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

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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]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 body wt and sodium phenytoin (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 HCl (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 H₂SO₄ 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/vt) 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 (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. 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 5998B (Hewlett Packard) quadrupole 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 phenylalanine purified from samples taken immediately before the tracer infusions commenced.

Calculations

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

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

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

\[ \frac{\left( \text{ conc}_\text{PORT} \times t/T_\text{PORT} \right) - \left( \text{ conc}_\text{ART} \times t/T_\text{ART} \right)}{\text{ PBF} \times 10^3} \]

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

\[ \text{Portal balance} = \frac{\text{input}}{\text{input}} \times 100 \]  

(3)

For the portal enteral tracer, input is the intake of \([\text{U-13C}]\)phenylalanine from the Spirulina. For the intravenous tracer, input is the arterial flux of \([2H_5]\)phenylalanine, i.e., PBF \times arterial concentration \times t/T of \([H_2]\)phenylalanine. The fractional extraction of the tracer by the intestinal tissues is 1 – Eq. 3.

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

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

(4)

where BW is body weight.

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

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

(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 \(\mu\text{mol·kg}^{-1} \cdot \text{h}^{-1}\)) was calculated next. Because the \([\text{U-13C}]\)-phenylalanine infusion rate and the arterial flux of \([H_2]\)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

\[ \frac{[\text{U-13C}]\text{phenylalanine incorporation}}{[\text{U-13C}]\text{phenylalanine infusion}} \times \text{dietary phenylalanine intake} \]  

(6)

and for the intravenous tracer as

\[ \frac{[H_2]\text{phenylalanine incorporation}}{\text{arterial flux} \times [H_2]\text{phenylalanine to the PDV}} \times \text{arterial phenylalanine mass flux} \]  

(7)

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

\[ \frac{\text{Eq. 6} + \text{Eq. 7}}{\text{mucosal protein-bound phenylalanine pool}} \times \frac{24}{6} \]  

(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

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

(9)

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

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

(10)

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

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

(11)

The two estimates for Eq. 11 are as follows: intravenous \(Q\) (\(Q_{IV}\)), where \(R\) is the rate of intravenous \([H_2]\)phenylalanine infusion, and intragastric \(Q\) (\(Q_{IG}\)), where \(R\) is the rate of intragastric \([U-13C]\)phenylalanine infusion. The third estimate (\(Q_{IG}\)) was calculated as

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

(12)

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

\[ Q_{IG} - Q_{IV} \]  

(13)

Hepatic extraction (in proportion of dose) was calculated as

Splanchnic extraction – Intestinal extraction  

(14)

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

\[ Q_{IG} – \text{phenylalanine intake} \]  

(15a)

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

\[ Q_{IG} – \text{phenylalanine portal mass balance} \]  

(15b)

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

\[ Q_{IV} – \text{phenylalanine portal mass balance} \]  

(15c)

Equation 15c approximates to proteolysis in the extraspacial 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 \(\mu\)mol \(\cdot \)kg\(^{-1} \cdot \)h\(^{-1}\)) was calculated with Eq. 16:

\[
\text{uptake from blood} = \frac{(0.75 \cdot t/T \text{ arterial amino acid}) + (0.25 \cdot t/T \text{ portal amino acid})}{\text{hepatic protein synthesis} + \text{uptake from blood}}
\]

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 \(7.62 \pm 0.31\) kg. The mass of mucosa isolated from the proximal 2 m of the jejunum was \(27.9 \pm 4.1\) g (3.66 \(\pm\) 0.35 g/kg), and the protein-bound phenylalanine in this sample was \(892 \pm 122\) \(\mu\)mol (117 \(\pm\) 10 \(\mu\)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 \(261 \pm 15\) g (34.3 \(\pm\) 1.3 g/kg), and the protein-bound phenylalanine was \(10,700 \pm 1,900\) \(\mu\)mol (1,400 \(\pm\) 150 \(\mu\)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 (intragastric tracer) and 2 (intravenous tracer). The data show that isotopic steady state in all three pools was achieved by \(\sim 3\) h of infusion. Data on the mass and tracer balances at steady state are summarized in Table 1. On average, the portal phenylalanine mass balance (59.6 \(\mu\)mol \(\cdot\)kg\(^{-1} \cdot\)h\(^{-1}\)) was only 40% of phenylalanine intake, and the portal balance of the intragastric tracer (7 \(\mu\)mol \(\cdot\)kg\(^{-1} \cdot\)h\(^{-1}\)) accounted for 46.7% of the infusion. Both values were also significantly less than the values reported previously (50 and 66%, respectively; Refs. 23, 24). The difference between the fractional mass and enteral tracer balances was significant (\(P < 0.05\)) and suggested a continuing utilization of arterial phenylalanine by the PDV. This was confirmed by direct measurements of the balance of the intravenous tracer across the PDV, which showed that 6.4% of the arterial tracer flux of the intravenous [\(^2\)H\(_5\)]phenylalanine tracer was removed across this tissue bed.

Whole body phenylalanine kinetics calculated with the two tracers are summarized 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
Table 1. Arterial and portal phenylalanine concentrations and tracer-to-tracee ratios and portal balance of intragastric [U-13C]phenylalanine and intravenous [3H]phenylalanine

<table>
<thead>
<tr>
<th>Blood Flow, l·kg⁻¹·h⁻¹</th>
<th>Concentration, µmol/l</th>
<th>Intragastric Tracer, mol%</th>
<th>Intraoperative Tracer, mol%</th>
<th>Balance of Intragastric Tracer</th>
<th>Balance of Intraoperative Tracer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>Intravenous</td>
<td>Arterial</td>
<td>Portal</td>
<td>Arterial</td>
<td>Portal</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>Arterial</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Portal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<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>

Values are means ± SD for 7 animals. * Extraction of arterial [3H]phenylalanine by the portal-drained viscera (PDV) as a whole.

splanchnic bed accounted for 63% of dose. Of this, 83% (54% of dose) represented first-pass intestinal tracer metabolism. The hepatic extraction of the tracer was 9.9% of dose, a value similar to our previous estimates (10.9% of dose; Ref. 23). Entry of phenylalanine from hepatic proteolysis, calculated from the difference between lines 2 and 3 of Table 2, was 34 ± 7 µmol·kg⁻¹·h⁻¹.

The labeling of mucosal phenylalanine is shown in Table 3. The relative contributions of the two tracers to the mucosal-free phenylalanine pool (intravenous-to-intragastric ratio of 0.20) were significantly (P < 0.05) different from their contributions to protein labeling (intravenous-to-intragastric ratio of 0.32), a result that suggests preferential utilization of the arterial phenylalanine for protein synthesis. The apparent fractional rates of mucosal protein synthesis calculated with the two tracers were also different (P < 0.01).

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 channelling 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 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 6, we used the data on hepatic protein synthesis and the isotopic enrichments of extracellular and intracellular phenylalanine to calculate the uptake of phenylalanine in first pass by the liver. The estimated rate of phenylalanine entry from hepatic proteolysis (27 ± 5 µmol·kg⁻¹·h⁻¹) was similar to that calculated from blood phenylalanine kinetics and shown in Table 2 (34 ± 7 µmol·kg⁻¹·h⁻¹). The calculated first-pass uptake of phenylalanine (11.6 µmol·kg⁻¹·h⁻¹) was 18% of the portal balance. Of the phenylalanine removed by the liver in first pass, we estimate that 64% was incorporated into hepatic constitutive protein.

**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...
to quantify the degree of first-pass utilization of enteral phenylalanine within the intestine and liver and to measure the relative contributions of dietary (or portal) and arterial phenylalanine to constitutive protein synthesis in the liver and the proximal jejunal mucosa.

**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 dose of phenylalanine by the intestine. These data were similar to those in our previous report. This reflected an apparently higher first-pass utilization (53% of dose) of phenylalanine in the intestine and liver and to quantify the degree of first-pass utilization of enteral phenylalanine within the intestine and liver and to measure the relative contributions of dietary (or portal) and arterial phenylalanine to constitutive protein synthesis in the liver and the proximal jejunal mucosa.

<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 intragastric 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).

In previous studies, we presented evidence (based on comparisons of the relative labeling of intra gastric and arterial phenylalanine to constitutive protein synthesis in the liver and the proximal jejunal mucosa) 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 gastric and arterial phenylalanine to constitutive protein synthesis in the liver and the proximal jejunal mucosa) 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.

**Table 4. Tracer-to-tracer ratios (mol%) of free and protein-bound [U13C]- and [2H5]phenylalanine in the mucosa from the proximal jejunum**

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Protein Bound Phe, µmol</th>
<th>Protein Tracer-to-Tracee 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>Intra gastric</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>82 ± 0.33</td>
<td>0.33 ± 0.05</td>
<td>2.9 ± 0.3</td>
<td>0.065 ± 0.005</td>
<td>7.9 ± 2.1</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).
in vivo 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 compartmentalization 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.

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 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.

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 extravascular 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.

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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 channelled 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 lumenal 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 as protein synthesis. The model and our results also imply that amino acids derived from hepatic proteolysis are specifically transported out of the hepatocyte. Interestingly, Vidrich et al. (25) also found that as rats approached the fasted state (a condition in which the rates of inward and outward transport are closer and hepatic proteolysis is increased), the equilibration between intracellular and aminoacyl-tRNA-bound valine labeling became more complete. This observation has been confirmed recently with regard to both leucine and phenylalanine in fasted pigs (2). In this context, it might be of significance that lysosomal proteolysis is particularly important in the liver and that the lysosome represents a compartment that is physically separated from the cytosolic amino acid pool. That being so, it is possible that release of amino acids and/or small peptides from the lysosome is predisposed toward outward transport rather than reentry into the aminoacyl-tRNA pool.

The other hepatic protein metabolic phenomenon that demands explanation is the evidence from the work of ourselves (23) and others (3, 4, 6) that portal, rather than arterial, amino acids are used preferentially for protein synthesis in the liver of fed animals and humans. These previous works concentrated on secretory protein synthesis, and it seemed to us entirely possible that the differences reflected functional rather than kinetic zonation. However, the present results suggest that the same compartmentation also applies to hepatic resident protein synthesis, in which case an argument based on functional zonation is more difficult to sustain.

There seems to us to be three possible explanations. First, the contribution of arterial blood flow to total hepatic blood flow could be much lower than is generally presumed. This seems unlikely largely because the hemoglobin oxygen saturation in the portal blood of fed piglets is low and a low hepatic arterial flow would compromise the energy status of the hepatocytes.

Second, although it is generally held that mixing of capillary blood derived from the portal and arterial circulations occurs within a very short distance of their respective entry into the acinar unit, hepatocytes may possibly show some degree of functional polarization, in which different amino acid transporters are exposed to the arterial and portal inputs. Although we know of no evidence for this proposition, it is noteworthy that the ratio of the two tracers in the hepatic-free amino acid pool was similar to the ratio found in portal blood, an observation that indicates preferential transport of amino acids derived from this input.

The third possibility is that the rate of protein synthesis in the perportal 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 perportal 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.

We are grateful to Leslie Loddeke for her sound editorial advice.

This work was supported in part by National Heart, Lung, and Blood Institute Grant RO1-HD-33920 (D. G. Burrin) and by federal funds from the USDA/ARS Cooperative Agreement 58-6250-6-001. B. Stoll was supported in part by the Alexander von Humboldt-Stiftung fund.
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