Intestinal lysine metabolism is driven by the enteral availability of dietary lysine in piglets fed a bolus meal

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STUDIES IN ANIMALS have shown that, during feeding, amino acid utilization by gut tissues is a major determinant of the systemic availability of both essential and nonessential dietary amino acids (27). Indeed, the intestine removes a substantial part of the absorbed dietary essential amino acids to fulfill its own metabolic needs for purposes of protein biosynthesis and energy production (37, 39, 43, 47). This phenomenon can decrease the systemic availability of essential amino acids to the body (39, 45, 49). However, the gut can also be considered as a buffer, sequestering dietary amino acids through protein synthesis during feeding when they are available in excess, and then releasing them in the fasted state to the peripheral pools when their availability is decreased (12, 33).

Nonessential amino acids are used by the intestine in synthetic pathways and energy production. They also undergo several metabolic interconversions (in particular, glutamate removal and arginine and alanine synthesis), producing a different pattern of amino acids released in the portal circulation than that of dietary protein (28, 29, 39, 41, 43, 48).

Although the intestine is mainly supplied with dietary amino acids during feeding, amino acids from the arterial circulation are also used in substantial amounts, particularly in the fasted state. The mechanisms that determine the relative contribution of luminal and arterial amino acids directed toward meeting intestinal amino acid requirements are of particular nutritional and physiological interest (1, 18, 38, 39, 43, 45). A clear picture of the partition between dietary and arterial amino acid utilization by the intestine is complicated by the fact that the relative contributions of these two sources vary continuously during the postprandial and interprandial periods, differ among the individual amino acids, and are affected by nutritional conditions (18, 28, 38, 39, 43, 45).

Data are available regarding the utilization of different amino acids by the portal-drained viscera (PDV; stomach, intestines, spleen, and pancreas) and the relative contributions of the luminal and arterial supplies obtained via a combination of portoarterial bal-

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ance techniques and isotope methodologies in animal models (18, 39, 45). However, all of these studies were conducted under steady-state conditions by providing either small, frequent meals or a continuous enteral infusion of nutrients. Although this experimental design provides a good model for the purpose of studying the effects of continuous enteral feeding, it is likely to conceal dynamic and temporary phenomena and may lead to different kinetic estimates of amino acid utilization when compared with a regimen in which nutrients are ingested in a large bolus meal (8, 11).

Therefore, the aim of the present work was to quantify the postprandial kinetics of dietary and systemic lysine metabolism by the PDV and whole body under non-steady-state conditions of the ingestion of a single mixed meal by young piglets. To accomplish this aim, we used our previously established arterial-portal venous balance approach (45) combined with the simultaneous administration of intrinsically labeled [15N]lysine in an oral bolus and systemic infusion of [13C]lysine. We conducted the study using young piglets, since they have been shown previously to be an excellent animal model for human infants (23).

MATERIALS AND METHODS

Animals. The study used five 4-wk-old female piglets (Large White × Hampshire × Duroc) purchased at the Texas Department of Criminal Justice, Huntsville, TX. The animals were received at the Children’s Nutrition Research Center (CNRC) at 2 wk of age. They were housed for a 1-wk adjustment period and fed a cow’s milk formula (Litterlife, Merrick, Union, WI) at a rate of 50 g dry matter·kg body wt⁻¹·day⁻¹, which supplied lactose (26.4 g·kg⁻¹·day⁻¹), protein (12.5 g·kg⁻¹·day⁻¹), and fat (5 g·kg⁻¹·day⁻¹). The protocol was approved by the Animal Care and Use Committee of the Baylor College of Medicine and conformed to current US Department of Agriculture guidelines.

Study design. At 3 wk of age, the pigs were prepared for surgery, as previously described (39). Food was removed overnight before surgery. The piglets were placed under general anesthesia (5% isoflurane), and catheters were implanted in a carotid artery, the jugular vein, and the portal vein (Transonic Systems, Ithaca, NY). After surgery, the piglets received parenteral nutrition for 24–36 h and were then progressively returned to their preceding level of dietary intake for >5 days before the experimental protocol. At a postnatal age of 4 wk, the animals were studied for a 10-h experimental period. Their mean body weight was 7.8 ± 1.4 kg.

Tracer protocol. The tracer protocol is depicted in Fig. 1. The pigs were fasted overnight before the experimental protocol. At 0700, [U-13C]lysine (99%, Cambridge Isotope Laboratory of the CNRC) was infused continuously at a mean rate of 7.1 ± 0.2 μmol·kg⁻¹·h⁻¹ via the jugular vein. At 0900 (t = 0), the animals received a single mixed meal providing one-third of their previous daily intake. The meal fed to the piglets consisted of 46 ml/kg body wt of a mix of Litterlife powder and water (1:3), to which was added a trace of a uniformly and intrinsically 15N-labeled soy protein isolate (150 mg/kg body wt, 98 atom %). The nutrient content of the meal was 3.9 g/kg body wt of protein, 1.6 g/kg body wt of fat, 8.4 g/kg body wt of lactose, and 250 kJ/kg. The amino acid composition of the ingested protein (formula + trace of soy protein) was determined after hydrolysis with 6 N HCl for 24 h at 110°C (in mg/g of protein): Asp/Asn 95, Glu/Gln 144, Ser/Thr 48, Gly/Gln 144, Ser 48, Gly 46, Pro 45, Arg 29, Pro 65, Tyr 29, Val 61, Met 22, Cys 11, Ile 58, Leu 108, Phe 34, and Lys 94. We used the published ratios between aspartic acid and asparagine (41/59) and between glutamic acid and glutamine (44/56), determined in casein, to estimate the individual amounts of these amino acids in the meal (7). The soy protein isolate was obtained from soybeans grown hydroponically using 15N-labeled salts of nitrate and ammonium at the Plant Physiology Laboratory of the CNRC (14). The 15N-labeled beans were flaked and extracted with hexane to remove oils, and the soy protein isolate was extracted, precipitated, and neutralized using standard techniques (25). The protein content of the isolate was 98%. The soy protein isolate was considered to have a digestibility close to that of milk protein (19) and, considering the small amount, to exhibit the same intestinal kinetics as the milk protein.

The total nitrogen and lysine contents of the mixed meal were 46 ± 1 and 2.59 ± 0.04 mmol/kg body wt, respectively ([15N₂]lysine enrichment: 2.06 mole%excess). The liquid meal was fed orally and ingested quickly within 45 min. A baseline blood sample was drawn at time 0. Portal and arterial blood samples (2.4 ml) were obtained every 15 min for 2 h, every 30 min for the next hour, and then every hour for the last 5 h. Hematocrit was measured at regular intervals in blood samples. The portal blood flow was measured and recorded continuously throughout the experimental period by transit-time ultrasound. An aliquot (0.2 ml) of arterial and portal plasma was mixed with an equal volume of methionine sulfone (0.5 mol/l) as an internal standard and stored at −70°C for later amino acid analysis. An aliquot of 0.2 ml of arterial and portal plasma was also taken for further isotopic analysis of [U-13C]lysine and [15N₂]lysine enrichments. At the end of the tracer protocol, animals were killed with an arterial injection of pentobarbital sodium, and their intestinal contents (stomach, small intestine, and cecum) were collected to assess the protein digestibility.

Sample analysis. The isotopic measurements and concentration determinations were made on plasma samples. Portal plasma flow (PPF) was derived from portal blood flow after correction for the hematocrit. Plasma glucose levels were assessed using a colorimetric assay based on the glucose oxidase method (Sigma-Aldrich, St. Louis, MO). Plasma amino acid concentrations were determined at each time point in arterial and portal samples by reverse-phase high-performance liquid chromatography of their phenylisothiocyanate derivatives (PicoTag System; Waters, Woburn, MA). The isotopic enrichment of lysine in plasma samples was determined by gas chromatography-mass spectrometry of the hexafluoroacetate derivatives. The analyses were performed with a 5890 series II gas chromatograph linked to a model 5989B (Hewlett-Packard, Palo Alto, CA) quadrupole mass spectrometer. We used methane negative chemical ionization, and the isotopic enrichment of lysine was determined by monitoring ions at mass-to-charge ratios of 560 and 562 mol/h.
for the $M+0$ and $M+2$ isotopomers of $[^{15}N_2]$lysine and of 566 for the $M+6$ isotopomer of $[U-^{13}C]$lysine. The relative amounts of $M+2$ isotopomers served to determine the isotopic enrichments of $[^{15}N_2]$lysine enrichments (tracing lysine of dietary origin), and the relative amounts of $M+6$ isotopomers served to determine the isotopic enrichments of $[U-^{13}C]$lysine enrichments (tracing systemic total body lysine).

Calculations. PPF (l·kg$^{-1}$·h$^{-1}$) was derived from the measured portal blood flow corrected for the hematocrit value. Dietary lysine used in first pass by the PDV and arterial lysine use were determined as previously described (45). The equations are detailed in the APPENDIX.

The gastric emptying rate of the cow's milk formula was calculated from measured values obtained in piglets of the same age ingesting the same amount of food (13–16 g dry matter/kg body wt) (24). These data showed that the half-time of gastric emptying was 76 min. The gastric emptying rate was fitted to a classical exponential decay equation: $y = a \cdot e^{-kt}$, where $y$ represents the fraction of the meal still present in the stomach, $a = 98.6$, and $k = 8.9 \times 10^{-3}$ min$^{-1}$. The gastric emptying curve was used to determine the fraction of the meal leaving the stomach and passing through the pylorus for each 1-h period. A delay of 30 min was applied between the maximum times of stomach release and intestinal absorption to account for the digestion step. The resulting points were fitted using a gamma equation (SigmaPlot 6.0, SPSS): $y = a \cdot t^b \cdot e^{-ct} + d$, where $y$ represents the absorption flux (AF) of dietary lysine (as a fraction of the ingested amount: %/min), $a = 3.072 \times 10^{-3}$, $b = 1.715$, $c = 0.025$, and $d = 0.0247$.

The cumulative flux of absorbed lysine accounted for the amount of truly digested and absorbed dietary protein (average 94%), which level was measured directly by subtracting the amount of dietary N recovered in the intestinal contents (stomach + small intestine + cecum) of each animal from the amount ingested. The stomach and small intestine retention times of the dietary nutrients were consistent with data obtained in 28- or 35-day-old piglets fed liquid milk diets, showing that the mean stomach retention time was 60–120 min, and the mean small intestine retention time was ∼120 min (46).

The systemic lysine fluxes were calculated using Steele’s equations (35) modified by Piroetto et al. (26). The use of two lysine tracers allowed us to calculate the total appearance rate ($R_a$) of lysine by using $[U-^{13}C]$lysine enrichments and the exogenous (dietary) $R_s$ of lysine by using $[^{15}N_2]$lysine enrichments (see equations used in the APPENDIX). The volume of distribution ($V$) of lysine was taken to be 0.5 l/kg and the pool fraction ($p$) for instant mixing 0.25. The product of $p$ and $V$ is thought to represent the truly active pool of lysine. The values of $p$ and $V$ were chosen to be identical to those used for leucine (4, 40) because of a lack of specific experimental data on lysine distribution volume and exchangeable pool size. The choice of these parameters is, however, of only minor relevance to the calculation of the $R_a$ of an amino acid, as previously discussed (5). This is not the case for the calculations of glucose kinetics by Steele’s equations because of the differences in the circulating glucose and amino acid concentrations.

Statistics. All results are expressed as means ± SD. Changes of plasma variables over time were tested using contrast analysis under a mixed model, with time as a repeated factor (SAS 8.01, SAS Institute, Cary, NC). Comparisons between cumulated lysine fluxes over periods (0–4 h and 4–8 h) were made with the paired Student’s $t$-test. A value of $P < 0.05$ was considered significant.

RESULTS

Glucose and amino acid portal mass balances after meal ingestion. The PPF rapidly and significantly increased between 30 and 300 min after the meal ($P < 0.05$) and then gradually returned to its baseline value (Fig. 2). Plasma glucose levels significantly increased after the meal ingestion, reaching a peak in portal plasma at 45 min (9.2 mmol/l) and in arterial plasma 15 min later (8.7 mmol/l). Lysine concentrations dramatically increased in both arterial and portal plasma, reaching up to five times their baseline levels at $t = 150$ min (Fig. 2).

The portal mass balances of total amino acids (essential and nonessential AA), lysine, and glucose are

![Fig. 2. Portal plasma flow (A) and arterial and portal concentrations of glucose (B) and lysine (C) in piglets fed a single mixed meal. Asterisks and horizontal lines (top) indicate time points at which values differ from baseline ($P < 0.05$, by use of a mixed model).](http://ajpendo.physiology.org/Downloaded.html)
shown in Fig. 3. Essential and nonessential AA exhibited the same time course of portal appearance after the meal ingestion, characterized by a rapid increase, as soon as 15–30 min after the start of the meal, and a peak between 75 and 150 min. However, the portal balance of essential AA declined less rapidly than that of nonessential AA. The lysine portal mass balance profile was very similar to that of the other essential AA. The time course of glutamine portal balance was the only one to differ from the other AA and was continuously negative (not shown). Branched-chain AA constituted the largest mass of essential AA released in the portal vein. The portal glucose balance was slightly negative before the meal and sharply increased immediately afterward (Fig. 3). From $t = 15–240$ min, it was relatively stable, at a level of $\sim 3.5 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, and then progressively returned to a zero balance for the last 3 h of the experimental period (not significant compared with the baseline value), indicating that the meal had been entirely absorbed at that time.

Over the 8-h postprandial period, the integrated portal balances of glucose and AA were compared with the dietary intake of each AA (Table 1). The portal appearance of dietary glucose amounted to $13.6 \pm 2.9 \text{ mmol/kg}$, which represented $59 \pm 12\%$ of the intake. The apparent gut utilization of essential AA was $22–28\%$ for the branched-chain AA, $30\%$ for methionine and histidine, but $39\%$ for phenylalanine and threonine and $51\%$ for lysine, or $31\%$ on average for essential AA. There was almost a complete utilization of glutamate, aspartate, and cysteine, whereas the portal output of alanine and arginine largely exceeded their intake ($190$ and $149\%$, respectively), implying net synthesis of these two AA. Overall, a net portal output of $2.43 \pm 0.49 \text{ g/kg}$ of free AA, representing $68 \pm 19\%$ of the amount ingested, appeared in the portal circulation.

**Lysine utilization by the PDV: fractional utilization of dietary and arterial lysine.** Figure 3 depicts the time course of portal and arterial lysine enrichments for the two lysine tracers used in the calculations of dietary and systemic lysine fluxes. After the meal ingestion, enrichments of $[U-^{13}\text{C}]$lysine fell rapidly for the first 30 min and remained stable until 300 min. The ratio of portal to arterial (portal/arterial) enrichment of $[U-^{13}\text{C}]$lysine was relatively constant ($0.72–0.83$).

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**Fig. 3.** Portal appearance of total amino acids (A), essential amino acids (EAA, C), nonessential amino acids (NEAA, E), lysine (B), and glucose (D) in piglets fed a single mixed meal. Asterisks and horizontal lines (top) indicate time points at which values differ from baseline ($P < 0.05$, by use of a mixed model).
lysine enrichments increased as early as 15 min after the meal and peaked at 300 min, when 63% of the portal lysine was of dietary origin. The mean portal/arterial ratio of $^{15}$N$_2$lysine enrichments was 1.23; it returned to 1 at 480 min (Fig. 4).

These data were used to assess the net utilization of both dietary (via both enteral and arterial sides) and systemic (via arterial side) lysine sources and the time course of their respective contributions to PDV lysine use after the single meal. The arterial lysine inflow was greatly enhanced by the meal ingestion, increasing from $263 \pm 81 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$ in the fasted state to $1,886 \pm 776 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$ at 180 min (Fig. 5A). The arterial lysine fractional use by the PDV was 9.7% of the input in the fasted state and remained relatively constant during the postprandial period, averaging $7.9 \pm 2.2\%$. Thus the absolute amount of lysine used from arterial origin paralleled the arterial lysine input, which increased significantly due to increases in both PPF and lysine concentration. The net portal appearance of dietary lysine, escaping the PDV first-pass use, remained stable at a level of ~250–330 $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$ between 75 and 300 min after the meal (Fig. 5B).

The fractional utilization of dietary lysine by the PDV differed substantially during the progression of the postprandial period (Fig. 5C). During the first 120 min, it ranged between 80 and 91% of the absorbed lysine and was essentially zero for the last 300 min because of a negative first-pass use. Over the total 8-h period, $32 \pm 18\%$ of the dietary lysine ingested was utilized by the PDV in first pass, and $15 \pm 10\%$ was used in second pass, as calculated from the areas under the curve. The flux of recycled lysine, calculated from that part of lysine entering the portal vein and coming neither from the meal nor from the arterial circulation, was $41 \pm 18 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$ in the fasted state, ranged from $56$ to $132 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$ until 4 h after the meal, and then became negligible for the last 4 h (Fig. 5C). Total net lysine use peaked at 1 h ($891 \pm 44 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$) and then gradually decreased to a value close to zero from 4 to 8 h after the meal.

The cumulative PDV fluxes of dietary and endogenous lysine are summarized in Fig. 6 for three distinct periods: before the meal (−4–0 h) and during the two halves of the postprandial period (0–4 h and 4–8 h). Before the meal, the PDV fluxes were in negative lysine balance, with a mean rate of lysine outflow of $57 \pm 21 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$. For the first 4 h after the meal ingestion, 55% of the dietary enteral entry of lysine was used by the PDV in first pass, and 9% was used in second pass. Arterial lysine was used at a rate of $451 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$, of which 44% was made of dietary lysine used in second pass. There was a slightly negative endogenous lysine balance, indicating a net release of $53 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$ on average. The total lysine use by the PDV amounted to $1,363 \pm 128 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$ during the next 4-h period, there was practically no more dietary lysine entering the

Table 1. Amino acid and glucose portal mass balances and net portal appearance throughout the 8-h postprandial period

<table>
<thead>
<tr>
<th></th>
<th>Intake, $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$</th>
<th>Portal Balance, $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$</th>
<th>Portal Appearance, % of Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>$23,078 \pm 295$</td>
<td>$13,581 \pm 2921$</td>
<td>$59 \pm 12$</td>
</tr>
<tr>
<td>Essential AA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>$2,432 \pm 31$</td>
<td>$1,201 \pm 288$</td>
<td>$49 \pm 11$</td>
</tr>
<tr>
<td>Threonine</td>
<td>$2,046 \pm 26$</td>
<td>$1,272 \pm 322$</td>
<td>$62 \pm 17$</td>
</tr>
<tr>
<td>Valine</td>
<td>$1,933 \pm 25$</td>
<td>$1,396 \pm 322$</td>
<td>$72 \pm 17$</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>$1,641 \pm 21$</td>
<td>$1,295 \pm 223$</td>
<td>$78 \pm 14$</td>
</tr>
<tr>
<td>Leucine</td>
<td>$3,059 \pm 39$</td>
<td>$2,269 \pm 420$</td>
<td>$74 \pm 14$</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>$767 \pm 10$</td>
<td>$467 \pm 37$</td>
<td>$61 \pm 5$</td>
</tr>
<tr>
<td>Methionine</td>
<td>$539 \pm 7$</td>
<td>$377 \pm 119$</td>
<td>$70 \pm 22$</td>
</tr>
<tr>
<td>Histidine</td>
<td>$436 \pm 6$</td>
<td>$305 \pm 37$</td>
<td>$70 \pm 9$</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>ND</td>
<td>$337 \pm 110$</td>
<td>ND</td>
</tr>
<tr>
<td>Total essential AA</td>
<td>$12,854 \pm 165$</td>
<td>$8,929 \pm 1,357$</td>
<td>$69 \pm 11$</td>
</tr>
<tr>
<td>Conditionally essential AA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>$625 \pm 8$</td>
<td>$929 \pm 317$</td>
<td>$149 \pm 52$</td>
</tr>
<tr>
<td>Proline</td>
<td>$2,087 \pm 27$</td>
<td>$1,834 \pm 586$</td>
<td>$88 \pm 29$</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>$594 \pm 8$</td>
<td>$569 \pm 104$</td>
<td>$96 \pm 18$</td>
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<tr>
<td>Cysteine</td>
<td>$353 \pm 6$</td>
<td>$60 \pm 43$</td>
<td>$17 \pm 12$</td>
</tr>
<tr>
<td>Total conditionally essential AA</td>
<td>$3,659 \pm 47$</td>
<td>$3,470 \pm 997$</td>
<td>$95 \pm 28$</td>
</tr>
<tr>
<td>Nonessential AA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>$2,127 \pm 27$</td>
<td>$4,026 \pm 1,643$</td>
<td>$190 \pm 80$</td>
</tr>
<tr>
<td>Glutamic acid*</td>
<td>$1,600 \pm 20$</td>
<td>$465 \pm 240$</td>
<td>$29 \pm 15$</td>
</tr>
<tr>
<td>Glutamine*</td>
<td>$2,036 \pm 26$</td>
<td>$584 \pm 510$</td>
<td>$29 \pm 26$</td>
</tr>
<tr>
<td>Aspartic acid*</td>
<td>$1,344 \pm 17$</td>
<td>$322 \pm 152$</td>
<td>$24 \pm 12$</td>
</tr>
<tr>
<td>Asparagine*</td>
<td>$1,292 \pm 17$</td>
<td>$394 \pm 156$</td>
<td>$77 \pm 12$</td>
</tr>
<tr>
<td>Serine</td>
<td>$1,687 \pm 22$</td>
<td>$1,418 \pm 315$</td>
<td>$84 \pm 19$</td>
</tr>
<tr>
<td>Glycine</td>
<td>$1,244 \pm 16$</td>
<td>$857 \pm 299$</td>
<td>$69 \pm 23$</td>
</tr>
<tr>
<td>Ornithine</td>
<td>$126 \pm 46$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrulline</td>
<td>$560 \pm 179$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total nonessential AA</td>
<td>$11,361 \pm 145$</td>
<td>$8,286 \pm 2,172$</td>
<td>$73 \pm 20$</td>
</tr>
</tbody>
</table>

Values are means ± SD. *The ratio of glutamic acid and glutamine was estimated to be 44/56, and the ratio of aspartic acid and asparagine was estimated to be 51/49 from the amino acid (AA) composition in casein (7). ND, not determined.
PDV by the luminal side (10% of the total absorbed amount). However, there was significant release of dietary lysine from the PDV (445 ± 189 μmol·kg⁻¹·4 h⁻¹). Endogenous lysine was used at a rate of 141 μmol·kg⁻¹·4 h⁻¹. Total lysine use by the PDV was negative during the first 4 h after the meal: −568 ± 664 μmol·kg⁻¹·4 h⁻¹. The PDV shifted from a strongly positive lysine balance during the first 4 h to a slightly negative balance for the last 4 h postprandially; during the total 8-h period, the total lysine use was 1,221 μmol/kg.

Systemic and splanchnic lysine kinetics. Systemic total, dietary, and endogenous lysine Ra values calculated from the arterial [U-¹³C]lysine (iv continuous infusion) and [¹⁵N₂]lysine (meal-derived) enrichments during the postprandial period are represented in Fig. 7. Total systemic lysine flux was 312 ± 36 μmol·kg⁻¹·1·h⁻¹ after the overnight fast and increased twofold during the 4-h period after the meal. This increase was due mainly to the entry of dietary lysine into the systemic circulation, but also to a slight increase in the endogenous lysine Ra, which amounted to −350 μmol·kg⁻¹·1·h⁻¹ between 0 and 4 h and returned to a level inferior to the fasting value during the last 4 h. Dietary lysine appearance in the systemic compartment was detected by 15 min after the meal, and it reached a plateau of −300 μmol·kg⁻¹·1·h⁻¹ between 2 and 6 h after the meal. The whole body and splanchnic lysine fluxes are summarized in Table 2 for the three following periods: after an overnight fast (4 h before the meal); the first 4 h after the meal; and the last 4 h of the 8-h experimental period. When expressed as the integrated area, the total lysine Ra over the 8 h after

Fig. 4. Time course of lysine enrichments in arterial and portal plasma samples in piglets infused continuously with [U-¹³C]lysine (7.1 ± 0.2 μmol·kg⁻¹·1·h⁻¹) and ingesting a single [¹⁵N₂]-labeled meal ([¹⁵N₂]lysine enrichment: 2.06 mole % excess (MPE)). MPE+2 and MPE+6 represent the enrichments of [¹⁵N₂]lysine (by dietary lysine rate of appearance (Ra) and [U-¹³C]lysine (by systemic lysine Ra), respectively. Asterisks and horizontal lines (top) indicate time points at which values differ from baseline (P < 0.05, by use of a mixed model).

Fig. 5. PDV metabolism of lysine from dietary and arterial sources in piglets fed a single mixed meal: arterial lysine input and net portal arterial lysine balance (A), enteral (dietary) lysine absorption flux and net dietary lysine portal balance (B), and time course of PDV lysine use from first-pass dietary lysine metabolism or arterial lysine supply (C). Recycled lysine flux refers to the lysine released by the PDV from endogenous (proteolysis) origin or lysine reabsorbed from hydrolysis of intestinal proteins. Note different scaling of the y-axis in A and B.
the meal ingestion was 4,304 μmol/kg, of which 1,934 μmol/kg (45%) was of dietary origin and 2,370 μmol/kg (55%) was of endogenous origin. At the systemic level, the appearance of dietary lysine was equally balanced between the early and late postprandial periods. The overall systemic availability of dietary lysine was higher than its portal availability, indicating a zero first-pass hepatic use.

**DISCUSSION**

This study represents the first simultaneous measurement of amino acid kinetics both across the PDV and at the whole body level under non-steady-state conditions after a single bolus meal ingestion. In addition, the study traced the metabolic fate of dietary lysine by using an intrinsically labeled protein (uniformly $^{15}$N-labeled soy protein), which is arguably a more valid approach than using a lysine tracer in the free amino acid form. Consistent with previous studies in continuously fed animals, we found that, even under bolus-feeding conditions, the PDV tissues consume a substantial portion of the dietary lysine intake (~47%) (18, 39, 45, 49). However, a novel observation was that, in the immediate postprandial period (0–4 h), PDV metabolism of dietary lysine was dominated by first-pass (73%) rather than second-pass (27%) arterial utilization. Interestingly, in the later postprandial period (4–8 h), the total rate of PDV lysine utilization decreased to near-fasting levels, and there was a small, but significant, net release of dietary lysine from the PDV tissues.

The present study was carried out using a non-steady-state protocol to better characterize the dynamic aspects of the first-pass PDV metabolism of dietary amino acids. Under the present conditions of a bolus meal, we derived the absorption flux from published gastric emptying rates obtained under similar conditions, and we found this absorption flux to be consistent with other data in piglets of the same age or in minipigs (3, 13, 46). The shape of this absorption flux had no influence on the final value of first-pass use, which was the result of the integrated amounts over the 8-h period. Under those conditions, the maximal rate of appearance of amino acids in the portal circulation occurred between 75 and 150 min postprandially, and the first-pass metabolism was concentrated in the first 3 h after the meal, in line with previous findings in older animals (29). As a result, the gut
removal of dietary lysine was greater during the early postprandial period (0–4 h), when we found that 55% of the dietary lysine absorbed was used in first pass. Interestingly, 37% of this early uptake reentered the portal circulation for the next 4 h, thus lowering the final 8-h average first-pass uptake to 32%. This value is markedly higher than that in continuously fed piglets, in which the first-pass utilization of dietary [13C]lysine was negligible (39, 45).

We suggest that two possible factors contributed to the large first-pass utilization of dietary lysine. The first is related to the fact that the dietary lysine tracer was an intrinsically labeled protein rather than a free amino acid, as in the previous study (45). Protein-bound amino acids tend to be absorbed more slowly than in free form, which may have increased the possibility for first-pass mucosal utilization. Indeed, previous studies have shown that protein-bound tracers result in reduced rates of oxidation and increased incorporation into splanchnic pools (5, 6, 20). However, the second and perhaps more likely reason for the increased first-pass lysine use was the greater stimulation of the PDV metabolism due to the bolus administration of nutrients. The fact that the single bolus may have enhanced the PDV metabolism is also supported by different findings, such as a higher portal appearance of arginine and higher fractional portal glucose uptake than reported previously in the same animal model during continuous enteral feeding (39, 43).

The fractional uptake of arterial lysine by the PDV appeared to be very constant when expressed on the arterial input basis, ranging from 6 to 13%, and agreed well with the values of 2–13% previously found for the fractional arterial essential amino acid uptake by the PDV under steady-state conditions (43, 45). However, because both the portal blood flow and the arterial lysine concentrations increased after the meal, the absolute amount of lysine used from the arterial source was sharply increased after the bolus meal. Moreover, an interesting result was that nearly half of the arterial supply was comprised of dietary lysine, reentering the PDV by the systemic circulation. Thus we were able to calculate that, overall, 47% of the ingested lysine was used by the PDV, either in first or second pass, and during the 8-h period, 93% of the lysine used by the PDV was of dietary origin. A crucial question regarding dietary availability is the metabolic fate of dietary lysine used in first pass by the PDV tissues. Previous results might indicate that the large first-pass utilization of dietary lysine in the present study resulted in a higher fractional rate of oxidation (10, 21, 22, 43). Further study is warranted to establish how feeding modality affects the proportion of dietary lysine that is retained via incorporation into protein or irreversibly lost via oxidative metabolism within the PDV tissues.

Regarding the dynamics of the global lysine use by the PDV after the bolus meal, total lysine use was remarkably variable during the progress of the postprandial period, ranging from a negative value in the fasting state, i.e., net PDV release of lysine, to a maximum rate of 891 μmol·kg⁻¹·h⁻¹ 1 h after the meal ingestion. For the last 4 h, the systemic lysine consumption was nearly balanced by the PDV release of both dietary and endogenous lysine. The rate of appearance of total systemic lysine was highly stimulated by the meal and was characterized by the importance of its dietary component. The basal systemic flux of lysine was low and increased more than twofold after the meal, mainly due to the entry of dietary lysine in this pool, reaching values consistent with previous measurements in steady-state studies (36, 45). The present results, obtained at the intestinal and systemic levels, were also highly consistent, in that at the end of the first 4-h postprandial period, we found that 45% of the meal-derived lysine had escaped first-pass metabolism by the PDV, whereas, during the same time period, 44% had appeared in the systemic compartment. At the end of the total study period, 80% of the absorbed dietary lysine had appeared in the systemic circulation. In humans, non-steady-state measurements of whole body leucine fluxes and exogenous leucine appearance after a single meal had shown equal or higher peripheral availability of meal-derived leucine, with 79–91% of the intake appearing in the peripheral circulation (4, 5, 40). For lysine, no such data exist, and the only estimate of lysine splanchnic

<table>
<thead>
<tr>
<th>Time Periods Before and After the Meal, μmol·kg⁻¹·h⁻¹</th>
<th>Total Postprandial, μmol·kg⁻¹·h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>–4–0 h</td>
<td>0–4 h</td>
</tr>
<tr>
<td>Systemic total lysine Rₜ</td>
<td>1.249 ± 143</td>
</tr>
<tr>
<td>Systemic endogenous lysine Rₑ</td>
<td>1.249 ± 143</td>
</tr>
<tr>
<td>Systemic dietary lysine Rₚ</td>
<td>973 ± 244</td>
</tr>
<tr>
<td>% of dietary lysine intake</td>
<td>44 ± 11</td>
</tr>
<tr>
<td>Splanchnic dietary lysine first-pass use</td>
<td>1.228 ± 241</td>
</tr>
<tr>
<td>% of dietary lysine intake</td>
<td>56 ± 11</td>
</tr>
<tr>
<td>PDV</td>
<td>1.219 ± 346</td>
</tr>
<tr>
<td>% of dietary lysine intake</td>
<td>55 ± 16</td>
</tr>
<tr>
<td>Hepatic</td>
<td>8 ± 209</td>
</tr>
<tr>
<td>% of dietary lysine intake</td>
<td>0.4 ± 9.6</td>
</tr>
</tbody>
</table>

Values are means ± SD for the postabsorptive (–4–0 h), early (0–4 h) and late (4–8 h) postprandial, and total postprandial (0–8 h) periods. Rₑ, appearance rate; PDV, portal-drained viscera. Significantly different from period 0–4 h (P < 0.05, Student’s t-test).
lysine metabolism in the case of bolus feeding compared with continuous enteral feeding in piglets. Indeed, it was previously assessed that, during continuous feeding, the rate of total lysine use amounted to 213 μmol·kg⁻¹·h⁻¹, which would correspond to a global amount of ~2,500 μmol/kg of lysine removed by the PDV during the 12-h feeding period (45). Based on a pattern of three meals of the same composition provided every 4 h, the current study indicates a global lysine use of ~4,000 μmol/kg for the feeding period of the day (3 times the amount used between 0 and 4 h after the meal, Fig. 6). Such a stimulation would be consistent with evidence suggesting that bolus vs. continuous enteral feeding not only increases small intestinal growth and development in piglets but also improves weight gain and feeding tolerance in preterm infants (32, 30). This effect is apparently not due to the stimulation of trophic peptide secretion in the gut, such as glucagon-like peptide 2 and peptide YY (44). However, it is conceivable that bolus feeding upregulates the expression of local growth factors, such as IGF-I, which in turn may increase mucosal lysine utilization for protein synthesis. The other possibility is that the additional lysine used is oxidized, but this is in contrast to studies in humans showing that bolus feeding produces a diminished whole body amino acid oxidation compared with continuous oral feeding (9).

The main conclusions of this study are that dietary lysine use by the PDV is driven by its luminal availability and is markedly stimulated during the first hours after a meal. We also found that not only is the quantity of PDV lysine utilization increased, but there is preferential first-pass use of dietary lysine rather than systemic arterial lysine during bolus compared with continuous feeding. Systemic lysine use is also stimulated in the postprandial period because of an increased arterial input of lysine. Thus PDV amino acid metabolism is acutely stimulated by feeding and shifts rapidly from the arterial to the enteral source as the dietary amino acid supply becomes available. Our results also confirm that a substantial part of the dietary lysine used in first pass by the PDV was recycled in the second half of the postprandial period. These observations provide further evidence of the important role of the pattern of feeding (single meals vs. continuous) with regard to the bioavailability of dietary lysine, and perhaps essential amino acids in general.

APPENDIX

Portal mass balances. Portal mass balances (μmol·kg⁻¹·min⁻¹) of amino acids and glucose were calculated by the following formula

\[ \text{portal mass balance} = \text{PPF} \times (C_p - C_a)/60 \quad (A1) \]

where PPF is the portal plasma flow (l·kg⁻¹·h⁻¹) and \( C_p \) and \( C_a \) are the portal and arterial plasma concentrations of amino acids or glucose (μmol/l). The integrated areas under the curves were calculated using the trapezoidal method for the 8-h period after the meal and were compared with the amount ingested.
Calculations of PDV lysine fluxes

arterial lysine input (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \text{arterial lysine concentration} \times \text{PPF}\] (A2)

in which lysine concentration is expressed in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\), and PPF is in \(\text{l} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\).

portal lysine output (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \text{portal lysine concentration} \times \text{PPF}\] (A3)

The tracer kinetics are obtained by substituting the tracer concentrations for the lysine concentrations

arterial \(\left[U^{13}\text{C}\right]\text{lysine input}\) (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \text{arterial} \left[U^{13}\text{C}\right]\text{lysine concentration} \times \text{PPF}\] (A4)

in which \(\left[U^{13}\text{C}\right]\text{lysine concentration}\) is expressed in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\), and PPF is in \(\text{l} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\).

portal \(\left[U^{13}\text{C}\right]\text{lysine output}\) (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \text{portal} \left[U^{13}\text{C}\right]\text{lysine concentration} \times \text{PPF}\] (A5)

arterial \(\left[\text{N}_{5}\right]\text{lysine input}\) (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \text{arterial} \left[\text{N}_{5}\right]\text{lysine concentration} \times \text{PPF}\] (A6)

portal \(\left[\text{N}_{5}\right]\text{lysine output}\) (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \text{portal} \left[\text{N}_{5}\right]\text{lysine concentration} \times \text{PPF}\] (A7)

portal \(\left[U^{13}\text{C}\right]\text{lysine balance}\) (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \text{Eq. 5} - \text{Eq. 4}\] (A8)

fractional arterial lysine use by the PDV (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \text{Eq. 8/Eq. 4}\] (A9)

amount of arterial lysine use by the PDV (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \text{Eq. 9} \times \text{Eq. 2}\] (A10)

The fractional use of enteral (dietary) lysine used in first pass by the PDV was assessed by the ratio of \(\left[\text{N}_{5}\right]\text{lysine portal balance} \times \text{PPF} \times \left[\text{N}_{5}\right]\text{lysine absorption flux (\(\left[\text{N}_{5}\right]\text{lysine AF}\)), expressed in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\); see MATERIALS AND METHODS for the calculation}, corrected for the amount of \(\left[\text{N}_{5}\right]\text{lysine reentering the PDV by the arterial site and assumed to be utilized to the same extent as arterial lysine. Thus the equations used were}

fractional use of dietary lysine (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \frac{\left[\text{N}_{5}\right]\text{lysine AF} - \text{Eq. 7} - (1 - \text{Eq. 9})}{\text{Eq. 6}}\times \text{Eq. 6/\left[\text{N}_{5}\right]\text{lysine AF}}\] (A11)

amount of PDV dietary lysine first pass use (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \text{Eq. 11} \times \text{dietary lysine AF}\] (A12)

Recycled lysine released by the PDV, derived neither from the dietary lysine nor from the arterial lysine but from PDV tissue proteolysis, was calculated as the difference between total lysine appearing in the portal vein and lysine from arterial or enteral origin

recycled lysine (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \text{Eq. 3} - (\text{AF} - \text{Eq. 12}) - (\text{Eq. 2} - \text{Eq. 10})\] (A13)

total lysine use by the PDV (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \text{Eq. 10} + \text{Eq. 12} - \text{Eq. 13}\] (A14)

Systemic lysine fluxes. Total rate of appearance (\(R_a\)) of lysine (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\)) was calculated according to the following formula

\[\text{total } R_a (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = [\text{IR} - \text{pV} \times C(t)]\]

\[\times (\frac{\text{d}E_{\text{tr}}(t)}{\text{d}t}/E_{\text{tr}}(t) - \text{IR})\] (A15)

where IR is the rate of \([U^{13}\text{C}]\text{lysine intravenous infusion corrected for isotopic purity, } p\) is the pool fraction for instant mixing, \(V\) is the volume of distribution of free lysine in the body (\(\text{kg}\)), \(C(t)\) is the mean arterial lysine plasma concentration between \(t\) and \(\Delta t\), and \(\text{d}E_{\text{tr}}(t)/\text{d}t\) is the variation of the plasma \([U^{13}\text{C}]\text{lysine enrichment (mole % excess, MPE)}:

\[\text{d}E_{\text{tr}}(t)/\text{d}t = [E(t + \Delta t) - E(t)]/\Delta t\]

Exogenous systemic lysine \(R_e\) was derived from total \(R_a\) by use of the arterial \([\text{N}_{5}]\text{lysine enrichments to evaluate the kinetics of dietary lysine, according to the transposition by Proietto et al.}\) (26)

\[\text{exogenous } R_e (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = [\text{Eq. 15} + \text{IR}] \times E_e(t)\]

\[+ (\text{pV} \times C(t) \times \text{d}E_{\text{tr}}(t)/\text{d}t)/\text{dietary lys MPE}\] (A16)

where \(E_e(t)\) is the mean arterial \([\text{N}_{5}]\text{lysine enrichment at time } t\)

\[E_e(t) = [E(t) + E(t + \Delta t)/2]\]

\[\text{d}E_{\text{tr}}(t)/\text{d}t\] is the variation of the plasma \([\text{N}_{5}]\text{lysine enrichment (MPE)}:

\[\text{d}E_{\text{tr}}(t)/\text{d}t = E(t + \Delta t) - E(t)/\Delta t\]

and dietary lysine MPE is the enrichment of dietary lysine (2.06 MPE).

Endogenous systemic lysine \(R_n\), representing the entry of lysine into the body pool from protein breakdown or the reabsorption of previously synthesized and secreted protein into the intestinal lumen, was assessed as the difference between the total lysine \(R_a\) (minus the tracer infusion rate) and the exogenous \(R_e\)

\[\text{endogenous } R_n = \text{Eq. 15} - \text{Eq. 16}\] (A17)

The combination of systemic lysine kinetics and PDV lysine use permitted us to calculate the hepatic and splanchic first-pass use of lysine as

hepatic first-pass use (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\)) = dietary lysine AF

\[\text{Eq. 12} - \text{Eq. 16}\] (A18)

splanchnic first-pass use (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[= \text{dietary lysine AF} - \text{Eq. 16}\] (A19)

To summarize the lysine fluxes of arterial/endogenous or dietary origin both across the PDV and entering the peripheral circulation, the integrated areas under the curves were calculated using the trapezoidal method for the 8-h period after the meal or for different periods of interest after the meal.

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DISCLOSURES

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