Parenterally Fed Neonatal Piglets Have a Low Rate of Endogenous Arginine Synthesis from Circulating Proline

Kristine L. Urschel, Amanda R. Evans, Craig W. Wilkinson, Paul B. Pencharz and Ronald O. Ball

Abstract

Parenterally fed neonatal piglets cannot synthesize sufficient arginine to maintain arginine status, presumably due to the intestinal atrophy that occurs with parenteral feeding. Parenteral feeding-induced atrophy can be reduced by the infusion of glucagon-like peptide 2 (GLP-2). GLP-2 infusion was hypothesized to increase the rate of endogenous arginine synthesis from proline, the major arginine precursor, in parenterally fed piglets receiving an arginine-deficient diet. Male piglets, fitted with jugular vein catheters for diet and isotope infusion, and femoral vein catheters for blood sampling (d 0), were allocated to a continuous infusion of either GLP-2 (n = 5; 10 nmol·kg\(^{-1}·d^{-1}\)) or saline (n = 5) for 7 d. Piglets received 2 d of a complete diet, followed by 5 d of an arginine-deficient (0.60 g·kg\(^{-1}·d^{-1}\)) diet. Piglets received primed, constant infusions of [guanido-\(^{14}\)C]arginine to measure arginine flux (d 6) and [U-\(^{14}\)C]proline (d 7) to measure proline conversion to arginine. Plasma arginine concentrations and arginine fluxes indicated a similar whole-body arginine status. Piglets receiving GLP-2 showed improvements in intestinal variables, including mucosal mass (P < 0.001), and villus height (P < 0.001), and a greater rate of arginine synthesis (\(\mu\)mol·kg\(^{-1}·h^{-1}\)) from proline (11.6 vs. 6.3) (\(P = 0.03\)) and mucosal mass (\(R^2 = 0.71\); \(P = 0.002\)) and villus height were correlated (\(R^2 = 0.66\); \(P = 0.004\)) with arginine synthesis. This study was the first to quantitate arginine synthesis in parenterally fed neonates and showed that although GLP-2 infusion increased arginine synthesis in a manner directly related to mucosal mass, this increased arginine synthesis was insufficient to improve whole-body arginine status in piglets receiving a low arginine diet. J. Nutr. 137: 601–606, 2007.

Introduction

The primary metabolic uses for arginine include protein synthesis, ammonia detoxification to urea via the urea cycle, and the synthesis of creatine, nitric oxide, and polyamines (1). Arginine synthesis and metabolism are well described in adult and juvenile mammals (1,2); however, research in neonates has found that neonatal arginine metabolism is very different from adult arginine metabolism (3–5). Low plasma arginine concentrations and arginine fluxes are frequently seen in preterm, compared with term, neonates (5), and preterm infants that develop necrotizing enterocolitis (NEC)<sup>6</sup> have lower plasma arginine concentrations than those infants that do not develop NEC (6). The provision of supplemental arginine to preterm infants reduced the incidence of NEC (7). Arginine conversion to nitric oxide is lower in infants during the acute phases of persistent pulmonary hypertension of the neonate compared with during recovery from the acute episode (8), and the provision of a bolus dose of arginine during persistent pulmonary hypertension of the neonate has been shown to improve infant oxygenation (9). Because of these critical roles of arginine, a complete knowledge of arginine metabolism in neonates is essential. However, because of ethical and practical constraints associated with experimentation in human neonates (10), a suitable animal model was needed to study arginine metabolism in neonates. The parenterally fed neonatal piglet has been validated as an experimental model to study amino acid metabolism in parenterally fed human neonates (10–13) and the majority of the research, both in vitro and in vivo, relating to arginine metabolism in neonates has been conducted in neonatal piglets (4,14–18).

Recently, we attempted to determine which tissues are important for arginine synthesis in neonatal piglets. Although arginine synthesis in adult mammals occurs via the intestinal-renal axis (19,20), the neonatal intestine contains all enzymes necessary for arginine synthesis (14,17) and we and others showed that arginine synthesis is primarily intestinal in neonates (16,17,21,22). First-pass intestinal metabolism, which is the
metabolism of dietary nutrients by the small intestine before they are released into the portal vein, accounted for 40–60% of whole-body endogenous arginine synthesis (4) and first-pass hepatic metabolism did not contribute to whole-body arginine synthesis (15). Therefore, the remaining 40–60% of arginine synthesis must be by either the peripheral tissues, such as the muscle or kidney, or via the intestinal metabolism of arterial substrates. We hypothesized that the intestinal metabolism of circulating precursors could make an important contribution to whole-body arginine synthesis.

Parenterally fed piglets receiving an arginine-free diet that contained a generous amount of proline, the major arginine precursor in neonatal piglets (4,17), experienced a rapid onset of hyperammonemia (16), indicating an extremely diminished capacity for endogenous arginine synthesis. However, a direct measurement of arginine synthesis in parenterally fed neonatal piglets has not been previously obtained. Parenteral feeding bypasses both first-pass intestinal and hepatic metabolism; therefore, parenterally fed piglets would be expected to have a lower rate of endogenous arginine synthesis than enterally fed piglets. Additionally, if the intestinal metabolism of arterial proline does make a contribution to whole-body arginine synthesis, then the decrease in intestinal blood flow (23) and the intestinal atrophy (24) that occur during parenteral feeding will likely result in a lower rate of arginine synthesis from circulating precursors in parenterally fed neonates than in enterally fed neonates.

Glucagon-like peptide 2 (GLP-2) is a 33-amino acid gut hormone that is released from the distal ileum in response to enteral feeding. Plasma GLP-2 concentrations are ~50% lower in parenterally than in enterally fed piglets (25,26). Intravenous infusion of supraphysiological (~10 nmol·kg⁻¹·d⁻¹) amounts of GLP-2 has been shown to improve intestinal structure (25,26), increase rates of intestinal cell proliferation and protein synthesis (26), and prevent the decrease in portal vein blood flow (27) normally observed in parenterally fed piglets. Therefore, we hypothesized that by improving intestinal structure and blood flow in parenterally fed neonatal piglets, using a continuous GLP-2 infusion, there would also be an increase in the ability of the intestine to endogenously synthesize arginine from circulating precursors such as proline.

The objectives of this study were: 1) to determine the rate of arginine synthesis in parenterally fed piglets; and 2) to determine how improving intestinal structure and increasing the intestinal mucosal mass, by using a continuous GLP-2 infusion, affects the use of circulating proline for arginine synthesis in parenterally fed piglets.

Materials and Methods

Animals and surgical procedures. All animal studies were conducted in accordance with the Canadian Council on Animal Care Guidelines and Policies with approval from the Faculty of Agriculture, Forestry and Home Economics Animal Policy and Welfare Committee for the University of Alberta. On d 0, 10 intact male Landrace/Large White/Duroc piglets (Hypor) (1.5–2.0 kg) were obtained from the University of Alberta Swine Research and Technology Centre at 1–2 d of age. Piglets were removed from the sow and immediately underwent surgical procedures to implant a jugular vein catheter for feeding and treatment solution infusion and a femoral vein catheter for blood sampling. The surgical procedures, postsurgical injections and care, and piglet housing were as previously described (15).

Experimental treatments. Immediately following surgery and upon initiation of intravenous diet infusion, piglets were allocated to 1 of 2 treatment groups: +GLP-2 (n = 5) or +Sal (n = 5). Piglets in the +GLP-2 group were continuously infused at a rate of 1 mL·kg⁻¹·h⁻¹ with a 9-g/L saline solution that contained 1.8 mg/L human GLP-2 (Bachem Bioscience) and 2.5 g/L bovine serum albumin (BSA, Sigma-Aldrich Canada). Therefore, these piglets received ~10 nmol·kg⁻¹·d⁻¹ GLP-2. A previous dose-response study in parenterally fed piglets (26) evaluated the effectiveness of 3 rates of GLP-2 infusion [2.5, 5, and 10 nmol·kg⁻¹·d⁻¹] in improving intestinal morphology and increasing intestinal protein synthesis and cell proliferation relative to parenteral feeding alone. The study found that a GLP-2 dose of 10 nmol·kg⁻¹·d⁻¹ was the optimal dose to improve all of these intestinal variables. The BSA was added as a carrier for the GLP-2 and to stabilize it in the infusion solution. Piglets in the +Sal group received a 1-mL·kg⁻¹·h⁻¹ infusion of a 9-g/L saline solution with 2.5 mg/L BSA. Both solutions were administered via the jugular vein catheter for the duration of the experiment.

Diets. Immediately following surgery, piglets received a complete elemental diet at 50% of targeted rate for 12 h, followed by another 12 h at 75% of targeted rate via the jugular vein catheter. By the morning of d 1, all piglets were being fed at the targeted rate of 13.5 mL·kg⁻¹·h⁻¹. Piglets were weighed each morning and diet and treatment solution infusion rates were adjusted accordingly. Diet composition and preparation were as previously described (11,15,28).

On the morning of d 3, all piglets were assigned to a low arginine diet. A low arginine diet was used, because we have previously shown that a deficient arginine intake results in the maximal stimulus for endogenous arginine synthesis (4), allowing us to more easily detect differences in arginine synthesis due to experimental treatment. The rate of arginine intake, 0.60 g·kg⁻¹·d⁻¹ (2.21 g/L arginine in base solution), was chosen because it represents ~50% of the estimated daily arginine use in neonatal piglets (1) but does not result in life-threatening hyperammonemia (21). The alanine (8.14 g/L) and glycine (2.34 g/L) concentrations of the base diet were modified to ensure that this diet and the base diet were isonitrogenous.

Blood sampling. Beginning the morning of d 3, immediately before piglets began receiving the arginine-deficient diet, blood samples (1.5 mL) were taken every 24 h until the morning of d 7. Whole blood was collected into heparinized tubes and immediately centrifuged at 9000 × g; 10 min and the plasma layer was removed and frozen at −20°C until the time of analysis. Daily plasma samples were used for the measurement of ammonia and urea concentrations, and amino acid concentrations were also determined in the d 7 sample. Additional blood samples were taken on d 6 and 7, as described below.

Constant tracer infusions. On d 6, arginine kinetics were measured in all piglets using a primed [111 kBq (3 μCi)/kg], constant [185 kBq (5 μCi)/kg·h] infusion of [L-14C]arginine (2.11 GBq/mmol; Moravek Biochemicals). The isotope was infused over 6 h and blood was sampled at 0, 60, 120, 180, 240, 270, 300, 330, and 360 min.

Proline kinetics and the conversion of proline to arginine were measured in all piglets on d 7, using a 7-h primed [740 kBq (20 μCi)/kg], constant [370 kBq (10 μCi)/kg·h] infusion of [L-14C]proline (9.32 GBq/mmol; Moravek Biochemicals). Blood was sampled at ~60, ~30, 0, 60, 120, 180, 240, 300, 330, 360, 390, and 420 min. We have successfully used these primed and constant infusion rates for both the arginine and proline isotopes in other studies using the neonatal piglet model (4,15). During both isotope infusions, the arginine-deficient diet was continuously infused.

Intestinal sampling. At the end of the d 7 infusion, piglets were anaesthetized with 5% isoflurane (AErrane; Baxter) and the entire small intestine was removed posterior to the ligament of Treitz. Piglets were then killed with a 500-mg pentobarbital sodium (Euthansol, 340 g/L; Schering Canada) injection into the femoral vein catheter. The extracted small intestine was rinsed with ice-cold saline and divided in half, with the proximal half designated as the jejunum and the distal half as the ileum. The jejunum was then divided in half again and a section of exactly 20 cm was excised from the center of this division. The mucosa from this 20-cm segment was scraped and the weight was recorded. A 5-cm segment immediately distal to the 20-cm segment was preserved in
a 10% buffered formalin solution (Histopaque; Fisher Scientific) for later histological analysis.

**Analytical procedures.** Histology samples from the mid-jejunum were prepared and analyzed by a certified veterinary pathologist at the University of Alberta, using previously described procedures (24).

Plasma ammonia (reference 200–02; Diagnostic Chemical) and urea nitrogen (Sigma Procedure no. 640; Sigma Diagnostics) concentrations were determined every 24 h during test diet infusion (d 3–d 7) using spectrophotometric assays.

Plasma amino acid concentrations and the specific activities of arginine and proline in the infusion plasma samples were measured by reverse-phase HPLC using phenylisothiocyanate derivatives, as previously described (29,30). The internal standards norleucine and l-[U-14C]leucine (10.81 GBq/mmol; American Radiolabeled Chemicals) were added to each 300-μL plasma sample. Postcolumn radioactive proline and arginine derivatives were collected in 3-mL fractions, 14 mL of scintillant (Biodegradable Counting Scintillant; Amersham Canada) was added, and samples were counted on a scintillation counter (Tri-Carb 4000 series, Canberra Packard).

**Calculations.** The proline and arginine fluxes, the fractional net conversion of proline to arginine, and the molar conversion of proline to arginine (Qproline to arginine) were all calculated, as recently described (28). The calculated flux values included the amino acids entering the plasma pool through the diet, de novo synthesis and protein breakdown, or leaving the pool through protein synthesis, oxidation, or conversion to other metabolites.

**Statistical analysis.** Unless specifically noted, all data were analyzed using the mixed model of SAS version 8.3 (SAS Institute) and differences were considered significant if \( P < 0.05 \). Statistical trends were considered at \( 0.05 < P < 0.10 \). When the fixed effects were significant \( (P < 0.05) \), least-squares means were compared using the pdiff test.

The dependent variables plasma ammonia and plasma urea nitrogen were analyzed using repeated-measures analysis, where the fixed effect was diet and the random variables were piglet nested in diet and day. The variance-covariance matrix was chosen for each analysis based on the lowest value for Schwarz’s Bayesian Criterion.

The remaining variables, including mucosal mass, plasma amino acid concentrations, arginine and proline fluxes, and Qproline to arginine, were analyzed using diet as the fixed effect and piglet nested in diet as the random variable.

The relations of the intestinal variables (mucosal mass and villus height) to Qproline to arginine and plasma citrulline concentrations were analyzed using the regression and correlation procedures of SAS.

**Results**

**Piglet performance.** All piglets remained healthy and active for the duration of the trial. The groups did not differ in [mean ± pooled SE] initial body weight (1.77 ± 0.09 kg), body weight on d 3 (2.13 ± 0.14 kg), rate of weight gain from d 3 to d 7 (81 ± 16 g·kg⁻¹·d⁻¹), and final body weight on d 7 (2.79 ± 0.16 kg).

**Intestinal variables.** Mucosal mass was greater in piglets receiving the GLP-2 infusion than in those receiving the control saline infusion \( (P < 0.05) \) (Table 1). Villus height was ∼40% greater in the +GLP-2 piglets \( (P < 0.001) \) and the number of villus cells tended to be greater in piglets in the +GLP-2 group \( (P < 0.1) \) (Table 1). The GLP-2 treatment did not affect crypt depth or crypt cell number (Table 1). The villus height:depth ratio was higher in the +GLP-2 than in the +Sal piglets \( (P < 0.01) \) (Table 1).

**Indicators of whole-body arginine status.** On d 7, concentrations of the plasma amino acids related to arginine synthesis and metabolism were particularly affected by the infusion of GLP-2 (Table 2). Although the treatment did not affect the plasma arginine concentration, plasma citrulline was greater \( (P < 0.05) \) in the piglets in the +GLP-2 group (Table 2). Plasma aspartate, glutamate, and glutamine concentrations tended to be lower \( (P < 0.10) \) and plasma ornithine concentration tended to be higher \( (P < 0.10) \) in piglets receiving the GLP-2 infusion (Table 2). With the exception of a trend \( (P = 0.08) \) for higher plasma glycine concentrations in piglets receiving the GLP-2 infusion \( (+GLP = 1510 \mu mol/L) ; +Sal = 961 \mu mol/L) \); pooled SE = 196 μmol/L), the plasma concentrations of the other amino acids were not affected by treatment (data not shown). The greater plasma concentrations of the 2 main intermediates in the arginine synthetic pathway, citrulline and ornithine, suggest an increase in the flux of substrates through this pathway. The trend for lower plasma aspartate and glutamate concentrations in the +GLP-2 piglets may reflect their use for arginine synthesis. Plasma glutamine concentrations previously have been shown to be greater in piglets with a poor whole-body arginine status and a limitation in urea cycle function (4,15,28) or in piglets receiving a diet deficient in an indispensable (31,32) amino acid, presumably due to its metabolic role as a nitrogen scavenger.

In previous studies (4,15,28), plasma ammonia concentrations were used as an indicator of urea cycle function, with high plasma ammonia concentrations indicating an impairment in urea cycle function. Day \( (P = 0.01) \), but neither treatment nor the interaction between day and treatment had a significant effect on plasma ammonia concentrations (Table 3). In piglets that received the GLP-2 infusion, there was no effect of day on plasma ammonia concentrations (Table 3); however, piglets in the +Sal group had significantly higher plasma ammonia concentrations on d 6 and d 7 than on d 3 (Table 3). There was no

### Table 1: Jejunal mass and morphology in parenterally fed piglets receiving a low arginine diet and a continuous infusion of either saline or GLP-2

<table>
<thead>
<tr>
<th>Variable</th>
<th>+GLP-2</th>
<th>+Sal</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunal mucosal mass, mg·cm⁻²·kg⁻¹</td>
<td>29</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>Villus height, μm</td>
<td>689</td>
<td>479</td>
<td>28</td>
</tr>
<tr>
<td>Villus cells, n</td>
<td>302</td>
<td>206</td>
<td>29</td>
</tr>
<tr>
<td>Crypt depth, μm</td>
<td>103</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>Crypt cells, n</td>
<td>43</td>
<td>42</td>
<td>2</td>
</tr>
<tr>
<td>Villus height:depth ratio</td>
<td>6.8</td>
<td>4.8</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Values are least-square means, \( n = 6 \). *Different from +GLP-2, \( P < 0.05 \).

### Table 2: Plasma concentrations of amino acids related to arginine metabolism in parenterally fed piglets receiving a low arginine diet and a continuous infusion of either saline or GLP-2 on d 7

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>+GLP-2</th>
<th>+Sal</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>31</td>
<td>34</td>
<td>8</td>
</tr>
<tr>
<td>Asparagine</td>
<td>21</td>
<td>28</td>
<td>5</td>
</tr>
<tr>
<td>Aspartate</td>
<td>27</td>
<td>43</td>
<td>6</td>
</tr>
<tr>
<td>Citrulline</td>
<td>63</td>
<td>62</td>
<td>6</td>
</tr>
<tr>
<td>Glutamate</td>
<td>126</td>
<td>208</td>
<td>30</td>
</tr>
<tr>
<td>Glutamine</td>
<td>178</td>
<td>269</td>
<td>33</td>
</tr>
<tr>
<td>Ornithine</td>
<td>24</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>Proline</td>
<td>397</td>
<td>328</td>
<td>69</td>
</tr>
</tbody>
</table>

Values are least-square means, \( n = 6 \). *Different from +GLP-2, \( P < 0.05 \).
effect of treatment, day, or the interaction between treatment and day on plasma urea concentrations (Table 3).

**Arginine and proline fluxes.** In addition to plasma amino acid, ammonia, and urea concentrations, whole-body arginine flux was also used as an indicator of whole-body arginine status. Similar to the other indicators studied (Tables 2 and 3), GLP-2 infusion, in conjunction with the low arginine diet, did not affect arginine flux (Table 4). Proline flux also did not differ between the groups (Table 4).

**Proline conversion to arginine.** Because proline, and not glutamate, is the major arginine precursor in neonatal piglets in vivo (4), Qproline to arginine is a measure of endogenous arginine synthesis. GLP-2 infusion significantly increased the fractional net conversion of proline to arginine ($P < 0.0001$) (Table 4) and absolute arginine synthesis from proline ($P = 0.03$) (Table 4). Because there were no differences in either proline flux (Table 4) or plasma proline concentrations (Table 2) between the 2 groups, we assumed that the size of the proline pool was similar in both groups and therefore was not the cause of the observed differences in arginine synthesis from proline.

**Relation between intestinal variables and Qproline to arginine and plasma citrulline concentration.** There was a strong, positive correlation ($r = 0.85; P = 0.002$) between mucosal mass and villus height. A strong, positive linear relation ($R^2 = 0.72; P = 0.002$) was found between mucosal mass and arginine synthesis from proline (Fig. 1). Villus height was also related to arginine synthesis from proline ($R^2 = 0.65; P = 0.004$). Although there was no relation between mucosal mass and plasma citrulline concentrations ($R^2 = 0.18; P > 0.10$), there was a significant linear relation between villus height and plasma citrulline concentration ($R^2 = 0.51; P = 0.02$).

**Discussion**

**Arginine synthesis in parenterally fed neonatal piglets.** To the best of our knowledge, this study is the first to directly measure arginine synthesis rates in parenterally fed neonates. We previously determined the basal and maximal rates of arginine synthesis from proline in enterally fed piglets receiving either a generous [1.80 g·kg$^{-1}·d^{-1}$] or deficient [0.20 g·kg$^{-1}·d^{-1}$] intake, respectively, of arginine (4,15). Enteral feeding piglets synthesized up to 0.19 g·kg$^{-1}·d^{-1}$ of arginine from circulating proline (15), which was 3 to 5-fold greater than the rate of arginine synthesis in the parenterally fed piglets [0.03–0.05 g·kg$^{-1}·d^{-1}$; Table 4]. Therefore, parenterally fed piglets synthesized substantially less arginine than enterally fed piglets. However, the finding that parenterally fed piglets are capable of even a small amount of endogenous arginine synthesis is a novel finding that is of both clinical and biochemical importance.

An important distinction between the enteral studies (4,15) and the present parenteral study is that the arginine intake from the arginine-deficient diet was lower [0.20 g·kg$^{-1}·d^{-1}$] vs. 0.60 g·kg$^{-1}·d^{-1}$] in the enteral studies. Parenterally fed piglets cannot be chronically maintained on <0.50–0.60 g·kg$^{-1}·d^{-1}$ of arginine (21); therefore, we could not feed the same deficient arginine diet as in our previous enteral studies. Compared with the enterally fed piglets (4,15), the parenterally fed piglets had similar plasma ammonia, urea, and arginine concentrations (Tables 2 and 3), indicating similar whole-body arginine status. Therefore, we do not think that the greater dietary arginine allowance in this study was the cause of the lower rate of arginine synthesis from circulating proline in the parenterally fed compared with the enterally fed pigs.

**Effects of GLP-2 infusion on intestinal variables and arginine synthesis.** Not only did piglets receiving the GLP-2 infusion have a greater mucosal mass and villus height than those receiving the saline infusion (Table 1), but the mid jejunum intestinal mass and villus height of piglets in both groups were similar to the values previously obtained from piglets of a comparable age (~10 d old) receiving enteral [~33 mg·cm$^{-1}·kg^{-1}$]; 534 μm] and parenteral [~20 mg·cm$^{-1}·kg^{-1}$]; 410 μm] diet administration, respectively (24). Therefore, the previously

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**Table 3** Plasma ammonia and urea concentrations in parenterally fed piglets receiving a low arginine diet and a continuous infusion of either saline or GLP-2.

<table>
<thead>
<tr>
<th>Day</th>
<th>+GLP-2</th>
<th>+Sal</th>
<th>Pooled SE</th>
<th>+GLP-2</th>
<th>+Sal</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td>Urea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>μmol/L</td>
<td>mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>110</td>
<td>63</td>
<td>47</td>
<td>1.92</td>
<td>2.69</td>
<td>0.57</td>
</tr>
<tr>
<td>4</td>
<td>199</td>
<td>127</td>
<td>47</td>
<td>2.17</td>
<td>1.71</td>
<td>0.40</td>
</tr>
<tr>
<td>5</td>
<td>123</td>
<td>139</td>
<td>47</td>
<td>2.50</td>
<td>1.69</td>
<td>0.51</td>
</tr>
<tr>
<td>6</td>
<td>138</td>
<td>230</td>
<td>47</td>
<td>1.95</td>
<td>1.39</td>
<td>0.42</td>
</tr>
<tr>
<td>7</td>
<td>198</td>
<td>220</td>
<td>47</td>
<td>2.49</td>
<td>2.03</td>
<td>0.58</td>
</tr>
</tbody>
</table>

1 Values are least-square means, $n = 5$. Means in a column with superscripts without a common letter differ, $P < 0.05$.

**Table 4** Plasma proline and arginine kinetics and absolute conversion of proline to arginine in parenterally fed piglets receiving a low arginine diet and a continuous infusion of either saline or GLP-2.

<table>
<thead>
<tr>
<th>[U-14C]proline infusion</th>
<th>+GLP-2</th>
<th>+Sal</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proline flux, μmol·kg$^{-1}·h^{-1}$</td>
<td>500</td>
<td>471</td>
<td>46</td>
</tr>
<tr>
<td>Fractional net conversion of proline to arginine, % of arginine flux</td>
<td>11.3</td>
<td>6.0</td>
<td>0.5</td>
</tr>
<tr>
<td>[Guanido-14C]arginine infusion</td>
<td>+GLP-2</td>
<td>+Sal</td>
<td>Pooled SE</td>
</tr>
<tr>
<td>Arginine flux, μmol·kg$^{-1}·h^{-1}$</td>
<td>102</td>
<td>106</td>
<td>12</td>
</tr>
<tr>
<td>$q_{arginine}$</td>
<td>11.7</td>
<td>6.3</td>
<td>1.4</td>
</tr>
</tbody>
</table>

1 Values are least-square means, $n = 5$. *Different from +GLP-2 value, $P < 0.05$.

2 Rates of synthesis were calculated, within piglet, by multiplying the fractional net conversion of proline to arginine by arginine flux.

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![Figure 1](https://example.com/figure1.png)

**Figure 1** Relation between arginine synthesis from proline and mucosal mass in parenterally fed piglets receiving either a GLP-2 (+GLP-2) or saline (+Sal) continuous infusion. There was a positive, linear relation between mucosal mass and arginine synthesis ($R^2 = 0.72; P = 0.02$).
reported differences in intestinal mass and structure (25,26) were achieved with GLP-2 infusion in this study and we could investigate whether the improvements in the intestinal variables in parenterally fed piglets also were associated with a greater rate of endogenous arginine synthesis.

In adults with intestinal atrophy or short bowel syndrome, plasma citrulline concentration was correlated with both the degree of intestinal villus atrophy \( (r = 0.81; P < 0.001) \) and short bowel length \( (r = 0.86; P < 0.0001) \) \((33,34)\) and was deemed a powerful indicator of whether the intestinal failure was permanent or transient \((34)\). In rats, the small intestine is the only tissue that releases large quantities of citrulline into circulation \((19)\). Therefore, plasma citrulline concentration appears to be a valuable tool to assess intestinal health and metabolic function. In agreement with the other studies, piglets receiving GLP-2, which had a greater mucosal mass \((Table 1)\), had plasma citrulline concentrations that were 50% higher than in piglets in the +Sal group \((Table 2)\). In addition, plasma citrulline concentration was linearly related to villus height \( (R^2 = 0.51; P = 0.02) \), although not to mucosal mass \( (R^2 = 0.18) \). The lack of an association between mucosal mass and plasma citrulline concentration may have been due to the relatively small sample size of this study. Therefore, similar to adults with intestinal dysfunction, plasma citrulline concentration also appears to be a reasonable indicator of villus height in parenterally fed neonatal piglets.

One of the primary objectives of this study was to evaluate the importance of the intestinal utilization of circulating proline for whole-body endogenous arginine synthesis. To address this objective, we compared the rates of arginine synthesis in parenterally fed piglets with either atrophied (+Sal) or non-atrophied (+GLP-2) small intestinal mucosa. Piglets receiving GLP-2 had a 100% greater rate of endogenous arginine synthesis from proline \((Table 4)\) compared with those receiving the control saline infusion. Moreover, there were strong linear relations between both villus height \( (not shown) \) and mucosal mass \((Fig. 1)\) and the rate of arginine synthesis, providing strong, although indirect, evidence that improvements in intestinal variables were associated with an increase in arginine synthesis. That the intestine is the tissue most likely to be responsible for the increase in arginine synthesis from proline due to GLP-2 infusion is supported by the literature. The trophic effects of GLP-2 administration are thought to be primarily intestinal, because the GLP-2 receptors are mainly located in the intestinal tissues \((35,36)\). A previous study in parenterally fed neonatal piglets, using the same infusion rate of GLP-2 as in this study, found an increased rate of protein synthesis in small intestine but not in other splanchnic and extra-splanchnic tissues, and it was intestinal mucosal thickness and not muscularis thickness that responded to GLP-2 administration \((26)\). In addition, the neonatal small intestine is the only known tissue, with the exception of the liver, to have all of the enzymes in the proline to arginine synthetic pathway \((14,17)\); therefore, differences between the +Sal and +GLP-2 groups in arginine synthesis from circulating precursors likely can be largely attributed to differences in intestinal mucosal metabolism. To directly confirm that it was the intestinal metabolism of arterial precursors that was responsible for the increased conversion of proline to arginine, a more invasive arterio-venous difference study using isotopic tracers would need to be conducted.

**Effects of GLP-2 infusion on whole-body arginine status.** The groups did not differ for any of the indicators of whole-body arginine status \((Tables 2–4)\), despite an almost 100% greater rate of arginine synthesis from proline in the +GLP-2 piglets \((Table 4)\). One explanation for this apparent paradox is that compared with arginine intake, the difference in the rate of endogenous arginine synthesis between the 2 groups was small, only 3% of arginine intake. Therefore, although significant, the higher rate of arginine synthesis from proline as a result of GLP-2 infusion appeared to be insufficient to be of physiological importance in improving the present measures of whole-body arginine status.

The low rates of arginine synthesis and the lack of differences in whole-body arginine status between the groups may also be related to the fact that there was a limitation in the metabolic pathway between proline and arginine that could not be overcome by GLP-2 administration. There is a maximum amount of proline that can be used for arginine synthesis in enterally fed piglets receiving an arginine-deficient diet, due to a limitation in citrulline formation \((28)\). Although plasma citrulline concentrations were higher in +GLP-2 piglets than in the +Sal piglets, the values were still lower than those previously observed in enterally fed piglets \((~100 \mu mol/L) \) \((4,15)\). Citrulline formation may have limited arginine synthesis in parenterally fed piglets in the present study to a greater extent than in the enterally fed piglets in previous studies \((4,15)\).

The low arginine intake may have limited the trophic effects of GLP-2. Arginine may be required for optimal GLP-2 effectiveness, because the increase in intestinal blood flow associated with GLP-2 administration is nitric oxide dependent \((27)\) and associated with an increase in endothelial nitric oxide synthase \((EC\ number\ 1.14.13.39)\) mRNA and protein expression and protein phosphorylation \((26,37)\). Because arginine is the precursor for nitric oxide synthesis \((38)\), a limitation in dietary arginine could limit nitric oxide formation and, subsequently, the effectiveness of GLP-2 administration on intestinal function. Although we did see improvements in villus height and mucosal mass with GLP-2 administration \((Table 1)\), they were not as dramatic as in the study by Burrin et al. \((26)\), which used the same rate of GLP-2 infusion. This may have been due to the low arginine intake in our study.

There was a trend \((P = 0.09)\) for lower plasma nitric oxide concentrations in piglets receiving a deficient vs. generous arginine diet \((4)\); however, the effect of arginine intake on the rate of nitric oxide synthesis has not been measured in neonatal piglets. Further research is also necessary to determine whether arginine intake affects the GLP-2-mediated intestinal response during parenteral feeding and whether increasing the dose of GLP-2 administered increases endogenous rate of arginine synthesis.

**Implications for neonatal nutrition.** Although there was no effect on whole-body arginine status as a result of GLP-2 administration to parenterally fed piglets, the finding that there was only a very small increase in arginine synthesis from proline in parenterally fed piglets receiving a low arginine diet and infused with GLP-2 has important implications for neonatal nutrition. It is critical that parenteral solutions provide enough arginine to satisfy daily metabolic needs \((1)\), because the contribution of endogenous synthesis is negligible. Arginine concentration in parenteral solutions is highly variable \((4.7–12.3\%\) of total amino acids by weight) \((39)\) and we think that some solutions do not provide enough arginine to maintain neonatal health \((39)\). Due to the direct relation between mucosal mass and arginine synthesis, efforts should be taken to ensure that neonatal mucosal mass is maintained, specifically during parenteral feeding. The finding that arginine metabolism was influenced by mucosal mass in parenterally fed neonates likely is applicable to the metabolism of other amino acids and this requires further investigation.
Parenterally fed piglets are capable of only a very small amount of endogenous arginine synthesis from circulating proline, ~25% of the amount that enterally fed piglets can synthesize from circulating proline. Although endogenous arginine synthesis was increased slightly in response to GLP-2 administration, presumably due to improvements in intestinal morphology, arginine status did not differ between the 2 groups. Therefore, arginine must be considered an indispensable amino acid in parenterally fed neonatal pigs.

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