Dietary and systemic phenylalanine utilization for mucosal and hepatic constitutive protein synthesis in pigs

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In a previous study of mucosal and hepatic protein synthesis (23), we infused [1-13C]phenylalanine intragastrically and [6-1H]phenylalanine intravenously in fed piglets. On the basis of the relative isotopic enrichments of the two tracers in hepatic-free and very low-density apolipoprotein B-100 (apoB-100) phenylalanine, we concluded that portal amino acids were preferential sources for the synthesis of proteins secreted by the liver. However, this previous study did not address two important issues. First, the isotopic enrichment of the intragastric 13C-labeled tracer was too low to make adequate measurements of its incorporation into albumin. Second, because we were interested in quantifying the uptake of arterial phenylalanine by the portal-drained viscera (PDV) in both the fasted and fed states within a single infusion, we infused the 1H-labeled intravenous tracer for twice as long as the intragastric tracer. This precluded any calculations of the relative contributions of the two extracellular sources of phenylalanine to constitutive protein synthesis.

The present study was performed to address these issues. We used [U-13C]phenylalanine (given as part of an intragastric [U-13C]protein infusion) to increase the mass spectrometric sensitivity of our measurements and infused the enteral and intravenous tracers for the same period of time. We hypothesize that there is preferential utilization of arterial phenylalanine for mucosal protein synthesis and portal phenylalanine for hepatic protein synthesis.

METHODS

Animals

The study was approved by the Baylor College of Medicine Animal Protocol Review Committee. Housing and care of the animals conformed to United States Department of Agriculture (USDA) guidelines. The study involved seven 28-day-old female crossbred (Large White × Duroc × Hampshire) piglets purchased from the Texas Department of Criminal Justice, Huntsville, TX. The pigs were received at the USDA/Agricultural Research Service (ARS) Children’s Nutrition Research Center when they were 2 wk old and were fed a powdered milk replacer (Litter Life, Merrick, Union, WI) at a daily rate of 60 g/kg body wt.

Study Design

The pigs were fed the diet in powdered form for 10 days. Surgery was performed on day 11 after an overnight fast. The surgery entailed the placement of catheters in the portal vein, in a carotid artery, in an external jugular vein, and in the stomach – 2 cm from the pyloric sphincter. An ultrasonic flow probe (either model 6S or 6R, Transonics, Ithaca, NY) was implanted around the common portal vein. The surgical
procedures have been described in detail in previous publications (9, 21, 23) and were performed under isoflurane anesthesia using strict sterile techniques.

After the surgery, the pigs were offered 25% of their preceding daily intake that night. This was followed by 50% intake on the first postoperative day, resuming full-feed intake on the second day after surgery. The 6-h tracer infusion protocol was carried out 5 days after surgery, by which time the animals had been growing at preoperative rates (200 to 250 g/day) for at least 2 days.

Infusion Protocol

The pigs were deprived of feed from 1800 to 0700. At 0700, baseline arterial and portal blood samples were taken and the pigs consumed a meal of liquid Litter Life that supplied 1/24th of the preceding daily intake. At the same time, an intragastric infusion of an aqueous suspension of U-13C-labeled Spirulina platensis (Martek, Malvern, MA) and an intravenous infusion of [3H3(phe)n]phenylalanine ([3H3]phenylalanine) were started. Both tracers were infused at a rate of ~0.1 ml/min. The Spirulina infusion supplied ~15 µmol [U-13C]phenylalanine·kg⁻¹·h⁻¹, and the intravenous infusion supplied ~7 µmol [3H3]phenylalanine·kg⁻¹·h⁻¹. Throughout the infusion, the pigs consumed hourly meals of Litter Life. Each meal supplied 600 mg protein/kg and 134 µmol phenylalanine/kg. 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 pentobarbital sodium (50 mg/kg body wt) and sodium pentobarbitone (5 mg/kg) (Beutanasia-D; Schering-Plough Animal Health, Kenilworth, NJ). Blood samples were collected in EDTA tubes and were frozen in liquid nitrogen.

Immediately after death, the abdomen was opened, and the proximal 2 m of small intestine and an aliquot (~5 g from a lateral lobe) of liver were removed, weighed, and frozen in liquid nitrogen and used for subsequent measurement of protein content, protein-bound phenylalanine isotopic enrichment, and amino acid composition. The remainder of the liver was removed and weighed.

Sample Preparation

Blood phenylalanine. The isotopic and concentration measurements of phenylalanine 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 room temperature, through a 10-kDa cutoff filter. The filtrate was dried, and the amino acids were analyzed by reverse-phase HPLC of their phenylisothiocyanate derivatives (Picotag, Waters, Woburn, MA). For isotopic analysis, 0.1 ml of a 10-kDa filtrate was mixed with 0.25 ml of HC1 (0.1 mol/l) and applied to a 1-ml bed volume column of Dowex 50 W × 8 (H⁺ form) at 4°C. The amino acids were eluted with 5 M NH₄OH and dried under vacuum.

Plasma proteins. Plasma (700 µl) was carefully layered under 700 µl of a solution of NaCl (0.195 mol/l) and Na₂EDTA (1 mmol/l) at pH 7.4 (final specific gravity 1.006 kg/l). The solution was centrifuged at 22°C for 3 h at 210,000 g in a 100.3 rotor in a Beckman (Palo Alto, CA) TL-100 ultracentrifuge. The very low-density lipoprotein fraction was removed by aspiration and apoB-100 was precipitated with isopropanol (10). The fibrin fraction of fibrinogen was isolated by mixing 0.1 ml of plasma with 40 µl of an aqueous thrombin solution (1 × 10⁵ U/l) and 40 µl of CaCl₂ (25 mmol/l). The fibrin was washed three times with saline (22). The total plasma protein in 10 µl of plasma was precipitated with TCA (0.6 mol/l), centrifuged, and washed repeatedly with TCA. Albumin was then extracted from the precipitate with 5 µl TCA (0.6 mol/l) and 1.0 ml of 100% ethanol (14). The supernatant was then dried. The dried protein samples were hydrolyzed in 1 ml of 6 M HCl at 110°C for 24 h and then analyzed using mass spectrometry.

Tissue Proteins and Amino Acids

The mucosa from the intestinal segment was isolated by freeze-thaw disruption of the mucosal structure (19). Weighed aliquots (~5 g) of mucosa and liver were homogenized (Ultra Turrax, Tekmar, Germany) with water (1:1 wt/wt) at 4°C. One milliliter of the homogenate was then treated with 1 ml of perchloric acid (1 mol/l) and centrifuged (15,000 g for 10 min) in a Microfuge. The supernatant was removed 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 of precipitate was used for determination of protein by the Biuret method. The remaining protein was reprecipitated with 0.15–0.2 ml of perchloric acid (12.7 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. A known aliquot of the hydrolysate was dried, redissolved in HCl (0.1 mol/l), and used for amino acid analysis. A second aliquot was purified by ion exchange chromatography and used for mass spectrometry.

Mass Spectrometry

Mass spectrometric analysis of the amino acids was carried out with the n-propyl ester heptafluorobutyramide derivative (13). Gas chromatography was performed on a 5890 series II gas chromatograph (Hewlett Packard, Palo Alto, CA), using a DB5 fused silica column (J & W Scientific, Folsom, CA). Mass spectrometric analysis was by methane-negative chemical ionization using a 5999B (Hewlett Packard) quadruple mass spectrometer. Ions with a mass-to-charge ratio of 383–392 were monitored. All runs were performed at least in triplicate. The crude ion spectra were converted to tracer-to-tracee ratios using, as baseline, the ion spectrum of phenylalaninecate. The crude ion spectra were converted to tracer-to-tracee ratios using, as baseline, the ion spectrum of phenylalaninecate. The isotopic and concentration measurements of phenylalanine 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 room temperature, through a 10-kDa cutoff filter. The filtrate was dried, and the amino acids were analyzed by reverse-phase HPLC of their phenylisothiocyanate derivatives (Picotag, Waters, Woburn, MA). For isotopic analysis, 0.1 ml of a 10-kDa filtrate was mixed with 0.25 ml of HC1 (0.1 mol/l) and applied to a 1-ml bed volume column of Dowex 50 W × 8 (H⁺ form) at 4°C. The amino acids were eluted with 5 M NH₄OH and dried under vacuum.

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Calculations

Portal mass balance (in µmol·kg⁻¹·h⁻¹) was calculated as follows

\[ (\text{conc}_{\text{PORT}} - \text{conc}_{\text{ART}}) \times \text{PBF} \]  

(1)

in which conc is the concentration in whole blood (in µmol/l), PORT and ART refer to portal and arterial blood, respectively, and PBF is portal blood flow (in l·kg⁻¹·h⁻¹).

Portal tracer balance (in µmol tracer·kg⁻¹·h⁻¹) was calculated as follows

\[ (\text{conc}_{\text{PORT}} \times t/T_{\text{PORT}}) - (\text{conc}_{\text{ART}} \times t/T_{\text{ART}}) \times \text{PBF} \]  

(2)

in which t/T is the tracer-to-tracee ratio of the U-13C-labeled or [2H5]-labeled isotopomers.
Fractional portal balance (in percent input) was calculated as follows:

\[
\frac{\text{Portal balance}}{\text{input}} \times 100 \quad (3)
\]

For the portal enteral tracer, input is the intake of \([\text{U-}^{13}\text{C}]\text{phenylalanine}\) from the Spirulina. For the intravenous tracer, input is the arterial flux of \([\text{H}^2]\text{phenylalanine}\), i.e., \(\text{PBF} \times \text{arterial concentration} \times t/T \) of \([\text{H}^2]\text{phenylalanine}\). The fractional extraction of the tracer by the intestinal tissues is 1 – Eq. 3.

Mucosal protein tracer incorporation (in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\)) was calculated as follows:

\[
\text{Mucosal protein-bound phenylalanine (\(\mu\text{mol/kg BW}\))} \times \frac{t/T \text{ protein-bound tracer}}{} \quad (4)
\]

where BW is body weight.

Hepatic protein tracer incorporation (in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\)) was calculated as follows:

\[
\text{Hepatic protein-bound phenylalanine (\(\mu\text{mol/kg BW}\))} \times \frac{t/T \text{ protein-bound tracer}}{} \quad (5)
\]

Note that Eqs. 4 and 5 do not measure protein synthesis but estimate the molar tracer incorporation into the respective protein pools.

Total phenylalanine incorporation into mucosal protein (in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\)) was calculated next. Because the \([\text{U-}^{13}\text{C}]\text{phenylalanine}\) infusion rate and the arterial flux of \([\text{H}^2]\text{phenylalanine}\) to the PDV are known, the incorporation of the two tracers into mucosal protein can be expressed as a proportion of the respective tracer inputs. On the assumption that both the tracer and tracee phenylalanine are metabolized identically, then the total incorporation of unlabeled (tracee) phenylalanine into mucosal protein can be calculated as follows for the enteral tracer:

\[
\frac{[\text{U-}^{13}\text{C}]\text{phenylalanine incorporation}}{[\text{U-}^{13}\text{C}]\text{phenylalanine infusion}} \times \text{dietary phenylalanine intake} \quad (6)
\]

and for the intravenous tracer as

\[
\frac{[\text{H}^2]\text{phenylalanine incorporation}}{\text{arterial} \ [\text{H}^2]\text{phenylalanine} \text{ to the PDV} \times \text{arterial phenylalanine mass flux}} \quad (7)
\]

The fractional rate of mucosal protein synthesis (day\(^{-1}\)) can then be calculated as:

\[
\text{Eq. 6} + \text{Eq. 7} \times \frac{24}{6} \quad (8)
\]

It should be noted that this estimate of mucosal protein synthesis is not dependent on knowledge of the labeling of the protein synthetic precursor pool and is enabled because both the input and the incorporation of tracer can be calculated in molar terms. In the present study, the result from Eq. 8 was compared with estimates derived from the standard precursor-product calculation of the fractional rate of protein synthesis:

\[
\frac{t/T \text{ protein-bound tracer}}{t/T \text{ mucosal-free tracer}} \times \frac{24}{6} \quad (9)
\]

Fractional rate of hepatic constitutive protein, albumin, and fibrinogen (day\(^{-1}\)) was calculated as follows:

\[
\frac{t/T \text{ protein-bound tracer (at 6 h)}}{t/T \text{ apoB-100-bound tracer}} \times \frac{24}{6} \quad (10)
\]

For whole body phenylalanine entry rate measurements, as previously discussed (23), three separate estimates of the rate of phenylalanine entry (flux, \(Q\), in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\)) can be calculated from the tracer-to-tracee ratios of arterial \([^{13}\text{C}]\text{phenylalanine}\) and \([\text{H}^2]\text{phenylalanine}\). Two of these use the standard steady-state equation

\[
Q = R \times (\frac{t/T \text{ infused phenylalanine}}{t/T \text{ blood phenylalanine}} - 1) \quad (11)
\]

The two estimates for Eq. 11 are as follows: intravenous \(Q\) (\(Q_{IV}\)), where \(R\) is the rate of intravenous \([\text{H}^2]\text{phenylalanine}\) infusion, and intragastric \(Q\) (\(Q_{GI}\)), where \(R\) is the rate of intragastric \([\text{U-}^{13}\text{C}]\text{phenylalanine}\) infusion. The third estimate (\(Q_{IG}\)) was calculated as:

\[
Q_{IG} = \frac{\text{portal tracer balance}}{t/T \text{ arterial phenylalanine}} \quad (12)
\]

First-pass splanchnic extraction (in proportion of dose) was calculated as:

\[
Q_{IG} = Q_{IV} \quad (13)
\]

Hepatic extraction (in proportion of dose) was calculated as:

\[
\text{Splanchnic extraction} - \text{intestinal extraction} \quad (14)
\]

Three estimates of the entry of phenylalanine from proteolysis in the fed state (phenylalanine appearance from body protein (in \(\mu\text{mol phenylalanine} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))) can also be calculated from the three estimates of \(Q\):

\[
Q_{IG} - \text{phenylalanine intake} \quad (15a)
\]

Equation 15a gives an estimate of whole body entry that includes gut and hepatic protein metabolism

\[
Q_{IG} - \text{phenylalanine portal mass balance} \quad (15b)
\]

Equation 15b approximates to the whole body proteolysis, excluding gut metabolism

\[
Q_{IV} - \text{phenylalanine portal mass balance} \quad (15c)
\]

Equation 15c approximates to proteolysis in the extra-splanchnic tissues.

Because we did not obtain samples of hepatic venous blood, we were unable to make direct measurements of the hepatic balance of phenylalanine. We therefore adopted an indirect approach to the calculation of the uptake of amino acids by the liver. The reasoning is outlined below.

For a nutritionally essential amino acid, the only sources of the intracellular free pool are transport from the blood and entry from tissue proteolysis. When the primary pool of tracer
is the blood (as in the present experiment), then the isotopic enrichment of the tissue-free amino acid is lower than that of the blood amino acid, and the degree of isotopic dilution is a function of the relative rates of transport from the blood and proteolysis. Thus, if the rate of proteolysis and the isotopic enrichment of the amino acid in the blood and tissue-free pools are known, then the rate of uptake of the labeled amino acid from the blood can be calculated from:

\[
\frac{t/T \text{ hepatic free amino acid}}{t/T \text{ extracellular amino acid}} = \frac{\text{uptake from blood}}{\text{entry from proteolysis} + \text{uptake from blood}}
\]

Although we know that under the circumstances of this experiment there was net protein deposition in the liver, the rate of hepatic protein deposition (~3% per day) is much less than the rate of hepatic protein turnover (~40% per day). Thus the measured rate of hepatic protein synthesis is a close approximation to that of hepatic proteolysis. The rate of uptake of the tracee amino acid (the hepatic first-pass phenylalanine uptake, in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\)) was calculated with Eq. 10:

\[
\frac{t/T \text{ hepatic free amino acid}}{(0.75 \cdot t/T \text{ portal amino acid})} + (0.25 \cdot t/T \text{ arterial amino acid})
\]

uptake from blood

hepatic protein synthesis + uptake from blood

In which hepatic protein synthesis is equal to the fractional rate of hepatic protein synthesis (Eq. 10) multiplied by hepatic protein-bound phenylalanine pool size.

Note that in this calculation we have assumed that 75% of total hepatic blood flow is portal and that the portal and arterial amino acids contribute to the hepatic-free pool in proportion to their respective flow rates. However, because the tracer-to-tracee ratios of arterial and portal phenylalanine differed by only 20%, the assumption with regard to the distribution of hepatic blood flow between the arterial and portal inputs in fact has a negligible effect on the final value for hepatic tracer amino acid uptake.

**RESULTS**

The mean body weight of the pigs was 7.62 ± 0.31 kg. The mass of mucosa isolated from the proximal 2 m of the jejunum was 27.9 ± 4.1 g (3.66 ± 0.35 g/kg), and the protein-bound phenylalanine in this sample was 892 ± 122 \(\mu\text{mol} (117 ± 10 \mu\text{mol/kg}). It is important to note that the mucosal mass, and hence the phenylalanine content of the protein of the proximal 2 m of the jejunum, was 36% (P < 0.01) higher than in the previous groups of pigs that we have studied (23). The mean liver weight of the animals was 261 ± 15 g (34.3 ± 1.3 g/kg), and the protein-bound phenylalanine was 10,700 ± 1,900 \(\mu\text{mol} (1,400 ± 150 \mu\text{mol/kg}).

The time courses of labeling of the two tracers in arterial and portal blood and in apoB-100 are shown in Figs. 1 and 2. The data show that isotopic steady state between the apparent phenylalanine flux measured with the two tracers are similar in the two tracers as shown in Table 2. All the values for phenylalanine entry rate were higher than our previous estimates in fed piglets, and the difference between the apparent phenylalanine flux measured with the two tracers suggested that first-pass utilization of the intragastric tracer by the tissues of the gut.

**Fig. 1.** Time course of intragastrically infused [U-\(^{13}\)C]phenylalanine labeling. Values are means ± SE for 7 animals for arterial, portal, and apoB-100-bound intragastrically administered [U-\(^{13}\)C]phenylalanine. ApoB-100-phenylalanine was slightly but not significantly less enriched with the \(^{13}\)C-labeled isotopomer than arterial phenylalanine.

**Fig. 2.** Time course of intravenously infused [\(^{2}\)H\(_5\)]phenylalanine labeling. Values are means ± SE for 7 animals for arterial, portal, and apoB-100-bound intravenously administered [\(^{2}\)H\(_5\)]phenylalanine. ApoB-100 phenylalanine was significantly less enriched than arterial phenylalanine.
Table 1. Arterial and portal phenylalanine concentrations and tracer-to-tracee ratios and portal balance of 
intragastric [U-13C]phenylalanine and intravenous [1H3]phenylalanine

<table>
<thead>
<tr>
<th>Blood Flow, l·kg⁻¹·h⁻¹</th>
<th>Arterial Concentration, µmol/l</th>
<th>Portal Concentration, µmol/l</th>
<th>Arterial Intraocular Tracer, mol%</th>
<th>Portal Intraocular Tracer, mol%</th>
<th>Arterial Intravenous Tracer, mol%</th>
<th>Portal Intravenous Tracer, mol%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.12 ± 0.49</td>
<td>100 ± 19</td>
<td>119 ± 20</td>
<td>4.41 ± 1.25</td>
<td>5.60 ± 1.74</td>
<td>5.64 ± 1.63</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intraocular Infusion, µmol·kg⁻¹·h⁻¹</th>
<th>Balance of Intraocular Tracer, µmol·kg⁻¹·h⁻¹</th>
<th>% Intraocular Tracer</th>
<th>Intraocular Arterial Flux, µmol·kg⁻¹·h⁻¹</th>
<th>Balance of Intraocular Tracer, µmol·kg⁻¹·h⁻¹</th>
<th>% Intraocular Flux</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.68 ± 0.32</td>
<td>7.03 ± 1.20</td>
<td>46.7 ± 7.9</td>
<td>17.0 ± 1.25</td>
<td>−1.04 ± 0.21*</td>
<td>6.41 ± 1.3</td>
</tr>
</tbody>
</table>

Values are means ± SD for 7 animals. *Extraction of arterial [1H3]phenylalanine by the portal-drained viscera (PDV) as a whole.

Table 5 summarizes the data on hepatic and plasma protein labeling. As we have observed previously, the steady-state isotopic enrichment of apoB-100-bound phenylalanine was approximately threefold higher than that of the bulked free phenylalanine pool. Furthermore, the ratios of the isotopic enrichments of the two tracers in arterial and portal phenylalanine were significantly different. The ratios of the tracers in apoB-100, hepatic constitutive protein, as well as in plasma albumin and fibrinogen were similar to one another and were not significantly different from the ratio of the two tracers in portal phenylalanine. The fractional rates of synthesis of the various proteins calculated with the two tracers were essentially the same when the calculation used the isotopic enrichment of apoB-100 phenylalanine as the denominator in the calculation.

In Table 4, we have used the data on mucosal protein labeling (Table 3) and the portal balances of the two tracers (Table 1) to calculate the absolute quantities of dietary and arterial phenylalanine incorporated into mucosal protein. These calculations suggest that, despite the channeling of arterial phenylalanine into mucosal protein synthesis, 59% of the mucosal protein-bound phenylalanine was derived from the diet. The estimated fractional rate of mucosal protein synthesis (70 ± 8% per day) calculated from these data was significantly (P < 0.05) lower than either of the estimates based on the relative labeling of the protein-bound and free pools of phenylalanine.

Table 2. Whole body phenylalanine kinetics and apparent rates of body protein breakdown calculated with the intravenous and intragastric tracer data

<table>
<thead>
<tr>
<th>Estimate</th>
<th>Apparent Phenylalanine Flux</th>
<th>Phenylalanine Intake</th>
<th>Body Protein Breakdown</th>
</tr>
</thead>
<tbody>
<tr>
<td>QIG</td>
<td>340 ± 96</td>
<td>150</td>
<td>190 ± 96</td>
</tr>
<tr>
<td>QIG</td>
<td>159 ± 36</td>
<td>59.4 ± 12</td>
<td>99 ± 23</td>
</tr>
<tr>
<td>QIV</td>
<td>124 ± 36</td>
<td>59.4 ± 12</td>
<td>65 ± 14</td>
</tr>
</tbody>
</table>

Values are means ± SD (in µmol·kg⁻¹·h⁻¹) for 7 animals.

DISCUSSION

Protein synthesis in the gastrointestinal tract and the liver makes a disproportionate contribution to whole body protein turnover. Exact quantification of the rates of protein synthesis in these two organs has proved difficult because of inexact knowledge of the sources of amino acids used to support protein synthesis and the problems that are posed by the metabolic compartmentation of the free amino acid pools of both tissues. This compartmentation has been revealed by a variety of approaches, including direct measurements of aminoacyl-tRNA labeling in the liver (2, 25), by measurements of the steady-state isotopic enrichments of rapidly turning over proteins of hepatic (6, 18, 20) and mucosal (7, 8) origin, and by the results of other dual-tracer studies (1, 3, 17). The aim of this study was...
from the proximal jejunum

present work, performed with a similar diet but using a 76% occurred in the intestine. These data were similar enteral tracer accounted for 45% of dose, and of this in fed piglets, first-pass splanchnic metabolism of the resulting from incomplete digestion of the U-13C-labeled phenylalanine, splanchnic first-pass tracer phenylalanine tracers indicated that, simultaneous intragastric (1-13C-labeled) and intrave-

First-Pass Metabolism of Enteral Amino Acids

There is now extensive literature on the first-pass splanchnic extraction of enteral amino acids, especially in humans (e.g., Refs. 5, 11, 12, 16). These studies revealed variations among amino acids with values ranging from 50% for phenylalanine (5) to <25% for leucine (12, 16). Our previous measurements with simultaneous intragastric (1-13C-labeled) and intravenous (2H5-labeled) phenylalanine tracers indicated that, in fed piglets, first-pass splanchnic metabolism of the enteral tracer accounted for 45% of dose, and of this 76% occurred in the intestine. These data were similar to earlier measurements in adult dogs (26, 27). In the present work, performed with a similar diet but using a U-13C-labeled protein as the source of enteral tracer phenylalanine, splanchnic first-pass tracer phenylalanine metabolism was 63% of dose, a value that is 40% higher than that in our previous report. This reflected an apparently higher first-pass utilization (53% of dose) of phenylalanine by the intestine.

Although it might be argued that the difference in apparent intestinal first-pass metabolism might have resulted from incomplete digestion of the U-13C-labeled protein tracer, we think this is unlikely because the portal balance of unlabeled phenylalanine (40% of intake) was also significantly lower, in relation to intake, than in our previous study (23). We believe that the difference between this study and our past results reflects differences in the husbandry of the animals used in the two studies. In our previous work, animals were purchased from a research facility (Texas A & M Univ., College Station, TX) where they had been held under relatively sterile conditions. The present animals were purchased from the Texas Dept. of Criminal Justice and had been housed outside. Hence, they had the opportunity to root and had probably ingested soil and fibrous material. This, we believe, underlies the fact that the pigs obtained from the Texas Dept. of Criminal Justice had a significantly (36%; P < 0.05 by paired t-test) greater mucosal mass than that of the pigs obtained from the research facility. It seems likely, therefore, that the increased first-pass metabolism of the enteral tracer and dietary phenylalanine resulted from a higher rate of enteral amino acid metabolism. This, in turn, implies that environmental factors that affect mucosal mass, including increased exposure to potential pathogens, have a measurable and nutritionally significant effect on the systemic availability of dietary amino acids.

In contrast to the results on intestinal metabolism, the estimates of first-pass hepatic utilization of dietary phenylalanine between the two studies were similar. In our previous study, the difference between total splanchnic first-pass extraction and intestinal extraction was 10.9% of dose, whereas the present results suggest a value of 9.9% of dose. Furthermore, calculations of hepatic proteolysis in the previous and the present study were similar.

Sources for Mucosal and Hepatic Protein Synthesis

In previous studies, we presented evidence (based on comparisons of the relative labeling of intra-arterial and

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Free Phenylalanine</th>
<th>Protein-Bound Phenylalanine</th>
<th>Protein-to-Free Ratio</th>
<th>Apparent Kp, day⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra gastric</td>
<td>3.98 ± 0.85</td>
<td>0.99 ± 0.37</td>
<td>0.23 ± 0.08</td>
<td>0.92 ± 0.32</td>
</tr>
<tr>
<td>Intravenous</td>
<td>0.79 ± 0.08</td>
<td>0.29 ± 0.08</td>
<td>0.37 ± 0.11 *</td>
<td>1.48 ± 0.44 *</td>
</tr>
<tr>
<td>Intravenous-to-intra gastric</td>
<td>0.198 ± 0.053</td>
<td>0.317 ± 0.089 †</td>
<td>1.61 ± 0.28</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD (in mol%) for 7 animals. Kp, fractional rate of protein synthesis. *Significantly different from intra gastric tracer (P < 0.05 by paired t-test). †Significantly different from the intravenous-to-intra gastric ratio in the free phenylalanine pool (P < 0.01 by paired t-test).

Table 4. Relative contributions of arterial and dietary phenylalanine to the protein synthesized by the proximal jejunal mucosa

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Mucosal Protein Phe, µmol</th>
<th>Protein Tracer-to-Tracer Ratio, mol%</th>
<th>Tracer Incorporation</th>
<th>Unlabeled Phe Input, µmol · kg⁻¹ · h⁻¹</th>
<th>Total Phe Incorporation, µmol · kg⁻¹ · h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intragastric</td>
<td>892 ± 122</td>
<td>0.99 ± 0.17</td>
<td>8.8 ± 1.8</td>
<td>0.198 ± 0.031</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Intravenous</td>
<td>633 ± 0.03</td>
<td>2.9 ± 0.3</td>
<td>0.055 ± 0.005</td>
<td>7.9 ± 1.1</td>
<td>20 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SD for 7 animals. Mucosal protein phenylalanine (Phe) was measured in the first 2 m of the jejunum and corresponds to a value of 117 ± 10 µmol/kg body wt. For the intravenous tracer, incorporation is expressed as a percent of the unidirectional removal of arterial [2H5]phenylalanine by the PDV (see Table 1). For the intragastric tracer, incorporation is expressed as the proportion of the tracer infused. For intragastric phenylalanine incorporation, input is phenylalanine intake. For arterial phenylalanine incorporation, input is the uptake of phenylalanine by the PDV, i.e., arterial phenylalanine flux × fractional extraction of [2H5]phenylalanine (see Table 1). Total phenylalanine incorporation (3.44 µmol · kg⁻¹ · h⁻¹) is equivalent to a fractional rate of mucosal protein synthesis of 0.70 ± 0.08 day⁻¹ [i.e., (3.44/117) × 24]. This is significantly lower than the values derived from the labeling of protein-bound and free phenylalanine (Table 3).
intravenous tracer phenylalanine in arterial and portal blood and in apoB-100 and fibrinogen) that suggested preferential use of portal phenylalanine for the production of hepatic secretory proteins. These data were similar to the results of other investigations of albumin synthesis in humans (6) and fibrinogen synthesis in dogs (3). Because of metabolic zonation in the liver, the results could have reflected compartmentation of secretory protein synthesis, i.e., preferential synthesis of secreted proteins in periportal hepatocytes. Therefore, a primary objective of the present study was to extend the data to the synthesis of hepatic constitutive proteins.

Table 6. Calculated first-pass utilization of portal phenylalanine for hepatic constitutive protein synthesis

| Tracer Route                | Hepatic Incorporation of [U-13C]phenylalanine, µmol/liver | Portal tracer-to-tracee ratio of protein-bound phenylalanine, mol% | K\textsubscript{a}, day\textsuperscript{-1} | Portal tracer-to-tracee ratio of apoB-100 | Hepatic uptake of blood phenylalanine, µmol·kg\textsuperscript{-1}·h\textsuperscript{-1} | Contribution of protein synthesis to first-pass utilization of portal phenylalanine | Hepatic uptake of phenylalanine, µmol·kg\textsuperscript{-1}·h\textsuperscript{-1} | Contribution of protein synthesis to first-pass utilization of phenylalanine | Hepatic free-to-blood phenylalanine labeling ratio | Hepatic protein synthesis from hepatic proteolysis, µmol·kg\textsuperscript{-1}·h\textsuperscript{-1} | Hepatic uptake of phenylalanine from hepatic proteolysis, µmol·kg\textsuperscript{-1}·h\textsuperscript{-1} | Phenylalanine entry from hepatic proteolysis, µmol·kg\textsuperscript{-1}·h\textsuperscript{-1} | Contribution of phenylalanine entry from hepatic proteolysis to hepatic protein synthesis, µmol·kg\textsuperscript{-1}·h\textsuperscript{-1} |
|----------------------------|-----------------------------------------------------------|------------------------------------------------------------------|--------------------------|----------------------------------------|-----------------------------------------|---------------------------------------------|---------------------------------------------|--------------------------|---------------------------------------------|-----------------------------------------------|---------------------------------------------|-----------------------------------------------|-----------------------------------------------|}

<table>
<thead>
<tr>
<th>Free phenylalanine</th>
<th>Protein-bound phenylalanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>10.708 ± 1.897</td>
</tr>
</tbody>
</table>

Values are means ± SD for 7 animals. The present results confirm our earlier observation that the steady-state isotopic enrichment of apoB-100 considerably exceeded that of the hepatic-free phenylalanine, suggesting channeling of extracellular phenylalanine to hepatic protein synthesis. In addition, on the basis of the relative isotopic enrichments of the two tracers in the various protein-bound pools of phenylalanine, we conclude that hepatic proteins, both secretory and constitutive, were derived almost exclusively from portal phenylalanine. The tracer incorporation results in the mucosa also confirmed earlier observations of simultaneous utilization of both arterial and enteral phenylalanine for mucosal protein synthesis (1, 17) and suggested that there was also channeling of arterial phenylalanine toward protein synthesis. Even so, when the molar rates of phenylalanine delivery from the diet and the arterial circulation were taken into account, 59% of the mucosal protein-bound phenylalanine was of direct dietary origin.

In the liver, firm calculations of the fractional rate of protein synthesis can, we believe, be made by using apoB-100 labeling to define that of the hepatic protein synthetic precursor pool. However, because of uncertain knowledge of the true isotopic enrichment of the mucosal protein synthetic precursor pool, the mucosal protein labeling cannot be used to calculate accurate estimates of the rate of mucosal protein synthesis. Indeed, because of the channeling of arterial phenylalanine, the two tracers gave significantly different estimates of the mucosal protein synthesis rate. In the present work, we were able to express the mucosal tracer incorporation data as a proportion of the unidirectional uptake of the enteral (tracer infusion rate) and arterial (portal [7H\textsubscript{3}]phenylalanine balance) tracers. This allowed the calculation of the absolute rate of mucosal protein synthesis, i.e., moles unlabeled phenylalanine incorporated per unit time. This, in turn, allows an estimate of the fractional rate of mucosal protein synthesis without knowledge of the isotopic enrichment of the protein synthetic precursor pool. It
was notable therefore that the value so derived was significantly less than the values that were based on conventional precursor-product calculations. This observation implies that the isotopic enrichment of the mucosal protein synthetic precursor pool of phenylalanine was significantly higher than that of the mixed acid, soluble-free pool. This conclusion is supported by the recent demonstration in fed piglets that showed that during a [1H]leucine infusion the steady-state isotopic enrichment of prolactase exceeds that of the mucosal-free leucine pool (8).

It appears, therefore, that in both the liver and the intestinal mucosa extracellular amino acids are channeled to protein synthesis without mixing freely with the intracellular pool of phenylalanine. The reverse is also implied, i.e., that phenylalanine derived from proteolysis is recycled to only a limited extent within the cell.

The cellular mechanisms that underlie these kinetic observations remain obscure. It may be important that both tissues are specialized with regard to the regulated extraction from and the release of amino acids to the extracellular compartment, and it is possible that inward and outward amino acid transport proceeds at different sites. Such a mechanism is easy to envision in the enterocyte because the arterial and luminal contributions to the free pool arise from transport at two functionally distinct surfaces (the brush-border and basolateral membranes) and there is no reason to believe that amino acids transported across the basolateral membrane necessarily suffer the same metabolic fate as newly transported dietary amino acids. Indeed, there is evidence that shows metabolic channeling of other dietary amino acids in the mucosa (19).

With regard to compartmentation of the free amino acid pool of the liver, Vidrich et al. (25) developed a statistical model for amino acid metabolic compartmentation in the liver in which they argued for the idea of specific activation of amino acids located on the inner face of the amino acid transporters. The implication of this model is that, in the fed state, in which inward transport exceeds outward transport, there is preferential use of newly transported amino acids for protein synthesis. The model and our results also imply that amino acids derived from hepatic proteolysis are specifically transported across the basolateral membrane necessarily suffer the same metabolic fate as newly transported dietary amino acids. Indeed, there is evidence that shows metabolic channeling of other dietary amino acids in the mucosa (19).

The third possibility is that the rate of protein synthesis in the periportal hepatocytes is considerably higher than in the perivenous cells, so that the apparent preferential utilization of portal amino acids reflects merely the fact that protein synthesis in periporal hepatocytes dominates the overall protein metabolic activity of the liver. There is some autoradiographic evidence to support this proposition (15).

However, irrespective of the underlying mechanism, the present data extend the growing body of evidence that suggests that (1) intestinal amino acid metabolism quantitatively dominates overall splanchnic metabolism and (2) dietary amino acids play a role as direct protein synthetic precursors for mucosal and hepatic protein synthesis.

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