Parenteral Administration of L-Arginine Prevents Fetal Growth Restriction in Undernourished Ewes$^{1,2}$

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Abstract

Intrauterine growth restriction (IUGR) is a major health problem worldwide that currently lacks an effective therapeutic solution. This study was conducted with an ovine IUGR model to test the hypothesis that parenteral administration of L-arginine (Arg) is effective in enhancing fetal growth. Beginning on d 28 of gestation, ewes were fed a diet providing 100% (control-fed) or 50% (underfed) of NRC-recommended nutrient requirements. Between d 60 of gestation and parturition, underfed ewes received i.v. infusions of saline or 155 μmol Arg·HCl/kg body weight 3 times daily, whereas control-fed ewes received only saline. The birth weights of lambs from saline-infused underfed ewes were 23% lower ($P < 0.01$) than those of lambs from control-fed dams. Administration of Arg to underfed ewes increased ($P < 0.01$) concentrations of Arg (69%), ornithine (55%), proline (29%), methionine (37%), leucine (36%), isoleucine (35%), cysteine (19%), and FFA (43%) in maternal serum, decreased maternal circulating levels of ammonia (18%) and triglycerides (32%), and enhanced birth weights of lambs by 21% compared with saline-infused underfed ewes. There was no difference in birth weights of lambs between the control-fed and the Arg-infused underfed ewes. These novel results indicate that parenteral administration of Arg to underfed ewes prevented fetal growth restriction and provide support for its clinical use to ameliorate IUGR in humans. The findings also lay a new framework for studying cellular and molecular mechanisms responsible for the beneficial effects of Arg in regulating conceptus growth and development. J. Nutr. 140: 1242–1248, 2010.

Introduction

Intrauterine growth restriction (IUGR)$^7$ is a major health problem worldwide, representing 11% of all newborns in developing countries and a large number of all newborns in developed nations, e.g. 3–5% in the US (1). Maternal undernutrition, which occurs under conditions such as inadequate supply of food or severe nausea and vomiting in pregnant women, is an important factor that adversely impacts fetal growth (2,3). IUGR results in emotional stress and contributes to extremely high costs of health care due to perinatal and life-long medical complications (4). For example, ~50% of nonmalformed stillbirths result from IUGR and 5% of premature deliveries are due to the poor growth of fetuses in utero (5,6). Also, infants who weigh <2.5 kg at birth have 5–30 times higher rates of perinatal mortality than newborns who have average birth weights, and these rates are 70–100 times higher for infants weighing <1.5 kg at birth (6). Furthermore, surviving infants with IUGR are at increased risk for neurological, respiratory, intestinal, and circulatory disorders (7). To date, there is no therapeutic means for preventing or ameliorating IUGR, the current management being empirical and primarily aimed at selecting a safe time for delivery (4,8).

L-Arginine (Arg), a nutritionally essential amino acid for the fetus (9), is a precursor for synthesis of nitric oxide (NO) and polyamines in cells (10). NO is a major endothelium-derived vasodilator, whereas polyamines are key regulators of DNA and protein synthesis (10). Consequently, Arg may play a critical role in placental growth (including vascular growth), utero-placental blood flow, and hence the transfer of nutrients from mother to fetus (11). We found that maternal undernutrition reduced Arg concentrations in maternal and fetal plasma of ewes (12) and decreased the availability of Arg, polyamines, and NO in the conceptus (fetus and associated membranes) (12–14). Interestingly, direct infusion of Arg into the fetal femoral vein for 3–4 h increased fetal whole-body protein accretion in an ovine model of IUGR induced by placental insufficiency (15).

We hypothesized that parenteral administration of Arg to underfed dams could ameliorate or prevent fetal growth.
receive either 10 mL of sterile saline solution (group 50% NRC, n = 5) or sterile Arg-HCl solution (155 μmol Arg/kg body weight; Sigma-Aldrich) (group 50% + Arg, n = 5) 3 times daily (0800, 1500, and 2200 h). This dose of Arg was chosen because it substantially increased concentrations of Arg in plasma of both mother and fetus (9). The amount of infused Arg provided 1.86 mmol N/(kg body weight-d), which was 11% of nitrogen intake from the feed [16.9 mmol N/(kg body weight-d)] in underfed ewes. We found that provision of 1.86 mmol N/(kg body weight-d) in the form of wheat protein did not affect fetal growth in 70- to 80-kg underfed ewes (our unpublished data). Therefore, effects of supplemental Arg on ewes were not likely due to a nonspecific action of increased nitrogen provision.

The Arg-HCl solution was prepared twice per week using sterile physiological saline (sodium chloride 0.9%, Hospira) with a final concentration of 300 g Arg/L. The pH was adjusted to 7.0 with 1 mol/L NaOH and the solution filtered through a 0.22-μm cellulose acetate filter (Corning) into reusable steril glass containers fitted with adjustable-sealing sterile rubber caps. The prepared Arg-HCl solution was kept at −20°C and thawed at 4°C the night before use. Disposable 10-mL syringes (latex free, luer-lok tip, Becton Dickinson) were marked with ewe identification numbers and filled in the laboratory with either saline or Arg-HCl solution before each infusion. The Arg-HCl solution was used throughout the day, with the rubber cap on the bottle being cleaned with 70% ethanol before insertion of the disposable needle. A bacteriological culture of randomly selected saline and Arg-HCl solution was performed by the Texas Medical Diagnostic Laboratory on 2 occasions to verify sterility. Between infusions, catheters were flushed and filled with 0.75 mL heparin solution (40 KU/L, American Pharmaceutical Partners) to prevent clotting and maintain potency.

Every 10 d from d 60 of pregnancy until parturition, ~7 mL of maternal blood was obtained from all ewes from the jugular vein contralateral to the catheterized jugular vein, using anticoagulant-free, sterile vacuum tubes (Vacutainer, Becton Dickinson) and 20 G × 3.8-cm blood collection needles (Vacutainer, Becton Dickinson). Blood was drawn in the morning immediately before the first infusion of either saline or Arg-HCl solution. Samples were placed on ice and immediately centrifuged at 3000 g for 15 min. Serum was separated and stored at −80°C until analyzed for amino acids, metabolites, and hormones.

At parturition, a portable scale was used to record birth weights of lambs. Care was taken to weigh the newborns immediately after birth.

### Materials and Methods

**Ewes.** Fifteen multiparous Suffolk crossbred ewes weighing 76.7 ± 2.8 kg (mean ± SEM) were mated to a single fertile Suffolk ram when detected in estrus (d 0) and 12 h later to minimize paternal genetic effects on size and weight of the fetuses. At d 21 postmating, ewes were transported to the Texas A&M Animal Science Teaching, Research and Extension Center, where they were individually housed in outdoor covered pens with free access to drinking water and allowed a 7-d period of acclimation. Transabdominal ultrasonography (7.5 MHz probe, Aloka console) was used to confirm pregnancy. Ewes were fed a wheat, cottonseed, rice mill, and alfalfa-based diet (Table 1; Producers Cooperative Association) to meet 100% of the NRC (20) nutrient requirements for pregnant sheep. Ewes were fed once daily from 0700 and 0800 h. This study was approved by the Texas A&M University Institutional Animal Care and Use Committee.

**Experimental design.** At d 28 of pregnancy, ewes were assigned randomly to be fed either 100% (n = 5) or 50% (n = 10) of the daily NRC nutrient requirements for pregnant sheep (20). The diet and daily feeding schedule were the same as provided during the acclimation period. Ewes were weighed weekly, before feeding, and diets were adjusted on an individual basis according to recorded live-weight changes. The 30% level of underfeeding was adopted, because it has been shown to reduce placental and fetal growth in sheep (12,18,19).

One to three days before d 60 of gestation, a 16 G × 13 cm polyurethane peripheral catheter (Millicath, MILA International) was placed into the jugular vein of the ewes, fixed to the skin of the neck, and fitted with a 30.5-cm microbore extension (Hospira) that was sutured close to the base of the head adjacent to the occipital region, allowing access from the back of the ewe. The extension was capped with an intermittent infusion plug (Kendall Argyle, Tyco Healthcare Retail Group) that permitted repeated insertion of regular disposable needles. The intermittent infusion plugs that capped the catheter extensions were periodically changed throughout the experimental period, whereas extensions and catheters were only replaced when damaged or pulled out.

Between d 60 of pregnancy and parturition, control-fed ewes (group 100% NRC, n = 5) received 10 mL of sterile saline solution as a bolus injection (sodium chloride 0.9%, Hospira) through the jugular catheter 3 times daily and underfed ewes were randomly divided into 2 groups to

### TABLE 1 Composition of the diet†

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat middlings</td>
<td>42.25 g</td>
</tr>
<tr>
<td>Cottonseed hulls</td>
<td>27.4 g</td>
</tr>
<tr>
<td>Rice mill feed</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Dehydrated alfalfa</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Liquid binder</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Ground limestone</td>
<td>1.76 g</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.50 g</td>
</tr>
<tr>
<td>Mineral mixture†</td>
<td>0.50 g</td>
</tr>
<tr>
<td>Vitamin mixture†</td>
<td>0.09 g</td>
</tr>
</tbody>
</table>

1 Provided the following (percent of diet): crude protein, 11.7; arginine, 0.60; crude fat, 3.4; crude fiber, 22.3; calcium, 1.0; phosphorus, 0.50; chlorine, 0.61; sodium, 0.40; potassium, 0.94; sulfur, 0.18; magnesium, 0.25.
2 Provided the following (mg/kg of the complete diet): manganese, 226; iron, 228; copper, 11.3; cobalt, 0.35; zinc, 146; iodine, 1.00; selenium, 0.53; and molybdenum, 1.00.
3 Provided the following (mg/kg of the complete diet): retinyl acetate, 4.46; a-tocopherol, 13.2; thiamin, 1.76; and menadione sodium bisulfate, 0.27.

### Determination of amino acids, other metabolites, and hormones in serum.

Deproteinated serum was used for the analyses for amino acids, ammonia, urea, glucose, lactate, glyceral, and β-hydroxybutyrate (BHB), whereas whole serum was assayed for FFA, triglycerides, insulin, and growth hormone (GH). Amino acids were determined by fluorometric HPLC methods involving precolumn derivatization with o-phthalaldialdehyde as described (12). BHB was measured enzymatically by a spectrophotometric method using 3-hydroxybutyrate dehydrogenase (21). FFA were quantified by an enzymatic colorimetric method using the assay kit from Waco Chemicals. Glucose was determined enzymatically using a spectrophotometric method involving hexokinase and glucose-6-phosphate dehydrogenase (22). Glyceral and lactate were quantified using enzymatic fluorometric methods, as described by Fu et al. (22) and Wu et al. (23), respectively. Triglycerides were determined enzymatically using the Infinity assay kit from Thermo Electron. Ammonia and urea were determined using fluorometric methods involving glutamate dehydrogenase and urease (23,24). Insulin was analyzed using the ovine insulin ELISA microplate kit from Mercodia, whereas GH was quantified using RIA validated for ovine serum (25,26).

### Statistical analyses.

Results are expressed as means ± SEM. Data on lamb birth weight were statistically analyzed by 1-way ANOVA using a variance-covariance matrix that considered the effects of number of lambs born (singleton or twins) (27). Data on concentrations of amino acids, other metabolites, and hormones in serum were analyzed by 2-way ANOVA for repeated measures to determine the effects of day of pregnancy, treatment, and day of pregnancy × treatment interactions (27). Where there was a significant treatment × day interaction, effects of day within nutritional treatment were analyzed by 1-way ANOVA. In 1-way

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TABLE 2  Maternal body weights on d 28 and 140 of pregnancy in ewes fed 100% NRC, 50% NRC, or 50% NRC + Arg diets and body weights of lambs at birth.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of pregnancy</th>
<th>Weight change (d 140 – d 28)</th>
<th>Birth weight of lambs</th>
<th>kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% NRC</td>
<td>d 28</td>
<td>71.1 ± 4.7</td>
<td>9.1 ± 3.2</td>
<td>3.99 ± 0.24</td>
</tr>
<tr>
<td>50% NRC</td>
<td>d 28</td>
<td>78.5 ± 2.7</td>
<td>–4.0 ± 1.8</td>
<td>3.06 ± 0.16</td>
</tr>
<tr>
<td>50% NRC + Arg</td>
<td>d 28</td>
<td>80.3 ± 5.7</td>
<td>–1.9 ± 0.7</td>
<td>3.70 ± 0.21</td>
</tr>
</tbody>
</table>

*Data are means ± SEM, n = 5. Means in a column with superscripts without a common letter differ, P < 0.01.*

or 2-way ANOVA, differences among treatment means were determined by the Student-Newman-Keuls multiple comparison test (27). Log transformation of variables was performed when variance of data were not homogenous among treatment groups, as assessed by the Levene’s test. P-values ≤ 0.05 were taken to indicate significance.

**Results**

**Body weights of ewes and newborn lambs.** Feed intake did not differ between saline- and Arg-infused underfed ewes throughout the experimental period. However, feed intake was 50% lower (P < 0.01) in the underfed ewes [11.3 ± 0.12 g/(kg body weight-d)] than in the control-fed ewes [22.2 ± 0.08 g/(kg body weight-d)]. On d 28 when nutrient restriction was initiated, the live weight of the ewes did not differ among control-fed (100% NRC requirement), underfed (50% NRC requirement), and Arg-treated underfed (50% NRC + Arg) groups (Table 2). At the end of pregnancy, control-fed ewes gained 13% in body weight over that at d 28, whereas the underfed ewes receiving either i.v. saline or Arg lost 6.7% and 2.1% of their body weight, respectively (Table 2). The length of gestation was 142.3 ± 1.2 d (n = 15) and did not differ among the 3 groups of ewes.

There were singleton and twin lambs born to ewes in all groups. At birth, both the control-fed and the 50% NRC + Arg groups had 3 singleton and 2 twin lambs each, whereas the 50% NRC group had 2 singleton and 3 twin lambs. Birth weights were greater (P < 0.01) for singleton lambs (4.03 ± 0.21 kg) than those for individual lambs born as twins (3.17 ± 0.14 kg). Thus, fetal number per ewe was included in the statistical analysis as a covariate to assess differences in birth weights between groups.

The birth weights of lambs from saline-infused underfed ewes were 23% lower (P < 0.01) than those for lambs born to control-fed dams (Table 2). Arg infusion to underfed ewes increased (P < 0.01) birth weights of lambs by 21% compared with underfed ewes receiving saline infusion (Table 1). There was no difference in birth weights between control-fed and 50% NRC + Arg groups.

We did not determine rates of postnatal growth in the lambs. However, we monitored their health status within 1 mo after

**TABLE 3**  Serum concentrations of amino acids on d 60, 80, 110, and 140 of pregnancy in ewes fed 100% NRC, 50% NRC, or 50% NRC + Arg diets.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Treatment (T)</th>
<th>60</th>
<th>80</th>
<th>110</th>
<th>140</th>
<th>SEM</th>
<th>T</th>
<th>D</th>
<th>T × D</th>
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<tbody>
<tr>
<td>Aspartate</td>
<td>7.9</td>
<td>4.6</td>
<td>6.0</td>
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<td></td>
<td></td>
<td>0.9</td>
<td></td>
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<tr>
<td>Glutamate</td>
<td>120</td>
<td>93</td>
<td>98</td>
<td>177</td>
<td>81</td>
<td>51</td>
<td>13</td>
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<tr>
<td>Asparagine</td>
<td>30</td>
<td>18</td>
<td>23</td>
<td>25</td>
<td>24</td>
<td>20</td>
<td>4</td>
<td></td>
<td></td>
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<tr>
<td>Serine</td>
<td>79</td>
<td>58</td>
<td>71</td>
<td>72</td>
<td>69</td>
<td>61</td>
<td>8</td>
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<tr>
<td>Glutamine</td>
<td>232</td>
<td>173</td>
<td>193</td>
<td>125</td>
<td>202</td>
<td>226</td>
<td>244</td>
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<td></td>
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<tr>
<td>Histidine</td>
<td>43</td>
<td>27</td>
<td>34</td>
<td>35</td>
<td>37</td>
<td>31</td>
<td>4</td>
<td></td>
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<tr>
<td>Glycine</td>
<td>394</td>
<td>381</td>
<td>442</td>
<td>362</td>
<td>379</td>
<td>409</td>
<td>472</td>
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<tr>
<td>Threonine</td>
<td>105</td>
<td>28</td>
<td>36</td>
<td>71</td>
<td>65</td>
<td>53</td>
<td>37</td>
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<tr>
<td>Citrulline</td>
<td>261</td>
<td>159</td>
<td>190</td>
<td>182</td>
<td>267</td>
<td>188</td>
<td>179</td>
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<tr>
<td>Arginine</td>
<td>295</td>
<td>178</td>
<td>300</td>
<td>204</td>
<td>301</td>
<td>265</td>
<td>262</td>
<td>26</td>
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<tr>
<td>β-Alanine</td>
<td>13</td>
<td>7.9</td>
<td>9.4</td>
<td>9.8</td>
<td>9.9</td>
<td>9.8</td>
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<tr>
<td>Taurine</td>
<td>142</td>
<td>77</td>
<td>92</td>
<td>48</td>
<td>144</td>
<td>110</td>
<td>113</td>
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<tr>
<td>Alanine</td>
<td>178</td>
<td>118</td>
<td>149</td>
<td>148</td>
<td>163</td>
<td>133</td>
<td>149</td>
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<tr>
<td>Tyrosine</td>
<td>52</td>
<td>27</td>
<td>33</td>
<td>33</td>
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<td>11</td>
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<tr>
<td>Valine</td>
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<td>77</td>
<td>106</td>
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<tr>
<td>Isoleucine</td>
<td>81</td>
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<td>70</td>
<td>51</td>
<td>65</td>
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<td>84</td>
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<td>Leucine</td>
<td>126</td>
<td>75</td>
<td>102</td>
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<td>98</td>
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<tr>
<td>Ornithine</td>
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<td>42</td>
<td>65</td>
<td>64</td>
<td>102</td>
<td>66</td>
<td>55</td>
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<tr>
<td>Lysine</td>
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<td>69</td>
<td>89</td>
<td>62</td>
<td>113</td>
<td>101</td>
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<tr>
<td>Cysteine</td>
<td>167</td>
<td>112</td>
<td>133</td>
<td>121</td>
<td>180</td>
<td>135</td>
<td>113</td>
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<tr>
<td>Proline</td>
<td>145</td>
<td>95</td>
<td>123</td>
<td>115</td>
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<td>122</td>
<td>124</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

*Data are means ± SEM, n = 15 observations/d (5 ewes × 3 treatments) and n = 20 observations/treatment (5 ewes × 4 d). Means in a row with superscripts without a common letter differ, P < 0.05 with regard to effects of treatment (T), day (D), or treatment × day (T × D) interaction.

2 Cysteine + 1/2 cystine.
birth. Two lambs from the underfed group died within the first week (d 2 and 6) after birth, but no deaths occurred in the control-fed or underfed + Arg group within 30 d after birth. All surviving lambs appeared to gain weight normally.

Concentrations of amino acids in maternal serum. Concentrations of amino acids in serum of control-fed ewes and underfed ewes with or without Arg are summarized in Table 3. Concentrations of all other amino acids were lower (P < 0.05) in saline-infused undernourished ewes compared with control-fed ewes. Concentrations of arginine, histidine, threonine, citrulline, tyrosine, tryptophan, valine, phenylalanine, and lysine were lower (P < 0.05) in the 50% NRC + Arg group than in control ewes but did not differ from control-fed ewes. Concentrations of asparagine, serine, histidine, and alanine in serum also increased (P < 0.05) for aspartate, citrulline, Arg, alanine, valine, isoleucine, lysine, cysteine, and proline in maternal serum between d 60 and 140 of gestation. Levels of cysteine in maternal serum also increased (P < 0.05) by 95, 63, 138, and 65%, respectively, in maternal serum between d 60 and 140 of gestation. In contrast, concentrations of glutamine, isoleucine, tyrosine, and lysine increased (P < 0.05) by 95, 63, 138, and 65%, respectively, in maternal serum between d 60 and 140 of gestation. Levels of cysteine in maternal serum also increased between d 60 and 80 of pregnancy but subsequently decreased gradually until d 140 of gestation. In addition, there was a treatment × day interaction (P < 0.05) for aspartate, citrulline, Arg, alanine, valine, isoleucine, lysine, cysteine, and proline in that concentrations in serum at d 60 of gestation were lower than those for control ewes.

Concentrations of Arg in maternal serum increased (P < 0.01) by 69% in the 50% NRC + Arg ewes compared with the 50% NRC ewes and were similar to values for 100% NRC ewes. Concentrations of arginine, histidine, and alanine in serum increased (P < 0.05) by 69% in the 50% NRC + Arg group to concentrations comparable to those for control ewes.

Except for asparagine, Arg, alanine, valine, lysine, cysteine, and proline in serum varied with day of pregnancy (Table 3). The most striking changes were observed for glutamate and threonine, whose concentrations progressively decreased (P < 0.01) by 71 and 48%, respectively, between d 60 and 140 of gestation. In contrast, concentrations of glutamine, isoleucine, tyrosine, and lysine increased (P < 0.05) by 95, 63, 138, and 65%, respectively, in maternal serum between d 60 and 140 of gestation. Levels of cysteine in maternal serum also increased between d 60 and 80 of pregnancy but subsequently decreased gradually until d 140 of gestation. In addition, there was a treatment × day interaction (P < 0.05) for aspartate, citrulline, Arg, alanine, valine, isoleucine, lysine, cysteine, and proline in that concentrations in serum at d 60 of gestation were lower than those for control ewes.
Concentrations of other metabolites and hormones in maternal serum. Undernourished ewes (with and without Arg infusion) had lower ($P < 0.01$) concentrations of glucose in serum than control-fed ewes, which were not affected by day of pregnancy (Table 5). Interestingly, levels of triglycerides in serum were $34\%$ lower ($P < 0.05$) and those of FFA were greater ($P < 0.05$) in $50\%$ NRC + Arg ewes than in both the $50\%$ NRC and $100\%$ NRC ewes. An i.v. infusion of Arg reduced ($P < 0.05$) concentrations of ammonia in serum from underfed ewes but had no effect on concentrations of BHB, glucose, lactate, urea, insulin, or GH.

Concentrations of BHB, FFA, glycerol, triglycerides, and GH increased ($P < 0.01$), whereas concentrations of insulin decreased ($P < 0.01$) in ewes between d 60 and 140 of gestation (Table 5). Day of pregnancy did not affect concentrations of glucose, urea, or ammonia in maternal serum. Among all metabolites and hormones measured, only glycerol and GH were affected by a treatment $\times$ day interaction ($P = 0.05$; Tables 4 and 5).

Discussion

Despite advanced technologies for prenatal care of both mothers and fetuses, IUGR remains a global health problem that causes severe perinatal complications and may contribute to adult-onset diseases (1). Because there is currently a lack of therapeutic treatment for IUGR in humans (4), animal models are used to test novel hypotheses that will provide the basis for development of effective therapies (17). Although there are several basic differences in pregnancy between sheep and humans, including time of implantation, type of placentation, and gestational length (28), the ewe is a well-established animal model for studying human placental-transfer of nutrients to the fetus for several reasons (29). First, the number of offspring and regulation of nutrient transfer from mother to fetus are similar between ewes and women (30). Second, ewes can be conveniently managed for easy access to blood and other sample collection. Third, pregnant ewes are tolerant of surgical procedures that include placement of catheters into maternal and fetal blood vessels without aborting (31). Fourth, there is a large database in the literature on placental and fetal development in normal and underfed ewes (32,33).

Among intrauterine environmental factors, nutrition plays the most decisive role in influencing placental and fetal growth (2). In fact, well-controlled animal studies have consistently demonstrated that maternal undernutrition during a critical period of pregnancy substantially reduces birth weight at term (34). Although enteral feeding to $100\%$ of NRC-recommended nutrient requirements is potentially effective to reverse IUGR caused by undernutrition (12), this means of intervention is not applicable under clinical conditions such as hyperemesis gravidarum, which is characterized by severe nausea and vomiting in gestating women (35). This life-threatening disorder occurs in 1–2% of all pregnancies and generally extends beyond wk 16 of gestation (3). Therefore, parenteral nutrition must be explored to improve pregnancy outcome in these women. Through bypassing intestinal catabolism (24), direct i.v. infusion of Arg into dams effectively increases its concentrations in maternal and fetal blood of underfed ewes (36). Consistent with this proposition, maternal and fetal concentrations of Arg in serum were increased by 170 and 40%, respectively, after an infusion of a large dose of arginine ($695\ \mu\text{mol/kg body weight over 280 min}$) into the femoral vein of well-fed sheep (37). Further, in an ovine model of IUGR induced by placental embolization, infusion of Arg into the fetal femoral vein increased fetal whole-body protein accretion (15). However, these studies involved only a short-term (3–4 h) infusion of Arg into ewes or fetuses, so the effects of Arg on fetal growth could not be evaluated.

Birth weight is one of the most sensitive and important measures of fetal growth (38), which depends on maternal uterine capacity but not simply maternal body weight (11). For example, recent evidence shows that a change in maternal body weight during gestation is not a valid indicator of fetal growth in Bagg's ewes with enhanced uterine capacity (39). Therefore, assessing fetal growth retardation based on the body weight of newborn lambs relative to maternal body weight could be potentially misleading and was not adopted for our study. Consistent with previous reports (12,18,19), results from the current study indicated that global nutrient restriction ($50\%$ of NRC requirements) beginning on d 28 of gestation resulted in IUGR (Table 2). In addition, marked reductions in concentrations of most amino acids (Table 3) and glucose (Table 5) were detected in maternal serum. Thus, ewes subjected to severe malnutrition were not able to maintain homeostasis of amino acids for normal fetal growth.

Whether pregnant ewes may carry singletons or twins was beyond our control in the current work due to the nature of ovine reproductive physiology. Thus, there was a variable number of singletons and twins among the 3 groups of ewes. Because the birth weight of singletons is usually heavier than each of the twins (11), we used a variance-covariance matrix as the statistical model (27) for analysis of lamb birth weight data. This model correctly considered the effects of number of lambs born (singletons or twins) but not simply the sum of birth weights for all newborn lambs in treatment groups. A novel and important finding of the present study is that i.v. infusion of Arg to underfed ewes ($155\ \mu\text{mol Arg-HCl/kg body weight}$) 3 times daily between d 60 of pregnancy and parturition enhanced the birth weight of lambs by $21\%$ and effectively prevented IUGR without affecting maternal body weight (Table 2).

Our results indicate that the Arg treatment increased the availability of nutrients to the conceptus to support fetal growth. Indeed, concentrations of several essential and conditionally essential amino acids (methionine, isoleucine, leucine, and cysteine) were higher in serum of Arg-treated than in saline-infused underfed ewes (Table 3). Moreover, concentrations of some nonessential amino acids (aspartate, serine, and alanine) in serum of Arg-treated underfed ewes were comparable to those for control-fed ewes (Table 3). In addition, concentrations of proline, a neutral amino acid that has been recently suggested to contribute to adult-onset disease (40), were $29\%$ higher in Arg-treated than in saline-infused underfed ewes. Because feed intake by saline-infused underfed ewes ($11.3 \pm 0.14\ g/(kg body weight \cdot d)$) was the same as that for Arg-infused underfed ewes ($11.3 \pm 0.11\ g/(kg body weight \cdot d)$), the higher concentrations of amino acids in serum from Arg-infused underfed ewes compared with saline-infused underfed ewes may have resulted from alterations in maternal nitrogen metabolism. In support of this view, there is evidence that NO
reduces the urea cycle activity and the oxidation of amino acids in hepatocytes (41).

Increasing concentrations of Arg in maternal plasma by 69% can enhance NO synthesis by endothelial cells (42) and uteroplacental blood flow (43). This, in turn, would be expected to promote the transfer of oxygen and nutrients from maternal to fetal circulations. Consistent with this theory, i.m. administration of Sildenafil citrate to underfed ewes between d 28 and 112 of gestation increased concentrations of most amino acids and polyamines in fetal plasma and fluids, as well as fetal growth (18). Sildenafil citrate, which acts through enhancing intracellular cGMP availability by inhibiting phosphodiesterase-5 (an enzyme that hydrolyzes cGMP), may have augmented uteroplacental blood flow via the protein kinase G signaling pathway (44). Because catheterization of fetal vessels for blood sampling could affect fetal growth, we chose not to perform this invasive procedure in the present study. Therefore, precise changes in concentrations of nutrients in fetal circulation as well as amniotic and allantoic fluids due to i.v. infusion of Arg into ewes were not determined. Additional studies are warranted to test the hypothesis that parenteral administration of Arg may increase utero-placental blood flow and, thus, the supply of nutrients from mother to fetus.

The changes in maternal serum concentrations of hormones and metabolites observed with advanced gestation and Arg infusion in this study deserve comments (Table 5). In contrast to a large pharmacological bolus of Arg (2 mmol/kg body weight) (44), i.v. infusion of a physiological dosage of Arg (155 μmol/kg body weight 3 times daily) did not affect the circulating levels of insulin or GH (Table 5). However, there seemed to be alterations in lipid metabolism as serum concentrations of triglycerides were lower, whereas concentrations of FFA were higher in Arg-treated ewes than in saline-infused underfed or control-fed ewes (Table 5). Of particular interest, physiological levels of NO stimulate the hydrolysis of triglycerides in adipose tissue (22), thereby increasing the availability of circulating FFA for oxidation by maternal tissues (e.g. skeletal muscle) as metabolic fuels (40,45,46). This, in turn, can spare the oxidation of amino acids (47), which may have contributed to elevated levels of some amino acids in maternal serum of Arg-treated underfed ewes (Table 3). In support of this suggestion, concentrations of ammonia were lower in serum of underfed ewes in response to i.v. infusion of Arg (Table 5). Additionally, Arg can regulate expression of key genes and signaling pathways involved in the metabolism of lipids, protein, and other energy substrates in multiple tissues, including skeletal muscle, liver, adipose tissue, and small intestine (48–53). Finally, there were progressive increases in concentrations of FFA, glycerol, and BHB in maternal serum during late gestation (Table 5). These results indicate mobilization of maternal fat stores to provide energy for mother and fetus because of inadequate feed intake by ewes. The findings are consistent with the progressive decrease in concentrations of insulin [an antilipolytic hormone (45)] and the progressive increase in concentrations of GH [a lipolytic-enhancing hormone (45)] in maternal serum between d 60 and 140 of gestation (Table 5).

In conclusion, parenteral administration of Arg to underfed ewes increased concentrations of Arg and related amino acids in maternal serum and prevented fetal growth restriction. These novel findings provide an experimental basis for the clinical use of Arg to eliminate or ameliorate IUGR in humans. The results also provide a new framework for studies of molecular mechanisms responsible for beneficial effects of Arg in regulating conceptus growth and development.

**Acknowledgments**


**Literature Cited**


