DE NOVO SYNTHESIS IS THE MAIN SOURCE OF ORNITHINE FOR
CITRULLINE PRODUCTION IN NEONATAL PIGS

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ABSTRACT

Citrulline is an amino acid synthesized in the gut and utilized for the synthesis of the conditionally-essential amino acid arginine. Recently the origin of the ornithine utilized for citrulline synthesis has become a matter of discussion. Multiple physiological factors may have contributed to the differences found among different researchers; one of these is the developmental stage of the subjects studied. To test the hypothesis that during the neonatal period de novo synthesis is the main source of ornithine for citrulline synthesis, neonatal piglets were infused intravenously or intragastrically with [U-13C6]arginine, [U-13C5]glutamine or [U-13C5]proline during the fasted and fed periods. (ureido)[15N]-citrulline and [2H2]ornithine were infused i.v. for the entire infusion protocol. During fasting plasma proline (13%) and ornithine (19%) were the main precursors for citrulline synthesis, whereas plasma arginine (62%) was the main precursor for plasma ornithine. During feeding enteral (27%) and plasma (12%) proline were the main precursors for the ornithine utilized in the synthesis of citrulline, together with plasma ornithine (27%). Enteral proline and glutamine were utilized directly by the gut to produce ornithine utilized for citrulline synthesis. Arginine was not utilized by the gut, which is consistent with the lack of arginase activity in the neonate. Arginine, however, was the main source (47%) of plasma ornithine and in this way contributed to citrulline synthesis. In conclusion, during the neonatal period the de novo pathway is the predominant source for the ornithine utilized in the synthesis of citrulline, and proline is the preferred precursor.
Keywords: Citrulline, arginine, neonate, stable isotope
Citrulline is a non-essential amino acid synthesized in hepatocytes and enterocytes by condensation of ornithine and carbamoyl phosphate. In the liver, citrulline functions as part of the urea cycle in the detoxification of ammonia and, because of the channeling of urea cycle intermediates (6), little or no citrulline escapes the liver. The citrulline produced in the small intestine, however, enters the portal vein and appears in the peripheral circulation serving as precursor for arginine synthesis. Recently the origin of the ornithine utilized for citrulline synthesis has become a matter of discussion (16, 17, 21). Multiple physiological factors may have contributed to the differences found among the different research groups. Feeding vs. fasting, luminal vs. arterial precursors together with species differences are just a few of the physiological factors that may affect the utilization of different precursors for citrulline synthesis. Other factors that add to the differences found by the different research groups are the choice of tracer to determine precursor-product relationships (21) and model employed to interpret the isotopic data (18).

A variable that has not been fully considered when determining the utilization of different precursors for citrulline and arginine synthesis is age. Developmental changes in enteral metabolism take place towards the end of the second week of life in mice, rats and piglets concurrent with the cortisol surge, the initiation of solid feed ingestion and gut colonization (25, 26). Among these changes, there are important differences in citrulline and arginine metabolism (for a review see (19)). The rapid increase in arginase expression (9, 12, 14), that coincides with a reduction in the expression and activity of argininosuccinate synthase and lyase
(9, 14) results in the loss of the ability of the gut to make arginine. Changes in proline oxidase and pyrroline-5-carboxylate (P5C) synthase activity during the pre-weaning period seem to reduce the contribution of proline and glutamine to the ornithine utilized for citrulline production (11, 12, 28, 40, 42). Furthermore, not only ornithine amino transferase (OAT) activity declines towards weaning (12, 28), but the direction of this bidirectional enzyme changes with age from synthesis to disposal of ornithine (36, 37). These enzymatic changes seem to suggest that there is a shift in the precursors utilized for the synthesis of ornithine, and thus citrulline, in the gut. During the neonatal period, the ‘de novo’ route of ornithine production from glutamine and proline seems to be favored, whereas later in life the ‘preformed’ route from arginine and extracellular ornithine may become predominant (Fig. 1). We have shown in adult mice that in fact arginine and plasma ornithine are the main precursors for citrulline synthesis (18, 21-23) indicating that the preformed pathway predominates in adulthood. The present experiments were conducted to test the hypothesis that the “de novo” pathway for ornithine synthesis is the predominant source of ornithine for citrulline synthesis during the neonatal period.

MATERIAL AND METHODS

Animals and treatments

General Newborn (≤1-day-old), crossbred female pigs (n = 6), obtained from the Texas Department of Criminal Justice (Huntsville, TX), were transported to the animal facility of the Children’s Nutrition Research Center (Houston, TX).
Upon arrival (day 1), piglets were implanted with silastic catheters into the jugular vein and stomach, and a tygon catheter into the carotid artery as previously described (30). After surgery, piglets were placed in individual cages in a heated room (~30°C) and fed every 3h at 50% of their requirement for 24 h. Their respective dietary intake was increased gradually to 100% within the next 3 days. Piglets were fed a cow’s milk–based formula for baby pigs (Litter Life; Merrick, Middleton, WI) at 50 g•kg\(^{-1}\)•d\(^{-1}\), suspended in 240 mL water, providing fat, protein and lactose at 5, 12.5 and 25 g•kg\(^{-1}\)•d\(^{-1}\), respectively, and including minerals and vitamins. All animal procedures were authorized by the Baylor College of Medicine Institutional Animal Care and Use Committee.

**Infusion and sampling** Piglets were infused in three different occasions (day 6, 8 and 10) to investigate the contribution of arginine, glutamine and proline to the synthesis of citrulline. The order of these three precursors was randomly assigned to each piglet, and there was not carryover isotopic enrichment as determined by the enrichment of background blood samples (time 0 h). On the day of the infusion piglets were feed deprived for 8 h. After collecting an arterial blood sample (0 h) for isotopic background determinations, a primed-continuous infusion was started. The infusion schedule consisted of a fasted (0-3 h) and fed (3-11 h) periods (Fig. 2). Feeding was accomplished by an oral bolus of 20 mL•kg\(^{-1}\) followed by an 8-h, intragastric continuous infusion of formula at 10 mL•kg\(^{-1}\)•h\(^{-1}\). During the fasted phase and the first 4 h of the fed period, U\(^{13}\)C arginine, glutamine or proline were infused i.v. to determine the contribution of plasma precursors. During the second part of the fed period (7-11 h) the labeled
precursors were infused i.g. to determine the contribution of enteral precursors to the synthesis of citrulline (Fig. 2). The rate of infusion of these precursors were (i.v prime, i.v continuous, i.g. prime, i.g. continuous): [U-13C6]arginine (20 µmol•kg⁻¹; 20 µmol•kg⁻¹•h⁻¹; 40 µmol•kg⁻¹; 40 µmol•kg⁻¹•h⁻¹), [U-13C5]glutamine (27 µmol•kg⁻¹; 27 µmol•kg⁻¹•h⁻¹; 135 µmol•kg⁻¹; 135 µmol•kg⁻¹•h⁻¹) and [U-13C5]proline (22.5 µmol•kg⁻¹; 22.5 µmol•kg⁻¹•h⁻¹; 30 µmol•kg⁻¹; 30 µmol•kg⁻¹•h⁻¹). Additional tracers were infused i.v. throughout the infusion protocol to determine rates of appearance and conversion. These i.v. tracers (prime and continuous infusion rates) were: (ureido)[¹⁵N]citrulline (4.8 µmol•kg⁻¹; 4.8 µmol•kg⁻¹•h⁻¹), [5,5-2H2]ornithine (5.6 µmol•kg⁻¹; 5.6 µmol•kg⁻¹•h⁻¹), (ring) [²H⁵]phenylalanine (10 µmol•kg⁻¹; 10 µmol•kg⁻¹•h⁻¹) and [3,5-2H2]tyrosine (3.8 µmol•kg⁻¹; 3.8 µmol•kg⁻¹•h⁻¹). The infused diet provided 82, 262, 300, 75 and 101 µmol•kg⁻¹•h⁻¹ of arginine, glutamine, proline, tyrosine and phenylalanine, respectively. All tracers were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA).

Arterial blood samples were collected at the end of the fasted period (2, 2.5 and 3 h), and during the fed phase (6, 6.5, 7, 10, 10.5, 11 h).

Sample analysis Plasma amino acid isotopic enrichments were determined as their dansyl derivatives by LC-MS/MS (20) utilizing a TSQ Quantum Ultra System (Thermo Finnigan, San Jose CA). The parent-daughter ion transitions monitored have been described elsewhere (18).

Calculations The calculations have been described in great detail in a previous publication (18). In brief, the rate of appearance and conversion of the different amino acids were determined by isotopic dilution and transfer of the
label between precursors and products, respectively. First pass splanchnic extraction of amino acids was calculated based on the disappearance of the i.g. tracer with respect to the i.v. infused tracer. The contribution of the different enteral and parenteral precursors for citrulline synthesis was determined utilizing a multifactorial approach that takes into account the contribution of all the precursors simultaneously. This is accomplished by solving a set of simultaneous equations in which the fractional contribution of each precursor is multiplied by the observed enrichment of the precursors to yield the observed enrichment of citrulline.

Data analysis Rate of appearance and conversion of the different amino acids were analyzed statistically as a complete randomized design utilizing the proc mixed procedure of SAS (v. 9.2, SAS Inst, Inc., Cary, NC), with fasting or fed as fixed effects and piglet and infusion day(age) as random effects of the model. The multiple equations used for the determination of the fractional contributions of the different precursors to citrulline synthesis were solved for each individual pig, and the data generated analyzed by ANOVA. Values presented in the text are least square means±standard error, and were tested for significance at the 5% level.

RESULTS

All piglets recovered well from the surgery and reached full feed by the third day. All animals gained weight and the averaged daily weight gain over the whole experiment was 122±18 g/d (60.4±3.4 g•kg⁻¹•d⁻¹). The infused tracers and their
products reached isotopic pseudo-plateau enrichment by the end of each sampling period (Figures 3-5) which allowed us to apply steady state models.

**Rate of amino acid appearance and conversion** The rate of appearance of the amino acids studied increased (P < 0.0001) during the feeding period with the exception of citrulline which remained unchanged (P = 0.432; Table 1). Of the three precursors studied glutamine had the highest first pass splanchnic extraction rate (64.3%), followed by proline (28.1%) and arginine (12.9%; Table 1).

During fasting the rate of conversion of phenylalanine into tyrosine (phenylalanine hydroxylation) accounted for a small percentage (2.9±0.2%) of the rate of appearance of phenylalanine. During feeding the rate of phenylalanine hydroxylation increased both in absolute and relative terms (P < 0.001), but still accounted for a small faction (<4%) of the phenylalanine rate of appearance (Table 2). Plasma ornithine contributed ~14 and 20 µmol•kg⁻¹•h⁻¹ to the synthesis of citrulline in the fasted and fed state, respectively; this contribution represented almost 19 and 22 % of the rate of appearance of citrulline and accounted for ~19 and 22% of the fate of circulating ornithine. The rate of conversion of citrulline to arginine (“de novo” arginine synthesis) increased (P < 0.0001) during feeding, but contributed a similar percentage (~15%; P = 0.915) of the rate of appearance of arginine during the fasted and fed periods. Plasma arginine was the fate of 55 and 73% of the rate of appearance of citrulline during fasting and feeding, respectively (P < 0.0001; Table 2). Plasma arginine to plasma arginine recycling
accounted for ~8 and 14 µmol·kg⁻¹·h⁻¹ during fasting and feeding, respectively, a small fraction (<4%) of the rate of appearance of arginine.

*Citrulline precursors* During the fasting period the contribution of the three arterial precursors studied accounted for ~28% of the rate of appearance of citrulline (Table 3). Proline was the main precursor for citrulline synthesis (P < 0.001), followed by glutamine and arginine. Whereas proline and glutamine made most of their contribution at the site of citrulline synthesis, arginine contribution was made through plasma ornithine. In fact plasma arginine was the main precursor for circulating ornithine accounting for ~62% of the rate of appearance of this amino acid.

During feeding, the contribution of the three precursors studied accounted for ~57% of the rate of appearance of citrulline. The contribution of arterial and enteral proline accounted for ~40% of the rate of appearance of citrulline (Table 3). Whereas the contribution of plasma glutamine was through plasma ornithine, enteral glutamine made its contribution directly at the site of citrulline synthesis. Arginine contributed to the synthesis of citrulline, but mainly through plasma ornithine (Table 3). Plasma arginine was the main precursor for circulating ornithine, accounting for ~47% of the rate of appearance of this amino acid, whereas the contribution of enteral arginine was not different from zero (P = 0.541). Plasma and enteral proline contributed to a similar extent to plasma ornithine; the contribution of glutamine to the synthesis of ornithine, however, was mainly from plasma sources (Table 3).
DISCUSSION

The differences in the contribution of the different precursors utilized for citrulline and arginine synthesis reported by different research groups has been due, at least in part, to the different tracers (21) and models (18) utilized to interpret the tracer data. Another overlooked factor in the utilization of the different precursors for citrulline synthesis is developmental stage. There are major enzymatic changes between the neonatal and postweaning periods in humans, piglets and rodents (12, 15, 28), which could translate in the utilization of different precursors in the synthesis of ornithine for citrulline production.

In the present work, all possible dietary and plasma precursors for citrulline synthesis were investigated during fasting and fed periods in neonatal pigs. This allowed for a direct comparison of the contribution of the different precursors to the synthesis of citrulline.

Rate of amino acid appearance and conversion As expected the rate of appearance of the amino acids studied increased during feeding. The only exception was citrulline which remained unchanged. In humans, citrulline production has been shown to be rather constant and independent from feeding or arginine content of the diet (5, 31). In mice, however, we have shown that feeding increased the rate of appearance of citrulline (18).

The rate of appearance of phenylalanine and tyrosine were similar to the ones reported in conventionally and parenterally fed piglets of similar age (7, 13). Neonatal metabolism is characterized by high rates of protein synthesis (8) and
dietary nitrogen retention (>85%, (13)). The low rate of plasma phenylalanine hydroxylation observed (~3 and 4% for the fasted and fed period, respectively) demonstrates the high efficiency of amino acid recycling, utilization and deposition during the neonatal period.

To the best of our knowledge there have been no reports on the rate of appearance of citrulline and ornithine in neonatal pigs. Urschel et al. (33), however, infused labeled citrulline and ornithine intragastrically and calculated “enteral” fluxes. Due to the first pass splanchnic disappearance of the tracers infused, these fluxes overestimate the real rate of appearance of these amino acids, which is consistent with our results.

The rate of appearance of arginine was within the wide range published (32, 34, 35); the first pass extraction measured in the current communication (12%), however, was lower than the 50% reported by others(35). Urschel et al. (35) reported no changes in first splanchnic extraction despite a 9-fold difference in arginine intake, which is surprising because the liver is the main site for (excess) arginine disposal (27).

The rate of appearance of glutamine and proline was similar to previous published observations (29, 34). Our data on the first pass splanchnic extraction of glutamine and proline agrees with the reduced extraction seen by others (for a review see (2)). Whereas the first pass extraction of dietary glutamine and proline is mostly by the gut (2), a reduced intestinal utilization of arginine by the gut is expected due to the lack of arginase during the neonatal period (9).
The contribution of plasma citrulline to "de novo" arginine production was ~16% of the rate or appearance of arginine, a value similar to the one reported in humans (11% (4)) and mice (16-26% (18)). Not all the circulating citrulline, however, was accounted for as plasma arginine, indicating that a fraction of the citrulline flux is used by different cell types to meet local arginine needs (10). The recycling of plasma arginine for the synthesis of arginine was lower than the one reported in piglets fed a high arginine (20%) or deficient arginine diets (35% (34)).

**Citrulline precursors** The multifactorial model utilized to integrate the tracer data takes simultaneously into account all the precursors for citrulline synthesis, thus avoiding some overaccounting seen by other models (18). We were able to account for 28 and 57% of the precursors for citrulline synthesis during fasting and feeding, respectively. The small contribution of other citrulline sources (nitric oxide synthesis, recycling of citrullinated proteins and catabolism of methylarginines) is not included in these calculation. The failure to account for 100% of the citrulline produced was due to the utilization of unlabeled precursors released from protein breakdown or synthesized within the enterocyte; as expected this contribution was greater during fasting than in the fed state.

Arginine contribution to the rate of appearance of citrulline (~7-8%) was done through plasma ornithine, with very little, if any, direct arginine utilization by the gut in both fasting and fed conditions. This is consistent with the lack of arginase activity during the neonatal period (9, 39), and with our previous observations in arginase II knockout mice (23). Thus ornithine generated from arginine in other
organs, can enter the circulation, be taken up by the enterocytes and contribute to the synthesis of citrulline. The utilization of extracellular ornithine by the gut has also been shown by the 40 and 75% recovery of intragastrically infused labeled ornithine as circulating citrulline in piglets fed an adequate and low arginine diet, respectively (33).

The contribution of glutamine for citrulline synthesis was also modest (~8-10%); glutamine provided roughly the same amount directly in the gut and through plasma ornithine during feeding. No reports on in vivo glutamine utilization for the synthesis of citrulline in the neonatal piglet are available, but glutamate has been shown to be a poor precursor (38). In vitro studies, however, seem to suggest that glutamine is in fact an important precursor (39, 41). This disagreement between the in vivo and in vitro data seems to indicate the challenge of in vitro systems to mimic complex multiprecursor interorgan processes.

Proline has been considered the main precursor for citrulline synthesis in the piglet (1, 3, 33). Whereas the incorporation of the proline tracer into citrulline and arginine in these reports is incontrovertible, the actual quantification of this contribution is confounded, due to the model used to integrate the tracer data (18). The utilization of this incorrect model, which ignores the first pass utilization of enteral precursors to the synthesis of citrulline, not only results in a greater contribution of proline, but also of glutamine (54% and 59% of circulating citrulline, respectively). In the present communication, we have demonstrated that dietary and plasma proline are in fact the main precursors for citrulline
synthesis in the neonatal piglet during fasting and fed conditions. Interestingly, it has been shown that enteral proline is a better precursor than arterial proline (3, 32) which we have also confirmed in this report. The contribution of the different enteral amino acids to the rate of appearance of ornithine indicates that not only arginase is absent in the neonatal gut, but that proline was the main substrate for enteral OAT.

The predominance of the de novo pathway of ornithine synthesis for citrulline production seems not only due to the lack of arginase activity in the neonatal gut, but probably to the activity of OAT, which during this period works toward the synthesis of ornithine. Clear evidence of the change of direction of OAT, from synthesis during the neonatal period to disposal of ornithine during adulthood, can be found in the ‘paradoxical’ neonatal hypo-ornithemia and hyper-ornithemia after weaning in OAT knockout mice, which mimics similar findings reported in humans with gyrate atrophy (37). In addition, the overexpression of OAT results in a decrease in the plasma concentration of ornithine in adult mice (36). The importance of OAT for the provision of ornithine during the neonatal period is evident in mice lacking OAT which require arginine supplementation for their survival during the first two weeks of life, but not thereafter (37).

Although enterocytes readily utilize extracellular ornithine for citrulline synthesis when available (33), ornithine generated from the disposal of arginine during this period is probably limited. The high arginine demand for protein synthesis, together with the reduced amount of arginine present in the diet fed compared to other studies, likely resulted in a small fraction of arginine being
disposed through arginase with the concomitant reduction in the production of ornithine by this route. For these reasons, it is likely that feeding diets with generous amounts of arginine may result in an increased arginine contribution to citrulline synthesis through plasma ornithine.

In conclusion, during the neonatal period the de novo pathway is the predominant source for the ornithine utilized in the synthesis of citrulline, and proline is the preferred precursor. The lack of enteral arginase precludes the direct utilization of arginine by the gut; plasma arginine, however, contributes to the synthesis of citrulline through plasma ornithine.
GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

JCM designed the study, conducted the experimental work, analyzed the samples and wrote the manuscript
BS conducted the experiments and reviewed the manuscript
ICD assisted in the conduct of the experiments and prepared the samples for analysis
DGB designed the study and reviewed the manuscript
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Figure 1. Pathways for the provision of ornithine for citrulline synthesis. The de novo pathway synthesizes glutamate semialdehyde (GSA) from glutamine/glutamate and proline by means of glutaminase (1), pyrroline-5-carboxylate (P5C) synthase (3), and proline oxidase (5). GSA can also be utilized for the synthesis of proline (P5C reductase, 6) and glutamate (P5C dehydrogenase, 4). In the preformed pathway ornithine originates from the hydrolysis of arginine (ARG arginase) or is from extracellular sources (ORN1, ornithine transporter 1). Ornithine aminotransferase (OAT) is bidirectional and can synthesize or dispose of ornithine. Finally, ornithine is utilized by ornithine transcarbamylase (OTC) for the synthesis of citrulline.

Figure 2. Infusion schedule. Piglets were feed deprived for 8h before the beginning of the infusion. The infusion schedule consisted of a fasted and fed period. During the fasted period (0-3h) U-\textsuperscript{13}C\textsubscript{n} labeled arginine, glutamine or proline were infused i.v. During the fed period the U-\textsuperscript{13}C\textsubscript{n} tracers were infused i.v. (3-7h) and i.g. (7-11h). In addition, citrulline, ornithine, phenylalanine and tyrosine tracers were infused i.v. for the entire infusion. Blood samples were collected for isotopic enrichment determination at the beginning of the infusion and at 2, 2.5, 3, 6, 6.5, 7, 10, 10.5 and 11h.

Figure 3. Plasma isotopic enrichment time course of infused \textsuperscript{13}C\textsubscript{6} arginine and its products (\textsuperscript{13}C\textsubscript{6} ornithine and \textsuperscript{13}C\textsubscript{6} citrulline) in neonatal piglets during fasting (0-3
Figure 4. Plasma isotopic enrichment time course of infused $^{13}C_5$ glutamine and its products ($^{13}C_5$ ornithine and $^{13}C_5$ citrulline) in neonatal piglets during fasting (0-3 h) and feeding (3-11 h). During feeding tracer were infused i.v. (3-7 h) and i.g. (7-11 h).

Figure 5. Plasma isotopic enrichment time course of infused $^{13}C_5$ proline and its products ($^{13}C_5$ ornithine and $^{13}C_5$ citrulline) in neonatal piglets during fasting (0-3 h) and feeding (3-11 h). During feeding tracer were infused i.v. (3-7 h) and i.g. (7-11 h).
Table 1. Rate of appearance and first pass extraction of selected amino acids in piglets during fasting and fed conditions

<table>
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<th>Fasted</th>
<th>Fed</th>
<th>P &lt;</th>
<th>FPE*</th>
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<td>RaPhe†</td>
<td>132 ± 7.0</td>
<td>173 ± 9.2</td>
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<td>RaTyr</td>
<td>121 ± 7.7</td>
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<td>RaCit</td>
<td>75 ± 6.9</td>
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<td>RaOrn</td>
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<td>91 ± 6.9</td>
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<tr>
<td>RaArg</td>
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<td>366 ± 56.1</td>
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<tr>
<td>RaPro</td>
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<td>313 ± 21.5</td>
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<td>RaGln</td>
<td>810 ± 85.9</td>
<td>1028 ± 94.2</td>
<td>0.0001</td>
<td>64.3 ± 3.7</td>
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</table>

Values are lsmeans ± SEM, n = 6, except for ornithine, citrulline, phenylalanine and tyrosine rate of appearance n = 18. *FPE, first pass splanchnic extraction, calculated from the disappearance of the i.g. tracer with respect to the i.v. infused tracer. †Rate of appearance, calculated from the isotopic dilution of the i.v. infused tracer.
Table 2. Rate of conversion of selected amino acids in piglets during fasting and fed conditions

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<td><strong>µmol•kg⁻¹•h⁻¹</strong></td>
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<td><strong>RcPhetoTyr</strong></td>
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<td>µmol•kg⁻¹•h⁻¹</td>
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<td>As%RaPhe</td>
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<td>µmol•kg⁻¹•h⁻¹</td>
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Values are lsmeans ± SEM, \( n=18 \), except for RcArgtoArg \( n=6 \)

*Rate of conversion
Table 3. Precursor contribution to the synthesis of ornithine and citrulline in fasted and fed piglets

<table>
<thead>
<tr>
<th></th>
<th>Precursor Contribution to the Synthesis of</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ornithine as %RaOrnithine</td>
<td>Citrulline as %RaCitrulline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>From Plasma</td>
<td>In gut Total</td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>61.6 ± 3.72</td>
<td>11.5 ± 0.71</td>
<td>-4.8 ± 0.40</td>
<td>6.7 ± 0.63</td>
</tr>
<tr>
<td>Glutamine</td>
<td>20.1 ± 3.55</td>
<td>3.7 ± 0.62</td>
<td>4.8 ± 0.80</td>
<td>8.5 ± 0.28</td>
</tr>
<tr>
<td>Proline</td>
<td>10.4 ± 1.35</td>
<td>2.0 ± 0.30</td>
<td>10.9 ± 2.0</td>
<td>12.9 ± 1.92</td>
</tr>
<tr>
<td>Ornithine</td>
<td>18.6 ± 0.56</td>
<td></td>
<td>18.6 ± 0.56</td>
<td></td>
</tr>
<tr>
<td><strong>Sum Fasting</strong></td>
<td><strong>92.1 ± 5.32</strong></td>
<td><strong>28.0 ± 2.04</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fed

Arterial

<p>| | | | |
|                |                |                |                |
| Arterial       |                |                |                |
| Arginine       | 47.0 ± 6.99    | 12.6 ± 1.93    | -5.5 ± 1.30    | 7.1 ± 0.67     |</p>
<table>
<thead>
<tr>
<th></th>
<th>Enteral</th>
<th>Sum Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>16.4 ± 2.55</td>
<td>4.3 ± 0.62</td>
</tr>
<tr>
<td>Proline</td>
<td>14.8 ± 1.12</td>
<td>12.2 ± 1.10</td>
</tr>
<tr>
<td>Ornithine</td>
<td></td>
<td>26.7 ± 0.72</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.4 ± 0.67*</td>
<td>1.2 ± 0.34</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2.4 ± 0.27</td>
<td>4.7 ± 0.88</td>
</tr>
<tr>
<td>Proline</td>
<td>18.6 ± 2.62</td>
<td>27.8 ± 2.44</td>
</tr>
<tr>
<td><strong>Sum Fed</strong></td>
<td><strong>99.5 ± 8.00</strong></td>
<td><strong>57.3 ± 2.98</strong></td>
</tr>
</tbody>
</table>

Values are lsmeans ± SEM. *Not different from zero
Glutamine $\rightarrow$ Glutamate $\rightarrow$ GSA $\rightarrow$ Ornithine $\rightarrow$ Citrulline

Glutamine $\rightarrow$ Glutamate $\rightarrow$ GSA $\rightarrow$ Ornithine $\rightarrow$ Citrulline

Proline $\rightarrow$ Ornithine

Ornithine $\rightarrow$ Arginine

Arginine $\rightarrow$ Ornithine

Citrulline $\rightarrow$ Ornithine

Glutamine $\rightarrow$ Glutamate $\rightarrow$ GSA $\rightarrow$ Ornithine $\rightarrow$ Citrulline

‘de novo’ pathway

‘preformed’ pathway
Fasted  Fed

$^{13}$C Precursors iv iv ig
-8  0  3  7  11

Blood Samples ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓
Fasted  Fed

i.v. tracer  i.g. tracer

Enrichment (mpe)

Time

$^{13}$C$_6$Arg
$^{13}$C$_5$Orn
$^{13}$C$_5$Cit
Fasted ⇄ Fed

i.v. tracer ⇄ i.g. tracer

Enrichment (mpe)

Time (h)

$^{13}$C$_5$Gln
$^{13}$C$_5$Orn
$^{13}$C$_5$Cit