Threonine Utilization Is High in the Intestine of Piglets[1,2]

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ABSTRACT The whole-body threonine requirement in parenterally fed piglets is substantially lower than that in enterally fed piglets, indicating that enteral nutrition induces intestinal processes in demand of threonine. We hypothesized that the percentage of threonine utilization for oxidation and intestinal protein synthesis by the portal-drained viscera (PDV) increases when dietary protein intake is reduced. Piglets (n = 18) received isocaloric normal or protein-restricted diets. After 7 h of enteral feeding, total threonine utilization, incorporation into intestinal tissue, and oxidation by the PDV, were determined with stable isotope methodology [U-^{13}C threonine infusion]. Although the absolute amount of systemic and dietary threonine utilized by the PDV was reduced in protein-restricted piglets, the percentage of dietary threonine intake utilized by the PDV did not differ between groups (normal protein 91% vs. low protein 85%). The incorporation of dietary threonine into the proximal jejunum was significantly different compared with the other intestinal segments. Dietary, rather than systemic threonine was preferentially utilized for protein synthesis in the small intestinal mucosa in piglets that consumed the normal protein diet ($P < 0.05$). Threonine oxidation by the PDV was limited during normal protein feeding. In protein-restricted pigs, half of the total whole-body oxidation occurred in the PDV. We conclude that, in vivo, the PDV have a high obligatory visceral requirement for threonine. The high rate of intestinal threonine utilization is due mainly to incorporation into mucosal proteins. J. Nutr. 135: 765–770, 2005.

KEY WORDS: • *amino acid* • *intestine* • *nutrition* • *piglets* • *threonine*

The small intestine is one of the most metabolically active tissues in the body. For example, the portal-drained viscera (PDV), i.e., the intestine, pancreas, spleen and stomach, in neonatal pigs account for only 4–6% of the whole-body mass, but are responsible for ~25% of the total whole-body CO_2 production, and for 20–50% of the total protein turnover (1–5).

Studies in pigs showed that >70% of the first-pass metabolism of some essential amino acids by the splanchnic tissues occurs in the intestine (6–9). In humans, the splanchnic tissues retain between 20 and 50% of the dietary intake of specific essential amino acids (5,10,11). For some dispensable amino acids, notably glutamate, the first-pass splanchnic extraction exceeds 90% of the dietary intake, in both humans and pigs (12,13). Together, these studies indicate that the small intestine has a substantial amino acid metabolism.

In this context, threonine is of critical nutritional importance, because it is the single most used indispensable amino acid by the metabolism of the PDV. The retention of threonine by the PDV in first pass ranges from 60 to 80% of the dietary intake under normal feeding circumstances, whereas the first-pass metabolism of other indispensable amino acids such as lysine and leucine accounts for roughly one third of the dietary intake (9,14,15). Consistent with the finding that so much threonine is utilized by the PDV, the whole-body threonine requirement is reduced by 60% in piglets receiving total parenteral nutrition compared with enteral nutrition (16). A key unresolved question is the metabolic fate of threonine used by the gut. A major metabolic fate of threonine is likely incorporation into mucosal proteins because some of these proteins contain a high amount of threonine in their peptide backbone (e.g., mucin) (17,18). However, another metabolic fate of threonine could be oxidation because previous studies showed that some indispensable amino acids including lysine and leucine are oxidized within the gut (14,19).

If the predominant metabolic fate of dietary threonine in the intestine is oxidation, then the first-pass metabolism by the intestine is a source of nutritional inefficiency. Therefore, determining the rate of intestinal oxidation and incorporation into mucosal proteins was the first objective of our study.

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Mucosal cells receive substrates directly from both the diet and the mesenteric circulation; previous studies showed a compartmentalization of amino acids by the intestinal tissues (8,13,14,20,21). The proportions of visceral threonine metabolism derived from the luminal and the arterial sites are not known. Thus, the second objective was to determine the relative rate of systemic threonine metabolism by the PDV.

The degree to which the first-pass utilization of amino acids is dependent on the nutrient composition and dietary protein intake is an important question. An obligatory high utilization rate of indispensable amino acids, which is independent of dietary protein intake, would result in a very low systemic availability of dietary amino acids at a low protein intake with subsequently impaired growth. Although there have been few investigations on this issue, the available data are contradictory. In a previous study with growing pigs, we showed that during protein restriction, intestinal growth is preserved, apparently at the expense of skeletal muscle growth (22,23). In addition, we found that a prolonged period of protein restriction lowers the fractional synthesis rate of total mucosal protein by only 25–40% (24,25). Determination of total threonine utilization by the PDV in piglets fed a low-protein diet was the last objective of our study.

We studied the utilization of systemic threonine by the PDV in 4-wk-old piglets fed isocaloric diets having either a normal protein (NP) or low-protein (LP) content. By using the stable isotope methodology, we were able to measure: *1*) the intestinal incorporation of threonine, *2*) systemic threonine uptake by the PDV, *3*) the systemic oxidation of threonine by the PDV, and *4*) the response of intestinal and whole-body threonine metabolism to protein restriction.

MATERIALS AND METHODS

Piglets. The Baylor College of Medicine Animal Protocol Review Committee approved the study. Housing and care of the piglets conformed to the USDA guidelines. The study involved 4-week-old female crossbred piglets ($n = 18$; Large White × Hampshire × Duroc) purchased from the Texas Department of Criminal Justice. The piglets were received at the CNRC when they were 2 wk old and were fed a liquid milk replacer (Litterlife, Merrick) at a rate of 50 g/(kg body weight · d). The composition (/kg dry matter) of the milk replacer was 500 g lactose, 100 g fat, and 250 g protein. The calculated energy density was 18 MJ gross energy/kg dry matter.

Study design. The study design was described previously (14,19,21,26). At a postnatal age of 3 wk, piglets ($n = 11$) were surgically implanted with catheters after overnight food deprivation. The surgery entailed the placement of catheters into the carotid artery, portal vein, and jugular vein. Eight piglets received a catheter into the duodenum for enteral tracer administration. One piglet received the threonine tracer enterally as well as i.v. In addition, an ultrasonic flow probe (Transonic Systems) was placed around the portal vein. After surgery, the piglets were administered complete i.v. nutrition for 24–36 h. They then were fed either regular Litterlife [NP diet: 12.7 g protein, 5.1 g lipid, and 25.5 g carbohydrates/(kg · d)] or a diet that contained only 40% of the protein content in Litterlife [LP diet: 5.1 g protein, 7.5 g lipid, and 30.4 g carbohydrates/(kg · d)]. Protein intake during LP feeding was deliberately set at a rate to provide enough protein to compensate for obligatory amino acid oxidation. The diets were made isocaloric by adding lactose (Sigma Chemical) and corn oil in the same ratio as in the control (NP) diet. The piglets were given ~4 times as much energy and protein compared with human infants to provide enough to maintain an adequate growth rate, which is 4 times as high in piglets as in human neonates. At postnatal d 28, whole-body CO_2 production was measured with an infusion of [1-^{13}C] bicarbonate. The [U-^{13}C] threonine infusion protocols were conducted on postnatal d 30 and 32, when the piglets had received full enteral feeding of the same diet (either NP or LP) for at least 8 d.

Isotope tracer protocol. After overnight food deprivation, the piglets consumed a meal that supplied one seventh of the preceding daily intake to restore intestinal motility. Immediately thereafter, a continuous gastric infusion of diet was started at a rate that provided one fourteenth of the preceding daily intake each hour. On postnatal d 28, [1-^{13}C] bicarbonate (99%, Cambridge Isotope Laboratories) was infused into the jugular catheter at a rate of 10 μmol/(kg · h). Arterial and portal blood samples (1 mL) were taken at 15-min intervals from min 75 to 120 of infusion. A methodological study in 4 piglets infused with $^{13}NaH^{13}CO_3$ for 7 h at d 28, 30, and 32 showed that bicarbonate kinetics per kilogram body weight remained the same although they were growing in that period. A plateau in enrichment was reached after 60 min. On postnatal d 30–32, [U-^{13}C] threonine 98%, Cambridge Isotope Laboratories) was infused via either the duodenal or the jugular catheter at a rate of 10.8 μmol/(kg · h) for 5 h. During the last hour of the tracer infusion, 4 arterial and portal blood samples were drawn at 15-min intervals. The piglets were killed with an arterial injection of sodium pentobarbital (50 mg/kg) and sodium phenytoin (5 mg/kg) (Beutanasia-D; Schering-Plough Animal Health). The abdomen was opened and the entire small intestine distal to the ligament of Treitz was immediately flushed with ice-cold saline. After flushing, the intestine was divided into 2 equal parts; the proximal half was designated the jejunum and the distal part, the ileum. These 2 parts were divided in half, resulting in a total of 4 segments: proximal jejunum, distal jejunum, proximal ileum, and distal ileum. The 4 segments were weighed and then the tissue samples were snap-frozen in liquid nitrogen and stored at −70°C until analysis for tracer enrichment, protein, and DNA. The first part of each intestinal segment was taken for analysis applying the same procedure.

Sample preparation

Blood samples. Small aliquots (0.2 mL) were taken for direct determination of the concentrations of blood gases (Chiron Diagnostics), glucose, and lactate (YSI analyzer). The isotopic and concentration measurements of the amino acids and $^{13}CO_2$ were made on whole blood as described in detail in a previous publication (14).

Intestinal tissue samples. Each intestinal tissue sample (200 mg) was homogenized in water and aliquots removed for analysis of protein and DNA as described previously (25). The protein fraction was isolated by adding 1 mL of 2.0 mol/L perchloric acid. The intestinal tissue/perchloric acid mixture was centrifuged at 1800 × g for 20 min. Pellets were washed 3 times with 3 mL of 0.2 mol/L perchloric acid to remove the remaining free amino acids. The washed pellets were hydrolyzed by adding 0.5 mL of 6 mol/L hydroxychloride and incubating at 110°C in sealed tubes for 24 h. The protein hydrolysates were dried under nitrogen at 55°C and the residue was dissolved in 0.5 mL water. Amino acids were isolated from protein hydrolysates by cation exchange separation as described previously for the blood amino acid fraction.

Derivatization. Threonine was converted to its *N*-ethoxycarbonylethylester derivative according to a modified method of Huşek (27).

Mass spectrometry

Blood samples. Whole-blood samples were prepared for amino acid and CO_2 analysis as described previously (13,14). Isotopic enrichment of threonine and CO_2 was measured by isotopic ratio MS (26). The atom percent enrichment was converted to mole percent threonine enrichment, after accounting for the 2.25-fold dilution of carbon in the derivative and the measured ^{13}C-abundance (98%) of the threonine tracer.

Intestinal tissues. A Thermo Finnigan Delta-XP isotope ratio MS coupled online with a trace GC (Thermo Electron) and a combustion interface type 3 (Thermo Finnigan) was used for of the $^{13}C/^{12}C$ ratio measurement of threonine. Aliquots of 1 μL of the chloroform suspension containing the amino acid derivatives were introduced into the GC system by a CTC PAL autosampler (CTC Switzerland). The flow was set at a constant rate of 1 mL/min and samples were introduced in splitless mode. A DB-225ms (Agilent)

capillary column 30 m in length with an i.d. of 0.25 mm was used for the chromatographic separations. The injector temperature was 250°C and the oven temperature was programmed starting at 160°C for 1 min, then increased from 160 to 230°C at a rate of 5°C/min and held at 230°C for 5 min.

After separation using capillary GC, amino acids were combusted online at 940°C and introduced as CO_2 into the isotope ratio MS, where the C^{13}/C^{12} ratio was measured.

Calculations. Previous studies from our laboratory and those of others used a steady-state, whole-body model of amino acid metabolism that was developed as described by Waterlow et al. (28) This model assumes a common metabolic amino acid pool through which all amino acids move, either as dietary or systemic amino acids or from protein breakdown, or to exit for protein synthesis or oxidation. This movement through the metabolic pool is called flux. From the measurements of arterial and portal enrichments of isotopically labeled tracers, arterial and portal amino acid concentrations, and portal blood flow, the total and systemic uptake of substrates across the PDV can be calculated (14,19,21,26).

The equations used for calculating the threonine metabolic fate were described previously (14,26). For the calculations of intestinal threonine incorporation, we used a value of 3.39 g threonine/100 g total amino acids measured in neonatal pigs as done by Wu et al. (29)

Statistics. Results concerning weight gain, CO_2 production, oxidation, and data for samples taken over the last hour of the tracer study are expressed as the mean ± the interanimal SEM. Other data are presented as the median (minimum-maximum). Differences between the balances of the piglets fed the NP or LP diet were tested by an independent samples *t* test or Mann-Whitney test (two-tailed). Significance between the incorporation of dietary threonine into different segments of the intestine was assessed using ANOVA and a paired *t* test (two-tailed). Differences between the incorporation of dietary threonine in different feeding groups were assessed using an independent samples *t* test (two-tailed). Differences with $P < 0.05$ were considered to be significant.

RESULTS

Before surgery, the piglets were gaining weight at a rate of 55 ± 2.3 g/(kg · d). Body weights of the 2 groups did not differ on the day of surgery (5.35 ± 0.09 kg). After surgery, weight gain in the NP piglets ($n = 9$) was 45 ± 2.6 g/(kg · d) and in the LP piglets 21 ± 2.2 g/(kg · d) ($n = 9$) ($P < 0.0001$), a difference that affected the body weight at postnatal d 30 and 32 when the piglets were killed (NP: 8.53 ± 0.29 vs. LP 7.07 ± 0.15 kg, $P < 0.0001$).

Neither the whole-body CO_2 production [57.8 ± 3.0 mmol/(kg · h) ($n = 11$)], nor the CO_2 production by the PDV [9.4 ± 1.4 mmol/(kg · h) ($n = 11$)], was affected by protein restriction. Overall, the PDV accounted for 16% of the total whole-body CO_2 production.

Table 1 summarizes the results obtained during the i.v. [U-^{13}C] threonine tracer infusions in both feeding groups. At 7 h after the start of feeding, the arterial and portal threonine concentrations were significantly lower in piglets fed LP than in those fed NP. The portal mass balance of threonine [(dietary intake + systemic intake) –portal outflow] tended to be lower ($P = 1.00$) in the protein-restricted group [median NP: 150 (−223 to 245) μmol/(kg · h) vs. median LP: 100 (−211 to 235) μmol/(kg · h)]. During the i.v. tracer administration, the portal isotopic enrichment of threonine was lower than the arterial isotopic enrichment, indicating that there was net intestinal uptake of systemic threonine during both feeding periods. The percentage of arterial threonine that was taken up by the PDV was 10% in piglets fed NP, whereas it decreased to 3% in piglets fed LP.

In piglets fed NP, two-thirds of the utilized threonine was sequestered by the PDV from the systemic circulation (**Table 2**). Of even more interest, the systemic threonine utilization was significantly affected by a lower protein intake. The total threonine utilization by the PDV was significantly lower in LP piglets [274 (139–585) μmol/(kg · h)] than in NP piglets [784 (689–1157) μmol/(kg · h)]. The equivalent of 85% of the total threonine intake was utilized in the PDV in piglets fed LP, whereas 91% of the threonine intake was utilized in piglets fed NP ($P > 0.05$).

The intestinal [U-^{13}C] threonine enrichment after 5 h of tracer infusion in each of the 4 intestinal segments was expressed as mole percent excess (**Fig. 1**). After the i.v. infusion, the values did not differ among the 4 intestinal segments and or between piglets fed NP or LP. During enteral tracer administration, the threonine enrichment was significantly higher in the proximal jejunum than in the other segments, indicating that the majority of the dietary threonine was taken up in the proximal part of the small intestine.

TABLE 1

Portal blood flow, arterial and portal enrichments of threonine and CO_2, arterial and portal concentrations of threonine and CO_2, flux, tracer balances and portal mass balances in piglets fed a NP or a LP diet in combination with an i.v. infusion of [U-^{13}C] threonine[1,2]

	NP ($n = 6$)	LP ($n = 5$)	*P*-value
Portal blood flow, *L/(kg · h)*	4.8 ± 0.8	4.9 ± 0.4	NS
Infusion tracer rate, *μmol/(kg · h)*	11.04 ± 0.42	11.54 ± 0.41	NS
Arterial [Threonine], *μmol/L*	1181 ± 69	698 ± 144	0.010
Portal [Threonine], *μmol/L*	1203 ± 68	710 ± 158	0.012
Arterial Threonine IE, *MPE*	0.776 ± 0.051	0.899 ± 0.049	NS
Portal Threonine IE, *MPE*	0.681 ± 0.041	0.863 ± 0.048	0.022
Tracer balance, *μmol/(kg · h)*	−4.87 ± 1.70	−0.68 ± 0.64	NS
Portal mass balance,[3] *μmol/(kg · h)*	150 (−223 to 245)	100 (−211 to 235)	NS
Flux (Q), *μmol/(kg · h)*	1434 ± 150	1243 ± 57	NS
Arterial [CO_2], *mmol/L*	25.57 ± 0.65	28.05 ± 0.38	0.021
Portal [CO_2], *mmol/L*	27.80 ± 0.46	29.63 ± 0.40	0.024
Arterial CO_2 IE, *MPE*	0.005 ± 0.001	0.001 ± 0.001	0.006
Portal CO_2 IE, *MPE*	0.004 ± 0.001	0.001 ± 0.000	NS

[1] Values are means ± SEM unless otherwise noted.
[2] Abbreviations: IE, isotopic enrichment; MPE, mole percent excess; NS, not significant.
[3] Values are medians (minimum–maximum).

TABLE 2

Threonine intake, systemic and total utilization of threonine by the PDV, the systemic oxidation by the PDV, and the dietary and systemic intestinal incorporation of threonine in piglets fed either a NP or a LP diet[1]

	NP (n = 6)	LP (n = 5)
Intake, μmol/(kg · h)	934	374
Systemic utilization, μmol/(kg · h)	467 (60–1334)	80 (0–235)*
Total utilization, μmol/(kg · h)	784 (689–1157)	274 (139–585)**
% of intake	91	85
Threonine incorporation, μmol/(kg · h)		
Dietary (n = 8)	439 (275–779)	250 (232–362)
Systemic (n = 8)	131 (91–136)#	83 (52–132)

[1] Values are medians (minimum–maximum) unless stated otherwise. Asterisks indicate different from NP: * $P < 0.05$, ** $P < 0.01$. # Different from NP dietary, $P < 0.05$.

The utilized threonine can be used for oxidation and protein synthesis. By measuring the isotopic enrichment of threonine in the intestinal mucosa, we were able to quantify the incorporation of threonine into mucosal proteins (Table 2). The incorporation rate of dietary threonine into intestinal protein was higher in piglets fed NP [439 (275–779) μmol/(kg · h)] than in those fed LP [250 (232–362) μmol/(kg · h)]. These amounts are 57% (NP-fed) and 86% (LP-fed) of the total PDV threonine utilization. In both NP- and LP-fed piglets, the intestine utilized both dietary and systemic threonine for intestinal protein synthesis, but dietary threonine was used predominately. Dietary and systemic threonine incorporation did not differ between piglets fed NP or LP, although dietary threonine incorporation tended to differ (P = 0,057) between the NP and LP groups.

The intestinal threonine oxidation did not differ between piglets fed NP [15 ± 7 μmol/(kg · h)] or LP [28 ± 10 μmol/(kg · h)]. Direct oxidation is therefore not a major metabolic pathway of threonine in the intestine. We measured ^{13}C-enrichment of glycine in arterial and portal blood samples to account for the threonine dehydrogenase degradation pathway, but [^{13}C] glycine enrichment did not differ from baseline values. The whole-body threonine oxidation as measured by the systemically infused threonine was 117 ± 18 μmol/(kg · h) during NP feeding. The systemic visceral threonine oxidation represented 13% of the whole-body threonine oxidation. Whole-body threonine oxidation was affected in piglets fed LP [61 ± 6 μmol/(kg · h) ($P < 0.05$)], and systemic visceral oxidation accounted for approximately half of the total oxidation rate.

DISCUSSION

Threonine is an important limiting amino acid for growth and maintenance in diets for pigs (30). In addition, threonine is of critical importance for intestinal function because it is essential to the structural protein mucous layer, which lines the gastrointestinal tract. Several studies showed that the utilization of a specific amino acid is not constant over a wide range of intakes (31–33). The aim in the present study was to investigate the effect of protein restriction on intestinal and whole-body threonine metabolism in piglets. We found that, irrespective of the dietary protein intake, the PDV extracted a very large amount of systemic threonine, which was incorporated mainly into intestinal mucosal proteins. Our results showed no effect of the LP diet on intestinal weight (data not shown). However, there was significant difference in body weight gain between the 2 feeding groups, with NP-fed piglets weighing more. These results suggest that during protein restriction, the intestine is spared in favor of other tissues.

In recent years, there has been growing recognition that a very large proportion of certain nonessential amino acids (e.g., aspartate, glutamate, and glutamine) from the diet are utilized by the intestine and do not appear in the systemic circulation (12,13). Similarly, we showed previously in piglets that the net portal balance of lysine is significantly less than the dietary intake, indicating that the PDV tissues utilize a considerable amount of dietary lysine (14). Moreover, we showed that in piglets fed high protein, most of the lysine used by the PDV is derived from the systemic circulation, but this shifts to an increased first-pass use during protein restriction. A similar phenomenon occurs for threonine. Two-thirds of the threonine utilization is derived from the systemic circulation in piglets fed NP, whereas the majority of the utilized threonine was derived from the intestinal lumen in piglets fed LP.

In a previous study, we showed that intestinal recycling of amino acids contributes significantly to their systemic availability (26). In the present study, we postulate that a small amount of threonine was recycled by the PDV because we only infused for 5 h. However, as discussed above, the amount of recycled threonine might be underestimated because the degradation of secreted proteins and subsequent absorption take more time. We therefore assume that the threonine utilized for intestinal protein synthesis eventually becomes at least partially available for the peripheral tissues through an efficient reabsorption process. The recycling of threonine might also explain the growth of the piglets during the study. We think it is likely that our results reflect the first process immediately after feeding was started because we measured for 5 h; thus, the intestinal net uptake of threonine might be an overestimation.

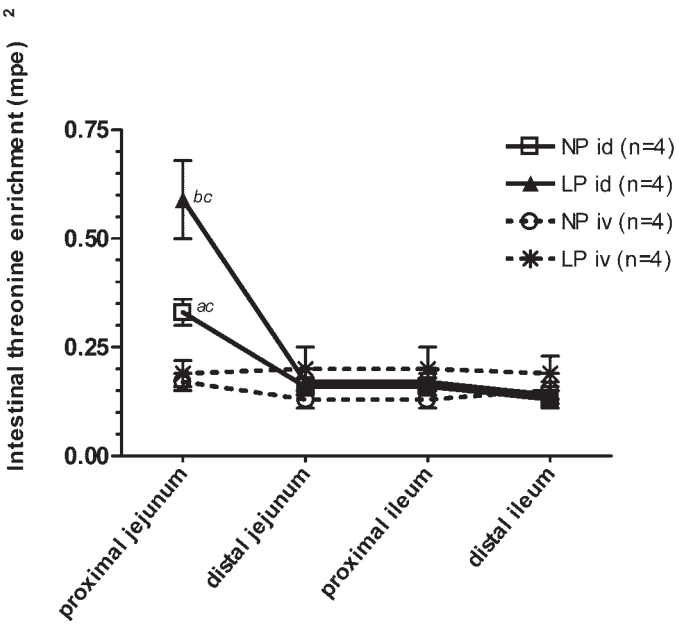

FIGURE 1 Intestinal threonine enrichment in piglets fed a NP or LP diet in combination with an intravenous (i.v.) or intraduodenal (i.d.) infusion of [U-^{13}C] threonine measured in the 4 segments of the small intestine. Values are means ± SEM. [a]Different from the other intestinal segments in the NP i.d. group ($P < 0.01$; [b]different from the other intestinal segments in the LP i.d. group ($P < 0.02$); [c]NP i.d. differed from LP i.d., $P < 0.05$.

Previous studies showed that threonine is one of the most important amino acids in (glyco-) proteins (e.g., mucins). Mucins contain large amounts of proline, threonine, and serine in their peptide backbone, together comprising 20–55% of the amino acid composition (17,18,34). These glycoproteins are the main constituents of the mucous layer. It appears that most threonine used by the intestine is for mucosal and secretory protein synthesis because threonine oxidation represents only 2–9% of the total threonine utilized. Total intestinal threonine incorporation contributed 71% to total threonine utilization. This incorporation can be within mucins and in goblet cells that are not yet secreted or into constitutive proteins in the intestine. We realize that this number is somewhat uncertain because we had to use a different set of piglets for the intraduodenal study. Variability among the piglets results in variability in the incorporation data. Because only 15 μmol/(kg·h) was oxidized in piglets fed NP (2%), 27% of the threonine utilized is unaccounted for. This is probably a reflection of the amount of threonine secreted (probably as mucins) into the lumen. Loss of apical cells (with proteins) also contributes. In piglets fed LP, slightly more than the total amount of utilized threonine was found incorporated within the intestine. This is probably due to variability among the different pigs. However, we are certain that the vast majority of the utilized threonine is used for protein synthesis within the intestine.

The last observation in this study that requires comment is the systemic and whole-body threonine oxidation. Our results indicate that the visceral oxidation of threonine under normal feeding conditions accounts for one eighth of the whole-body threonine oxidation, and that this visceral threonine oxidation is not suppressed during protein restriction. In contrast to our previous findings regarding intestinal lysine oxidation, the PDV oxidizes threonine that is taken up from the mesenteric artery (14). However, we measured visceral threonine oxidation, which included the oxidation of threonine by the pancreas, spleen, and stomach. These findings challenge the traditional concept that threonine is not catabolized by the intestinal mucosa, and further enzymological work is therefore required to establish the biochemical origins of intestinal catabolism of dietary threonine.

In examining whole-body threonine oxidation, Chu and Hegsted (33,35) observed no changes in threonine dehydratase activity in rats fed protein-free or threonine-free diets, and concluded that no adaptation occurs in whole-body protein metabolism when threonine is specifically lacking. In contrast to their findings, we found substantially decreased whole-body threonine oxidation during protein restriction. According to Ballevre et al. (36) who developed and validated a multitracer method in pigs allowing the calculation of the partition of both threonine pathways, the measurement of ^{13}C-labeled CO_2 probably underestimates threonine oxidation. In addition, we did not find a significant enrichment in glycine during the administration of labeled threonine. Nevertheless, whole-body threonine oxidation was significantly lowered by protein restriction, suggesting a protein-sparing adaptive mechanism.

For effective nutritional support for neonates to achieve normal growth, it is necessary to evaluate the amount of amino acids that is actually available from the diet for absorption. This is not an easy task because the flow of amino acids delivered to the small intestines is comprised of microbial proteins and proteins secreted by the intestinal mucosal cells. The neonatal piglet is considered to be an appropriate model for the human infant due to similarities in gastrointestinal physiology and functions and metabolism. The high intestinal threonine utilization might have important nutritional value, especially in children with impaired gut function. These infants probably need large amounts of dietary threonine to maintain the mucous layer that protects the whole gastrointestinal tract.

In conclusion, the present study demonstrates that during protein restriction the PDV maintain a high rate of metabolism and continue to utilize a very high amount of dietary threonine during the first period of feeding. Threonine is incorporated mainly into intestinal mucosal proteins, and the level of protein intake affects the site of threonine utilization by the PDV, as it switches from dual threonine use (i.e., dietary and systemic) to predominantly luminal utilization of threonine when dietary protein becomes limiting.

Taken together, the results highlight the important role of the intestine in modulating dietary amino acid availability to the body and point to the obligatory requirement of threonine for maintaining intestinal integrity.

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