First-Pass Metabolism Limits the Intestinal Absorption of Enteral α-Ketoglutarate in Young Pigs

Barry D. Lambert, Rafał Filip, Barbara Stoll, Peter Junghans, Michael Derno, Ulf Hennig, Wolfgang B. Souffrant, Stefan Pierzynowski, and Douglas G. Burrin

Abstract

Our results in a previous study indicated that the portal absorption of intragastrically fed α-ketoglutarate (AKG) was limited in young pigs. Our aim was to quantify the net portal absorption, first-pass metabolism, and whole-body flux of enterally infused AKG. In study 1, we quantified the net portal nutrient absorption in young pigs (n = 9) given an intraduodenal infusion of milk replacer [10 mL/(kg·h)] and either saline (control) or 930 μmol/(kg·h) AKG for 4 h. In study 2, we quantified the luminal disappearance of a duodenal AKG bolus in young pigs (n = 7). In study 3, we quantified the whole-body kinetics of 13C-AKG metabolism when infused either enterally (n = 9) or intravenously (n = 9) in young pigs. In study 1, when compared with the control group, enteral AKG infusion increased (P < 0.01) the arterial (13.8 ± 1.7 vs. 27.4 ± 3.6 μmol/L) and portal (22.0 ± 1.4 vs. 64.6 ± 5.9 μmol/L) AKG concentrations and the net portal absorption of AKG [19.7 ± 2.8 vs. 95.2 ± 12.0 μmol/(kg·h)]. The mean fractional portal appearance of enterally infused AKG was 10.23 ± 1.3%. In study 2, the luminal disappearance of AKG was 663 μmol/(kg·h), representing 63% of the intraduodenal dose. In study 3, the whole-body 13C-AKG flux [4685 ± 666 vs. 801 ± 67 μmol/(kg·h)] was higher (P < 0.05) when given enterally than intravenously, but 13CO2 recovery was not different (37.3 ± 1.0 vs. 36.2 ± 0.7% dose). The first-pass splanchnic 13C-AKG utilization was ~80%, of which 30% was oxidized to 13CO2. We conclude that the intestinal absorption of AKG is limited in young pigs largely due to substantial first-pass gastrointestinal metabolism.

Introduction

Since the in situ rat studies of Windmueller and Spaeth (1,2), it has been known that glutamate and glutamine are important metabolic fuels for the small intestine. Windmueller and Spaeth were the first to report the large fractional metabolism of glutamate (>95%) and glutamine (>70%) by the intestinal tract during absorption. Their results have since been confirmed in vivo in both piglets (3,4) and humans (5–7). During glutamate oxidation, the first step is transamination by any number of enzymes or deamination by glutamate dehydrogenase (GDH), all of which are expressed in the gastrointestinal tract (8). During transamination by branched-chain amino transferase (BCAT),7 glutamate donates an amino moiety to a branched-chain α-keto acid, forming α-ketoglutarate (AKG) and the corresponding branched-chain amino acid. Because AKG is an intermediate in the oxidation of both glutamate and glutamine, as well as a TCA cycle intermediate, it is conceivable that AKG could serve as a metabolic/oxidative fuel, thus sparing glutamate and glutamine carbon. Additionally, we were interested in the effects of enteral AKG on ammonia and urea metabolism. AKG could serve to decrease free ammonia via a decrease in glutamate and glutamine oxidation and/or via incorporation of ammonia into glutamate by the reversible enzyme GDH.

Previously, studies have shown that the portal appearance of intragastrically infused AKG was limited in young pigs (9,10), representing only 10% of intake. In our first study, it was not possible to determine whether lack of intestinal transport or metabolism within intestinal tissue led to low portal appearances of AKG. Additionally, the intragastric infusion of AKG and a bolus feeding of a corn-based diet used in our previous study could have interfered with the continuous flow of AKG to the small

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References


7 Abbreviations used: AKG, α-ketoglutarate; BCAT, branched chain amino transferase; NOLD, nonoxidative leucine disposal; PDV, portal drained viscera.

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intestine. However, subsequent studies have shown that there is excess capacity for sodium-dependent dicarboxylic acid transport (NaDC-1) in the small intestine and stomach to handle the AKG intake administered in our pig studies (11). Thus, it seemed apparent that the low rate of dietary AKG absorption is due to intestinal mucosal metabolism. To address this issue, we conducted studies in growing pigs to examine the metabolic fate of AKG when it is presented to the gut either intraduodenally or intragastrically and also in tracer form ([13C]-AKG) systemically or enterally. We hypothesized that the low rate of intestinal absorption of dietary AKG was due to extensive metabolism and oxidation by the gastrointestinal tract.

Materials and Methods

Studies 1 and 2 were approved by the Baylor College of Medicine Animal Protocol Review Committee. Housing and care of the animals conformed to USDA guidelines. Study 3 was approved by the Animal Care and Use Committee of the Ministry of Nutrition, Agriculture, Forestry, and Fishery, Schwerin, State Mecklenburg-Vorpommern, Germany.

Study design

In studies 1 and 2, female piglets (n = 9) were purchased from the Texas Department of Criminal Justice, Huntsville, TX. Pigs (14 d of age) arrived at the Children’s Nutrition Research Center and for a 7-d adjustment period were fed a liquid milk replacer diet (Litter Life, Merrick) at a rate of 50 g/kg·h. The composition of the milk replacer (per kg dry matter) was 500 g lactose, 100 g fat, and 250 g protein. After 7 d, food was withdrawn from the piglets overnight and they were prepared for surgery as described previously (3). Briefly, under isoflurane anesthesia and aseptic conditions, the piglets were implanted with a polyethylene catheter (o.d., 1.27 mm, Becton Dickinson) in the common portal vein, and silastic catheters (o.d., 1.78 mm) in an external jugular vein and a carotid artery. An ultrasonic flow probe (8–10 mm i.d., Transonic) was placed around the portal vein. A silicone catheter (o.d., 2.17 mm, Baxter Healthcare) was implanted into the lumen of the duodenum. The catheters were filled with sterile saline containing heparin (25 kU/L), and exteriorized on either the left flank (portal and duodenal catheters, flow probe lead) or between the scapula (jugular and carotid catheters). Immediately preoperatively, animals received an intramuscular injection of antibiotic (20 mg/kg enrofloxacin, Bayer) and an intramuscular injection of analgesic (0.1 mg/kg butorphenol tartrate, Fort Dodge Labs). Before enteral feeding was resumed postoperatively, pigs were maintained on total parenteral nutrition for 24 h at a rate of 5 mL/kg·h. Pigs were allowed 7 d to recover from surgery. In all piglets, intakes and rates of weight gain had returned to preoperative levels.

Study 1. Piglets were deprived of food for 15 h before initiation of the experiment. On the day of the experiment, at time −1 h, a primed (10 mL/kg; 25% w/w aqueous solution; oral), continuous duodenal infusion of milk replacer (10 mL/kg·h, Litter Life, Merrick) was prepared as a 25% w/w aqueous solution that provided −920 kJ and 12.5 g protein (kg·d). The treatments were dissolved in water and contained either NaCl (control; 930 mmol/L) or sodium-AKG (930 mmol/L; Sigma-Aldrich); the solutions were infused intraduodenally at 1 mL/kg·h, such that the AKG infusion rate was 930 μmol/kg·h. The level of AKG was chosen based on previous data from our laboratory, where intakes of >2.5% of diet dry matter was required to observe a detectable portal balance of AKG (10). Pigs also received an intravenous (200 μmol/kg), continuous, 6-h infusion of 15N2-urea [20 μmol/kg·h; 99%; Cambridge Isotope Laboratories]. At time 0 h, a primed (15 μmol/kg), continuous, 2-h infusion of NaH13CO3 [15 μmol/kg·h; 99%; Cambridge Isotope Laboratories] was initiated. Arterial samples were obtained at 0, 90, 105, and 120 min after initiation of NaH13CO3 infusion to determine whole-body CO2 production.

At time 2 h, the NaH13CO3 infusion was terminated and a primed (40 μmol/kg), continuous, 4-h infusion of [1-13C]Leucine [40 μmol/kg·h; 99%; Cambridge Isotope Laboratories] was initiated. Arterial and portal venous samples were obtained at time 4, 5, and 6 h for determination of leucine and urea kinetics as well as mass balance of ammonia, AKG, glucose, and amino acids. All pigs received both control and AKG treatments in a completely randomized design with at least 24 h between treatment periods.

Study 2. After completion of study 1, the following day some of the pigs (n = 7) were given a duodenal bolus infusion [7.75 mL/kg; 25% (w/w) aqueous solution] of liquid milk replacer (Litter Life, Merrick) containing 25 g/L sodium AKG (1040 μmol/kg body weight). After 1 h, pigs were killed by i.v. injection of 50 mg/kg pentobarbital. The small intestine was carefully clamped at the proximal duodenum and distal ileum, removed, and flushed with 2 × 50 mL of saline to wash the intestine. The washes were collected, pooled, and a 15 mL aliquot was flash frozen in liquid N2 and stored at −80°C for later AKG analysis.

Study 3. Portions of the experimental design, diets, and protocol have been described previously (12). Eighteen castrated male pigs (German Landrace) with initial body weight of −15 kg and 8 wk of age were housed in individual metabolic cages. Pigs were surgically implanted with a 3 catheters into the jugular vein, the stomach/duodenum for infusion of nutrients and AKG, and into the carotid artery for blood sampling. The catheters were filled with sterile saline containing heparin. Immediately after surgery, pigs received an intramuscular injection of 4 mL antibiotic (gentamycin, Vepha-Gent, Veyx-Pharma GmbH) and 1 mL Rimadyl (carprofen, Pfizer Animal Health) i.m. After surgery the pigs were fed intraduodenally an elemental diet composed of free amino acids, dextrose, Intralipid, electrolytes, and vitamins for 7 d. The diet provided the following approximate daily nutrient intakes per kg body weight: 4 g protein, 12 g glucose, and 5.5 g lipid. Three days postsurgery, pigs began receiving a continuous infusion of AKG (5.93 mmol sodium AKG/kg·d), equivalent to 1 g/kg·d either intraduodenally or intragastrically. At 7 h postsurgery, pigs received a continuous infusion of 15 mg/kg[1-13C]AKG (Chemotrade) dissolved in 50 mL isotonic saline solution for 3 h. The AKG was infused either intravenously (jugular vein) or via the enteral catheters (intraduodenal or intragastric). The number of pigs studied in each group was as follows: intraduodenal[13C]-AKG intraduodenal (ID-fed, n = 4), diet intraduodenal[13C]-AKG intravenous (ID-fed, n = 5), diet intragastric[13C]-AKG intragastric (IG-fed, n = 4), diet intragastric[13C]-AKG intravenous (IG-fed, n = 5). Blood samples were taken 1 h before (−1) and 1, 1.5, 2, 2.5, and 3 h after the start of [1-13C]AKG infusion.

Sample analysis

Blood samples were immediately placed on ice and centrifuged at 3000 × g for 10 min. Plasma was collected, immediately frozen in liquid N2, and stored at −80°C until analysis. For plasma amino acid analysis, a 0.2 mL aliquot of plasma was mixed with an equal volume of an aqueous solution of methionine sulfone (4 mmol/L) and centrifuged at 10,000 × g for 120 min through a 10-KDa cutoff filter. A 50 μL aliquot of the filtrate was dried and the amino acids were analyzed by reverse-phase HPLC of their phenylisothiocyanate derivatives (Pico Tag, Waters).

Plasma AKG was determined by the method of Bergmeyer and Bernt (8) with minor modifications. The assay was carried out in 0.5 mL of working solution consisting of 100 mmol/L phosphate buffer (pH 7.6), 4 mmol/L ammonium chloride, and 50 μmol/L NADH. To the working solution, an appropriate amount of plasma containing 1–10 nmol of AKG was added. An initial absorbance reading was obtained at 340 nm. Following the initial absorbance reading, ∼6 units (in a volume of 10 μL) of bovine GDH (G2501; Sigma-Aldrich) was added to each tube. After a 10-min incubation, a second absorbance reading was taken at 340 nm. The amount of AKG in the sample is directly proportional to the decrease in absorbance between the first and second reading. The AKG concentration was calculated by the use of a standard curve. Plasma ammonia was determined using a spectrophotometric assay kit (171-C, Sigma-Aldrich). Plasma glucose was determined using a spectrophotometric assay kit (315–100; Sigma-Aldrich).

To estimate the enrichment of blood bicarbonate, an aliquot of whole blood (1.0 mL) was placed in a 10-mL vacutainer (Becton Dickinson), and 0.5 mL of perchloric acid (10% w/v) was added. Room air (10 mL) filtered through soda lime (SodaRabs; Grace Container Products) was injected into the vacutainer, removed into a gas-tight syringe, and
transferred to a second vacutainer. The isotopic enrichment of the carbon dioxide in the gas sample was measured on a continuous flow gas isotope mass spectrometer (Thermo Finnigan Gasbench-II coupled with DeltaPlusXL Isotope Ratio MS).

Plasma ketoisocapric acid (KIC) was isolated by cation exchange chromatography (AG-50W resin, Bio-Rad). Eauls were treated with sodium hydroxide (100 µL; 10 mol/L) and hydroxylamine HCl (200 µL; 0.36 mol/L) and heated (60°C; 30 min). After cooling, the pH of the samples was adjusted to <2. The keto acids were extracted in 5 mL of ethylacetate and dried under nitrogen at room temperature. Derivatization of KIC was accomplished by adding 30 mL of N-methyl-N-t-butyldimethylsilyl-trifluoroacetamide plus 1% t-butyldimethylchlorosilane. The isotopic enrichment of KIC was determined by EI GC-MS (GC-MS model HP-6890/5973 MSD, Hewlett Packard) by monitoring ions at 316 m/z and 317 m/z.

Plasma urea isotopic enrichments were determined by EI GC-MS analysis. Proteins were precipitated from 50 µL of plasma with 200 µL of ice-cold acetone. After vortexing, the protein was separated by centrifugation (3000 x g for 20 min), and the supernatant was removed and dried under nitrogen. To the dried supernatant, 250 µL of a 1:20 dilution of malonaldehyde bid (dimethyl acetal) and concentrated HCl (30 w %) was added, the sample was incubated at room temperature for 2 h, and then completely evaporated (Speedvac, Savant Instruments, Forma Scientific). The urea was derivitized with 50 µL of N-methyl-N-t-butyldimethylsilyl-trifluoroacetamide + 1% t-butyldimethylchlorosilane and the isotopic enrichment in plasma was determined using EI GC-MS analysis by monitoring ions at 153 to 155 m/z.

Whole-body leucine oxidation [µmol/(kg · h)] was calculated as follows:

\[
\text{Whole-body leucine oxidation} = [\text{IE L}]/\text{CONC. ART} \times \text{PBH},
\]

(Eq. 1)

where CONC. is the concentration in the blood (µmol/L), PORT and ART refer to portal and arterial blood and PBF is portal blood flow (L/kg · h).

Whole-body leucine flux [µmol/(kg · h)] was calculated as follows:

\[
Q = R \times ([\text{IE}_{\text{infusate}}/\text{IE}_{\text{plasma}}] - 1)
\]

(Eq. 2)

where R is the tracer infusion rate [µmol/(kg · h)] and IEinfusate and IEplasma are the isotopic enrichments (expressed as mol%) of the infused tracer and plasma KIC, respectively. Whole-body AKG and urea flux were calculated using Eq. 2 based on the steady-state plasma IE of [13C]AKG and [15N]urea, respectively.

In study 1, whole-body CO2 production was calculated as follows:

\[
\text{Whole Body CO2 production} = \left( \frac{\text{IE}_{\text{infusate}}}{\text{IE}_{\text{arterial bicarbonate}}} \right) \times \text{tracer infusion rate} \times 0.81
\]

(Eq. 3)

where IEinfusate is the enrichment of H13CO3- in the infused (mol % excess), IE arterial bicarbonate is the enrichment in arterial blood (mol % excess), and tracer infusion rate [µmol/(kg · h)] during the i.v. bicarbonate infusion that preceded each treatment period. The entire equation was divided by 0.81 to correct for recovery of infused labeled carbon in bicarbonate (13).

Whole-body leucine oxidation [µmol/(kg · h)] was calculated as follows:

\[
\text{Whole-body leucine oxidation} = \text{IE}_{\text{CO2}}/\text{IE}_{\text{L}} \times 3,
\]

(Eq. 4)

where IE CO2 is the isotopic enrichment of bicarbonate during the [1-13C]leucine infusion and IE L is the isotopic enrichment of 1-13C-KIC during the [1-13C]leucine infusion.

Whole-body nonoxidative leucine disposal (NOLD) is an estimate of leucine incorporation into muscle. NOLD [µmol/(kg · h)] was calculated by the following equation:

\[
\text{NOLD} = \text{Eq. 2} - \text{Eq. 4}.
\]

(Eq. 5)

Whole-body endogenous leucine appearance rate (Rα) [µmol/(kg · h)] is an estimate of leucine released into the plasma pool by tissue proteolysis and was calculated as:

\[
\text{Rα} = \text{Eq. 2} - \text{leucine intake}.
\]

The fractional first-pass splanchnic extraction of enterally infused [13C]AKG was calculated as follows:

\[
f = 1 - \left( \frac{\text{PE}_{\text{enteral}}}{\text{PE}_{\text{intravenous}}} \right)
\]

where PE represents the steady-state plasma enrichments of [13C]AKG during enteral and intravenous infusion. The fraction of enteral [13C]AKG that was oxidized on first pass by the splanchnic tissues was calculated as follows:

\[
\text{First-pass fractional}[13C]AKG oxidation} = \text{fox}_{\text{intestinal}} - (1 - f) \times \text{fox}_{\text{intravenous}}
\]

where fox represents the fractional oxidation of [13C]AKG given either enterally or intravenously as described previously.

Statistical analysis
For all statistical tests, a P-value of 0.05 was considered to be significant. In study 1, the effects of AKG on the arterial, portal, and net portal appearance of individual amino acids, AKG, glucose, ammonia, and leucine kinetics were analyzed using the General Linear Model procedure (Minitab). The model contained the effects of AKG supplementation and pig. Pig was included as a random variable. In study 3, whole-body kinetic data were analyzed using a 2-way ANOVA with diet (ID vs. IG fed) and tracer infusion route (IV vs. Enteral) as main effects. A 1-way Student’s t test was used to test whether AKG net portal balance was significantly greater than zero during control treatments. Values in the text are means ± SEM.

Results
Expt. 1. The body weights of pigs in the control (7.45 ± 0.3) and AKG- infused group (7.29 ± 0.4) did not differ. AKG infusion increased (P < 0.01) arterial and portal AKG concentration and the net portal balance of AKG (Table 1). Even when no AKG was infused into the duodenum, the net portal absorption of AKG was significantly greater than zero. However, the net portal absorption of AKG was increased (P < 0.001) with AKG treatment compared with control. The net portal balance of AKG represented only 10.23% of the amount infused. The net portal balance of 10.23% was actually a slight overestimate of infused AKG absorption, because when only saline was infused, there was a significant absorption of AKG. When we corrected the data for absorption of AKG from the control diet, the proportion of infused AKG appearing in the portal venous drainage was 8.12%.

Interestingly, net portal balance of glucose was decreased (P < 0.05) with AKG treatment. Portal blood flow, ammonia net portal balance, and whole-body urea flux were not affected by AKG treatment (Table 1). Whole-body flux, NOLD, Ra, and...
TABLE 1 Effects of enteral AKG infusion on net portal nutrient balance and on whole-body [15N]urea and [13C]leucine kinetics.

<table>
<thead>
<tr>
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<th>Control</th>
<th>AKG</th>
<th>1-Way ANOVA</th>
</tr>
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<tbody>
<tr>
<td>AKG infusion rate, µmol/(kg · h)</td>
<td>0</td>
<td>930</td>
<td>—</td>
</tr>
<tr>
<td>Portal blood flow, L/(kg · h)</td>
<td>3.21 ± 0.28</td>
<td>3.36 ± 0.27</td>
<td>0.34</td>
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<tr>
<td>Arterial AKG, µmol/L</td>
<td>13.8 ± 1.7</td>
<td>27.4 ± 3.6</td>
<td>&lt; 0.01</td>
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<tr>
<td>Portal AKG, µmol/L</td>
<td>22.0 ± 1.4</td>
<td>64.6 ± 5.9</td>
<td>&lt; 0.001</td>
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<tr>
<td>AKG net portal balance, µmol/(kg · h)</td>
<td>19.7 ± 2.8</td>
<td>95.2 ± 12</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AKG net portal balance, % of infused</td>
<td></td>
<td>10.23 ± 0.57</td>
<td>—</td>
</tr>
<tr>
<td>Glucose net portal balance, mmol/(kg · h)</td>
<td>1.863 ± 0.34</td>
<td>1.132 ± 0.38</td>
<td>&lt; 0.05</td>
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<tr>
<td>Ammonia net portal balance, µmol/(kg · h)</td>
<td>520 ± 66</td>
<td>561 ± 53</td>
<td>0.91</td>
</tr>
<tr>
<td>Whole-body urea flux, µmol/(kg · h)</td>
<td>398 ± 35</td>
<td>378 ± 39</td>
<td>0.56</td>
</tr>
<tr>
<td>Whole-body leucine flux, µmol/(kg · h)</td>
<td>918 ± 68</td>
<td>1073 ± 91</td>
<td>0.26</td>
</tr>
<tr>
<td>Whole-body leucine oxidation, µmol/(kg · h)</td>
<td>150 ± 37</td>
<td>209 ± 46</td>
<td>0.51</td>
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<tr>
<td>Whole-body leucine NOLD, µmol/(kg · h)</td>
<td>770 ± 45</td>
<td>814 ± 57</td>
<td>0.27</td>
</tr>
<tr>
<td>Whole-body leucine Ra, µmol/(kg · h)</td>
<td>497 ± 81</td>
<td>570 ± 72</td>
<td>0.26</td>
</tr>
<tr>
<td>Whole-body leucine balance, µmol/(kg · h)</td>
<td>272 ± 98</td>
<td>244 ± 41</td>
<td>0.27</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 9. Ra, endogenous rate of leucine appearance.

Expt. 2. An AKG bolus of 1040 µmol/kg was infused. The luminal disappearance rate was 663 ± 38 µmol/(kg · h). This represented 63.8% of the 1040 µmol/kg of infused AKG. A potential metabolic fate of AKG, which may have occurred within the gut lumen, is spontaneous, nonenzymatic decarboxylation. However, in preliminary experiments, we found that AKG was stable for several weeks after being spiked into plasma and frozen at −80°C and also when mixed in aqueous solutions and stored at 4°C.

Expt. 3. Plasma isotopic enrichments of [13C]AKG in 18 pigs infused with [13C]AKG either enterally or intravenously for 3 h and fed an elemental diet either intraduodenally or intragastrically are presented in Figure 1. Body weights did not differ among the 4 groups studied (overall mean 17.2 ± 0.3 kg). The [13C]AKG enrichment (MPE) was significantly higher in pigs after intravenous compared with enteral [13C]AKG infusion (Fig. 1). The whole-body [13C]AKG flux of was higher in pigs after enteral than intravenous [13C]AKG infusion (tracer infusion route effect, P < 0.05) (Table 3). However, the whole-body flux of [13C]AKG was also higher after in the IG compared with ID fed groups (diet effect, P < 0.05). There was a significant (P < 0.05) diet × tracer infusion route interaction such that the increase in whole-body [13C]AKG flux associated with the IG diet was greater during the enteral tracer infusion mode. CO2 recovery was not affected by either diet (ID vs. IG) or tracer infusion route (IV vs. ENT 13C-AKG infusion) and was ~37% of the dose administered. The calculated rates of first-pass splanchnic [13C]AKG utilization and oxidation to 13CO2 were ~80 and 30% among the 2 modes of enteral AKG infusion. First-pass splanchnic oxidation of [13C]AKG represented ~80% of the whole-body [13C]AKG rate. The pigs used in study 3 (9 wk of age) were older than those used in Studies 1 and 2 (~4 wk of age) and thus may have experienced some developmental changes in gut metabolism. This may explain why the splanchnic first-pass [13C-AKG] utilization was only 80% in study 3, yet the net intestinal AKG utilization determined in study 1 was ~90%.

Discussion

AKG is an intermediate in the TCA cycle and an intermediate in the oxidation of key gut fuels, glutamate and glutamine; thus, we were interested in the absorption and metabolism of AKG in the young pig. Our previous study suggested that AKG absorption...
was limited in young pigs when it was infused intragastrically (10). However, we questioned whether the observed low rate of AKG absorption was due to poor intragastric emptying, reduced mucosal absorption, or metabolism within the stomach or intestinal mucosa. The current findings indicate that only 10% of the intraduodenally infused AKG was absorbed into the portal circulation, consistent with previous studies (9,10). We also found that, following a duodenal bolus, 64% of the AKG disappeared from the small intestinal lumen within 1 h, which suggests rapid mucosal AKG uptake. Finally, in vivo tracer studies demonstrated that 80% of the enterally fed $^{13}$C-AKG was metabolized in first-pass by the splanchnic tissues. The observation that only 10% of infused AKG appeared in the portal plasma raises several possibilities as to the fate of luminal AKG transport is limited. Sodium-bolus of 1040 mol/(kg h) in AKG treated pigs, and that over 800 mol/(kg h) of AKG was unaccounted for in the portal circulation, it is possible that the increase in portal release can be completely accounted for by conversion from AKG. Such a large conversion of AKG to proline in the enterocyte could have led to a decrease in portal ammonia balance, but portal ammonia balance remained unchanged. The lack of effect on portal ammonia balance was also reflected in similar rates of whole-body urea synthesis in the 2 groups.

Branched-chain amino acid (BCAA) aminotransferase (BCAT) catalyzes the reaction between AKG and BCAA (leucine, isoleucine, and valine). The presence of BCAT in the intestine has been shown in pigs and other species based on enzyme activity, immunohistochemistry, and in vivo metabolism (17–20). The BCAA is transaminated, forming glutamate from AKG and the respective keto-acid from each of the BCAA. We had hypothesized that supplemental AKG would lead to a decrease in the net release of BCAA from the PDV by stimulating the transamination of BCAA to form glutamate. In contrast, however, the portal release of leucine and isoleucine was increased by AKG, yet this did not affect whole-body leucine kinetics. The net portal balance of proline, and yet oxidation to CO$_2$ is quantitatively important. If the absorbed AKG was converted to glutamate via transamination, it could either be released into the portal blood or converted to other amino acids. However, it might be expected that the release of glutamate would not be increased by AKG even if substantial conversion to these amino acids occurred, given that very little dietary glutamate is released by the PDV under normal feeding conditions (3). Alternatively, proline can be synthesized from enteral glutamate by the intestinal mucosal tissue (15,16). Moreover, we found that net portal proline balance was 60% higher in pigs supplemented with AKG. Given that the net increase in proline balance was 138.1 mol/(kg h) in AKG treated pigs, and that over 800 mol/(kg h) of AKG was unaccounted for in the portal balance, it is possible that the increase in proline release can be completely accounted for by conversion from AKG. Such a large conversion of AKG to proline in the enterocyte could have led to a decrease in portal ammonia balance, but portal ammonia balance remained unchanged. The lack of effect on portal ammonia balance was also reflected in similar rates of whole-body urea synthesis in the 2 groups.

### TABLE 3

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<tr>
<td>ID-fed</td>
<td>IG-fed</td>
</tr>
<tr>
<td>White-body $^{13}$C-AKG flux,$^2$</td>
<td>653 ± 37$^a$</td>
</tr>
<tr>
<td>$^{13}$CO$_2$ recovery, % dose</td>
<td>36.9 ± 1.1</td>
</tr>
<tr>
<td>First-pass splanchic $^{13}$C-AKG utilization, % intake</td>
<td>—</td>
</tr>
<tr>
<td>First-pass splanchic $^{13}$C-AKG oxidation, % intake</td>
<td>—</td>
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1 Values are means ± SEM, n = 4–5. Means in a row with superscripts without a common letter differ, P < 0.05.
2 Effects of diet, tracer route, and their interaction were significant, P < 0.05.
of AKG oxidation, representing ~80% of whole-body AKG oxidation.

In conclusion, our results indicate that a substantial amount of dietary AKG disappeared from the lumen of the small intestine, but is not absorbed into the portal blood stream. It is likely that some of the AKG in the diet was metabolized to proline, accounting for the 60% increase net portal proline appearance. In vivo tracer results suggested that large amounts of AKG are metabolized in first-pass by the splanchnic tissues (~80% intake), however, nonoxidative pathways predominate over complete metabolism to CO₂. We found no effect of dietary AKG on either whole-body urea or leucine fluxes. These results generally agree with previous data where AKG was provided intragastrically and suggest that gastric metabolism of dietary AKG is not significant. We conclude that the net intestinal absorption of AKG is limited in young pigs due to extensive metabolism by mucosal epithelial cells or by bacteria in the intestinal lumen. However, the fractional rate of AKG oxidation to CO₂ in the gut is less than other major gut fuels, namely, glutamate and glutamine.

**Literature Cited**