Coadministration of Ornithine and α-Ketoglutarate Is No More Effective Than Ornithine Alone As an Arginine Precursor in Piglets Enterally Fed an Arginine-Deficient Diet

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Abstract

Simultaneous administration of α-ketoglutarate and ornithine, in a 1:2 molar ratio, may improve the effectiveness of ornithine as an arginine precursor in neonatal piglets by shifting ornithine metabolism away from oxidation and toward the synthesis of arginine and other metabolically important compounds. To study this proposed mechanism, enterally fed piglets were allocated to receive 1 of 4 diets for 5 d: an arginine-deficient [1.2 mmol/(kg · d)] arginine diet (basal), or the basal diet supplemented with either α-ketoglutarate [4.6 mmol/(kg · d)] (+α-KG), ornithine [9.2 mmol/(kg · d)] (+Orn), or both ornithine and α-ketoglutarate (+α-KG + Orn, molar ratio 1:2). Primed, constant infusions of [1-14C]ornithine given both intragastrically and intraportally were used to measure ornithine kinetics and determine the role of first-pass intestinal metabolism in ornithine metabolism. Whole body arginine and glutamate kinetics were measured using a primed, constant intragastric infusion of [guanido-14C]arginine and [3,4-3H]glutamate. The diets did not affect plasma arginine or ammonia concentrations, arginine flux, or arginine synthesis from ornithine. Therefore, arginine synthesis was not increased by the simultaneous infusion of ornithine and α-ketoglutarate. Piglets that received dietary ornithine had a 2-fold greater rate of proline synthesis from ornithine \( (P < 0.05) \) and oxidized a greater \( (P < 0.05) \) portion of the infused ornithine than piglets in the basal and +α-KG groups. Overall, ornithine addition to an arginine deficient diet had a greater effect on ornithine and arginine metabolism than the addition of α-ketoglutarate. First-pass intestinal metabolism was critical for ornithine synthesis and conversion to other metabolites but not for ornithine oxidation. J. Nutr. 137: 55–62, 2007.

Introduction

When equimolar amounts of the arginine precursors proline, ornithine, and citrulline were individually added to an arginine-deficient diet given to enterally fed neonatal piglets, only citrulline was as effective as arginine in improving whole-body arginine status (1). These data suggested that there was a limitation in the ability of enterally administered ornithine to be used as an arginine precursor and that the supplemental ornithine was being converted to metabolites other than arginine (1). Other than arginine, ornithine may be converted to numerous other metabolites including: polyamines and pyrroline-5-carboxylate (P5C) (2). P5C can subsequently be converted to either proline or to glutamate, which can then be converted to either glutamine or α-ketoglutarate, which enters the tricarboxylic acid cycle (2).

Ornithine α-ketoglutarate (OKG) is an ionic salt that dissociates in solution into 2 mol of ornithine and 1 mol of α-ketoglutarate and has been used clinically in many situations to improve nitrogen retention (for review, see (3)). A study in rats following fracture trauma (4) found that OKG had greater positive effects on nitrogen retention than either of the OKG components, α-ketoglutarate or ornithine, separately. Moreover, in healthy adults receiving a bolus dose of either OKG, ornithine-HCl, or calcium α-ketoglutarate, only OKG administration resulted in a significant increase in plasma arginine and proline
carboxylate. OKG has been used in the treatment of trauma (4), total parenteral nutrition (5), and renal disease (6). In a study examining the use of OKG in healthy adults (7), OKG supplementation increased plasma arginine concentrations by 40% and proline concentrations by 20%. A follow-up study in this group of healthy adults (8) reported a modest effect of OKG on plasma arginine concentrations and no effect on plasma proline concentrations.

Abbreviations used: +α-KG, α-ketoglutarate supplemented diet; +α-KG + Orn, α-ketoglutarate and ornithine supplemented diet; +Orn, ornithine supplemented diet; OAT, ornithine amino transferase; OKG, ornithine α-ketoglutarate; P5C, pyrroline-5-carboxylate.

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concentrations, whereas the increase in plasma glutamate concentrations was pronounced only when ornithine or α-ketoglutarate alone were administered (5).

The proposed mechanism for the biochemical effects of OKG in humans is that, by simultaneously administering 2 components that are metabolically related by a series of reversible reactions, the equilibrium of ornithine metabolism is shifted away from glutamate and α-ketoglutarate production and toward the production of the other ornithine metabolites, including proline, arginine, and polyamines (3,5). To our knowledge, neither OKG metabolism nor this proposed mechanism has been tested in vivo in neonates using isotopically labeled compounds. If the proposed mechanism of OKG metabolism occurs in neonatal piglets, then ornithine coadministered with α-ketoglutarate (OKG) should improve whole-body arginine status in enterally fed piglets receiving an arginine-deficient diet.

The first objective of this study was to examine ornithine metabolism in piglets receiving an arginine-deficient basal diet or the basal diet supplemented with either α-ketoglutarate (+α-KG), ornithine (+Orn), or OKG as α-ketoglutarate and ornithine (+α-KG/+Orn) in a 1:2 molar proportion. Specifically, we wanted to determine whether the OKG improved the use of ornithine for arginine synthesis relative to ornithine administration alone.

Ornithine aminotransferase (OAT; EC number 2.6.1.13) is the enzyme responsible for the conversion between P5C and ornithine and is primarily localized to the small intestine of piglets (6). In 7-d-old suckling piglets, the activity of OAT is >100% greater in the direction of P5C than in the direction of ornithine (7,8); this may further explain why ornithine does not seem to be used effectively as an arginine precursor (1). First-pass intestinal metabolism is an important site for both ornithine (9) and arginine (10) synthesis in enterally fed neonatal piglets. Furthermore, only ~10% of enterally administered α-ketoglutarate is absorbed into the portal vein, suggesting extensive first-pass intestinal α-ketoglutarate metabolism (11). First-pass intestinal metabolism was hypothesized to be an important site, not only for ornithine metabolism, but also for the metabolic interaction between α-ketoglutarate and ornithine. The second objective of this study was to elucidate the role of first-pass intestinal metabolism in ornithine metabolism in neonatal piglets by infusing ornithine radioisotopes both intragastrically and intraportally.

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. Twenty intact male Landrace/Large White/Duroc piglets (Hypro) (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 gastric catheter for diet and intragastric isotope infusion, an umbilical vein catheter advanced to the portal vein for intraportal isotope infusion, and a femoral vein catheter for blood sampling.

Twenty minutes prior to surgery (d 0), piglets were given an intramuscular injection of atropine sulfate (0.05 mg/kg; MTC Pharmaceuticals), an intramuscular injection of ampicillin sodium (20 mg/kg; Novopharm), and a subcutaneous injection of meloxicam (0.2 mg/kg; Metacam 0.5% injection, Boehringer Ingelheim). Piglets were then masked and preoxygened, followed by initial anesthesia with halothane (halothane, B.P.; MTC Pharmaceuticals). Once lightly anesthetized, piglets were intubated and anesthesia was maintained throughout surgery using 1.5–2.5% isoflurane (AErrane; Baxter). This pressurization procedure is a modification to previously described methods (12) and has resulted in more stable piglet vital signs during surgery. Surgical procedures, postoperative care, and piglet housing were recently described (12).

Diets and treatment groups. A complete elemental diet, designed to meet the nutrient requirements of neonatal piglets (12), was continuously infused enterally via the gastric catheter using pressure-sensitive infusion pumps. Diet composition, preparation, and piglet adaptation to the enteral diet were all as previously described (1,12,13).

On the morning of d 3, piglets were randomly assigned to 1 of 4 test diets (5/15 diet): an arginine-deficient basal diet (basal; 0.73 g/L arginine in the base diet), or either +α-KG (2.47 g/L α-ketoglutaric acid), +Orn (5.70 g/L ornithine-HCl), or α-KG/+Orn (2.47 g/L α-ketoglutaric acid and 5.70 g/L ornithine-HCl). Details of the basal diet composition were recently described (1). The α-KG/+Orn diet had a molar ratio of ornithine to α-ketoglutarate of 2:1; this is equivalent to OKG because OKG dissociates in solution into its 2 components. To ensure that the diets were isonitrogenous, the concentrations of alanine (9.64 g/L in the basal and +α-KG diets; 6.63 g/L in the +Orn and +α-KG/+Orn diets) and glycine (3.61 g/L in the basal and +α-KG diets; 1.07 g/L in the +Orn and +α-KG/+Orn diets) were adjusted. The α-KG, +Orn, and +α-KG/+Orn base solutions were used within 1–2 wk of being mixed to ensure the stability of the ornithine and α-ketoglutarate in solution. Previous work has shown that both ornithine and α-ketoglutarate are stable in solution for at least 21 d when stored at 4°C (14).

Blood sampling. Blood samples (2 mL) were collected every 24 h, beginning immediately prior to the allocation to test diets on d 3 and continuing until the end of the trial. The daily blood samples were used for the determination of plasma ammonia and urea nitrogen concentrations, and the blood sample taken on d 7 was also used for the determination of plasma amino acid concentrations. As described in the next section, additional blood samples were taken during tracer infusions on days 5, 6, and 7.

Constant tracer infusions. On the morning of d 5, ornithine kinetics were determined by a primed [481 kBq (13 μCi)/kg], constant [370 kBq (10 μCi/kg)·h] infusion of L-[1-14C]ornithine (1.96 GBq/mmol; Moravek Biochemicals). One-half of the piglets in each dietary treatment were given an intragastric infusion of the isotope via the gastric catheter, and the other piglets received an intraportal infusion via the umbilical catheter. On d 7, piglets were infused with L-[1-14C]ornithine via the route of infusion that they did not receive on d 5. To enable 14CO2 collection, for the measurement of L-[1-14C]ornithine oxidation, piglets were contained in plexi-glass boxes (30 cm × 60 cm × 45 cm high) for the duration of both of these isotope infusions. The isotope was infused over a 6.5-h period, and blood (1 mL) was sampled at 0, 60, 120, 180, 240, 270, 300, 330, 360, and 390 min. On d 7, additional samples were taken 1 h (~60 min) and 30 min (~30 min) prior to the start of isotope infusion to correct for the background specific activity of arginine in the blood. Breath samples were collected every 30 min for the duration of the isotope infusion for the determination of 14CO2 using a previously described procedure (15). On d 7, an additional hour of breath sampling was completed prior to the initiation of isotope infusion to correct for background 14CO2 production. Infusing the ornithine isotope both intragastrically and intraportally enabled us to examine the effects of first-pass intestinal metabolism on ornithine metabolism (9,10).

On the morning of d 6, arginine kinetics were determined by a primed [111 kBq (3 μCi/kg)], constant [185 kBq (5 μCi/kg)·h] infusion of L-[guanido-14C]arginine (2.11 GBq/mmol; Moravek Biochemicals). Glutamate kinetics were simultaneously determined using a primed [740 kBq (20 μCi/kg)], constant [370 kBq (10 μCi/kg)·h] infusion of L-[3,4-14C]glutamate (1480 GBq/mmol; Moravek Biochemicals). Both isotopes were infused intragastrically for 6.5 h. Blood (1 mL) was sampled at ~60, ~30, 0, 60, 120, 180, 240, 270, 300, 330, 360, and 390 min. The infusion doses and periods for all infusions were based on previous experiments (1,9,10,12). Diets were infused continuously throughout all isotope infusions.
At the end of the d 7 infusion, piglets were killed with an injection of 500 mg of pentobarbital sodium (Euthansol, 340 g/L; Schering Canada) into the femoral vein catheter.

**Analytical procedures.** The radioactivity of the breath samples collected during the [1-14C]ornithine infusions was determined by combining a 1-mL aliquot of the collected breath sample with 6 mL of scintillant (Atomlight; Perkin Elmer Life and Analytical Sciences), and counting the samples on a scintillation counter (Tri-Carb 4000 series).

Plasma amino acid concentrations and the SA of arginine, glutamate, ornithine (1-14C)ornithine infusion samples only), and proline (1-14C]ornithine infusion samples only) were measured by reverse-phase HPLC using phenylisothiocyanate derivatives and postcolumn collection of the radioactive derivatives of arginine, glutamate, leucine, ornithine, and proline, as previously described (1,16,17).

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 (days 3–7) using spectrophotometric assays.

**Calculations.** The fractional net conversions of the precursor (ornithine) to product metabolite (either arginine, glutamate, or proline), whole-body fluxes for the intragastrically infused ornithine, glutamate, and arginine and the intraportally infused ornithine, and the absolute conversion of precursor (ornithine) to product (arginine, glutamate, or proline) (Qornithine to product aa) were all calculated using recently described formulas (1).

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. The intragastric fluxes are influenced by both first-pass splanchnic metabolism and the metabolism by other peripheral tissues (such as muscle, kidney, lung, and intestinal metabolism of arterial substrates), whereas intraportal fluxes exclude the influences of first-pass intestinal metabolism. Whereas the intragastric proline flux was not measured in this study, it was previously determined in a similar study, using similar piglets receiving the identical basal and +Orn diets and identical methods (1). Therefore, the previously determined proline flux value of 511 μmol/kg · h was used for piglets receiving the basal and +α-KG diets and a proline flux value of 649 μmol/kg · h was used for piglets receiving the +Orn and +α-KG/+Orn diets.

First-pass intestinal conversion of ornithine to product amino acid (arginine, glutamate, proline) was calculated within piglet by subtracting the fraction of the intragastrically infused ornithine. The only plasma amino acid concentrations that were affected by diet were ornithine and glutamate (P < 0.05). Piglets receiving the 2 diets containing ornithine had plasma ornithine concentrations that were 2.7–4.8 fold higher (α-KG, 213 μmol/L; +α-KG/+Orn, 168 μmol/L) than in piglets not receiving dietary ornithine (basal, 46 μmol/L; +α-KG, 37 μmol/L; pooled SE, 17 μmol/L). Plasma glutamate concentrations were greatest in piglets receiving the +Orn diet (102 μmol/L) and lowest in the +α-KG piglets (52 μmol/L), with intermediate values in piglets receiving the basal (73 μmol/L) and +Orn/+α-KG (58 μmol/L; pooled SE, 8 μmol/L) diets. The diets tended (P = 0.08) to affect the plasma proline concentration, with +Orn piglets having the highest concentration (886 μmol/L), followed by the +α-KG/+Orn (785 μmol/L), basal (624 μmol/L), and +α-KG (478 μmol/L; pooled SE, 112 μmol/L) piglets.

The use of plasma ammonia and urea concentrations as indicators of whole-body metabolism has been recently discussed (1). There was an effect of diet (P = 0.0002), but not for interaction (P = 0.42), on plasma ammonia concentrations (Table 1). Piglets receiving the +Orn and +α-KG/+Orn diets experienced only transient increases in plasma ammonia concentrations relative to the d 3 levels, whereas piglets in the other 2 groups had elevated plasma ammonia concentrations from d 4 onwards.

Diet (P < 0.0001) and day (P < 0.0001), but not their interaction (P = 0.78), affected plasma urea concentrations (Table 1). The plasma urea concentrations were higher from d 4 onwards than on d 3 (P < 0.05) in piglets in the basal, +α-KG, and +α-KG/+Orn groups. In addition, piglets receiving the +Orn diet had lower plasma urea concentrations than piglets receiving the +α-KG diet from d 4 onwards (P < 0.05).
Ornithine flux and first-pass intestinal ornithine extraction. In all groups, the intragastric flux was greater than the intraportal flux (P < 0.05), but the difference between the intragastric and intraportal fluxes was greater in the piglets not receiving any dietary ornithine than in those piglets in the +Orn and +α-KG/+Orn groups (P < 0.05) (Table 2). For both intragastric and intraportal routes of infusion, ornithine flux was greater in the +Orn and +α-KG/+Orn piglets than in the other 2 groups (P < 0.05) (Table 2). A greater percentage of ornithine was extracted during first-pass intestinal metabolism in basal and +α-KG piglets than in the +Orn and +α-KG/+Orn piglets (Table 2) (P < 0.0001).

Ornithine oxidation. Intragastric ornithine oxidation, as a percentage of ornithine dose oxidized, differed among all 4 groups (P < 0.05); ornithine oxidation was lowest in piglets receiving the +α-KG diet, followed by the basal and the +Orn groups, and was greatest in piglets receiving the +Orn/+α-KG diet (Table 2). Intraportal ornithine oxidation was higher in piglets receiving dietary ornithine than in the other 2 groups of piglets (P < 0.05) (Table 2). The intraportal oxidation of ornithine was greater than the intragastric rate in all 4 groups (P < 0.05) (Table 2).

Ornithine conversion to other metabolites. With the exception of the ornithine to glutamate conversion in piglets receiving the +Orn and +α-KG/+Orn diets, the intragastric fractional net conversions of ornithine to arginine, glutamate, and proline were greater than the intraportal conversions (P < 0.05) (Table 3). Both the intraportal and intragastric conversions of ornithine to proline were greater in the +Orn and +α-KG/+Orn piglets than in piglets receiving the other diets (P < 0.05) (Table 3). The fractional net conversion of ornithine to arginine was not affected by diet, regardless of route of infusion (Table 3), whereas the interaction between diet and route of infusion tended to affect this conversion (P = 0.06). Diet did not affect the intragastric fractional net conversion of ornithine to arginine; however, for the intraportal infusion, this conversion was lowest in the +α-KG group, intermediate in the basal group, and was greatest in the +Orn and +α-KG/+Orn groups (P < 0.05) (Table 3).

Similar to the fractional net conversion values, the intraportal rates of synthesis of ornithine metabolites were lower than the intragastric synthetic rates (P < 0.05), with the exception of the glutamate synthetic rate in piglets receiving +Orn and +α-KG/+Orn diets, where there was no effect of route of infusion (Table 3). Day was only significant as a covariate (P < 0.001) (Table 3). The intraportal conversions of ornithine to other metabolites were greater than the intragastric conversions at days 7, 14, and 21 (P < 0.0001). This was due to a greater fractional net conversion of ornithine to arginine, glutamate, and proline in the intraportal route of infusion relative to the intragastric route (Table 3). The intraportal rate of synthesis of ornithine to glutamate was greater than the intragastric rate (P < 0.0001). The intraportal rate of synthesis of ornithine to proline was greater than the intragastric rate (P < 0.0001). The intraportal rate of synthesis of ornithine to arginine was greater than the intragastric rate (P < 0.0001). The intraportal rate of synthesis of ornithine to proline was greater than the intragastric rate (P < 0.0001). The intraportal rate of synthesis of ornithine to arginine was greater than the intragastric rate (P < 0.0001). The intraportal rate of synthesis of ornithine to proline was greater than the intragastric rate (P < 0.0001). The intraportal rate of synthesis of ornithine to arginine was greater than the intragastric rate (P < 0.0001).
intragastric rates of arginine synthesis from ornithine (Table 3). For both the intraportal and intragastric rates of glutamate synthesis, piglets in the +α-KG/Orn group had the greatest rate and piglets in the +α-KG group had the lowest synthetic rate (P < 0.05) (Table 3), which corresponded to the fact that these 2 groups had the highest and lowest intragastric glutamate fluxes. For the intragastric infusion, the rate of glutamate synthesis in the piglets in the basal group was not different from the rates in the +α-KG and +Orn groups. Intragastric glutamate synthesis in the +Orn group was greater than in the +α-KG group (P < 0.05) but was not different from the other 2 groups (P > 0.05) (Table 3). For the intraportal rate of glutamate synthesis, piglets in the basal and +α-KG groups had lower rates of synthesis than piglets in the +Orn group (P < 0.05), which had a lower rate of synthesis than the piglets in the +α-KG/+Orn group (P < 0.05) (Table 3). Proline synthesis from ornithine was higher in piglets receiving ornithine in their diets (P < 0.05) than in piglets receiving either the basal or +α-KG diet, regardless of the route of isotope infusion (Table 3).

First-pass intestinal metabolism played a large role in ornithine conversion to its various metabolites (Table 3). The rate of glutamate synthesis occurring during first-pass intestinal metabolism in the piglets in the +Orn and +α-KG/+Orn groups was not different from 0, because there were no significant differences between the intragastric and intraportal rates of glutamate synthesis (Table 3), whereas there was a significant intestinal conversion in piglets in the +α-KG and basal groups. Proline synthesis from ornithine during first-pass intestinal metabolism was greater in piglets receiving the +Orn and +α-KG/+Orn than in the other 2 groups (P < 0.05) (Table 3).

**Discussion**

**Ornithine α-ketoglutarate vs. ornithine as an arginine precursor.** This study is the first, to the best of our knowledge, to use the infusion of isotopes to quantify the effects of OKG on metabolism and assess its effectiveness as an arginine precursor in neonates. Because there is no previous research in neonates relating to the metabolism of OKG, the only basis of comparison for our results is to literature in adults and in rodents; therefore, these comparisons should be interpreted carefully with the understanding that the piglet is not an experimental model for adult metabolism. The coadministration of ornithine and α-ketoglutarate to neonatal piglets in this study did not affect whole-body arginine status (Tables 1 and 2) or arginine synthesis during first-pass intestinal metabolism to be affected by diet (P = 0.08).

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**Table 3** Ornithine conversion to arginine, glutamate, and proline in piglets enterally fed either the basal diet or the +α-KG, +Orn, or +α-KG/+Orn diets

<table>
<thead>
<tr>
<th>Ornithine conversion to product amino acids</th>
<th>Basal</th>
<th>+α-KG</th>
<th>+Orn</th>
<th>+α-KG/+Orn</th>
<th>Diet</th>
<th>Route of infusion</th>
<th>Diet × Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractional net conversion, % of arginine from ornithine</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Intragasric</td>
<td>33.1 ± 5.0</td>
<td>47.2 ± 5.0</td>
<td>35.0 ± 5.0</td>
<td>39.7 ± 5.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraportal</td>
<td>8.9 ± 5.0*</td>
<td>6.1 ± 5.0*</td>
<td>21.0 ± 5.0*</td>
<td>18.1 ± 5.0*</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>Arginine synthesis from ornithine, μmol/(kg · h)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Intragasric</td>
<td>147 ± 22</td>
<td>153 ± 22</td>
<td>134 ± 22</td>
<td>125 ± 22</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Intraporal</td>
<td>36 ± 22*</td>
<td>18 ± 22*</td>
<td>80 ± 22*</td>
<td>65 ± 24*</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>First-pass intestinal arginine synthesis, μmol/(kg · h)</td>
<td>106 ± 21b</td>
<td>131 ± 21b</td>
<td>59 ± 21b</td>
<td>48 ± 23b</td>
<td>&lt;0.05</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Glutamate synthesis from ornithine, μmol/(kg · h)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Intragasric</td>
<td>6.09 ± 1.37</td>
<td>6.49 ± 1.37</td>
<td>7.46 ± 1.37</td>
<td>8.14 ± 1.37</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Intraporal</td>
<td>2.19 ± 1.37**</td>
<td>1.72 ± 1.37b</td>
<td>6.13 ± 1.37b</td>
<td>9.38 ± 1.54b</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Proline synthesis from ornithine, μmol/(kg · h)</td>
<td></td>
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<tr>
<td>Intragasric</td>
<td>273 ± 61b</td>
<td>213 ± 61b</td>
<td>392 ± 61bc</td>
<td>620 ± 61b</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>Intraporal</td>
<td>121 ± 61b**</td>
<td>65 ± 61b**</td>
<td>298 ± 61b</td>
<td>720 ± 67b</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>First-pass intestinal glutamate synthesis, μmol/(kg · h)</td>
<td>152 ± 67</td>
<td>148 ± 67</td>
<td>93 ± 67</td>
<td>-105 ± 75</td>
<td>NS</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fractional net conversion, % of glutamate from ornithine</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Intragasric</td>
<td>9.9 ± 0.7a</td>
<td>10.3 ± 0.7a</td>
<td>23.0 ± 0.7b</td>
<td>22.8 ± 0.7b</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Intraporal</td>
<td>3.1 ± 0.7b*</td>
<td>2.9 ± 0.7b*</td>
<td>12.3 ± 0.7a*</td>
<td>12.0 ± 0.8a*</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Proline synthesis from ornithine, μmol/(kg · h)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intragasric</td>
<td>51 ± 5b</td>
<td>53 ± 5b</td>
<td>149 ± 5b</td>
<td>148 ± 5b</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Intraporal</td>
<td>16 ± 5a*</td>
<td>15 ± 5a*</td>
<td>80 ± 5a*</td>
<td>78 ± 5a*</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>First-pass intestinal proline synthesis, μmol/(kg · h)</td>
<td>35 ± 6b</td>
<td>38 ± 6b</td>
<td>69 ± 6b</td>
<td>69 ± 6b</td>
<td>&lt;0.0001</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Notes:
1. Values are least square means ± SEM, n = 5, except for +α-KG/+Orn for the intraportal rates of synthesis, n = 4. Means in a row with superscripts without a common letter differ, P < 0.05. *Different from the intragastric rate, P < 0.05. **Different from the intragastric rate, P < 0.0001.
2. Rates of synthesis were calculated, within piglet, by multiplying intragastric or intraportal fractional net conversion value by intragastric flux value for the product amino acid (Table 2 1).
3. Day was significant as a covariate for the arginine synthetic rate (P = 0.0001) and piglets in the orn groups were lower rates of synthesis than the piglets in the -KG groups, regardless of the route of isotope infusion (Table 3).
4. There was no significant difference between the intragastric and intraportal rates of glutamate synthesis of arginine for the +Orn and +α-KG/+Orn diets; therefore, the first-pass intestinal rate of synthesis is not significantly different from 0.
from ornithine (Table 3) compared with piglets receiving ornithine alone. We have previously shown that the addition of an effective arginine precursor, citrulline, to the arginine-deficient basal diet resulted in lower plasma ammonia and urea concentrations and a higher plasma arginine concentration and whole-body ornithine flux (1). We did not observe any effects on these variables in piglets receiving the +\(\alpha\)-KG/+Orn diet compared with those receiving the +Orn diet; therefore, we conclude that OKG was no more effective than the molar equivalent of ornithine as an arginine precursor.

One of the fundamental differences between this study and the previous study in adult humans (5) is that the piglets in our study were in a state of chronic arginine deficiency. We have previously shown that the primary limitation in arginine synthesis in neonatal piglets receiving an arginine-deficient diet was citrulline formation (1), whereas the proposed mechanism of action of OKG (5,20) in adult humans both assumed and seemed to demonstrate that ornithine availability was the limiting factor for arginine synthesis. For this reason, we formulated the objective of comparing ornithine and ornithine + \(\alpha\)-ketoglutarate in neonatal piglets to determine whether this could be a secondary limitation for arginine synthesis in the neonate.

There are several other possible reasons why the proposed mechanism (3,5) whereby OKG may be an effective arginine precursor in adult humans does not appear to be the case in neonatal piglets (5). First, studies in humans showed that a bolus infusion of OKG was necessary to achieve an increase in plasma arginine concentrations (5,20). The authors proposed that a bolus dose was necessary to rapidly saturate the ornithine to \(\alpha\)-ketoglutarate pathway and allow for the diversion of ornithine to arginine synthesis (20). A continuous infusion of arginine was used in the present study to allow steady-state isotope kinetics to be used and this raises the question of whether a bolus dose may be necessary to observe effects on arginine synthesis. In this study, the rate of OKG infusion was \(\approx 1.9\) g/kg-h), or more than 10 times greater than the bolus doses given to humans [\(-0.15\) g/kg (5,20)]. This very high infusion rate, representing \(\approx 80\%\) of the intragastric ornithine flux (Table 2), was used to ensure saturation of the metabolic pathways. Therefore, it is unlikely that the use of a continuous infusion was the reason for the lack of effect of OKG administration on arginine synthesis. Second, the metabolism of OKG may be different in neonatal vs. older mammals. However, in both 1-wk-old suckling piglets and 58-d-old weaned piglets, the activity of OAT is substantially higher in the direction of PSC vs. ornithine formation (19,21); therefore, this does not support OKG being used more effectively as an arginine precursor in weaned piglets than in suckling piglets. Finally, the addition of OKG in the adult studies was in the form of a neutral ionic salt (5), whereas in this study, 3 acidic molecules (2 ornithine-HCl and 1 \(\alpha\)-ketoglutaric acid molecules) were used. However, because OKG is an ionic salt, it readily dissociates in solution. Both diets still contained the same molar proportions of the ornithine and \(\alpha\)-ketoglutarate and therefore the only difference would have been the pH of the diet. Because the diet was infused enterally into the acidic environment of the stomach, the lower pH of the mixture compared with the salt would not have affected the ability of the +\(\alpha\)-KG/+Orn to act as an arginine precursor.

**Ornithine metabolism and the effects of first-pass intestinal metabolism.** The following stochastic model was used for both the intragastric and intraportal routes of ornithine infusion to calculate the amount of de novo ornithine synthesis and nonoxidative ornithine disposal (Table 4):

<table>
<thead>
<tr>
<th>Table 4</th>
<th>A stochastic model of the effect of first-pass intestinal metabolism on ornithine metabolism in piglets enterally fed either the basal diet or the +(\alpha)-KG, +Orn, or +(\alpha)-KG/+Orn diets1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dietary intake</td>
</tr>
<tr>
<td></td>
<td>Intragastric infusion</td>
</tr>
<tr>
<td></td>
<td>Basal</td>
</tr>
<tr>
<td>Ornithine entry into the plasma pool, (\mu)mol/(kg·h)</td>
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<td>Intraportal infusion</td>
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</tr>
<tr>
<td>Oxidation3</td>
<td>Intragastric infusion</td>
</tr>
<tr>
<td>Intraportal infusion</td>
<td>188</td>
</tr>
<tr>
<td>Nonoxidative disposal4</td>
<td>Intragastric infusion</td>
</tr>
</tbody>
</table>

1 For both routes of infusion, ornithine flux (Table 2) was equivalent to ornithine intake + de novo ornithine synthesis or to ornithine oxidation + nonoxidative ornithine disposal.

2 For the intraportal ornithine metabolism, the first-pass intestinal ornithine extraction value (Table 2) was multiplied by ornithine intake to calculate the amount of ornithine extracted during first-pass intestinal metabolism so that the intraportal ornithine intake could be calculated by subtracting first-pass intestinal extraction from dietary intake.

3 The molar rate of ornithine oxidation was calculated for both the intraportal and intragastric routes of ornithine infusion by multiplying the percentage of ornithine dose oxidized (Table 2) by the respective ornithine flux (Table 2).

4 Nonoxidative ornithine disposal represents the conversion of ornithine to other metabolites including arginine, glutamate, and proline.

Ornithine flux = ornithine intake + de novo ornithine synthesis = ornithine oxidation + nonoxidative ornithine disposal.

Because ornithine is not a component of protein, ornithine use for protein synthesis and ornithine entering the plasma pool from protein breakdown were not included in this model. The difference between the intragastric and intraportal values represents the contribution of first-pass intestinal metabolism.

Whole-body (intragastric) metabolism of ornithine differed among the groups; the addition of ornithine, as either ornithine-HCl or as ornithine-HCl + \(\alpha\)-ketoglutaric acid, to an arginine-deficient diet resulted in a 43–63% decrease in de novo ornithine synthesis, a 3-fold increase in oxidative ornithine disposal, and an \(\approx 70\%\) increase in nonoxidative ornithine disposal (Table 4). Similar to arginine intake regulating arginine synthesis in piglets (10,12), ornithine intake also regulated ornithine synthesis, with increasing intake resulting in decreased synthesis. To the best of our knowledge, the effects of ornithine intake on ornithine oxidation have not been previously studied in vivo in neonatal piglets; however, studies in adult males showed that ornithine oxidation increases with ornithine flux (22,23). Therefore, similar to adult humans, ornithine oxidation in neonatal piglets appears to be regulated, at least in part, by ornithine intake or status. There was an increase in whole-body proline synthesis as a result of ornithine supplementation (Table 3), which agrees with results from previous studies in humans and rodents, where proline concentrations in the plasma (5,20,24–26) and tissues...
(24) were consistently higher in the subjects receiving OKG vs. an ornithine-free solution.

First-pass intestinal metabolism is a major site of the regulation of ornithine metabolism in response to ornithine intake. For piglets in the basal and +α-KG groups, where de novo ornithine synthesis was the greatest, first-pass intestinal metabolism accounted for more than one-half of whole-body ornithine synthesis (Table 4). This confirms previous in vivo tracer research that found first-pass intestinal metabolism was the primary site of ornithine formation in enterally fed piglets (9). Unlike ornithine synthesis, however, there was little difference between the intragastric and intraportal rates of ornithine oxidation, regardless of ornithine intake, indicating that first-pass intestinal metabolism made little contribution to ornithine oxidation (Table 4). This is in agreement with in vitro data, which found that in wk-old piglet enterocytes, ornithine oxidation was negligible (27).

For all 4 groups of piglets, ~100 μmol/(kg·h) of ornithine was converted to other metabolites during first-pass intestinal metabolism (Table 4); however, the metabolite products differed depending on the diet (Table 3). Piglets receiving the basal and +α-KG diets synthesized more arginine and glutamate than the piglets receiving the +Orn and +α-KG/+Orn diets, who synthesized more proline during first-pass intestinal metabolism. One of our previous isotopic studies in neonatal piglets showed that proline synthesis from ornithine is dependent on small intestinal metabolism (9) and the results from this study further demonstrate the importance of small intestinal metabolism for proline synthesis. Although whole-body (intragastric) rates of arginine and glutamate synthesis from ornithine were quite constant between diets, the intraportal conversion rates were significantly lower in piglets receiving the diets without ornithine (Table 3), resulting in a greater calculated rate of first-pass intestinal synthesis. Therefore, when the ornithine tracer bypassed first-pass intestinal metabolism in the arginine-deficient piglets not receiving dietary ornithine, other tissues, possibly the liver, extracted more ornithine tracer without a resulting increase in arginine or glutamate release when than ornithine was included in the diet. The first-pass hepatic extraction of ornithine was not determined in this study, but a study using perfused rat livers found that there was hepatic uptake of ornithine that was not affected by α-ketoglutarate addition to the perfusate (28).

The hepatic metabolism of ornithine and arginine is complex (28) and additional research is necessary to quantify in vivo the relation between the hepatic metabolism of these amino acids and their dietary intake.

The primary objective of this study was to determine whether OKG was a more effective arginine precursor than ornithine. Based on whole-body arginine status and arginine synthesis from ornithine, OKG was no better than ornithine alone as an arginine precursor. Furthermore, ornithine administration in general did not affect arginine metabolism differently than feeding diets devoid of ornithine. This supports the data of our previous study, which found that the limitation in arginine synthesis was citrulline formation (1). Ornithine, but not α-ketoglutarate, intake had profound effects on ornithine metabolism by decreasing de novo synthesis and increasing whole-body oxidative and nonoxidative ornithine disposal. First-pass intestinal metabolism was important for ornithine synthesis and nonoxidative ornithine disposal but not for ornithine oxidation. Arginine is an important amino acid for neonatal health and survival (29–31), and whereas further research is still required to complete our understanding of its endogenous synthesis, we now have a better understanding of ornithine metabolism, which is one of the major intermediates in de novo arginine synthesis.

Acknowledgments
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Literature Cited


