Arginine synthesis is regulated by dietary arginine intake in the enterally fed neonatal piglet

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Wilkinson, Dana Lee, Robert F. P. Bertolo, Janet A. Brunton, Anna K. Shoveller, Paul B. Pencharz, and Ronald O. Ball. Arginine synthesis is regulated by dietary arginine intake in the enterally fed neonatal piglet. Am J Physiol Endocrinol Metab 287: E454–E462, 2004. First published May 18, 2004; 10.1152/ajpendo.00342.2003.—Arginine is conditionally indispensable in the neonate, and its synthesis in the intestine is not sufficient to meet requirements. It is not known how neonatal endogenous arginine synthesis is regulated and the degree to which proline and glutamate are used as precursors. Primed, constant intraportal and intragastric infusions of L-[U-14C]proline and L-[3,4-3H]glutamate, and intragastric L-[guanido-14C]arginine were used to measure whole body and first-pass intestinal arginine synthesis in 10 neonatal piglets fed generous (1.80 g·kg−1·day−1) levels of arginine. Glutamate tracer was not detected in arginine, indicating a biologically insignificant conversion of <1% of arginine flux. Endogenous arginine synthesis from proline had obligatory (0.36 g·kg−1·day−1) and maximal (0.68 g·kg−1·day−1) levels (P < 0.05, pooled SE 0.05). Although first-pass gut metabolism is responsible for 42–63% of whole body arginine synthesis, the gut is incapable of upregulating proline to arginine conversion during arginine deficiency, compared with a more than threefold increase without first-pass gut metabolism. These data suggest that upregulation of proline-to-arginine conversion occurs via increased arterial extraction of proline by the gut or in nonintestinal tissues. This study demonstrates that dietary arginine is an important regulator of endogenous arginine synthesis in the neonatal piglet and that proline, but not glutamate, is an important precursor for arginine synthesis in the neonate.

proline: glutamate: arginine requirement; biosynthesis; amino acid kinetics; first-pass metabolism

Our laboratory has previously shown that arginine and proline are “co-indispensable” (7), and that proline is a significant precursor for small intestinal arginine synthesis in the neonate (3). Glutamate, via pyrroline-5-carboxylate (P5C), may also be a precursor for the synthesis of arginine in the neonate, although the data are limited. In vitro studies of arginine synthesis from glutamine in enterocytes of preweaning piglets suggest that this conversion is limited by low P5C synthase (EC no. not assigned) activity in the neonatal intestine (36). In support of these in vitro data, Murphy et al. (21) did not detect any conversion of intragastrically administered glutamate to arginine in 10-day-old piglets. In milk-fed piglets, however, 17% of mucosal arginine flux originated from intragastrically infused glutamate (26). Because the activity of P5C synthase (32, 35) and other urea cycle enzymes undergoes marked changes throughout early development (33), the importance of glutamate as a precursor for arginine must be distinctly characterized throughout this period.

In a previous study (7), we demonstrated that feeding arginine-free diets to neonatal piglets led to severe life-threatening hyperammonemia in hours. In that study, we also determined that proline, but not glutamate, was an effective precursor in ameliorating arginine deficiency-induced hyperammonemia. In preliminary studies, we were able to demonstrate that the gastrically fed piglet could be fed arginine at a minimum level of 0.20 g·kg−1·day−1 with only a moderate, but steady, increase in plasma ammonia over 5 days (unpublished observations). We hypothesized that this level of arginine intake would lead to a maximal rate of endogenous arginine synthesis over days. The arginine requirement for piglets has been estimated at 0.40 g·kg−1·day−1 by the National Research Council (NRC) (22), and we have generally employed a standard diet with 0.90 g·kg−1·day−1 without compromising growth or nitrogen balance. As a result, we fed arginine at a generous level of 1.80 g·kg−1·day−1 to determine the basal or obligatory rate of endogenous arginine synthesis.

The mechanisms governing the rate of endogenous arginine synthesis in the neonate are not known. Studies in a mature rodent model suggest that renal arginine synthesis is independent of dietary arginine intake (14). Similarly, isotopic investigations conducted in healthy men fed arginine-adequate or arginine-free diets suggest that de novo arginine synthesis is not increased in response to a reduced level of dietary arginine (10). Arginine homeostasis in the adult human, therefore, appears to be maintained via altering arginine degradation relative to dietary intake and metabolic state. This hypothesis is supported by a significantly reduced rate of conversion of arginine to ornithine measured in the plasma, combined with a 46% reduction in ornithine oxidation in healthy men fed arginine-free diets (11). Because the major site of arginine synthesis differs between the adult (kidney) and the neonate (small intestine) (35), the regulation of arginine synthesis should be examined separately in the neonate. Furthermore, to the authors’ knowledge, the relative contributions made by proline and glutamate to arginine synthesis have never been examined under different dietary intakes of arginine in the neonate.

The present study was designed to elucidate whether the neonatal intestine has a basal and a maximal arginine synthetic
capacity, and whether the dietary arginine intake affects the degree to which proline and glutamate are used as precursors for arginine synthesis.

MATERIALS AND METHODS

Animals and surgical procedures. Ten male Yorkshire piglets were obtained from the University of Alberta’s swine research unit. All piglets were kept with the sow until 1–2 days of age and were then transferred to the metabolic unit for immediate surgical implantation of catheters. The procedures used in this study received the approval of the University of Alberta Animal Care and Welfare Committee.

Piglets were initially anesthetized with an intramuscular injection of acepromazine (0.5 mg/kg Atarvet; Ayerst Laboratories, Montreal, QC, Canada) and ketamine hydrochloride (10 mg/kg Rogarsetic; Rogar STB, Montreal, QC, Canada) and subsequently maintained under a mixture of oxygen and halothane (Fluthoane; Ayerst Laboratories, Montreal, QC, Canada) throughout surgery (day 0). Three Silastic catheters (Ed-Art, Don Mills, ON, Canada) were added to complete the infusion regimen. Ten male Yorkshire piglets were given the IP primed, constant infusion [370 kBq (10 μCi/kg), constant infusion [370 kBq (10 μCi/kg) -[1-11C]proline (American Radiolabeled Chemicals). IP glutamate kinetics were also determined via a simultaneous primed [185 kBq (5 μCi/kg)] and constant infusion [370 kBq (10 μCi/kg) -[1-11C]arginine (American Radiolabeled Chemicals). IP glutamate kinetics were also determined via a simultaneous primed [185 kBq (5 μCi/kg)] and constant infusion [370 kBq (10 μCi/kg) -[3-2H]glutamate (American Radiolabeled Chemicals). Tracers were infused via the umbilical catheter over a 7-h period, with blood sampled every hour for 4 h and every 30 min thereafter. The 1 ml/h infusion of 0.9% saline was discontinued during tracer infusion.

On the morning of day 6, intraportal (IP) proline kinetics were determined by a primed [370 kBq (10 μCi/kg)] and constant infusion [370 kBq (10 μCi/kg) -[1-11C]arginine (American Radiolabeled Chemicals) on day 6 for the SRA values at plateau were used to calculate the relative rates of proline and glutamate metabolism.

Analytical procedures. Plasma amino acid concentrations and specific radioactivities (SRA) of glutamate, proline, arginine, hydroxyproline, glutamine, citrulline, ornithine, and urea were measured by reverse-phase HPLC using phenylisothiocyanate derivatives, as previously described (18). Two internal standards, norleucine and l-[4,5-3H]leucine (Amersham Pharmacia Biotech, St. Louis, MO), were added to each 300-μl plasma sample. Postcolumn radioactive derivatives were collected in 2-ml fractions to which 14 ml of scintillant were added. Radioactive fractions were counted on a liquid scintillation counter using the dual-isotope counting method for 3H and 14C. On days 6 and 7 of the infusions, SRA values at plateau were corrected for background SRA values measured immediately before the respective infusion.

Plasma ammonia concentrations were determined every 24 h during test diet infusions. The colorimetric assay used in the analysis was based on the amination of 2-oxoglutarate to glutamate with simulta-
neous oxidation of NADPH (Sigma Procedure No. 171-UV, Sigma Diagnostics, St. Louis, MO). Plasma urea concentrations were measured daily and during tracer infusions by use of a spectrophotometric assay kit in which ammonia was liberated from urea by enzymatic hydrolysis (Sigma Chemical).

Plasma NOx concentrations were determined every 24 h during test diets via chemical reduction of nitrate to nitrite, followed by spectrophotometric analysis of total nitrite with Griess reagent (Oxis International, Portland, OR).

Calculations. Plasma SRAs were calculated as plasma SRA (Bq/mmol) / amino acid radioactivity (Bq/l)/amino acid concentration (mmol/l). Plateau in plasma SRA was calculated as the mean of the SRA for the time points in the plateau period. All plateaus included at least four time points except for the conversion of arginine to urea, which included three. At plateau, SRA ratios of product to precursor were used to determine the fractional net conversion of precursor to product:

\[
\text{fractional net conversion} = \left( \frac{\text{SRA}_{\text{amino acid product}}}{\text{SRA}_{\text{amino acid precursor}}} \right) \times 100.
\]

Values for flux and fractional net conversion were used in the calculation of molar rates of conversion of precursor (proline, glutamate) to arginine, following the approach of Beaumier et al. (2):

\[
Q_{\text{precursor to product}} = \frac{\text{fractional net conversion} \times \text{flux}_{\text{amino acid product}} \times \text{constant infusion rate}}{\text{SRA}_{\text{plasma amino acid at plateau}}}. 
\]

Small intestinal (gut) first-pass contribution to \(Q_{\text{precursor to product}}\) was calculated as the difference between values for \(Q_{\text{precursor to product}}\) during IG and IP isotope infusions.

Statistical analyses. The dependent variables NO, ammonia, and BUN were analyzed using the Mixed-model procedure of SAS version 8.3 (SAS Institute, Cary, NC) with treatment (generous or deficient arginine diet) as a fixed effect, days as a fixed repeated effect, and pig (treatment) as a random error term to test treatment. The variance-covariance matrix was chosen for the statistical model using an iterative process wherein the best fit was based on Schwarz’s Bayesian Criterion. The Kenward-Roger option was used to determine denominator degrees of freedom. Least square means were separated using the pdiff option for significant \((P < 0.05)\) fixed effects and are presented throughout the paper. A one-tailed test was used to compare plasma ammonia and BUN values between diet groups, because we expected these values to be significantly higher in arginine-deficient animals compared with those receiving a generous quantity of dietary arginine. Given the lack of comparable studies, a prediction could not

Fig. 1. Experimental design. IG, intragastric infusion; IP, intraportal infusion.
be made regarding the results for the nitric oxide (NO) analysis; hence, a two-tailed test was employed.

The dependent variables of plasma net flux from proline to arginine ($Q_{\text{pro-Arg}}$) and the plasma fractional net conversion of proline to hydroxyproline, citrulline, glutamine, and ornithine were analyzed using a two-by-two factorial design with interaction using the Mixed model procedure of SAS (SAS version 8.3). The fixed effects were treatment (generous or deficient arginine diet) and route of isotope infusion (IP or IG). A two-way analysis of variance with interaction was used to test whether the SRA values calculated during the tracer infusions on day 7 were significantly affected by the order of isotope infusion on days 5 and 6 (SAS version 8.3). All other comparisons between diet groups were performed using a two-tailed $t$-test (SAS version 8.3). Data are presented as means with pooled SE and were considered significantly different if $P < 0.05$.

RESULTS

The health status of the animals was not visibly impacted by the feeding of a diet with a deficient or a generous quantity of arginine. There were no significant differences between the two diet groups in body weight upon arrival or at the time of initiation of the test diets (Table 1). Total and rate of weight gain were not different during feeding of complete diet from days 0 to 3; however, total ($P = 0.03$) and rate ($P = 0.01$) of weight gain were significantly higher from days 3 to 7 in pigs receiving the arginine-deficient diet (Table 1).

During test diet infusion, plasma ammonia (Fig. 2) and BUN (Fig. 3) concentrations were significantly greater in piglets receiving the deficient vs. the generous quantity of arginine in the diet ($P < 0.05$). Arginine-deficient piglets had significantly lower plasma concentrations of arginine, ornithine, and hydroxyproline; these changes reflect the reduced supply of arginine. Deficient pigs also had higher plasma concentrations of glutamine, which is an alternative ammonia carrier when urea cycle metabolism is lower due to arginine deficiency (8). Serine and glycine concentrations were also higher in deficient pigs ($P < 0.01$), reflecting their higher inclusion in the diet to balance total nitrogen (Table 2). In addition, methionine and threonine concentrations were higher in the generous-arginine pigs; because glycine is involved in the catabolism of both these amino acids, it is possible that the changing glycine concentrations may have affected disposal of these amino acids. In any event, plasma concentrations of either amino acid were not lower than those in sow-fed reference pigs in either treatment group. Plasma amino acid concentrations were not different over time during infusion protocols within pigs ($P > 0.05$). SRA plateau for infused [14C]proline and [guanido-14C]arginine included at least four time points with coefficients of variation of <10\%. Plateaus for the SRA of all [14C]proline products included at least four time points, and all coefficients of variation for the product plateaus were <15\%. The plateaus

### Table 1. Growth performance of neonatal piglets intragastrically fed generous or deficient quantities of arginine for 5 days

<table>
<thead>
<tr>
<th></th>
<th>Generous Arginine, 1.80 g·kg⁻¹·day⁻¹</th>
<th>Deficient Arginine, 0.20 g·kg⁻¹·day⁻¹</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (day 0), kg</td>
<td>1.62</td>
<td>1.66</td>
<td>0.05</td>
</tr>
<tr>
<td>Weight gain on complete diet (days 0–3), kg</td>
<td>0.31</td>
<td>0.29</td>
<td>0.03</td>
</tr>
<tr>
<td>Rate of weight gain on complete diet, g·kg⁻¹·day⁻¹</td>
<td>93</td>
<td>85</td>
<td>10</td>
</tr>
<tr>
<td>Weight at day 3, kg</td>
<td>1.93</td>
<td>1.95</td>
<td>0.07</td>
</tr>
<tr>
<td>Weight gain on test diets (days 3–7), kg</td>
<td>0.72</td>
<td>0.89*</td>
<td>0.05</td>
</tr>
<tr>
<td>Rate of weight gain on test diets, g·kg⁻¹·day⁻¹</td>
<td>82</td>
<td>99*</td>
<td>4</td>
</tr>
</tbody>
</table>

Data represent means of $n = 10$ piglets with pooled SE. *Data are different compared with piglets fed generous arginine diets ($P < 0.05$).
in plasma SRA achieved on day 7 of the protocol were not significantly impacted by the order of isotope infusion on days 5 and 6.

The fluxes for proline, glutamate, and arginine are presented in Table 3. Only arginine flux, determined from IG tracer infusion, was significantly lower in animals receiving deficient arginine (P < 0.01). In another study using the same diets and similar piglets, we also determined arginine flux from intravenous tracer infusions (unpublished data). In that study, the intravenous arginine flux was 256 μmol·kg⁻¹·h⁻¹ for arginine-deficient piglets (n = 4) and 499 μmol·kg⁻¹·h⁻¹ for piglets fed the generous amount of arginine (n = 4); similar flux values were determined from IP infusion in the same pigs.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Generous Arginine, 1.80 g/kg⁻¹·day⁻¹</th>
<th>Deficient Arginine, 0.20 g/kg⁻¹·day⁻¹</th>
<th>Pooled SE</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>83</td>
<td>87</td>
<td>11</td>
<td>47–121</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>106</td>
<td>116</td>
<td>10</td>
<td>81–143</td>
</tr>
<tr>
<td>Leucine</td>
<td>299</td>
<td>264</td>
<td>24</td>
<td>102–175</td>
</tr>
<tr>
<td>Lysine</td>
<td>649</td>
<td>684</td>
<td>41</td>
<td>77–317</td>
</tr>
<tr>
<td>Methionine</td>
<td>129</td>
<td>52*</td>
<td>16</td>
<td>33–74</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>119</td>
<td>105</td>
<td>9</td>
<td>34–86</td>
</tr>
<tr>
<td>Threonine</td>
<td>1,445</td>
<td>536*</td>
<td>113</td>
<td>214–500</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>58</td>
<td>57</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>271</td>
<td>254</td>
<td>22</td>
<td>175–318</td>
</tr>
</tbody>
</table>

Table 3. Plasma proline, glutamate, and arginine fluxes of neonatal piglets intragastrically fed generous or deficient quantities of arginine for 5 days

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Generous Arginine, 1.80 g/kg⁻¹·day⁻¹</th>
<th>Deficient Arginine, 0.20 g/kg⁻¹·day⁻¹</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP prolineflux</td>
<td>441</td>
<td>388</td>
<td>22</td>
</tr>
<tr>
<td>IG prolineflu x</td>
<td>431</td>
<td>394</td>
<td>24</td>
</tr>
<tr>
<td>IP glutamateflu x</td>
<td>298</td>
<td>516</td>
<td>147</td>
</tr>
<tr>
<td>IG glutamateflu x</td>
<td>447</td>
<td>549</td>
<td>215</td>
</tr>
<tr>
<td>IG arginineflu x</td>
<td>1011</td>
<td>590*</td>
<td>62</td>
</tr>
</tbody>
</table>

Data represent means in μmol·kg⁻¹·h⁻¹ of n = 10 piglets with pooled SE. *Data are different compared with piglets fed generous arginine diets (P < 0.05). Boldface nos. indicate that these values were outside the sow-fed reference range (38).

These data, when combined with those in the present study, result in a mean splanchnic extraction of 55% for arginine-deficient pigs and 51% for pigs fed generous arginine. The net Qₘₚₒ₋ₚᵢᵣᵦg was presented in Table 4 for IP and IG infusions using IG flux rates. The differences between the net flux calculated by these routes of infusion represents the first-pass small intestinal contribution to net flux. The net Qₘₚₒ₋ₚᵢᵣᵦg was substantially greater in arginine-deficient pigs (P < 0.01) during both IP and IG tracer infusion. The arginine content of the diet did not, however, significantly affect first-pass intestinal contribution to Qₘₚₒ₋ₚᵢᵣᵦg. Fractional net conversions of proline to hydroxyproline, citrulline, glutamine, and ornithine are presented in Table 5. Regardless of the quantity of dietary arginine provided, the fractional net conversions of proline to ornithine and glutamine (P < 0.03) were significantly greater in arginine-deficient piglets with a significant interaction between treatment and route of infusion. The fractional net conversion of proline to ornithine was 0.23 ± 0.05.

Table 4. Plasma net Qₘₚₒ₋ₚᵢᵣᵦg of neonatal piglets intragastrically fed generous or deficient quantities of arginine for 5 days

<table>
<thead>
<tr>
<th>Conversion Product</th>
<th>Generous Arginine, 1.80 g/kg⁻¹·day⁻¹</th>
<th>Deficient Arginine, 0.20 g/kg⁻¹·day⁻¹</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP</td>
<td>Hydroxyproline</td>
<td>11±2</td>
<td>13±1</td>
</tr>
<tr>
<td>Glutamine</td>
<td>5±0</td>
<td>2±1*</td>
<td></td>
</tr>
<tr>
<td>Citrulline</td>
<td>22±2</td>
<td>26±3</td>
<td></td>
</tr>
<tr>
<td>Ornithine</td>
<td>5±3</td>
<td>16±1*</td>
<td></td>
</tr>
<tr>
<td>IG</td>
<td>Hydroxyproline</td>
<td>12±1</td>
<td>15±1</td>
</tr>
<tr>
<td>Glutamine</td>
<td>11±1‡</td>
<td>3±2*‡</td>
<td></td>
</tr>
<tr>
<td>Citrulline</td>
<td>62±3†</td>
<td>61±5†</td>
<td></td>
</tr>
<tr>
<td>Ornithine</td>
<td>20±0†</td>
<td>37±1*†</td>
<td></td>
</tr>
</tbody>
</table>

Data represent means ± SE of n = 10 piglets. *P < 0.05 for IG compared with IP fractional net conversion.
also significantly greater during IG infusions ($P < 0.01$), although there was no interaction between treatment and route of infusion.

Because the fluxes of the metabolic intermediates were not quantified, a comparison of the fractional net conversions of proline to hydroxyproline, citrulline, glutamine, and ornithine must be interpreted with caution between diet groups, as the fluxes of these metabolites may have been affected by diet. On the other hand, because metabolite fluxes are constant within a diet group, the IG and IP comparisons, including the intestinal contribution data, are valid (Table 5). The fractional net conversion of arginine to urea was significantly greater in piglets receiving a generous dietary supply of arginine (68 vs. 14%, SE 3; $P < 0.01$).

The recovery of radioactive label from glutamate in arginine was not different from zero when corrected for background. The net flux from glutamate to arginine ($Q_{Glu→Arg}$) was therefore deemed to be biologically insignificant (<1% of arginine flux). As a result, fractional net conversions of glutamate to hydroxyproline, glutamine, citrulline, and ornithine were not calculated.

Plasma NOx concentrations were not affected by the level of arginine provided in the diet ($P < 0.09$; Fig. 4).

**DISCUSSION**

The first major objective of this work was to quantify the maximum arginine synthetic capacity of the neonatal intestine. To quantify maximum synthesis, we had to provide the maximum stimulus to synthesis, namely, a deficient level of dietary arginine that would still enable long-term survival of the animal. These data confirm that 0.20 g·kg$^{-1}$·day$^{-1}$ of arginine is indeed a deficient arginine intake for the neonatal piglet. Piglets fed this diet had higher plasma ammonia concentrations than animals fed a generous quantity of the amino acid, while sustaining sufficient urea cycle function to maintain moderate ammonia levels. Because arginine was limiting for protein synthesis, arginine-deficient piglets had to dispose of more ammonia than arginine-adequate animals. This disposal was accomplished by increased generation of urea and glutamine. The rate of ammonia disposal was not commensurate with its rate of production, however, as evidenced by several transient instances of hyperammonemia. Because past IG administration of 0.15 g·kg$^{-1}$·day$^{-1}$ arginine led to severe, sustained hyperammonemia within 8 h of feeding (unpublished observations), 0.20 g·kg$^{-1}$·day$^{-1}$ appears to be acceptable as the minimal dietary inclusion level for arginine.

Despite twofold greater whole body arginine synthesis in deficient compared with arginine-supplemented animals (Table 4), the total available arginine (intake, 0.20 g·kg$^{-1}$·day$^{-1}$ + synthesis, 0.68 g·kg$^{-1}$·day$^{-1}$) in the deficient animals was not sufficient to satisfy the metabolic requirement, as evidenced by elevated plasma ammonia (Fig. 2) and BUN levels (Fig. 3). Therefore, intestinal arginine synthesis was not sufficiently upregulated to compensate for the dietary arginine deficiency and must represent the maximum arginine synthetic capacity of the neonatal piglet intestine in this model. Plasma proline concentrations indicate that arginine synthesis was not limited by supply of dietary proline but by limited de novo synthetic capabilities. In conditions that increase the metabolic demand for arginine beyond this maximum arginine synthetic capacity, dietary arginine supplementation is necessary.

Our second objective was to quantify whether there is a basal level of arginine synthesis that occurs even when there is an adequate supply of arginine. This objective was achieved by providing a generous quantity of arginine in the diet (8, 30). Theoretically, arginine synthesis should be minimized under such conditions. These piglets, however, synthesized 0.36 g·kg$^{-1}$·day$^{-1}$ arginine, about one-half as much as deficient pigs.

Recent results from our laboratory (3, 8, 30) have demonstrated that indexes of arginine adequacy reach a plateau when arginine is provided between 1.20 and 2.00 g·kg$^{-1}$·day$^{-1}$, suggesting that the total metabolic demand for arginine is satisfied at ~1.20 g·kg$^{-1}$·day$^{-1}$, a level three times greater than the current NRC-estimated dietary requirement of 0.38 g·kg$^{-1}$·day$^{-1}$ (22) and almost double the arginine intake from sow’s milk (0.66 g·kg$^{-1}$·day$^{-1}$) (13). On the basis of these previous observations, we are reasonably certain that 1.80 g·kg$^{-1}$·day$^{-1}$ arginine is metabolically sufficient and likely a generous intake for the neonatal piglet. We have therefore demonstrated that there is a basal and obligatory rate of arginine synthesis in the neonatal piglet.

The dietary regulation and partitioning of precursors for arginine synthesis formed the third objective in this study. Although renal arginine synthesis does not appear to be under dietary regulation in the adult human (9, 10) or in the rat (14), our results suggest that whole body $Q_{Pro→Arg}$ in the neonate is under dietary regulation. When challenged by an inadequate supply of dietary arginine, piglets were able to upregulate net synthesis of arginine from proline. On a whole body level, the net quantity of arginine synthesis from proline doubled in

![Fig. 4. Mean plasma nitric oxide concentrations (μmol/l) of piglets fed a generous (1.80 g·kg$^{-1}$·day$^{-1}$) or deficient (0.20 g·kg$^{-1}$·day$^{-1}$) quantity of arginine for 5 days. There was a trend ($P < 0.09$) for plasma nitric oxide concentrations to be affected by the level of arginine provided in the diet, as determined by repeated-measures analysis. Data are means ± SE; n = 10.](attachment:image.png)
deficient compared with adequately fed piglets and tripled during IP infusions (Table 4). The quantity of arginine synthesized by the gut on first pass, however, was not different between diet levels. These data suggest that first-pass gut arginine synthesis occurred at its maximum capacity regardless of the dietary inclusion level of arginine. Because first-pass gut conversion of proline to arginine was already operating at its maximum capacity, piglets provided with a deficient quantity of arginine in the diet upregulated conversion of proline to arginine by some other mechanism to match the level of conversion occurring on first pass by the gut.

The IP infusion of nutrients was developed in our laboratory to isolate first-pass gut metabolism (4). We have previously used this method to demonstrate that conversion of proline to arginine is dependent on first-pass gut metabolism in the neonate (3); however, we did not previously investigate the contribution of the liver or of other tissues to arginine synthesis. Increased conversion of proline to arginine during IP infusion between test diets may result from increased arterial extraction of proline by the healthy gut for arginine synthesis. This arterial extraction of proline may explain why, in an earlier study, piglets fed IG an arginine- and/or proline-free diet exhibited a less severe rise in plasma ammonia than piglets fed the same diet intravenously (7). Because our data suggest that dietary glutamate is not an important precursor for arginine synthesis in piglets of this age, it is possible that the healthy gut of the IG-fed animals was extracting arterial proline for conversion to arginine.

Although these data suggest that the liver and the kidney are not involved in neonatal arginine synthesis under normal conditions, urea cycle enzyme and cationic amino acid transporter activities may be altered under abnormal dietary conditions. There is evidence that factors such as starvation, high-protein feeding, and glucagon treatment can increase the hepatic activities and mRNA levels of all urea cycle enzymes (19). In the rat kidney, high-protein feeding causes a doubling in the activity of “arginine synthetase” [argininosuccinate synthase (ASS) + argininosuccinate lyase (ASL)] (27) and in the relative abundance of mRNA for the enzymes of arginine synthesis (20). Furthermore, a number of urea cycle enzymes have extrarenal and extrahepatic locations (25, 31). Windmueller and Spaeth (31) observed net citrulline release from the skeletal muscle in the rat and also found evidence of extrarenal and extrahepatic citrulline utilization. Whether or not these other tissues can make a significant contribution to arginine synthesis is not known. However, it must be noted that, although the kidney is the major site of arginine synthesis in the adult and renal arginase activity is low relative to the liver (15), the activity of ASS and ASL is low in the kidney of the neonate (35).

The recovery of $^3$H in arginine from $[^3$H]glutamate was not detectable by our methods; our detection limits correspond to a conversion of no more than 1% of arginine. Our conclusions regarding the effectiveness of glutamate as a precursor for arginine concur with findings by Murphy et al. (21) in neonatal piglets and by Wu et al. (36) and Blachier et al. (5) in neonatal piglet enterocytes. Furthermore, piglets fed a deficient level of dietary arginine had 0.88 g·kg$^{-1}$·day$^{-1}$ of available arginine from a combination of diet and intestinal synthesis from proline; however, these animals still experienced episodes of hyperammonemia, a phenomenon that would not have occurred if they were synthesizing significant quantities of arginine from glutamate. Because of the marked changes in the activities of the enzymes that synthesize arginine from glutamate and glutamine during the course of early development (33), our conclusions regarding the suitability of glutamate as a precursor for arginine synthesis are age specific and may apply only to a young piglet.

IG arginine fluxes were used in this study for calculations of intestinal arginine synthesis. Because arginine is synthesized in the gut of neonates, it is not reasonable to exclude the effects of the gut from our conversion estimates. IG flux represents whole body flux, and oral dosing includes metabolism by the gut, which contributes to the central plasma pool. In particular, this inclusion is necessary because the “extraction” of amino acids by the gut is not irreversible, as is often assumed for a short infusion period. In fact, van der Schoor et al. (28) have recently showed that almost 50% of the lysine in the portal vein is derived from recycling of glycoproteins by the gut within a 6-h period; so orally delivered isotope is “extracted” by the gut, incorporated into proteins to be secreted into the lumen, and then degraded such that the extracted amino acids eventually enter the systemic circulation and central plasma pool. Therefore, subtracting this extraction from the infusion dose is not appropriate, given that this isotope is eventually reintroduced into the plasma pool. Regardless of whether intravenous, IP, or IG fluxes are used for calculation purposes, the relative differences between diets in $Q_{Pro→Arg}$ are real. Indeed, if splanchnic extraction is subtracted from the IG infusion dose to determine intravenous flux rates in the pigs of the present study (2), then the data would be approximately one-half in absolute value but would not change the statistical comparisons made. The major findings of this research confirm the existence of a basal and a maximal rate of arginine synthesis and of dietary regulation of arginine synthesis from proline. These findings are independent of specific flux values.

In this study, there was a significant difference in the rate of body weight gain in animals infused IG between diets containing generous or deficient levels of arginine. This difference was not, however, in the expected direction, because the piglets fed a deficient diet gained significantly more body weight than those fed an adequate arginine diet. The higher plasma ammonia and BUN levels in these deficient animals may have induced more net catabolism of protein in this group. The data of House et al. (17) are instructive in this respect. In that study, glutamine supplementation to total parenteral nutrition tended to increase live body weight gain in neonatal piglets via an increase in total body water and, in particular, extracellular fluid expansion. The formation of glutamine from glutamate and ammonia is an important physiological means of scavenging excess ammonia. Because the urea cycle function of the arginine-deficient diet group was challenged by an inadequate arginine intake, the animals exhibited plasma glutamine concentrations that were fourfold greater than those of piglets fed an adequate level of arginine. Therefore, high plasma glutamine concentrations in deficient animals may have induced an expansion in the extracellular fluid, resulting in greater body weight gain.

Animals fed a deficient diet had plasma arginine concentrations that were 90% lower than those observed in the animals fed a generous quantity of the amino acid, similar to the pattern of the decline in the dietary arginine concentration. Despite
plasma arginine concentrations that were much lower than those in the sow-fed reference group. Plasma ornithine concentrations of the arginine-deficient piglets did not drop below levels characteristic of sow-fed piglets, although they were significantly lower than those of adequately fed animals. These observations are similar to those made by Castillo et al. (11), who observed a decline in ornithine oxidation in healthy men fed arginine-free diets. A reduction in ornithine oxidation could help to preserve urea cycle substrates in the absence of sufficient dietary arginine. Plasma citrulline concentrations were unaffected by dietary arginine deficiency, a phenomenon that has also been documented in rats (16), 20- to 50-kg pigs (24), and adult humans (12).

There may have been a trend suggestive of impaired NO synthesis in arginine-deficient animals in the present study. In rats, Wu et al. (34) observed a reduction in NO synthesis by constitutive NO synthase and inducible NO synthase during arginine deficiency, although the magnitude of the response was smaller in the rats than in the present study. To the authors’ knowledge, no comparable studies of the influence of dietary arginine on NO synthesis exist in healthy neonates. That dietary arginine deficiency may have an impact on NO production is of extreme importance to the health of neonates, because decreased NO production is associated with neonatal diseases, such as persistent pulmonary hypertension of the newborn and necrotizing enterocolitis (NEC). Indeed, plasma arginine concentrations are lower in infants affected with these conditions (1, 23, 29, 39), and arginine supplementation can significantly reduce the incidence of NEC (1). Conversely, overdosage of NO can contribute to tissue injury, hypotension, and myocardial failure (6).

In conclusion, although first-pass gut metabolism is responsible for 42–63% of whole body arginine synthesis, the gut is incapable of upregulating proline-to-arginine conversion (on first pass) during arginine deficiency. Therefore, the role of first-pass gut metabolism in the synthesis of arginine, although substantial, seems to be limited to an obligatory function. This lack of dietary response compares with the more than threefold increase in proline-to-arginine conversion when first-pass gut metabolism is bypassed (i.e., IP). These data suggest that upregulation of proline-to-arginine conversion occurs via increased arterial extraction of proline by the gut or in nonintestinal tissues. This study has demonstrated that dietary arginine is an important regulator of endogenous arginine synthesis in the neonatal piglet. In addition, because glutamate was not a significant precursor for arginine synthesis, proline is the primary precursor for neonatal arginine synthesis, further supporting the coindispensability (7) of these amino acids in the neonate.

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REFERENCES


