Catabolism Dominates the First-Pass Intestinal Metabolism of Dietary Essential Amino Acids in Milk Protein-Fed Piglets[1,2]

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ABSTRACT To investigate the extent of first-pass intestinal metabolism of dietary amino acids, seven female pigs (28 d old, 8.0 kg) were implanted with arterial, venous, portal and gastric catheters and with an ultrasonic portal blood flow probe. The pigs were fed a milk-based diet once hourly and infused intragastrically with [U-^{13}C]algal protein. On average, 56% of the essential amino acid (EAA) intake appeared in the portal blood. However, the net portal balance of methionine (48% of intake) and threonine (38% of intake) tended ($P = 0.08$) to be lower than the mean of all EAA. The net portal balance (expressed as a percentage of intake) of alanine (205%), tyrosine (167%) and arginine (137%) exceeded their intake. Net portal outflow of ammonia accounted for 18% of total amino acid nitrogen intake. As a percentage of the enteral tracer input, there was substantial first-pass metabolism of lysine (35%), leucine (32%), phenylalanine (35%) and threonine (61%). However, only 18, 21, 18 and 12% of the total first-pass metabolism of lysine, leucine, phenylalanine and threonine, respectively, were recovered in mucosal protein. We conclude that roughly one third of dietary intake of EAA is consumed in first-pass metabolism by the intestine and that amino acid catabolism by the mucosal cells is quantitatively greater than amino acid incorporation into mucosal protein. J. Nutr. 128: 606–614, 1998.

KEY WORDS: • *dietary protein metabolism* • *stable isotopes* • *intestinal metabolism* • *mucosal protein synthesis* • *pigs*

Questions related to nutrient absorption and systemic availability remain important areas of uncertainty in the formulation of recommended intakes. When these recommendations concern protein and amino acid nutrition, there are two problems to be considered. The first is the extent to which dietary protein is digested into amino acids and taken up by the mucosal enterocytes. Traditional approaches to the determination of digestibility, such as the measurement of apparent fecal nitrogen digestibility or of the ileal outflow of amino acids, are complicated by bacterial nitrogen metabolism and the secretion of endogenous proteins into the small intestinal lumen, respectively. Recent work aimed at circumventing these problems by using either ^{15}N-labeled dietary protein (e.g., Mahé et al. 1994) or prolonged intravenous infusions of ^{15}N-amino acids (de Lange et al. 1990, Gaudichon et al. 1996, Lien et al. 1997, Souffrant et al. 1993) has shown that many commonly consumed dietary proteins are virtually completely digested. The results of these studies imply that differences in the apparent digestibility of proteins among diets are largely a function of differences in the rate of endogenous protein loss.

The second problem in determining the availability of dietary amino acids relates to their fate in the enterocyte. Experiments based on the direct measurement of the net portal appearance of amino acids in fed pigs suggest that <100% of the dietary amino acids appear as free amino acids in the portal blood (Ebner et al. 1994, Rerat et al. 1988 and 1992).

There are three possible explanations for the difference between the disappearance of luminal protein and the portal appearance of amino acids. First, it could reflect the portal appearance of nutritionally significant quantities of oligopeptide products of digestion. However, although peptide transport across the enterocyte brush border (Mathews and Adibi 1976) is an established phenomenon, there is little quantitative data obtained in vivo (see Seal and Parker 1991) to confirm that oligopeptides derived from the diet survive intracellular proteolysis and that they make a nutritionally significant contribution to the portal outflow of the products of dietary protein digestion in monogastric animals. Second, it is possible that the transfer of dietary amino acids from the intestinal lumen to the portal blood is highly efficient, but because there is a simultaneous removal of mesenteric arterial amino acids

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TABLE 1

The amino acid composition of dietary and [U-^{13}C]-labeled Spirulina *protein* [1]

Amino acid	Amino acid concentrations			
	Dietary	*Spirulina*	Dietary	*Spirulina*
	g/kg protein		*mmol/kg protein*	
Aspartate + asparagine	104	102	787	772
Glutamate + glutamine	145	105	993	719
Serine	63	38	600	362
Glycine	42	112	560	1493
Histidine	16	14	103	90
Threonine	74	60	622	504
Alanine	76	150	854	1685
Arginine	20	8	115	46
Proline	69	52	600	452
Tyrosine	14	8	77	44
Valine	69	83	590	709
Methionine	19	9	128	60
Isoleucine	62	70	473	534
Leucine	104	100	794	763
Phenylalanine	27	37	164	224
Lysine	82	48	562	329

[1] The values presented are the mean of at least four separate determinations. Between-preparation CV was 7%.

by the portal-drained viscera (PDV)[4] (Yu et al. 1990 and 1992), the net portal balance of amino acids underestimates the quantity of dietary amino acids that are actually transported to the portal blood.

The third possibility, and that with which this paper is primarily concerned, is that there is substantial metabolism of dietary amino acids in the enterocytes. It has been known for many years that some dietary amino acids, with glutamate and aspartate as notable examples (Reeds et al. 1996, Windmueller and Spaeth 1980), are catabolized extensively in the gut. Furthermore, recent studies in humans have shown considerable first-pass splanchnic metabolism of intragastrically administered, stable isotope-labeled amino acids (Biolo et al. 1992, Hoerr et al. 1991 and 1993, Matthews et al. 1993). Investigations of the splanchnic metabolism of intravenously administered leucine (Yu et al. 1990 and 1992) also suggest that the majority of the splanchnic metabolism of amino acids occurs in the portal-drained visceral tissues (mainly intestine) rather than in the liver.

The first-pass intestinal metabolism of essential amino acids (EAA), derived from dietary protein, poses two important nutritional questions. First, what is their metabolic fate within the enterocytes? Second, is the degree of first-pass metabolism different among amino acids? The first question is important because if the predominant fate of dietary amino acids in the enterocyte is catabolism, then first-pass metabolism by the intestine is a source of nutritional inefficiency. The second question is equally important because if differences exist among amino acids, then measurements of the pattern of amino acids in the diet will not reflect their availability to the extra-intestinal tissues.

This paper, in which we discuss the intestinal metabolism of dietary amino acids, is one part of a study of the splanchnic metabolism of dietary amino acids. We will present data related to hepatic amino acid utilization in a separate paper. The experiments were based on the combination of the intragastric infusion of protein-bound ^{13}C-labeled amino acids with the quantification of the amino acid mass (to measure net absorption) and tracer (to measure total absorption) balance across the PDV of fed piglets. To address the question of potential differences in first-pass metabolism among EAA, we used uniformly [U-^{13}C]-labeled algal protein (Berthold et al. 1995) as the isotopic tracer and determined the labeling of threonine, leucine, lysine and phenylalanine. We also measured the incorporation of [U-^{13}C]-labeled amino acids into mucosal protein to quantify the contribution of mucosal protein synthesis to total first-pass intestinal metabolism. In designing the study, we hypothesized first, that there would be consistent differences among the first-pass utilization of different dietary amino acids and second, that mucosal protein synthesis would represent the predominant metabolic fate of EAA absorbed from the lumen.

MATERIALS AND METHODS

Animals. The study was approved by the Baylor College of Medicine Animal Protocol Review Committee. Housing and care of the animals conformed to U.S. Department of Agriculture guidelines. The isotopic study involved seven female crossbred piglets. Three animals were purchased from the Department of Animal Science, Texas A & M University, College Station, TX and four from the Texas Department of Criminal Justice (TDCJ), Huntsville, TX. Although the two groups of animals were of similar genetic background and had been suckled by their dams until transferred to the laboratory, they had been reared under different conditions. The pigs from Texas A & M had been housed indoors in a research facility, whereas those from the TDCJ had been kept in the field. This difference had no apparent effect on the health status of the piglets but, as we discuss below, was associated with differences in the contribution of the small intestine to body weight. In a subsequent study, 10 female piglets, purchased from the TDCJ, were used to quantify portal ammonia production.

Study design. The animals were received at the Children's Nutrition Research Center (CNRC) when they were 2 wk of age. For the next 10 d, they were offered a powdered milk replacer (Litterlife, Merrick, Union, WI) at a daily rate of 60 g/kg body weight (BW) supplying 14.4 g protein/(kg · d) and 969 kJ gross energy/(kg · d). After 10 d, food was withdrawn from the animals overnight and they were prepared for surgery as described previously in detail (Ebner et al. 1994, Reeds et al. 1996). In brief, under isoflurane anesthesia and strict aseptic conditions, the pigs were implanted with a polyethylene catheter (o.d., 1.27 mm, Becton Dickinson, Sparks, MD) in the common portal vein, and Tygon catheters (o.d., 1.78 mm) in an external jugular vein and a carotid artery. An ultrasonic blood flow probe (6 mm i.d., Transonic, Ithaca, NY) was placed around the portal vein. A silicone catheter (o.d., 2.17 mm, Baxter Healthcare, McGaw Park, IL) was implanted into the stomach lumen, ~2 cm from the pyloric sphincter. The catheters were filled with sterile saline containing heparin (2.5×10^4 U/L), and exteriorized on either the left flank (portal and gastric catheters, flow probe leads) or between the scapulae (jugular and carotid catheters). All of the catheters were protected with gauze pads and secured with an elastic bandage. Immediately postoperatively, the animals received an intramuscular injection of analgesic (0.1 mg/kg butorphenol tartrate, Fort Dodge Labs, Fort Dodge, IA) and antibiotic (20 mg/kg enrofloxacin, Bayer, Shawnee Mission, KS); pigs received a second antibiotic dose 1 d postoperatively. After the surgery, the pigs were offered 25% of their preceding daily intake that night, followed by 50% intake for the first postoperative day, resuming full feed intake on the second day after surgery. The infusion protocol was carried out 5 d after surgery, by which time the animals had been growing at preoperative rates (200–250 g/d) for at least 2 d.

[4] Abbreviations used: BW, body weight; CNRC, Children's Nutrition Research Center; EAA, essential amino acids; PBF, portal blood flow; PDV, portal-drained viscera; TDCJ, Texas Department of Criminal Justice.

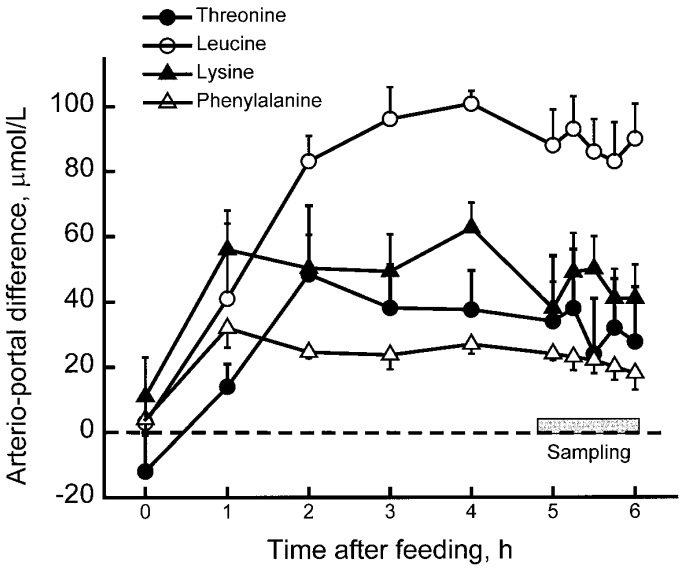

FIGURE 1 The difference between the portal and arterial whole-blood concentrations of threonine, leucine, lysine and phenylalanine in seven piglets fed once hourly with a milk protein–based diet. Portal balance results are based on samples collected between 5 and 6 h after the start of the feeding protocol. Values are means ± SEM.

Infusion protocol. The piglets were deprived of feed from 1800 to 0700 h. At ~0645 h, base-line arterial and portal blood samples were taken. At 0700 h, the pigs were offered a meal of liquid milk replacer (Litterlife) supplying ~600 mg protein and 40 kJ gross energy/kg BW. The meal was the equivalent of one twenty-fourth of the daily intake. Immediately after the first meal, piglets received a constant infusion of [U-^{13}C]*Spirulina platensis* (purchased from the Martek Corporation, Malvern, MA), suspended in water (~150 g/L) via the gastric catheter at a rate of ~0.2 mL/min. The *Spirulina* supplied ~60 mg of [U-^{13}C]protein/(kg·h). After the tracer infusion was started, the piglets continued to receive hourly meals of liquid milk replacer to supply 600 mg protein/(kg·h). Arterial and portal blood samples (3 mL) were taken at hourly intervals until 5 h of tracer infusion, and then at 15-min intervals until the animals were killed with an arterial injection of sodium pentobarbital (50 mg/kg BW) and sodium phenytoin (5 mg/kg) (Beutanasia-D; Schering-Plough Animal Health, Kenilworth, NJ).

Immediately after death, the abdomen was opened and the proximal 2 m of small intestine were isolated, either by excision or by ligation and excision (4 animals). A sample (~10 g) of liver was also removed and frozen in liquid nitrogen. The 2-m segment of small intestine was quickly flushed with ice-cold saline. The mucosa was immediately isolated in three piglets by scraping the small intestinal segment with a microscope slide. In the other four piglets, the ligated small intestine was immediately frozen in liquid nitrogen, and the mucosa was isolated subsequently from the ligated intestine by freeze-thaw disruption of the mucosal structure. The remainder of the small intestine and liver was removed and weighed.

[U-^{13}C]Algal protein and dietary amino acid analysis. Weighed samples of the diet and *Spirulina* were incubated overnight at pH 7.4 and 37°C with Pronase E (Sigma Chemical, St Louis, MO; 2 mg/100 *Spirulina*). After centrifugation at 10,000 × g for 5 min a known aliquot of the supernatant was hydrolyzed in 5 mol/L HCl at 110°C for 24 h. Amino acid standards were also hydrolyzed in parallel to account for any losses of amino acids, especially threonine and tyrosine, on hydrolysis. The amino acid compositions were then measured. The amino acid compositions of the diet and the tracer protein are shown in **Table 1.**

The *Spirulina platensis* algae used in this study were grown hydroponically in an atmosphere of 98% $^{13}CO_2$ to obtain amino acids with highly isotopically enriched carbon atoms. An aliquot of the hydrolyzed *Spirulina* protein was also derivatized and taken for the determination of the isotopomer spectrum of the amino acids. Eighty-five percent of the [U-^{13}C]threonine was present as the $^{13}C_4$-isotopomer, [U-^{13}C]leucine and lysine contained 79% $^{13}C_5$, and [U-^{13}C]-phenylalanine contained 70% $^{13}C_9$. These fractional isotopic abundances were used in the calculations of the rates of administration.

Sample analysis. The isotopic and concentration measurements of the amino acids were made on whole blood. Samples (0.5 mL) for amino acid concentration measurements were mixed with an equal volume of an aqueous solution of methionine sulfone and centrifuged at 10,000 × g for 5 min at room temperature through a 3-kDa cut-off filter. The filtrate was dried and the amino acids were analyzed by reverse-phase high performance liquid chromatography of their phenylisothiocyanate derivatives (PicoTag, Waters, Woburn, MA). For isotopic analysis, blood samples were brought to 4°C, and 0.1 mL was mixed with 1 mL of ice-cold acetic acid (0.5 mol/L). The solution was then applied to a 1-mL bed volume column of Dowex 50 Wx8 (H+ form) at 4°C. The amino acids were eluted with 3 mol/L NH_4OH and dried under vacuum.

The mucosal sample was homogenized (Ultra Turrex, Tekmar, Germany) with water (wt/wt) at 4°C. One milliliter of the homogenate was then treated with 1 mL of perchloric acid (1 mol/L). The homogenate was centrifuged (15,000 × *g* for 10 min) in a microfuge. The supernatant was taken and brought to pH 4–6 with KOH (5 mol/L). After removal of the potassium perchlorate, the amino acid fraction was isolated by cation exchange chromatography as described for the blood amino acid fraction. The protein precipitate was redissolved in 5 mL of NaOH (0.3 mol/L), and 1 mL was taken for determination of protein by the biuret method. The remaining protein was reprecipitated with 0.15–0.2 mL of perchloric acid (11.7 mol/L), washed with two changes of ice-cold ethanol and suspended in 2 mL water. An aliquot of the homogenate was mixed with an equal volume of HCl (10.8 mol/L) and hydrolyzed at 110°C for 24 h in a sealed tube. The hydrolysate was dried, redissolved in HCl (0.1 mol/L), and an aliquot was taken for amino acid analysis. The remainder was dried under vacuum, redissolved in water, redried and finally suspended in HCl (0.1 mol/L). The amino acid fraction was then isolated by cation exchange chromatography and taken for mass spectrometry.

Mass spectrometric analysis of the amino acids was conducted with the *n*-propyl ester heptaflurobutyramide derivative (Jahoor et al. 1994) by using a modified procedure designed to minimize the deamidation of glutamine (Reeds et al. 1996). Mass spectrometry was by methane negative chemical ionization. Analysis was performed on a 5890 Series II (Hewlett Packard, Palo Alto, CA) quadrupole gas chromatograph/mass spectrometer.

Calculations. The crude ion abundances of the uniformly labeled isotopomers were converted to molar tracer:tracee ratios (mol isotopomer per mol ^{12}C-amino acid) (Brauman 1996).

Net portal amino acid balance [μmol/(kg·h)]

$$(\text{Conc.}_{\text{PORT}} - \text{Conc.}_{\text{ART}}) \times \text{PBF} \quad (1)$$

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

Portal tracer amino acid balance (μmol[U-^{13}C]isotopomer/kg·h)

$$\{(\text{Conc.}_{\text{PORT}} \times t/T_{\text{PORT}}) - (\text{Conc.}_{\text{ART}} \times t/T_{\text{ART}})\} \times \text{PBF} \quad (2)$$

in which t/T is the tracer:tracee ratio of the [U-^{13}C]isotopomer (mol [U-^{13}C]amino acid:mol [U-^{12}C]amino acid).

Fractional portal balance (percentage of input)

$$\text{Portal balance}/(\text{Input} \times 100) \quad (3)$$

For the net portal amino acid balance calculations, input is the intake of the dietary amino acid (diet + *Spirulina*) and for the portal tracer amino acid balance calculations, input is the rate of infusion of the tracer amino acid. Note that for glutamine utilization by the PDV, the values are expressed in proportion to the arterial flux of glutamine assuming that the PBF out equals the total arterial blood flow to the PDV.

$$\text{Arterial GLN flux} = \text{Conc.}_{\text{ARTERIAL GLUTAMINE}} \times \text{PBF} \quad (4)$$

Fractional first-pass intestinal utilization (percent)

$$(1 - \text{Fractional portal tracer balance}) \times 100 \quad (5)$$

Total mucosal protein-bound amino acid (μmol/kg BW)

TABLE 2

Intake, arterial and portal concentrations and portal mass balance of amino acid in seven piglets fed once hourly with a milk replacer diet supplying ~660 mg protein/(kg·h)[1]

	Intake	Concentration, μmol/L		Portal balance	
Amino acid	μmol/(kg·h)	Arterial	Portal	μmol/(kg·h)	% of intake
		Essential amino acids			
Threonine	334 ± 34	576 ± 46	608 ± 46	127 ± 37	38 ± 11
Leucine	486 ± 43	319 ± 17	403 ± 20	292 ± 30	57 ± 5
Lysine	299 ± 31	347 ± 28	391 ± 29	160 ± 29	54 ± 10
Phenylalanine	132 ± 14	82 ± 3	104 ± 4	80 ± 6	60 ± 8
Valine	314 ± 32	376 ± 42	429 ± 33	191 ± 30	61 ± 9
Methionine	69 ± 7	64 ± 6	73 ± 9	33 ± 7	48 ± 10
Isoleucine	252 ± 26	250 ± 25	298 ± 32	176 ± 29	69 ± 11
		Conditionally essential amino acids			
Arginine	61 ± 6	75 ± 12	98 ± 10	84 ± 14	137 ± 20
Proline	308 ± 32	368 ± 34	419 ± 38	188 ± 26	62 ± 9
Tyrosine	41 ± 5	93 ± 17	112 ± 17	68 ± 14	167 ± 33[2]
Cystine	ND[3]	65 ± 3	68 ± 3	10 ± 19[4]	ND
		Nonessential amino acids			
Alanine	449 ± 47	766 ± 63	1014 ± 63	901 ± 96	205 ± 28[2]
Serine	307 ± 32	267 ± 39	317 ± 38	181 ± 35	58 ± 10
Glutamate[5]	532 ± 56	347 ± 14	357 ± 16	37 ± 21	7 ± 4[3]
Aspartate[6]	426 ± 44	48 ± 7	52 ± 8	16 ± 8	4 ± 2[3]
Glycine	298 ± 31	806 ± 90	850 ± 83	161 ± 49	52 ± 16
Glutamine	—	498 ± 25	461 ± 24	−135 ± 31	−8 ± 2[7]

[1] Values are means ± SEM (n = 7). Mean portal blood flow was 3.57 ± 0.36 L/(kg·h).
[2] Significantly >100% ($P < 0.05$ by paired t test).
[3] Not determined.
[4] Not significantly different than zero ($P > 0.2$ by paired t test).
[5] Dietary analysis of glutamate represents both glutamate and glutamine.
[6] Dietary analysis of aspartate represents both aspartate and asparagine.
[7] Expressed as a percentage of the mean arterial glutamine flow to the portal-drained visceral tissues, which was 1778 μmol/(kg·h).

$$\text{Mucosa (g/kg BW)} \times \text{protein/mucosa (g/kg mucosa)} \times \text{amino acid/protein (}\mu\text{mol/g protein)} \quad (6)$$

These calculations were based on the measured total small intestinal mucosal mass.

Tracer incorporation into mucosal protein [μmol/(kg·h)]

$$\text{Total mucosal amino acid} \times t/T \text{ of protein-bound amino acid} \times \tfrac{1}{6} \quad (7)$$

in which $\frac{1}{6}$ accounts for the 6-h infusion.

Statistics. All concentrations, tracer:tracee ratios and portal balances are presented as the mean values for samples taken over the last hour of the study (five samples for mass balance and three samples for tracer balance) ± the between-animal SEM. Balances were tested against zero by one-tailed t tests. For balances that are expressed as a proportion of input, the values shown are the means of the ratios. Differences between the balances of threonine, leucine, lysine and phenylalanine (proportion of input) were assessed by one-way ANOVA, with amino acid as the independent variable, followed by a post-hoc t test with the appropriate Bonferroni adjustment for four comparisons. A value of P (two-tailed) < 0.05 was taken as significant.

RESULTS

The differences between the portal and arterial concentrations of the four test amino acids are shown after overnight food deprivation (0 h) and each hour after the start of the hourly feeding protocol (**Fig. 1**). At the start of the study, the portal-arterial concentration differences did not differ significantly from zero, although the balance was nominally positive for leucine, lysine and phenylalanine and negative for threonine. Within 1 h after the start of feeding, the portal-arterial balance became significantly positive and remained so throughout the study. For threonine and leucine, the portal balance after 1 h of feeding was significantly ($P < 0.05$) lower than that over the last hour of the study, whereas lysine and phenylalanine apparently achieved a steady state of absorption within 1 h of feeding. Over the last hour, there was no significant difference in the portal-arterial concentration between samples.

The net portal balances of all analyzed amino acids over the last hour of the study are shown in **Table 2.** On average, the net portal balance of the EAA accounted for 56% of their intake. There was a trend ($P = 0.08$) for a lower proportion of the intake of methionine and threonine to be found in the portal circulation. The interanimal variation of the fractional portal balance was substantial (average CV 35%).

There were also systematic differences in the net portal balance among the nonessential and conditionally essential amino acids. The net portal balance of cystine, glutamate and aspartate did not differ significantly from zero. Glutamine was the only amino acid with a significantly negative net portal balance, indicating net uptake by the PDV tissues. It is difficult

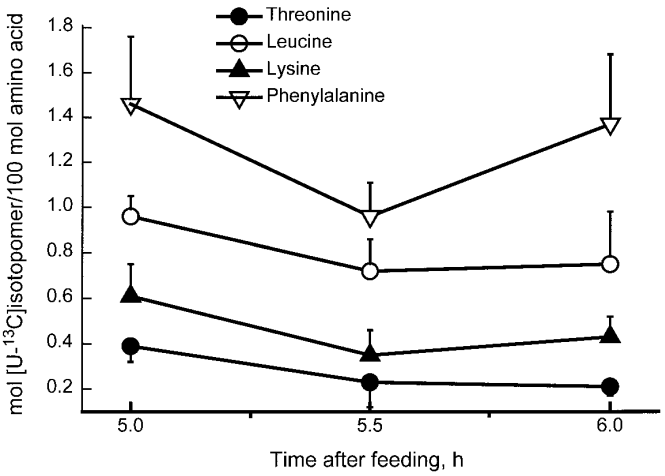

FIGURE 2 The difference between the tracer:tracee ratios of arterial and portal [U-^{13}C]threonine, leucine, lysine and phenylalanine in seven piglets fed once hourly and infused via the stomach with [U-^{13}C]*Spirulina* for 6 h. Results are shown only for samples collected between h 5 and 6 of isotope infusion. Values are means ± SEM.

to estimate the fractional portal glutamine balance because we did not have a valid estimate of the dietary glutamine content. However, the net portal glutamine uptake represented 8% of the arterial glutamine input. The fractional portal balances of tyrosine (167% of intake) and alanine (205% of intake) were significantly ($P < 0.05$) > 100%, and there was a trend ($P = 0.07$) for the portal outflow of arginine (137% of intake) to exceed its intake.

The differences between the tracer:tracee ratios of portal and arterial [U-^{13}C] threonine, leucine, lysine and phenylalanine over the last hour of infusion are shown in **Figure 2.** Although there was some variation between sampling times, there was no systematic upward or downward trend in the portal-arterial difference in the tracer:tracee ratio. The portal tracer balances for these four amino acids are summarized in **Table 3.** The fractional portal balance of [U-^{13}C] threonine was significantly ($P < 0.05$) lower than that of the other tracer amino acids, and the proportion of labeled threonine appearing in the portal blood was the same as the proportion of threonine intake that was absorbed. The fractional tracer balances of the other labeled amino acids were higher ($P < 0.05$) than their respective net portal mass balances.

The incorporation of the tracer amino acids into small intestinal mucosal protein is summarized in **Table 4.** The mucosal protein-bound amino acid level (μmol/kg BW) was also variable (between animal CV = 32%), but the variation reflected differences in the mucosal protein mass of different pigs. Of particular note was the fact that the mucosal protein mass of the pigs that had been reared in the Texas A & M research facility (1.53 ± 0.24 g mucosal protein/kg BW) was significantly ($P < 0.05$) less than that of the pigs that had been reared in the field (2.34 ± 0.61 g mucosal protein/kg BW). There was readily measurable labeling of mucosal protein with each amino acid, and a significant ($r = 0.82$; $P < 0.01$) linear relationship between total first-pass utilization of the amino acids and their utilization for mucosal protein synthesis (**Fig. 3**). Nevertheless, incorporation into mucosal protein synthesis accounted for only 18–21% of the metabolized lysine, leucine and phenylalanine and a lower ($P < 0.025$) proportion of the dose of threonine (11%; Table 4).

The low proportional incorporation of the [U-^{13}C]amino acids into mucosal protein suggests that their major fate in the mucosa was catabolism; thus we made measurements of portal and arterial ammonia concentrations in a separate group of pigs, obtained from the TDCJ facility. These pigs were the same breed and age, and were studied after identical surgical procedures while they were receiving hourly meals of identical amounts of the same diet as that used in the tracer study. The results for 10 pigs are shown in **Figure 4.** Within 1 h of feeding, there was a substantial (2.5-fold) increase in the difference between portal and arterial ammonia. The portal ammonia balance [0.76 mmol N/(kg·h)] accounted for 18% of total nitrogen intake; when taken in conjunction with the net synthesis of alanine [0.4 mmol N/(kg·h)] and arginine [0.09 mmol N/(kg·h)], it appeared that nitrogen derived from amino acid catabolism within the PDV accounted for 28% of total nitrogen intake. Taken together, ~80% of the nitrogen fed to the piglets could be accounted for in the net portal output of amino acids and ammonia.

DISCUSSION

Net portal amino acid balance. The degree to which dietary amino acids are utilized in first pass by the intestine and the liver determines their availability for the support of peripheral tissue metabolism. Although recent isotopic experiments (de Lange et al. 1990, Gaudichon et al. 1996, Lien et al. 1997, Mahé et al. 1994, Roos et al. 1995) suggest that the luminal digestion of most dietary proteins is virtually complete, other literature (Ebner et al. 1994, Rerat et al. 1988 and 1992)

TABLE 3

The doses, arterial and portal whole-blood tracer:tracee ratios and portal balances of [U-^{13}C]threonine, leucine, lysine and phenylalanine in seven fed piglets over the last hour of a 6-h intragastric infusion of [U-^{13}C]Spirulina platensis *supplying ~60 mg [U-^{13}C]protein/(kg·h)*[1]

Amino acid	[U-^{13}C] amino acid infusion rate[2], μmol/(kg·h)	Tracer:tracee ratio, mol/100 mol amino acid		Portal/arterial tracer:tracee ratio	Portal tracer balance	
		Arterial	Portal		μmol/(kg·h)	% of dose
Threonine	33 ± 2	5.61 ± 1.91	5.85 ± 2.00	0.27 ± 0.04	12.02 ± 5.28	38.6 ± 15.6
Leucine	48 ± 3	5.98 ± 2.28	6.65 ± 2.47	0.77 ± 0.09	31.10 ± 4.53	68.5 ± 10.6
Lysine	24 ± 2	3.36 ± 1.06	3.52 ± 1.27	0.42 ± 0.06	13.80 ± 2.87	65.4 ± 11.9
Phenylalanine	16 ± 1	6.32 ± 2.24	8.19 ± 1.14	1.23 ± 0.16	10.10 ± 2.09	65.3 ± 11.0

[1] Values are means ± SEM ($n = 7$).
[2] Represents the actual mean infusion rate of only the uniformly labeled isotopomer of each amino acid in the isolated *Spirulina* mixture. All tracer:tracee ratios and tracer balance values represent only the uniformly labeled isotopomer of each amino acid.

TABLE 4

The mucosal protein-bound content, tracer:tracee ratios and incorporation of threonine, leucine, lysine and phenylalanine in seven fed piglets at the end of a 6-h intragastric infusion of [U-^{13}C]Spirulina platensis[1]

Amino acid	Total tracer metabolized in first pass[2]	Mucosal protein amino acid content	Mucosal protein labeling		
			Tracer:tracee ratio	Tracer incorporation into protein	
	μmol/(kg·h)	*mmol/kg body weight*	*mol/100 mol*	*μmol/(kg·h)*	% of total first-pass metabolism
Threonine	21.3 ± 6.1	1.08 ± 0.13	1.14 ± 0.20	2.2 ± 0.4	11.6 ± 1.6
Leucine	17.1 ± 7.8	1.58 ± 0.19	1.33 ± 0.20	3.5 ± 0.6	20.5 ± 2.6
Lysine	10.3 ± 3.0	1.04 ± 0.13	1.02 ± 0.13	1.8 ± 0.2	17.5 ± 2.1
Phenylalanine	6.8 ± 1.8	0.47 ± 0.06	1.51 ± 0.18	1.2 ± 0.2	17.6 ± 1.8

[1] Values are means ± SEM, $n = 7$.
[2] The difference between the rate of infusion and portal balance (see Table 3).

indicates that <100% of ingested amino acids appear in the portal blood after a single meal. The present results, based on steady-state values measured in pigs receiving frequent protein-containing meals, confirm the general conclusion of these papers and suggest that in toto, 64% of the protein consumed by the animals appeared as free amino acids in the portal blood. This value, however, concealed marked differences in the portal appearance of different amino acids; in this respect, the present results are also in agreement with previous findings (Ebner et al. 1994, Rerat et al. 1992).

The most notable observation regarding the EAA in this study was the particularly low portal appearance of threonine and methionine. The nutritional result was that although the first limiting amino acid in the milk-protein diet consumed by the piglets on the basis of chemical score was probably lysine (Davis et al. 1994), on the basis of the net portal amino acid balance, the limiting EAA for extra-intestinal protein deposition were threonine and methionine.

The low portal appearance of threonine has been a consistent observation in studies of this nature (Rerat et al. 1988 and 1992) and presumably reflects the high concentration of threonine in the core protein of the intestinal mucins (Roberton et al. 1991). However, the low net portal balance of methionine was surprising in view of the fact that there was no significant portal appearance of cystine. Both observations have been made in previous studies in pigs fed milk protein–based diets (Ebner et al. 1994) and received specific comment by Rerat et al. (1988). Taken together, the observations imply that in pigs, the majority of circulating cystine derives from endogenous synthesis (an observation that has important implications for methionine and serine metabolism), and that the sulfur amino acids apparently have a specific metabolic role in the intestinal mucosal cells. In this context, it is of note that we have shown (Jahoor et al. 1995) that in piglets of this age, the concentration and turnover rate of mucosal reduced glutathione are both very high. Additional isotopic evidence (Reeds et al. 1997b) suggests that the glutamate and glycine in the mucosal glutathione pool of fed piglets derive directly from the diet. We are currently investigating whether enteral cysteine is also channeled into mucosal glutathione synthesis.

Moreover, there were major differences between the portal balances of other amino acids. These results confirmed previous observations (Windmueller and Spaeth 1975 and 1980) of the almost complete first-pass removal of dietary glutamate and aspartate as well as the substantial net synthesis of alanine by the intestinal tissues (see also Jungas et al. 1992). However, we also found that the portal balance of tyrosine and arginine exceeded their intake. It is reasonable to presume that the additional tyrosine in portal blood derived from the hydroxylation of phenylalanine. These data suggest that mucosal tyro-

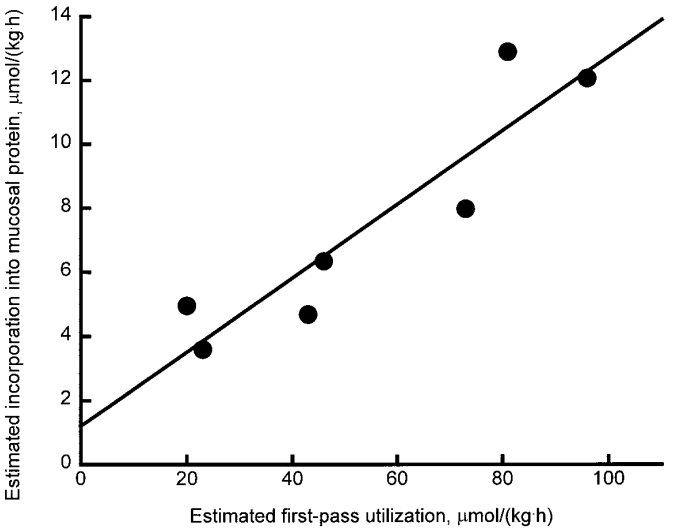

FIGURE 3 The relationship between the total intestinal utilization and incorporation into small intestinal mucosal protein of intragastric [U-^{13}C] threonine, leucine, lysine and phenylalanine in piglets. Each point shows data obtained in a single animal. The slope of the line is 0.15 and the relationship ($r = 0.82$) is significant ($P < 0.01$).

TABLE 5

Portal amino acid and ammonia nitrogen balance in fed piglets[1]

Component	Intake	Balance	
	mmol N/(kg·h)	*mmol N/(kg·h)*	% of intake
Essential	2.13 ± 0.22	1.11 ± 0.18	52 ± 8
Conditionally essential	0.59 ± 0.06	0.59 ± 0.08	100 ± 18
Nonessential	2.01 ± 0.21	1.30 ± 0.18	64 ± 13
Ammonia	—	0.76 ± 0.19	—
Total	4.74 ± 0.49	3.89 ± 0.21	82 ± 18

[1] Values are means ± SEM, $n = 7$.

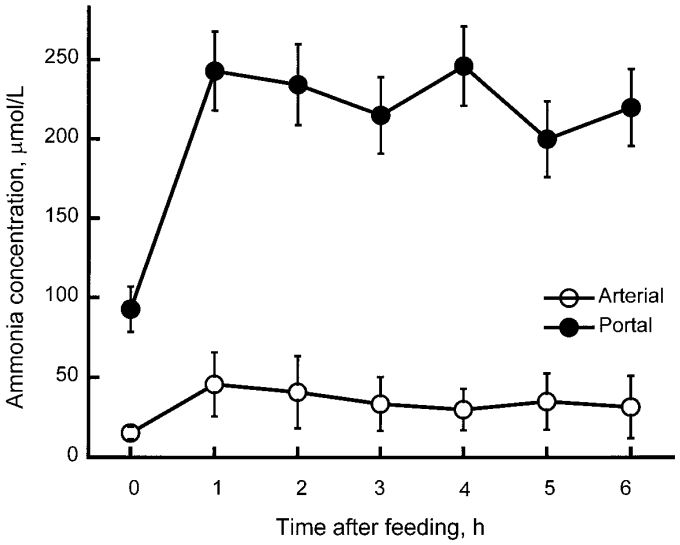

FIGURE 4 Arterial and portal ammonia concentrations in 10 piglets fed a milk protein–based diet once hourly. Values are means ± SEM.

sine synthesis [27 μmol/(kg·h)] could have accounted for ~50% of the difference [52 μmol/(kg·h)] between phenylalanine intake and the portal balance of phenylalanine.

The observation regarding arginine is particularly interesting. Because milk proteins contain insufficient arginine to support maximum rates of protein deposition (Davis et al. 1994), it seems likely that arginine synthesis plays an important role in the infant animal (Flynn and Wu 1996). It is generally held (Featherstone et al. 1973) that arginine synthesis in mammals involves the initial synthesis of citrulline in the intestinal mucosa followed by renal conversion of this citrulline to arginine. However, this metabolic scheme was developed from experiments in adult rats. There is evidence obtained in vitro (Blachier et al. 1993, Wu and Knabe 1995) that, at least in neonates, porcine enterocytes are capable of the complete synthesis of arginine. Because the net portal balance of arginine exceeded the arginine intake, it is suggested that the intestinal mucosal cells of 4-wk-old piglets are also able to synthesize arginine in vivo. We have recently obtained evidence from a study with intragastrically administered [U-^{13}C]-glutamate that directly demonstrates arginine synthesis by the mucosa of piglets in vivo (Reeds et al. 1997a).

Intestinal utilization of enteral amino acids. A major objective of this study was to identify the underlying factors that govern the net portal appearance of dietary amino acids. There is the likelihood that the PDV continue to use arterial amino acids even in the fed state (Rerat et al. 1992, Yu et al. 1990 and 1992), so that the net portal balance underestimates the true rate of absorption of dietary amino acids into the portal circulation. This was observed for lysine, leucine and phenylalanine, but not for threonine. We estimate that the arterial uptake of lysine, leucine and phenylalanine by the PDV amounted to 11, 11 and 5% of their respective intake and was the equivalent of 2.6, 4.6 and 2.6% of the arterial flux of the three amino acids. Presumably, the large difference in the fractional extraction of amino acids presented on the luminal (~100%), vs. arterial capillary interface (2–5%), reflects the difference in the surface areas and amino acid transport capacities of the respective plasma membranes. Even so, it should be emphasized that because the arterial flux of EAA to the gut greatly exceeds their dietary intake, the low fractional extraction of arterial EAA (2–4%) still represents a nutritionally important molar uptake of amino acids.

The major determinant governing the net appearance of dietary amino acids in the portal blood however, was metabolism by the mucosal enterocytes. Recent studies in humans and animals with intragastrically administered, stable isotope–labeled free amino acids (Basile-Filho et al. 1997, Biolo et al. 1992, Hoerr et al. 1991 and 1993, Yu et al. 1992) have shown that nutritionally important quantities of EAA are removed in first pass by the tissues of the splanchnic bed. In addition, it appears that the dominating tissue in the splanchnic utilization of leucine (Yu et al. 1990 and 1992) and phenylalanine (Stoll et al. 1997) is the intestine rather than the liver. This study generally confirms these phenomena and has further shown that for leucine, lysine and phenylalanine, first-pass intestinal metabolism accounts for roughly one third of the dietary intake.

Despite the significance of first-pass metabolism, it is perhaps of greater importance to establish the metabolic fate of amino acids taken up by the intestinal mucosa. In this regard, work carried out some years ago (Alpers 1972) established that the villous cells utilize luminal as well as systemic amino acids for protein synthesis. In designing this study, we hypothesized that mucosal protein synthesis would be responsible for the majority of the first-pass intestinal metabolism of dietary EAA. Under the circumstances of this experiment, however, this did not seem to be the case, because the direct mucosal protein incorporation of enteral [U-^{13}C] lysine, leucine, phenylalanine and threonine was a remarkably low proportion of their total measured first-pass intestinal utilization.

Before discussing the implication of this result, key points underlying the calculation require emphasis. First, the method that we used to quantify the utilization of the enteral tracer amino acids in mucosal protein is not a measure of the mucosal protein synthetic rate. Rather, it is a direct calculation of the mass of each amino acid incorporated into mucosal protein. As such, the calculation requires only information about the total quantity of protein-bound amino acids and the tracer:tracee ratio of the mucosal protein-bound amino acids. It does not require information on the isotopic enrichment of the mucosal protein synthetic precursor pool. Second, the values presented in Table 4 are based on the labeling of protein isolated from the mucosa of the proximal 2 m of the small intestine. In calculating the incorporation into total mucosal protein, we assumed that the tracer:tracee ratio of each amino acid in mucosal protein was constant throughout the small intestine. This assumption would have led to an overestimate rather than an underestimate of the total quantity of tracer amino acid incorporated into mucosal protein as a whole.

Finally, the small intestinal mucosa was presented with the luminal isotopic tracers for a 6-h period. Because of this, the incorporation of amino acids into the resident proteins of the mucosa would have likely underestimated their total incorporation, to the extent that a portion of the protein synthesized during the tracer infusion would have been secreted into the intestinal lumen. In this context, it is noteworthy that the incorporation of threonine into mucosal protein, expressed in proportion to total intestinal threonine metabolism, was significantly lower than that of the other amino acids. As we pointed out above, threonine is a major component of the mucosal mucins, and thus it is likely that a higher proportion of the incorporated threonine was secreted into the lumen. In fact, if we take the difference between threonine incorporation and the incorporation of the other amino acid tracers as an estimate of the error introduced by the failure to account for secreted proteins, then we calculate that the contribution of

mucosal protein synthesis to total first-pass intestinal amino acid utilization might have been subject to twofold underestimation. However, even if this were correct, no more than 40% of the intestinal metabolism of the enteral lysine, leucine and phenylalanine was directed to protein synthesis. The remainder was presumably catabolized.

The potential role of amino acids as energy sources for the mucosal cells is not a new proposition. It has been known for many years that the catabolism and oxidation of enteral glutamate, aspartate (Windmueller and Spaeth 1980) and arterial glutamine (Windmueller and Spaeth 1975) by the intestinal tissues are extensive. Indeed, in this experiment, the catabolism of glutamine, glutamate and aspartate alone amounted to 660 μmol N/(kg·h). However, on the basis of the measurements of tracer balance and incorporation into mucosal protein, it would appear that at least 60% of the first-pass metabolism of the EAA was also catabolized. This is the equivalent of at least 20% of the EAA that the piglets consumed.

Because this conclusion was based entirely on tracer data, we also estimated total amino acid catabolism from calculations of amino acid nitrogen balance and ammonia outflow. The difference in portal ammonia balance between the postabsorptive and fed states was 760 μmol N/(kg·h), and was the equivalent of at most 18% of total protein-nitrogen intake. The net portal ammonia balance could also result from the hydrolysis of circulating urea N taken up by the intestine. Thus, the net portal ammonia balance or release represents a maximal rate of dietary amino acid catabolism and should be verified further by determining the actual contribution of intestinal urea hydrolysis to net portal ammonia release. When the portal ammonia release was taken in conjunction with the net synthesis of arginine [92 μmol N/(kg·h)] and alanine [452 μmol N/(kg·h)], it appeared that the portal-drained viscera were catabolizing the amino acid equivalent of 1300 μmol N/(kg·h) or 27% of the piglets' total protein-nitrogen intake.

Finally, the net portal nitrogen balance (i.e., the sum of amino acid and ammonia nitrogen outflow) accounted for 82% of the known protein-nitrogen intake. Adding this figure to the estimated incorporation of dietary amino acids into mucosal protein (7% of intake) would account for at least 90% of the dietary protein. If one assumes that the dietary protein was nearly 100% digestible, it is likely that the remaining dietary nitrogen could be accounted for by mucosal protein secreted into the lumen and, therefore, not recovered in our samples.

In conclusion, these results indicate that substantial first-pass metabolism of dietary protein in the intestinal mucosa is not confined to nonessential amino acids. It appears that approximately one third of the dietary EAA intake may be metabolized in the first pass by the intestinal mucosa. If indeed one assumes complete oxidation of the metabolized EAA, this represents not only an important source of dietary amino acid loss, but also indicates that the dietary EAA of well-fed piglets may be an important source of energy for the small intestinal mucosa. We believe this to be a novel and nutritionally important finding with implications for the interpretation of dietary amino acid availability and, as such, requiring further confirmation. Finally, the quantities of dietary amino acids that were utilized in the first pass by the intestine were closely related to the mucosal mass of the piglets, implying that environmental conditions that affect intestinal mass have an important effect on the overall nutritional efficiency of dietary protein utilization.

It is unclear whether the catabolism of amino acids represents a functional requirement by the intestine for purposes of growth and function or if it is driven simply by the local availability of these substrates to the mucosal enterocyte. At this stage, it is also uncertain whether the high rate of mucosal amino acid catabolism in the intestine suggested by these results is under nutritional regulation, particularly by the habitual protein intake of the animals; we are currently investigating this question in detail.

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