	167^{a}	5	0.001
Ornithine	35^{bc}	33^{c}	36^{bc}	44^{ab}	49^{a}	47^{a}	53^{a}	52^{a}	8	< 0.001

[1] Values are means with pooled SEM, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$.

min and concentrations of proline between 15 and 240 min but did not affect other amino acids at any time point (**Supplemental Table 4**).

Pharmacokinetics of arginine and citrulline in ewes. The AUC values were greater ($P < 0.05$) in maternal plasma after administration of an i.v. bolus of citrulline compared with arginine (**Table 4**). Accordingly, CL values were lower ($P < 0.05$) and the $T_{1/2}$ greater after i.v. administration of citrulline. C_{max} of arginine and citrulline in maternal plasma did not differ after their i.v. infusion into ewes (Table 4).

Discussion

Arginine is a nutritionally essential amino acid for optimal growth and development of the fetus (27) due to underdevelopment of the intestinal-renal axis for endogenous arginine synthesis from glutamine, glutamate, and proline (28). The fetus obtains arginine directly from the maternal circulation via uteroplacental blood flow, as well as indirectly from citrulline via reactions catalyzed by argininosuccinate synthase and argininosuccinate lyase (21). Because citrulline is a neutral amino acid and its administration does not disturb the acid-base balance of the animal (16), there is increasing interest in the use of citrulline as a precursor for arginine in nonpregnant animals and humans (29–31). However, there are no reports of studies to determine the efficacy of conversion of exogenous citrulline into arginine in the mother or fetus. The major finding of the present study is that i.v. administration of L-citrulline was more effective than L-arginine in achieving and sustaining a prolonged increase in concentrations of arginine in the fetal circulation (Tables 2 and 3). Consistent with this observation, the $T_{1/2}$ of citrulline in maternal plasma of ewes was twice that of arginine (Table 4). These results can be explained by higher activities for arginase than for argininosuccinate synthase and argininosuccinate lyase (the enzymes responsible for citrulline catabolism) in extrahepatic tissues of adult mammals (19) and by the lower rate of transport of citrulline by animal cells (e.g. endothelial cells and macrophages) than that of arginine (32–34). Thus, in contrast to the i.v.-infused arginine, which is rapidly taken by maternal tissues for catabolism, i.v.-infused citrulline has a longer half-life and sustains a prolonged increase in arginine concentrations in maternal plasma. To our knowledge, this is the first report on citrulline metabolism in any pregnant mammal.

There is a high rate of arginine turnover in pregnant ewes due to its rapid clearance from the maternal plasma (18). To confirm and extend this observation, we found that concentrations of arginine and ornithine, a product of arginine metabolism, in plasma of ewes on d 135 ± 1 of gestation increased to peak values within 5 min after i.v. administration of a bolus of arginine and declined rapidly thereafter to baseline values (Table 2). Because NO production is quantitatively a minor pathway for arginine catabolism in healthy mammals (19), arginine administration did not affect concentrations of citrulline (a coproduct of NO synthase) in maternal plasma (Table 2). In contrast, i.v. infusion of citrulline into ewes markedly increased concentrations of both citrulline and arginine, but not ornithine, in maternal plasma (Table 3). Notably, concentrations of citrulline and arginine in maternal plasma remained elevated throughout a 4-h period after administration of a single bolus of citrulline into the maternal venous circulation. Furthermore, even at 4 h, the concentrations of citrulline and arginine in the fetus were 49 and 25% greater, respectively, than baseline values (Table 3).

TABLE 4 Pharmacokinetics of arginine and citrulline in the maternal plasma of late pregnant ewes receiving a single i.v. bolus injection of either L-arginine or L-citrulline[1]

	Amino acid administered		
Parameters	Arginine	Citrulline	*P*-value
AUC, *(mmol· min)/L*	29.0 ± 6.3	70.8 ± 13.3	0.017
CL, *mL/(min·kg)*	6.60 ± 1.41	2.64 ± 0.6	0.031
$T_{1/2}$, *min*	45.3 ± 7.0	89.2 ± 11.6	0.009
C_{max}, *μmol/L*	453.4 ± 78.1	530.9 ± 40.1	0.398

[1] Values are means with pooled SEM, $n = 6$.

Results of the present study indicate that citrulline is readily converted into arginine in maternal tissues. In support of this view, aspartate (a substrate of argininosuccinate synthase in the pathway of arginine synthesis from citrulline) was the only amino acid for which concentrations in maternal plasma were reduced due to its extensive utilization for arginine formation in response to citrulline administration (Supplemental Table 2). The findings of the present study also suggest that citrulline-derived arginine is not substantially degraded to generate ornithine via arginase in pregnant ewes. This further provides evidence for complex metabolism of arginine via its compartmentalized pathways in animals (19). Thus, whether i.v. administration of arginine or citrulline into pregnant ewes is capable of increasing arginine availability in the fetus must be determined experimentally, as we did in the present study.

A substantial increase in concentrations of amino acids (including citrulline and arginine) in the maternal plasma can enhance their uptake into the fetal circulation (35–37). However, the rate of delivery of an amino acid from the uterus to the fetus depends not only on its transport from the maternal circulation to the fetal-placenta circulation but also on its utilization and metabolism by placental cells and subsequent efflux from the placenta into the fetus (27,38). These critical events involve placental transport systems at both maternal and fetal surfaces of the placenta (38). In sheep, the y^+ and y^+L cationic amino acid transport systems on both surfaces of the placenta are responsible for net transport of arginine to the fetus (19,39). A high activity of arginase in ovine placentomes rapidly hydrolyzes arginine into urea and ornithine (4). Thus, although peak concentrations of arginine in maternal plasma occurred within 5 min of its i.v. administration to ewes and were 320% greater than baseline values (Table 2), concentrations of arginine in fetal plasma increased only 52% at 30 min and returned to baseline values at 60 min (Supplemental Table 2). In contrast, i.v. administration of citrulline to ewes resulted in progressive increases in concentrations of both citrulline and arginine in fetal plasma between 5 and 60 min and, importantly, these elevated concentrations were sustained throughout the remainder of the sampling period, i.e. to 240 min. Indeed, concentrations of citrulline, arginine, ornithine, and proline in fetal plasma at 4 h were 49, 25, 48, and 17% greater than for baseline values, respectively. Collectively, these are the first results to demonstrate that i.v. administration of a bolus of citrulline into pregnant ewes results in a protracted period of increased arginine availability in the fetus.

Changes in concentrations of amino acids other than arginine, citrulline, ornithine, and aspartate in maternal and fetal circulations after i.v. administration of arginine or citrulline deserve comments. Basic amino acids share the same transport systems in plasma membranes (40). Moreover, the systems $b^{o,+}$, $B^{o,+}$, and y^+L can transport both basic and large neutral amino acids into

cells (41). Thus, with increased concentrations of arginine in plasma of ewes, uptakes of lysine (a basic amino acid) as well as proline and methionine (large neutral amino acids) by maternal tissues were reduced, leading to transient increases in concentrations of these amino acids in the maternal circulation (Supplemental Table 2). An increase in concentrations of glutamine in maternal plasma after arginine infusion is likely due to the formation of glutamine via the pathway involving arginase, ornithine aminotransferase, and glutamine synthetase (19). Interestingly, i.v. administration of both arginine and citrulline increased concentrations of proline in fetal plasma but had no significant or prolonged effects on concentrations of other neutral amino acids (Supplemental Table 3 and 4). This finding is exciting, because proline has been suggested to have an important role in conceptus metabolism and development (21). Additionally, these results indicate that the dosages of arginine or citrulline administered to pregnant ewes do not alter the availability of other amino acids in the fetus that are required to promote fetal and placental growth.

Due to technical difficulties to obtain allantoic and amniotic fluids from ewes at d 135 of gestation, we did not have data on amino acid concentrations in these 2 fluids. Because arginine in these 2 fetal fluids is derived from maternal and fetal plasma (4), there is a positive correlation between arginine concentrations in fetal circulation and those in allantoic and amniotic fluids (42). Thus, it is reasonable to conclude that an increase in arginine concentrations in fetal plasma in response to maternal infusion of arginine or citrulline reflects an increase in arginine availability in the fetus. Lassala (43) reported that i.v. infusion of arginine to underfed ewes (155 μmol/kg body weight) between d 60 and 147 of gestation prevented fetal growth restriction. Thus, the increased concentrations of arginine in fetal plasma brought about by maternal infusion of arginine or citrulline are nutritionally meaningful.

In summary, results of this study reveal that the $T_{1/2}$ of citrulline in plasma of pregnant ewes is approximately twice that for arginine. Therefore, i.v. administration of citrulline into ewes is more effective than arginine in increasing concentrations of arginine in both maternal and fetal circulations, without reducing concentrations of other amino acids in the fetus. These novel findings should aid in the design of strategies for effective delivery of L-arginine or L-citrulline solutions into underfed, obese, or preeclamptic dams to ameliorate intrauterine growth restriction (44,45), an important problem in both human medicine and animal production (5,23).

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