Arginine synthesis does not occur during first-pass hepatic metabolism in the neonatal piglet

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In week-old piglets, it is proline and not glutamine/glutamate that is the major precursor for the carbon backbone required for arginine synthesis (7, 25, 31). Addition of proline to an arginine-free diet prevented severe hyperammonemia when neonatal piglets were enterally, but not parenterally, fed (7). These findings suggested that proline can act as an arginine precursor only when first-pass splanchnic metabolism is intact (7). Indeed, Bertolo et al. (4) showed that first-pass intestinal, and not hepatic or peripheral, metabolism was responsible for the conversion of proline to ornithine. These observations were presumably due to the primary localization of ornithine aminotransferase (OAT, EC 2.6.1.13) to the small intestine (18). However, a subsequent study found that although whole body arginine synthesis was twofold greater in enterally fed piglets receiving a deficient arginine diet relative to piglets fed a generous arginine diet, first-pass intestinal metabolism was necessary for 42–63% of whole body arginine synthesis and was not affected by arginine intake (31). Therefore, there must be sites other than first-pass intestinal metabolism involved in arginine synthesis in the neonatal piglet, and it is the synthesis at these sites that is regulated by arginine intake. One such site is first-pass hepatic metabolism, which is also bypassed during parenteral feeding.

The neonatal porcine liver has detectable activity of the two enzymes necessary to convert proline to ornithine, proline oxidase (EC No. not assigned) and OAT (18, 29). Because the piglet liver also has complete urea cycle enzymatic activity (8, 15, 16), the piglet liver has all the enzymes necessary to synthesise arginine from proline; however, whether this actually occurs and can release measurable quantities of arginine to the rest of the body has not been quantified. We hypothesized that first-pass hepatic metabolism in enterally fed neonatal piglets could account for the difference between whole body arginine synthesis and first-pass intestinal arginine synthesis. In the present study, our first goal was to examine arginine synthesis under dietary conditions previously shown to result in both basal and maximal rates of endogenous arginine synthesis (31). Arginine synthesis in adult men (10) and rats (14) appears to be independent of arginine intake, and in men the metabolic response to an arginine-free diet is a decrease in the rate of ornithine oxidation (12). Neonatal piglets are able to increase arginine synthesis in response to arginine deficiency (31), although their ability to decrease arginine hydrolysis during arginine deficiency has not been examined. The portion of the plasma urea that is derived from dietary arginine is five times higher in piglets fed a generous arginine diet than in piglets fed a deficient arginine diet (31), suggesting that the hydrolysis of dietary arginine is decreased, as a conservation mechanism, during times of inadequate arginine intake. We hypothesized that, if piglets are able to conserve arginine during times of deficiency, this will result in a lower relative conversion of arginine to other urea cycle intermediates, such as ornithine and citrulline, compared with piglets fed a generous amount of arginine.

The conversion of arginine to either citrulline or ornithine can only be measured with a label that is not removed during urea formation, such as [4,5-3H]arginine. Because the label remains in the urea cycle, this results in an accumulation of the

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label in arginine as well as the other urea cycle intermediates. We hypothesized that the label recycling of the [4,5-\textsuperscript{3}H]arginine isotope would result in an underestimation of arginine flux and an overestimation of the conversion of arginine to urea cycle intermediates.

**MATERIALS AND METHODS**

*Animals and surgical procedures.* All procedures in this study were approved by the Faculty Animal Policy and Welfare Committee at the University of Alberta. Sixteen intact male Landrace/Large White piglets (Hypor, Regina, SK, Canada) (1.5–2.0 kg) were obtained from the University of Alberta Swine Research and Technology Centre on 1–2 days of age. Piglets were removed from the sow and immediately underwent surgical procedures to implant catheters.

Before surgery (day 0), piglets were given an intramuscular (im) injection of ampicillin sodium (20 mg/kg Novopharm, Toronto, ON, Canada) and then preanesthetized with an im injection of acepromazine maleate (0.22 mg/kg Atravet, Wyeth Laboratories, Guelph, ON, Canada) and atropine sulfate (0.05 mg/kg; MTC Pharmaceuticals, Cambridge, ON, Canada). Piglets were then intubated, and anesthesia was maintained throughout surgery using 1% halothane. Each piglet was implanted with gastric, jugular vein, femoral vein (TiCell Bioservices, Thornhill, ON, Canada), and umbilical vein (0.040 in. ID x 0.085 in. OD Silastic tubing; Dow Corning, Midland, MI) catheters using previously described aseptic techniques (37). A Stamm gastrostomy (28) was performed to implant the gastric feeding catheter. The femoral catheter used for blood sampling was inserted into the left femoral vein and then advanced to the inferior vena cava just caudal to the heart. The jugular catheter used for isotope infusion was inserted into the left jugular vein and then advanced to the superior vena cava just cranial to the heart (37). The portal catheter used for isotope infusion was implanted by introducing the catheter transperitoneally into the umbilical vein and then advancing it to the portal-hepatic junction (4). To ensure the patency of the umbilical vein catheter, a 0.9% saline solution was infused into this catheter at a rate of 1.2 ml/h throughout the entire trial.

After surgery, piglets were given an im injection of the analogase buprenorphine hydrochloride (0.03 mg/kg Buprenex; Rekitt and Colman Pharmaceutical, Richmond, VA). Piglets were given additional im injections of buprenorphine hydrochloride 8 and 16 h later. Ampicillin sodium was given iv into the jugular vein catheter (10 mg/kg) every 12 h on days 1 and 2, and gentamicin sulfate (2.5 mg/kg Garasol; Schering-Plough Animal Health, Pointe-Claire, PQ, Canada) was given im on the mornings of days 1 and 2. A topical antibacterial and antifungal creme (Hibitaine Veterinary Ointment, Wyeth Laboratories) was used on all incision sites.

Piglets were housed individually in circular wire mesh cages, ~75 cm in diameter. These cages allowed for visual and audio contact between the piglets, and toys were provided for additional environmental enrichment. Lighting was on a 12:12-h light-dark schedule, and the room temperature was maintained between 21 and 27°C, with localized supplemental heat provided by heat lamps.

*Diet.* A complete elemental diet, designed to meet the nutrient requirements of neonatal piglets (37), was continuously infused parenterally via the jugular catheter at 50% of targeted intake until the morning of day 1. Diet infusion was then continued via the gastric catheter at 50% of targeted intake for 12 h, 75% of targeted rate for 12 h, and then at a full target rate (13.5 ml·kg⁻¹·h⁻¹) for the remainder of the trial. On the morning of day 3, piglets were randomly assigned to receive a diet containing either a generous (1.80 g·kg⁻¹·day⁻¹ or 6.62 g/l in base solution; n = 8) or deficient (0.20 g·kg⁻¹·day⁻¹ or 0.74 g/l in base solution; n = 8) concentration of arginine. These diets were chosen to represent diets that would result in minimum and maximum rates of arginine synthesis (31) without creating adverse metabolic effects (7). To ensure that the diets were isonitrogenous, the concentrations of alanine (generous arginine diet: 1.37 g/l; deficient arginine diet: 9.64 g/l) and glycine (generous arginine diet: 0.44 g/l; deficient arginine diet: 3.61 g/l) in the base solution were adjusted.

*Blood sampling.* Beginning on the morning of day 3, before the diet allocation to test diets, blood samples (2 ml) were collected every 24 h until the end of the trial on the evening of day 7. The daily blood samples were used for the determination of plasma ammonia and urea nitrogen levels, and the blood sample taken on the morning of day 7 was also used for the determination of plasma amino acid concentrations. As described under *Constant tracer infusions*, additional blood samples were taken during tracer infusions on days 5, 6, and 7.

*Constant tracer infusions.* On the morning of day 5, proline kinetics were determined by a primed [740 kBq (20 μCi/kg), constant [370 kBq (10 μCi/kg)] infusion of L-[U-14C]proline (8.58 GBq/mmol; Amersham Biosciences, Baie d’Urfe, PQ, Canada). One-half of the piglets in each dietary treatment were given an iv infusion of the isotope via the jugular vein catheter, and the other piglets received an intraportal (ip) infusion via the umbilical vein catheter. The routes of infusion were crossed over on day 7, and piglets were infused with L-[U-14C]proline via the route of infusion that they did not receive on day 5. The isotope was infused over a 7-h period, and blood (1 ml) was sampled at 0, 60, 120, 180, 240, 270, 300, 330, 360, and 420 min. On day 7, an additional sample was taken 1 h (~60 min) before the start of isotope infusion to correct for the background specific radioactivity (SRA) of arginine, citrulline, glutamate, glutamine, hydroxyproline, ornithine, and proline in the blood. During the ip infusions, the 1.2 ml/h infusion of 0.9% saline was discontinued.

On day 6, whole body arginine kinetics were determined using a primed [111 kBq (3 μCi/kg), constant [185 kBq (5 μCi/kg)] infusion of either L-[guanido-\textsuperscript{14}C]arginine (n = 4 per treatment group; 1.9 GBq/mmol; NEN Life Science Products, Boston MA) or L-[4,5-\textsuperscript{3}H]arginine (n = 4 per treatment group; 1,480 GBq/mmol; American Radiolabeled Chemicals, St. Louis MO). Isotope was infused iv into the jugular catheter for 6.5 h. Blood (1 ml) was sampled at ~60, 0, 60, 120, 180, 240, 270, 300, 330, 360, and 390 min. The infusion doses and lengths for all infusions were based on previous experiments (4, 31).

After the isotope infusion on day 7, piglets were anesthetized with halothane and killed by the injection of 1,000 mg of pentobarbital sodium into the jugular vein catheter.
Analytic procedures. Plasma amino acid concentrations and the SRAs of arginine, citrulline, glutamate, glutamine, hydroxyproline, proline, and ornithine were measured by reverse-phase HPLC using phenylisothiocyanate derivatives as previously described (6, 23). The internal standards norleucine and L-[4,5-3H]Hleucine (1,920 GBq/mmol; Amersham Pharmacia Biotech, St. Louis MO) were added to each 300-μl plasma sample. Postcolumn radioactive derivatives were collected in 2-ml fractions, 14 ml of scintillant (Biodegradable Counting Scintillant; Amersham Canada, Oakville, ON, Canada) was added, and samples were counted on a scintillation counter. The determination of the urea concentration and radioactivity was as previously described (4).

Plasma ammonia (Reference 200–02; Diagnostic Chemical, Charlotte, PEI, Canada) and urea nitrogen (Sigma Procedure no. 640; Sigma Diagnostics, St. Louis MO) concentrations were determined every 24 h during test diet infusion (day 3 to day 7) using spectrophotometric assays.

Calculations. The formulas and procedures that were used to calculate the plasma SRAs of the postcolumn radioactive derivatives, the fractional net conversions of the precursor (either proline or arginine) to the product amino acids, the whole body fluxes of the infused amino acids (proline and arginine), and the absolute conversions of proline to arginine (Qproline to arginine) were as previously reported (31). The calculated flux values included the amino acids entering the plasma pool through all sources: dietary, de novo synthesis, and protein breakdown. The first-pass hepatic contributions were calculated within piglet by subtracting the iv value from the ip value for both the proline fractional net conversions and the Qproline to arginine. A similar approach has been previously used to isolate the effects of first-pass intestinal metabolism (4, 31).

Statistical analyses. All data were analyzed using the mixed model of SAS version 8.3 (SAS Institute, Cary, NC), and data were considered statistically significant if P < 0.05.

The dependent variables plasma ammonia and plasma urea nitrogen were analyzed using repeated-measures analysis, where the fixed effect was diet (generous or deficient arginine diet) and the random variables were piglet nested in diet and day. The Kenward-Roger option was used to estimate the denominator degrees of freedom. The variance-covariance matrix was chosen for each analysis on the basis of Schwarz’s Bayesian Criterion. When the effects were significant (P < 0.05), least square means were separated using the pdiff option. We expected the plasma ammonia and urea nitrogen levels to be greater in piglets fed the deficient arginine diet (33); therefore a one-tailed t-test was used to compare values between treatment groups.

The results from the iv and ip proline infusions and the iv and ip Qproline to arginine were analyzed using a 2 × 2 factorial design with the diet, route of infusion, and interaction between diet and route of infusion as the fixed effects. Day of isotope infusion was tested as a covariate for the proline infusion data and was included in the model only when the effect was significant (P < 0.05). When there were no significant differences within a diet between the iv and ip values, the first-pass hepatic contribution was considered to be not significantly different from zero. 2 × 2 Factorial analysis was also used to analyze the arginine flux data with the diet, isotope infused, and interaction between diet and isotope infused as the fixed effects. All other data were analyzed using diet as the fixed effect. For all statistical analyses, piglet nested in diet was used as the random term, and when the model P value was <0.05, least square means were separated using the pdiff option, and the two-tailed P values were used to assess significance.

RESULTS

All piglets remained active throughout the entire trial. The umbilical catheter was not patent for two pigs per treatment group; therefore, these piglets received only the iv infusions. Thus, for all results involving the ip infusion, only six values per treatment group were available. Based on a priori power calculations, using our previous data (31), six values per treatment group is more than adequate to detect significant differences between the two diets for ip fluxes, fractional net conversions. Qproline to arginine and first-pass hepatic contributions.

There were no differences between the two treatment groups for piglet weight at initiation of test diet infusion (pooled mean = 2.08 kg, SE = 0.08 kg), rate of weight gain on test diet (pooled mean = 91 g·kg⁻¹·day⁻¹, SE = 10 g·kg⁻¹·day⁻¹), and final piglet weight (pooled mean = 2.91 kg, SE = 0.16 kg).

Both diet (P = 0.0004) and the interaction between day and diet (P = 0.004) had a significant effect on plasma ammonia concentrations (Fig. 1). Piglets fed the deficient arginine diet had higher plasma ammonia concentrations (P < 0.05) than those piglets fed the generous arginine diet from the morning of day 4 onward (Fig. 1). Diet (P < 0.0001) and the interaction between day and diet (P = 0.05) also significantly impacted plasma urea nitrogen concentrations (Fig. 1). From the morning of day 5 onward, piglets fed the deficient arginine diet had higher plasma urea nitrogen concentrations (P < 0.02) than the piglets fed the generous arginine diet (Fig. 1).

On the morning of day 7, piglets fed the arginine-deficient diet had significantly lower plasma concentrations of arginine and ornithine (P < 0.01; Table 1) and higher concentrations of asparagine and glutamine (P < 0.05; Table 1). Plasma threo-
Arginine flux (niv) and proline flux (niv) were separated using the pdiff option. iv, intravenous; ip, intraportal. NS, 0.20. *P < 0.05 vs. piglets fed the generous arginine diet.

Arginine 257 27* 38 <0.01
Aspartate 10 14 2 <0.15
Asparagine 15 40* 3 <0.0001
Citrulline 66 86 8 <0.15
Glutamate 143 144 17 NS
Glutamine 103 347* 33 <0.05
Hydroxyproline 100 85 9 NS
Ornithine 170 39* 15 <0.0001
Proline 627 647 86 NS

Data represent least square means in μmol/l. Least square means were separated using the pdiff option. NS (not significant), P > 0.20. *P < 0.05 vs. piglets fed the generous arginine diet.

The whole body arginine fluxes determined using both the L-[14C]arginine and the L-[4,5-3H]arginine were significantly greater in the piglets fed a generous or deficient arginine diet (Table 2).

There were no other differences in plasma amino acid concentrations between the two treatment groups (data not shown).

Route of infusion and dietary treatment did not affect proline flux (P > 0.05; Table 2). The whole body arginine fluxes determined using both the L-[guanido-14C]arginine and the L-[4,5-3H]arginine were significantly greater in the piglets fed the generous arginine diet (P < 0.0001, Table 2). However, the calculated fluxes using the L-[4,5-3H]arginine isotope were significantly lower for both treatment groups (P = 0.01) compared with the values determined using the L-[guanido-14C]arginine isotope (Table 2).

Day of isotope infusion was found to significantly (P = 0.009) affect only the conversion of proline to glutamate and thus was included as a covariate in the statistical model to analyze these data only. Proline fractional net conversion to ornithine in piglets fed the deficient arginine diet, these values were not different from zero, because there were no differences in the conversions between the iv and ip routes of infusion.

For the L-[4,5-3H]arginine isotope infusion, very little label was detected in glutamate, glutamine, and hydroxyproline, leading to highly variable SRA values. In addition, the proline SRA did not reach plateau in the 6.5-h infusion for four of the eight piglets. Therefore, plateaus in SRA values for these amino acids were not obtained, and fractional net conversions could not be calculated. Arginine conversion to citrulline, ornithine, and urea was significantly greater (P < 0.0005; Table 4) in piglets fed the generous arginine diet.

Table 1. Plasma concentrations of amino acids involved in the urea cycle and arginine synthesis in piglets enterally fed a generous or deficient arginine diet for 5 days.

Table 2. Plasma proline and arginine fluxes of neonatal piglets enterally fed a generous or deficient arginine diet.

Table 3. Fractional net conversion of L-[U-14C]proline to arginine, citrulline, glutamate, glutamine, hydroxyproline, and ornithine in piglets fed a generous or deficient arginine diet.
There was a difference in the arginine flux calculated using the two arginine isotopes (Table 2). The $^3$H on the 4,5 positions of the arginine is not lost with each turn of the urea cycle, unlike the $^{14}$C on the guanido group. Therefore, the lower arginine flux calculated using $^{1-}[4,5-^3$H]arginine is probably due to labeled arginine being recycled through the urea cycle. As a result, we used the mean arginine fluxes derived from the $^{1-}[4,5-^3$H]arginine isotope infusion (Table 2) to calculate the $Q_{\text{proline to arginine}}$ (Table 5). This approach to calculating the $Q_{\text{proline to arginine}}$ is valid because of the significant difference in arginine fluxes between diets (Table 2; $P < 0.0001$) and confirms that the arginine flux is affected primarily by the level of dietary arginine. $Q_{\text{proline to arginine}}$ was greater in the piglets fed the deficient arginine diet both in the presence (ip: $P = 0.0002$) and absence (iv: $P < 0.0001$) of first-pass hepatic metabolism (Table 5). However, first-pass hepatic metabolism did not contribute to the net whole body $Q_{\text{proline to arginine}}$ ($P > 0.05$), regardless of the diet (Table 5).

**DISCUSSION**

To our knowledge, this is the first time that a multi-isotope, multisite amino acid infusion has been used in vivo to separate hepatic metabolism from intestinal, peripheral, and whole body metabolism in neonatal piglets. This research builds on similar approaches to fractional net conversion that have been successfully used to isolate the effects of first-pass intestinal (4, 31) and splanchnic (11) arginine metabolism. This study has clearly demonstrated that the entire conversion of proline to arginine does not occur during first-pass hepatic metabolism, regardless of arginine intake, as evidenced by a lack of difference between the ip and iv fractional net conversions of proline to arginine, or $Q_{\text{proline to arginine}}$ (Tables 3 and 5). Because the ip infused proline tracer was exposed to first-pass metabolism by the liver and the iv infused proline tracer was not, the differences in the fractional net conversions and $Q_{\text{proline to arginine}}$ between these two routes can be attributed to first-pass hepatic metabolism.

Although the neonatal piglet intestine contains all of the enzymes necessary to convert both glutamine and proline to arginine (18, 35, 36), and in vitro studies have shown that, in week-old piglet enterocytes, the net production of arginine from proline is less than that from glutamine (33), whole body studies in week-old piglets have clearly shown that it is proline that is the major precursor for arginine in vivo (7, 25, 31). A potential reason for the discrepancy between the in vitro and in vivo data is that glutamine is a source of the nitrogen moieties incorporated into arginine (24, 33). These uses of glutamine/glutamate in arginine synthesis would not have been accounted for with the $[^{14}$C]glutamate used in the in vivo studies (25, 31). Furthermore, Wilkinson et al. (31) found that, when piglets are fed an arginine-deficient diet, proline contributes to 27% of whole body arginine flux whereas glutamate makes no contribution. Because the in vivo data suggest that in week-old piglets proline is the major source of the carbon backbone for arginine synthesis (25, 31), the $[U-^{14}$C]proline used in the present study was a valid choice for a tracer to measure endogenous arginine synthesis.

Our finding that the liver does not release arginine to the rest of the body is in agreement with the adult mammalian data. Although urea cycle enzymes are abundant in the liver, hepatic release of arginine is low or absent in adult and growing rats (17, 20, 32), mice (19), and humans (3), even when fed an arginine-free diet (20, 21). Adult mammalian hepatocytes have high arginase (EC 3.5.3.1) activity (15, 16, 22), and there is tight channeling of urea cycle between the cytoplasmic urea cycle enzymes (13), presumably resulting in efficient ammonia detoxification to urea. On the basis of the present results, it appears that the metabolic priority of the urea cycle enzymes in the neonatal piglet liver is ammonia detoxification and not endogenous arginine synthesis.

We have previously used the same generous and deficient arginine diets to examine both whole body and first-pass intestinal arginine synthesis (31). On the basis of the similar plasma ammonia (Fig. 1), urea nitrogen (Fig. 1), and plasma amino acid (Table 1) concentrations and the ip fractional net conversions of proline to arginine (Table 3; Ref. 31) between the two studies, the metabolic status of these piglets was similar between the present and previous (31) studies, and therefore the results will be considered together to gain a more complete understanding of the sites of endogenous arginine synthesis in neonatal piglets.

Our previous study calculated $Q_{\text{proline to arginine}}$ using an intragastrically (ig) infused arginine isotope to determine arginine flux (31), whereas the present study used an iv infused fraction of proline to arginine isotope to calculate $Q_{\text{proline to arginine}}$. Because the ig and iv infusions of arginine are closely correlated (Fig. 1), it is possible that $Q_{\text{proline to arginine}}$ determined by the ig tracer is a valid tracer to determine the flux of proline to arginine in vivo.

**Table 4. Fractional net conversion of infused arginine isotope to other urea cycle intermediates in piglets fed a generous or deficient arginine diet**

<table>
<thead>
<tr>
<th>Conversion Product</th>
<th>Generous Arginine Diet</th>
<th>Deficient Arginine Diet</th>
<th>Pooled SE</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>73</td>
<td>16*</td>
<td>4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Citrulline</td>
<td>57</td>
<td>8*</td>
<td>2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ornithine</td>
<td>128</td>
<td>74*</td>
<td>9</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data represent least square means in percentages. Least square means were separated using the pdiff option. *$P < 0.05$ vs. piglets fed the generous arginine diet.

**Table 5. Synthesis of arginine from proline determined during ip and iv infusions of $[U-^{14}$C]proline in piglets enterally fed a generous or deficient arginine diet**

<table>
<thead>
<tr>
<th>Arginine Synthesis from Proline</th>
<th>Generous Arginine Diet</th>
<th>Deficient Arginine Diet</th>
<th>Pooled SE</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$mol$/\text{kg}^{-1} \cdot \text{h}^{-1}$</td>
<td>16.0</td>
<td>40.0*</td>
<td>3.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$g$/kg $^{-1} \cdot \text{day}^{-1}$</td>
<td>0.07</td>
<td>0.17*</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$\mu$mol$/\text{kg}^{-1} \cdot \text{h}^{-1}$</td>
<td>15.5</td>
<td>47.1*</td>
<td>3.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$g$/kg $^{-1} \cdot \text{day}^{-1}$</td>
<td>0.06</td>
<td>0.20*</td>
<td>0.01</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data represent least square means. Least square means were separated using the pdiff option. First-pass hepatic conversion of proline to arginine was calculated within piglet by subtracting the iv conversion from the ip conversion. NS, $P > 0.20$. *$P < 0.05$ vs. piglets fed the generous arginine diet. †Not different from zero, on the basis of no significant difference between ip and iv amounts of arginine synthesized from proline.
arginine isotope. Both approaches are valid as long as the distinction is made as to what the $Q_{\text{proline}}$ to arginine value represents. Flux calculated with the iv-infused isotope includes the effects of first-pass splanchnic metabolism of the isotope; therefore, the resulting $Q_{\text{proline}}$ to arginine is the total endogenous arginine synthesis. $Q_{\text{proline}}$ to arginine calculated with the iv-infused arginine isotope excludes the arginine that is synthesized and subsequently extracted by the splanchnic tissues during first-pass metabolism and thus represents net arginine synthesis. Arginine synthesis calculated using both the iv (Table 2) and the ig (31) arginine flux values is presented in Table 6. First-pass intestinal metabolism is necessary for 60 and 40% of whole body arginine synthesis when piglets are fed a generous or deficient diet, respectively (31). However, first-pass hepatic metabolism does not contribute to whole body arginine synthesis (Tables 3 and 5). Therefore, the remainder of whole body arginine synthesis must require sites not associated with first-pass splanchnic metabolism. In the present study, the synthesis of arginine from proline during peripheral metabolism was threefold higher in the piglets fed the deficient vs. the generous arginine diet (Table 5), suggesting that these are potential sites for the increased arginine synthesis during arginine deficiency.

Peripheral metabolism includes metabolism by other tissues, such as muscle and kidney, as well as second-pass splanchnic metabolism, which is the metabolism of arterially derived substrate by the intestine and liver. Although Windmueller and Spaeth (32) observed a net release of citrulline from rat muscle, the muscle of neonatal piglets lacks proline oxidase activity (29) and has very low OAT activity (18) relative to the intestine. Because muscle does not appear to be able to convert proline to ornithine, the synthesis of arginine from proline by the muscle would not have been detected by the [U-14C]proline infused in the present study. The kidney is crucial for arginine synthesis in adult mammals; however, it requires an exogenous source of citrulline (14), presumably due to the absence of the enzyme ornithine transcarbamoylase (OTC; EC 2.1.3.3) (16). The piglet kidney also has the necessary enzymes to convert citrulline to arginine (35), as well as proline oxidase activity that is in the same order of magnitude as in the small intestine (29). However, the renal activity of OAT is 40 times lower than in the jejunum (18), and in growing piglets there is no detectable renal OTC activity (16), which is likely preventing the conversion of substantial amounts of pyrroline-5-carboxylate to citrulline by the kidney. These findings do not preclude the possibility that the kidneys and muscles are involved in some portion of arginine synthesis in neonatal piglets, only that they are not likely capable of the entire proline-to-arginine synthetic pathway. The roles of these tissues in arginine synthesis in piglets require further investigation. Blood and nutrients from both the portal vein and the hepatic artery flow through the liver sinusoids (1); therefore, the effects of second-pass hepatic metabolism would be expected to be similar to those of first-pass hepatic metabolism. Thus second-pass hepatic metabolism cannot be a site of endogenous arginine synthesis (Tables 3 and 5).

On the basis of the results from the present study and of previous studies by our group (4, 5, 7, 31), we hypothesize that second-pass intestinal metabolism is the site of increased arginine synthesis during arginine deficiency. The intestine has the enzymatic capability to convert proline to arginine (33), and the findings of Brunton et al. (7) suggest that intact first-pass splanchnic metabolism is necessary for the conversion of proline to arginine. However, the results from the present experiment and that by Wilkinson et al. (31) eliminate first-pass metabolism by both gut and liver as potential sites of increased arginine synthesis during arginine deficiency. The discrepancy in these observations may be explained by the importance of the enteral intake of nutrients in maintaining gut morphology (4) and splanchnic blood flow (27), both of which are negatively affected by parenteral feeding. The absence of healthy gut morphology and lower blood flow may have reduced the ability of the parenterally fed gut to extract proline from arterial circulation to use in second-pass arginine synthesis (7). Although an arterial-venous difference study, in 2-wk-old fasted piglets, concluded that there was no uptake of arterial proline by the jejunum (34), it is still possible that arterial proline may be extracted by the gut of the fully fed week-old piglets used in the present study. The activities of the enzymes involved in endogenous arginine synthesis and the interconversions between arginine precursors and urea cycle intermediates both undergo changes between 1 and 2 wk of age in neonatal porcine enterocytes (33, 35, 36). Furthermore, week-old piglet enterocytes synthesize a greater amount of arginine from proline than those from 2-wk-old piglets (33), and thus arterial proline extraction may be necessary to sustain this synthesis. Research directly examining at the importance of second-pass intestinal metabolism in arginine synthesis is necessary.

The lower iv whole body arginine fluxes observed in piglets fed the arginine-deficient diet were expected (10, 31) and were primarily due to the differences in arginine intake, which has also been observed in humans fed arginine-free vs. arginine-supplemented diets (9, 10, 12). Approximately 80–95% of the difference in arginine fluxes between the two diets was accounted for by arginine intake, when arginine intake was

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**Table 6. Sites of total and net arginine synthesis in piglets enterally fed a generous or deficient arginine diet for 5 days**

<table>
<thead>
<tr>
<th>Site</th>
<th>Generous Arginine Diet</th>
<th>Deficient Arginine Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Route of proline infusion</td>
<td>Total arginine synthesis</td>
</tr>
<tr>
<td>Whole body</td>
<td>ig</td>
<td>0.34±1.07</td>
</tr>
<tr>
<td>Whole body, excluding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>first-pass intestinal</td>
<td>ip</td>
<td>0.13±0.06</td>
</tr>
<tr>
<td>First-pass intestinal</td>
<td>ig – ip</td>
<td>0.21±0.11</td>
</tr>
<tr>
<td>Peripheral tissues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>second-pass splanchnic</td>
<td>iv</td>
<td>0.13±0.06</td>
</tr>
<tr>
<td>First-pass hepatic</td>
<td>ip – iv</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

Data are in g·kg⁻¹·day⁻¹. *Route of [U-14C]proline infusion used to measure the fractional net conversion of proline to arginine for use in calculation of arginine synthesis at each site. †Calculated using intragastric (ig) [U-14C]proline flux of 1,011 μmol·kg⁻¹·h⁻¹ (33). ‡Calculated using iv [U-14C]proline flux of 499 μmol·kg⁻¹·h⁻¹ (Table 2 and present study). |

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corrected for first-pass splanchnic extraction by use of the approach of Castillo et al. (11). By use of the iv arginine fluxes from the present study, in combination with the ig arginine fluxes of Wilkinson et al. (31) (arginine deficient: 590 μmol·kg⁻¹·h⁻¹; arginine generous: 1,011 μmol·kg⁻¹·h⁻¹), ~57 and 51% of the dietary arginine from the arginine-deficient and -generous diets, respectively, was extracted during first-pass splanchnic metabolism. First-pass intestinal extraction of dietary arginine in piglets is 40% (4); therefore, first-pass intestinal metabolism makes a larger contribution than first-pass hepatic extraction (11–17%) to total splanchnic extraction of dietary arginine. The conversions of arginine to urea during the iv infusion (Table 4) were similar to the results obtained from an ig infusion (generous: 68%; deficient: 14%; pooled SE = 3) (31), indicating that first-pass splanchnic metabolism is not a major site of dietary arginine hydrolysis in week-old piglets. Why piglet intestinal tissue extracts a substantial amount of arginine during first-pass metabolism is unknown. Other possible fates of the arginine extracted by the intestine include protein synthesis or the synthesis of metabolically active compounds such as polyamines or nitric oxide.

Arginine fractional conversion to urea, ornithine, and citrulline was 4.5-, 2-, and 11-fold greater, respectively, in piglets fed the generous vs. deficient arginine diet (Table 4); therefore, arginine makes a smaller contribution to the fluxes of these metabolites in times of arginine deficiency. Although arginine is the immediate precursor to urea, circulating arginine accounted for only 16 and 73% of the whole body urea flux (Table 4), when measured using the [4,5-3H]arginine tracer, in the arginine-deficient diets. This response agrees with our previous conclusion that arginine is conserved when it is limiting in the diet. The fractional net conversion of arginine to ornithine, calculated using the [4,5-3H]arginine tracer, in the piglets fed the generous arginine diet was greater than 100% (Table 4), which further supports our hypothesis of label recycling. In a study in men fed an arginine-enriched diet, and using an arginine isotope that was recycled, the portion of plasma ornithine flux derived from the arginine was determined to be 150% (2). Those authors proposed that plasma arginine might be sequestered in a distinct intracellular pool where ornithine is synthesized and then released back into the plasma, resulting in an overestimation of whole body arginine to ornithine conversion (2). Our data do not allow us to comment on their proposal of a distinct intracellular pool that sequesters ornithine; however, our data clearly indicate that recycling probably accounts for a substantial portion of the ornithine flux that they observed.

In conclusion, the neonatal porcine liver in vivo is not capable of synthesizing arginine from proline during first-pass metabolism, even when fed a limiting arginine diet. Whole body arginine synthesis is upregulated when a deficient arginine diet is fed; however, because first-pass intestinal arginine synthesis was not affected by arginine intake (31), extrasplanchnic tissues or second-pass intestinal metabolism are likely responsible for this additional synthesis. In addition, piglets fed a deficient arginine diet also appear able to conserve circulating arginine by decreasing its conversion to other urea cycle intermediates. This study clearly demonstrates that, in neonatal piglets, whole body arginine synthesis is upregulated when arginine is deficient but not by first-pass intestinal or hepatic metabolism. The site of increased arginine synthesis during arginine deficiency requires further investigation.

**REFERENCES**


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