Phenylalanine utilization by the gut and liver measured with intravenous and intragastric tracers in pigs

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Stoll, Barbara, Douglas G. Burrin, Joseph Henry, Farook J ahooor, and Peter J. Reeds. Phenylalanine utilization by the gut and liver measured with intravenous and intragastric tracers in pigs. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G1208–G1217, 1997.—To investigate intestinal and hepatic metabolism of phenylalanine, four conscious pigs (7.5 kg), bearing arterial, venous, and hepatic portal catheters, were fasted for 12 h and infused with [phenyl-2H5]phenylalanine via a peripheral vein and [carboxyl-13C]phenylalanine via the stomach. During the first 6 h of the infusion, the pigs remained fasted and received only the intravenous tracer. During the second 6 h, they received an intragastric infusion of milk replacer and both tracers. In the fasted state, the portal-drained viscera extracted 10% (P < 0.025) of the arterial [2H5]phenylalanine flow of the pigs. In the fed state, the splanchic tissues metabolized 45% of the enteral tracer and intestinal metabolism accounted for 76% of the total splanchnic extraction. The tracer-to-tracer ratio of both tracers in apolipoprotein B-100 (apo B-100) phenylalanine was twofold (P < 0.001) higher than that of hepatic free phenylalanine. The ratios of the two tracers in portal (2H5/13C; 1.66) and apo B-100 (1.76) phenylalanine were similar but higher (P < 0.05) than that of arterial phenylalanine (1.29). We conclude that intestinal metabolism dominates the splanchnic extraction of enteral phenylalanine and that in the fed state, the hepatic protein synthetic precursor pool derives from portal phenylalanine.

THE INABILITY TO ACCURATELY determine the source of amino acids used for protein synthesis poses particular difficulties for studies of the pathways of hepatic and intestinal mucosal amino acid utilization. The degree to which tracer amino acids are diluted in the intracellular pool of both organs is substantial (1, 15, 19, 26). Moreover, in the fed state, the amino acid supply of the intestine and liver derives in part from dietary amino acids, so that the cells of both tissues are exposed to two extracellular inputs of amino acids with different isotopic enrichments. Furthermore, there is substantial first-pass utilization of enteral amino acids within the splanchnic bed as a whole (7, 20, 21, 25); this reflects metabolism in both the intestine (35) and liver (35, 36), and the intestinal mucosa can use both enteral and arterial amino acids for protein synthesis (2).

The most direct method of identifying the sources of amino acids used for cellular protein synthesis is to measure the isotopic enrichment of the appropriate amino acyl tRNA. This is not a simple undertaking, as shown strikingly by the very limited literature on this subject. Moreover, much of the literature has been concerned with skeletal muscle (5, 34), a tissue in which kinetic compartmentation of the free amino acid pool may well be relatively minor. With the exception of radioisotopic studies published in the 1970s (1, 33), there is little information on the liver and, to our knowledge, no measurements of amino acyl tRNA labeling in the mucosa.

Various approaches have been used either to avoid the compartmentation problem or to infer, indirectly, the isotopic labeling of the protein synthetic precursor pool. The so-called “flooding-dose” method, designed to minimize the differences between the labeling of the intracellular and extracellular amino acid pools, has been used extensively in animals and applied to the measurement of albumin synthesis in humans (3). We (6, 22, 28) and Cayol et al. (9), Cryer et al. (10), Lichtenstein et al. (24), and Parhofer et al. (27) have proposed that the equilibrium labeling of apolipoprotein B-100 isolated from the very low density lipoprotein fraction (VLDL apo B-100) can be used to estimate the isotopic enrichment of the hepatic protein synthetic precursor pool. The plateau isotopic enrichments of both prosucrase and prolactase have also been used as the basis for calculating protein synthesis in the small intestinal mucosa (14, 15).

The apo B-100 method is particularly useful for studies in humans. It is minimally invasive and lends itself to studies of the kinetics of the hepatic amino acid pool. The approach has been used to investigate the relationship between both feeding status (6, 28) and protein intake (9) and hepatic amino acid labeling. A recent study involving dual intragastric and intravenous infusions of leucine (9) applied the method to the determination of plasma protein synthesis in humans. The results of these studies suggest that extracellular amino acids in general may be used disproportionately for apo B-100 synthesis and that in the fed state there may be preferential incorporation of amino acids derived from the diet.

Although the kinetic basis of using the steady-state labeling of apo B-100 to measure the isotopic enrichment of its own precursor pool is theoretically sound given the metabolic and circulatory zonation of the liver, it is possible that the steady-state labeling of apo B-100 is not reflective of the precursor pool used for the synthesis of other hepatic proteins. On the basis of comparisons of albumin synthesis determined with different amino acids (22) or with the same tracer given either intravenously or intragastrically (9), it has been concluded that apo B-100 is a good index of the labeling of the hepatic protein synthetic precursor pool. How-
ever, as far as we can ascertain, there is no direct in vivo information on the relationship between apo B-100 labeling and the hepatic free amino acid pool.

We undertook the present study with the following objectives: 1) to measure the first-pass utilization of arterial and portal phenylalanine by the portal-drained viscera, 2) to partition total splanchnic utilization of enteral phenylalanine between the intestine and the liver, 3) to quantify the incorporation of the intragastroscopic tracer into mucosal and hepatic protein, 4) to investigate the relationship between the labeling of phenylalanine in portal and arterial blood, in VLDL-apo B-100, and in the bulked hepatic free amino acid pool, and finally, 5) to investigate further whether apo B-100 is a valid index for calculating the synthesis of other plasma proteins of hepatic origin.

To achieve these objectives, we carried out dual intravenous and intragastric infusions of [carboxyl-13C]- and [phenyl-2H5]phenylalanine in fasted and fed piglets bearing catheters in the arterial and portal circulation. Because this approach allowed the quantification of tracer and tracee balances across the portal-drained viscera (see also Refs. 35, 36), as well as the measurement of the apparent rates of whole body phenylalanine turnover measured with intragastric and intravenous tracers, we were able to quantify the contributions of the gut and the liver to splanchnic phenylalanine utilization. Furthermore, by examining the relationship between the tracer-to-tracee ratios of the two tracers in different phenylalanine compartments, we were able to examine in more detail the precursor product relationships between the various amino acid pools and their potential protein products. On the basis of the literature, we hypothesized that intestinal metabolism would account for the majority of the splanchnic extraction of enteral phenylalanine (36), that extracellular amino acids would make a major contribution to the hepatic protein synthetic precursor pool (6, 28), and that in the fed state this pool would derive predominantly from the portal blood (9).

**MATERIALS AND METHODS**

The protocol was approved by the Animal Protocol Review Committee of Baylor College of Medicine. All animal housing and care complied with United States Department of Agriculture policies and practices.

**Animals and Surgery**

The study involved four piglets (Duroc × Large White × Hampshire) that were purchased from the Department of Animal Science of Texas A & M University (College Station, TX). The piglets were received at 2 wk of age and were weaned to a high-protein milk-based diet (Litter Life; Meredith, Middletown, WI) given at 60 g (dry matter) · kg⁻¹ · day⁻¹. After 10 days of feeding, the animals were implanted with catheters in the portal vein, the right external carotid artery, the jugular vein, and the stomach. An ultrasonic blood flow probe (Transonic, Ithaca, NY) was placed around the common portal vein. The surgery was performed under strict aseptic conditions, and the procedures have been described in detail previously (16, 29). The animals reestablished full feed intake and presurgery weight gain within 2 days of surgery and were allowed to recover for 4–5 days before receiving the tracer infusions. At the time of the infusions, the animals weighed 7.4 ± 0.5 kg.

**Tracer Amino Acids**

L-[carboxyl-13C]phenylalanine ([13C]phenylalanine) and [phenyl-2H5]phenylalanine ([2H5]phenylalanine) were purchased from Cambridge Isotope Laboratories (Andover, MA). We confirmed their chemical purity (>99%) by amino acid analysis. The measured fractional molar abundance of [1-13C]phenylalanine was 80 ± 0.4% and that of [phenyl-2H5]phenylalanine was 76.7 ± 0.3%. These values were used in the calculations of tracer balance and phenylalanine flux.

**Infusion Procedure**

The animals were fasted from 6:00 PM the night preceding the infusion. At 6:50 AM the next day, all the catheters were flushed with NaCl (154 mmol/l) containing heparin (25 U/ml), and arterial and portal baseline blood samples were taken. [2H5]phenylalanine in sterile NaCl (154 mmol/l) was then infused via the jugular catheter at a rate of 7.4 ± 0.3 µmol · kg⁻¹ · h⁻¹ at a rate of 5 ml/h for 12 h. During the first 6 h of the infusion, the animals remained fasted, and arterial and portal blood samples (5 ml) were taken at hourly intervals until 5 h of infusion. Arterial and portal samples were then taken at 5, 5.5, and 6 h.

After 6 h had elapsed, a dietary infusion consisting of an aqueous suspension of Litter Life (1.4, wt/wt) containing [13C]phenylalanine was started. The diet and tracer were infused at 1.25 ml/min and supplied 2.5 g dry diet, 625 mg protein, and 130 µmol phenylalanine · kg⁻¹ · h⁻¹. The infusion rate of [13C]phenylalanine was 14.5 ± 1.4 µmol · kg⁻¹ · h⁻¹. Arterial and portal blood samples were taken at hourly intervals until 11 h of infusion (6 h of intragastric tracer infusion) and then at 11.5 and 12 h.

Immediately after the last blood sample was taken, the animals were euthanized by injection of pentobarbital sodium (50 mg/kg body wt) and sodium phenytoin (5 mg/kg) (Beuthanasia-D; Schering-Plough Animal Health, Kenilworth, NJ) via the arterial catheter. The abdomen was opened, and a sample of liver (–5 g) was immediately excised and frozen in liquid nitrogen. The proximal 2 m of the small intestine were then rapidly excised, flushed with ice-cold saline, blotted, weighed, and opened, and the mucosa was removed by scraping with a microscope slide. The crude mucosal sample was immediately frozen in liquid nitrogen. The remaining liver and small intestine were removed and weighed.

**Sample Preparation**

Blood samples were collected into 10-ml Vacutainers containing Na2EDTA. Two 0.5-ml aliquots were immediately frozen, the remainder of the blood sample was centrifuged at 3,000 g at 4°C for 10 min, and the plasma was frozen. All samples were then held at –78°C until analyzed.

The isotopic and concentration measurements of the amino acids were made on whole blood. Samples for amino acid concentration measurements were mixed with an equal volume of an aqueous solution of methionine sulfone and centrifuged through a 3-kDa cutoff filter. The filtrate was dried and the amino acids were analyzed by reverse-phase high-performance liquid chromatography of their phenylisothiocyanate derivatives (PicoTag; Waters, Woburn, MA). For isotopic analysis, blood samples were brought to 4°C and 0.1 ml was mixed with 1 ml of ice-cold acetic acid (0.5 mol/l). They were then applied to a 1-ml bed volume column of Dowex 50 W×8
in which 

\[ C = \text{the concentration (\(\mu\text{mol/l}\)), } BF_{pv} = \text{portal blood flow (l·kg\(^{-1}\)·h\(^{-1}\))}, \text{ and the subscripts art and pv refer to the arterial and portal venous data, respectively. Portal tracer balance (\(\mu\text{mol·kg}^{-1}·\text{h}^{-1}\)) was calculated as:}

\[ \text{Portal tracer balance} = [(C_{pv} \times t/T_{pv}) - (C_{art} \times t/T_{art})] \times BF_{pv} \tag{2} \]

\[ \text{in which } t/T \text{ is the tracer-to-tracee ratio of the tracer (mol tracer/mol tracer) in portal and arterial blood. Note that in this study, a positive portal balance signifies an addition of unlabeled or tracer phenylalanine to the portal blood and a negative value signifies net removal from the arterial inflow by the portal-drained visera.}

\[ \text{Fractional portal appearance (proportion of input)} = \frac{\text{Portal balance}}{\text{Rate of input}} \tag{3} \]

\[ \text{For the intravenous tracer the denominator is the arterial tracer flow} (C_{art} \times t/T_{art}) \text{, for the intragastric tracer the denominator is the gastric tracer infusion rate, and for the mass balance calculations in the fed state the denominator is the known phenylalanine intake.}

\[ \text{Fractional portal extraction of the enteral tracer (proportion of dose)} = 1 - \text{fractional portal balance} \tag{4} \]

\[ \text{Systemic phenylalanine flux (Q; \(\mu\text{mol·kg}^{-1}·\text{h}^{-1}\)) was calculated as:}

\[ Q = R \times \frac{t/T \text{ infused phenylalanine}}{t/T \text{ blood phenylalanine}} - 1 \tag{5} \]

\[ \text{In the fed state three separate estimates of the rate of phenylalanine entry were calculated from the tracer-to-tracee ratio of arterial [\(^{13}\text{C}\)]- and [\(^{2}\text{H}\)]phenylalanine: } Q_{ig} \text{, in which } R \text{ is the rate of intragastric [\(^{13}\text{C}\)]-tracer infusion, } \text{ and } Q_{iv} \text{, in which } R \text{ is the rate of intravenous [\(^{2}\text{H}\)]tracer infusion. The splanchnic extraction of the intragastric tracer was calculated as:}

\[ \text{First-pass splanchnic extraction (proportion of dose)} = \frac{Q_{ig} - Q_{pv}}{Q_{ig}} \tag{6} \]

\[ \text{Hepatic extraction (proportion of dose) equals splanchnic extraction minus portal extraction (i.e., Eq } 6 \text{ – Eq } 4\text{).}

\[ \text{Phenylalanine appearance from body protein (\(\mu\text{mol phenylalanine·kg}^{-1}·\text{h}^{-1}\)) was calculated from three estimates of the entry of phenylalanine from proteolysis in the fed state, as follows:}

\[ Q_{iv} = \text{Phenylalanine intake} \tag{7a} \]

\[ \text{This gives an estimate of proteolysis that includes the gut and the liver.}

\[ Q_{iv} = \text{Phenylalanine portal mass balance} \tag{7b} \]

\[ \text{This approximates to the whole body rate excluding gut proteolysis.}

\[ Q_{iv} = \text{Phenylalanine portal mass balance} \tag{7c} \]
This approximates to proteolysis in the extrasplanchnic tissues.

Whole body degradation (g protein·kg⁻¹·day⁻¹) was calculated using Eq. 7 as follows

\[ t/T (t) = A - Ae^{-k_{equil}(t-d)} \]  

in which \( A \) is the predicted steady-state tracer-to-tracee ratio of circulating phenylalanine, \( k_{equil} \) is the rate constant of equilibration, \( t \) the time of infusion, and \( d \) a delay. Apo B-100 kinetics were fitted using the equation

\[ t/T (t) = (A_{apo} - A_{apo}e^{-k_{equil}(t-d)}) \times (1 - e^{k_{equil}t}) \]

in which \( A_{apo} \) is the predicted steady-state value for apo B-100 labeling and \( k \) the fractional synthetic rate. In the fasted state, \( k_{equil} \) was that of arterial phenylalanine. In the fed state, the equilibration kinetics of portal phenylalanine were used in the calculation.

For the calculation of fibrinogen and albumin synthesis it was assumed that the steady-state tracer-to-tracee ratio of arterial apo B-100 defined the steady-state labeling of their precursor pool. Thus the fractional synthesis rate was calculated as

\[ \Delta t/T \ (fibrinogen \ or \ albumin) = 1 - e^{-k_{equil}(t-d)} \]

For both proteins, separate estimates of fasting and fed values were calculated from the intravenous tracer. Fasting values were calculated using the increase in the ratio of tracer to tracee in the protein product from 3 to 6 h of infusion, with the mean tracer-to-tracee ratio of apo B-100 over 4–6 h as the denominator. Fed values were calculated from fibrinogen and albumin labeling from 7 to 12 h, using the mean tracer-to-tracee ratio of apo B-100 from 10 to 12 h as the denominator. A separate estimate of fibrinogen synthetic rate in the fed state was made with the intragastric tracer. Unfortunately, the tracer-to-tracee ratio of [13C]phenylalanine in albumin was too low to make accurate calculations of albumin synthesis from intragastric phenylalanine.

Mucosal and hepatic protein synthesis. The fractional rate of mucosal and hepatic constitutive protein synthesis (%/day) was calculated with the simplified equation

\[ \frac{t/T \ mucosal \ or \ hepatic \ protein \ \times 1,440}{t/T \ \text{precursor}} \]

in which \( t/T \) is the tracer-to-tracee ratio of [13C]phenylalanine (mol/mol). For mucosal protein, the denominator was the tracer-to-tracee ratio of mucosal free phenylalanine; for hepatic protein the denominator was the plateau tracer-to-tracee ratio of apo B-100 phenylalanine.

In both tissues, total incorporation of the intragastric tracer (µmol [13C]phenylalanine incorporated/6 h) was calculated as the product of the measured protein-bound phenylalanine content of the mucosa of the proximal 2 m of the intestine (650 ± 21 µmol) or the liver (6,900 ± 1,160 µmol) and the absolute tracer-to-tracee ratio of protein-bound phenylalanine (mol/mol).

Statistics

All portal balance and phenylalanine flux data were based on the mean values obtained from 4 to 6 h and from 9 to 12 h of infusion. All data are presented as means ± SD. Where ratios (e.g., 13C/2H in a given pool) have been calculated, the values shown are the means of the ratios. Portal balance data were tested against zero using a two-tailed t-test. The statistical significance of differences between the fasted and fed states was tested with paired two-tailed t-tests. \( P < 0.05 \) was considered statistically significant.

RESULTS

Figure 1 shows the labeling kinetics of arterial and apo B-100 phenylalanine during the fasting phase of the study. Both pools had reached steady state by 3 h of infusion, and at plateau the ratio of tracer to tracee in apo B-100 was 53 ± 12% of that of arterial phenylalanine. Model-predicted and measured plateau values did not differ significantly. Figure 2 shows similar data for the labeling of arterial, portal, and apo B-100 phenylalanine from the intragastric tracer. The tracer-to-tracee ratio of portal phenylalanine rose more rapidly than either arterial or apo B-100 phenylalanine, and apo B-100 achieved steady state by between 4 and 5 h of infusion. The plateau tracer-to-tracee ratio of the intragastric tracer in apo B-100 was 92 ± 5% of that of arterial phenylalanine and 76 ± 4% of portal phenylalanine.

Table 1 summarizes the data on the portal phenylalanine mass and tracer balance. After a 16-h fast, the portal-drained viscera were at phenylalanine mass equilibrium, but there was a significant (\( P < 0.01 \)) net removal of 10 ± 4% of the arterial flux of [2H]phenylalanine. In the fed state, the tracer-to-tracee ratio of
[2H]phenylalanine was lower in portal than in arterial blood, but once the increase in phenylalanine concentration was taken into account, the portal [2H]phenylalanine tracer balance was nominally negative but not significantly different from zero.

In the fed state, the portal-drained viscera were in a highly significant net positive portal phenylalanine balance, and the appearance of unlabeled phenylalanine accounted for 50 ± 5% of the phenylalanine intake. The tracer-to-tracer ratio of the intragastric tracer rose significantly (P < 0.05) across the portal-drained viscera, and the portal [13C]phenylalanine balance accounted for 66 ± 11% of the intragastric tracer infusion. Thus 34% of the enteral tracer was utilized by the intestinal tissues in first pass.

Table 2 shows the various calculated values for blood phenylalanine flux (rate of appearance) as determined with the two tracers in the fed and fasted states.

As expected, if it was assumed that all the intragastric tracer was absorbed into the portal circulation, the apparent phenylalanine flux determined with [13C]phenylalanine (i.e., Qiv, 199 µmol phenylalanine·kg⁻¹·h⁻¹) was much higher (P < 0.01) than that estimated with the intravenous tracer (i.e., Qiv, 109.3 µmol phenylalanine·kg⁻¹·h⁻¹). Comparison of the two values suggested a total splanchnic extraction of 45 ± 6% of the enteral tracer in first pass. Of this, 34 ± 11% was accounted for by first-pass intestinal metabolism (Table 1), so that the liver accounted for a removal of 11 ± 3% of the enteral tracer, i.e., 25% of the total splanchnic uptake.

In Table 2, we also show estimates of the entry of phenylalanine from tissue protein degradation. The fasted phenylalanine flux (89.9 µmol·kg⁻¹·h⁻¹) was the equivalent of the rate of whole body protein turnover of 8.5 ± 1.1 g protein·kg⁻¹·day⁻¹. Whole body proteolytic entry in the fed state, as determined with the intragastric tracer (Qig, phenylalanine intake), was the equivalent of 6.5 g protein·kg⁻¹·day⁻¹ and was significantly lower than the fasted value (P < 0.01). The value for proteolytic entry determined with the intravenous tracer (Qiv, portal phenylalanine mass balance) was the equivalent of 4.1 g protein·kg⁻¹·day⁻¹. On the basis of the difference between the two estimates, protein degradation in the splanchnic tissues was 2.4 g protein·kg⁻¹·day⁻¹ or 36% of the whole body. The difference between Qig and Qiv, which we take as an estimate of intestinal tissue protein degradation, sug-

Table 1. Portal phenylalanine mass and tracer balance in fasted and fed piglets receiving intravenous [phenyl-2H₅]phenylalanine and intragastric [carboxyl-¹³C]phenylalanine

<table>
<thead>
<tr>
<th>State</th>
<th>Blood flow, l·kg⁻¹·h⁻¹</th>
<th>Arterial Conc, µmol/l</th>
<th>Portal Conc, µmol/l</th>
<th>Balance, µmol·kg⁻¹·h⁻¹</th>
<th>Fractional balance, %input</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted</td>
<td>2.21 ± 0.13</td>
<td>76 ± 25</td>
<td>77 ± 24</td>
<td>0.4 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Fed</td>
<td>2.87 ± 0.42</td>
<td>120 ± 10</td>
<td>143 ± 11</td>
<td>65 ± 14</td>
<td>50 ± 5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tracer Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial tracer-to-tracer ratio, mol/100 mol</td>
</tr>
<tr>
<td>[2H₅]phenylalanine fasted</td>
</tr>
<tr>
<td>[2H₅]phenylalanine fed</td>
</tr>
<tr>
<td>[¹³C₁]phenylalanine fasted</td>
</tr>
</tbody>
</table>

Values are means ± SD for 4 animals. Fractional balance is expressed as %arterial flow with intravenous tracer, as a percentage of the phenylalanine intake (mass balance) or of the rate of intragastric tracer infusion (tracer balance) with intragastric tracer. NS, not significant.
Table 3. Labeling in blood: apo B-100 and hepatic-free phenylalanine

<table>
<thead>
<tr>
<th>Pool</th>
<th>Tracer-To-Tracee Ratio, mol/100 mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal free</td>
<td></td>
</tr>
<tr>
<td>Arterial free</td>
<td></td>
</tr>
<tr>
<td>Hepatic free</td>
<td></td>
</tr>
<tr>
<td>Apo B-100</td>
<td></td>
</tr>
</tbody>
</table>

\[
\begin{array}{cccc}
[1^{13}C]\text{phenylalanine, ig} & 9.1 \pm 1.1^* & 7.9 \pm 0.9 & 3.01 \pm 0.72^+ & 7.29 \pm 1.26^\dagger \\
[2H_5]\text{phenylalanine, iv} & 5.5 \pm 1.1^* & 6.4 \pm 0.9 & 2.11 \pm 0.23 & 4.20 \pm 0.49^\dagger \\
[1^{13}C]{/2H_5}\text{phenylalanine} & 1.66 \pm 0.24^* & 1.26 \pm 0.21^* & 1.42 \pm 0.47 & 1.76 \pm 0.25^* \\
\end{array}
\]

Values are means ± SD for 4 animals. *Determined with intragastric tracer. †Determined with intravenous tracer. ‡Significantly different from value determined in fasted state (P < 0.05). ND, not determined.

gested that the liver and intestine made equal contributions to splanchnic proteolysis.

Table 3 summarizes the steady-state tracer-to-tracee ratios of portal, arterial, hepatic, and apo B-100 phenylalanine during the fed phase of the study. As a result of the rise in the tracer-to-tracee ratio of \([1^{13}C]\)phenylalanine and a fall in that of \([2H]\)phenylalanine across the portal-drained viscera, the ratios of the two tracers in portal (1.66 ± 0.24) and arterial (1.26 ± 0.21) phenylalanine were significantly (P < 0.05) different. \([1^{13}C]{/2H_5}\) in apo B-100 (1.76 ± 0.25) was similar to that of portal phenylalanine but significantly (P < 0.05) higher than that of arterial phenylalanine. The \([1^{13}C]{/2H_5}\) ratio in hepatic free phenylalanine (1.42 ± 0.47) lay between the two blood values. However, the most striking result was that the tracer-to-tracee ratio of apo B-100 \([(1^{13}C)]\)phenylalanine, 7.29 ± 1.62; \([2H_5]\)phenylalanine, 4.20 ± 0.49 was between 2- and 2.3-fold higher than that of hepatic-free phenylalanine \((1^{13}C)\)phenylalanine, 3.01 ± 0.72; \([2H_5]\)phenylalanine, 2.11 ± 0.23.

Calculations of free phenylalanine and plasma protein kinetics are summarized in Table 4. In the fasted state, arterial phenylalanine approached isotopic equilibrium with a rate constant of 126%/h (t1/2, 0.55 h). With the intragastric tracer, the calculations suggested that there was an average delay of 0.59 h before significant quantities of intragastrically infused labeled phenylalanine appeared in the portal circulation, but thereafter the tracer-to-tracee ratio rose with a rate constant of 221%/h (t1/2, 0.31 h). In the fasted state, the average turnover rate of VLDL apo B-100 was 73%/h, with a delay of 0.46 h before labeled apo B-100 appeared in the arterial circulation. On the basis of the data from the intragastric tracer, the calculated fractional rate of apo B-100 synthesis fell to 55%/h in the fed state.

The changes with time in the labeling of plasma fibrinogen expressed in terms of the tracer-to-tracee ratios of the intravenous and intragastric tracers in apo B-100 are depicted in Fig. 3. The fibrinogen labeling kinetics (Table 4) derived from the intravenous tracer suggested little change in the fibrinogen synthesis rate between the fasting (2.6%/h) and fed (2.6%/h) states. In the fed state, the rate of fibrinogen synthesis calculated with the intragastric tracer (3.4%/h) was on average 27% higher than that determined with the intravenous tracer, but the difference did not achieve statistical significance (P = 0.09).

Figure 4 shows the appearance of tracer in plasma albumin. Unfortunately, the high baseline of the \([1^{13}C]\)isotopomer of the heptafluorobutyramide n-propyl ester of phenylalanine prevented accurate measurements of albumin labeling from the intragastric tracer, so Fig. 4 shows data only for the intravenous tracer. In the fasted state, the fractional rate of synthesis of albumin was 0.48 ± 0.08%/h. It is evident from Fig. 4 that on feeding, there was a change in the rate of isotope incorporation into albumin, and the calculated synthesis rate of albumin (Table 4) in the fed state (0.67 ± 0.04%/h) was 40% (P < 0.05) higher than the fasting value.

Table 4 summarizes the data on mucosal and hepatic protein synthesis as determined with the intragastric tracer. The calculated fractional protein synthetic rates

Table 5 summarizes the data on mucosal and hepatic protein synthesis in fed and fasted states.

<table>
<thead>
<tr>
<th>Pool</th>
<th>Fractional turnover, %/h</th>
<th>Delay, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>126 ± 39</td>
<td>None</td>
</tr>
<tr>
<td>Portal</td>
<td>73 ± 4</td>
<td>0.46 ± 0.16</td>
</tr>
<tr>
<td>Apo B-100</td>
<td>2.4 ± 0.3</td>
<td>0.95 ± 0.18</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.48 ± 0.08*</td>
<td>ND</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.67 ± 0.04†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD for 4 animals. *Determined with intravenous tracer. †Determined with intragastric tracer. ‡Significantly different from value determined in fasted state (P < 0.05). ND, not determined.
have suggested that the combined digestion and luminal absorption of most dietary proteins is at least 90% complete and for some proteins approaches 100% (13). On the other hand, detailed documentation of the portal mass balance of amino acids in fed pigs (29) has shown that ~50% of the essential amino acids known to have been taken up from the intestinal lumen by the enterocytes appear in the portal blood. The difference between the luminal disappearance of dietary amino acids and their portal appearance could either reflect substantial first-pass utilization by the enterocytes or highly efficient absorption that has been balanced by the simultaneous utilization of arterial amino acids by the portal-drained viscera. Distinguishing between these two possibilities requires measurements of portal mass balance in combination with isotopic tracers, and this approach formed one part of the present study. The results suggest that metabolism of the enteral phenylalanine by the intestinal cells was the main contributor to the failure to account for the luminal disappearance of dietary amino acids in the portal outflow. Nevertheless, because the proportion of the phenylalanine intake that appeared in the portal blood (66% of dose), it appeared that, as observed by Yu et al. (36) with leucine, utilization of arterial phenylalanine by the portal-drained viscera continued in the fed state. On the assumption that dietary phenylalanine and the intragastric tracer phenylalanine were metabolized identically, the difference between the fractional portal mass balance (percentage of intake) and the fractional portal tracer balance (percentage of infusion) allows the uptake of arterial phenylalanine by the portal-drained viscera to be estimated. The difference between fractional mass balance and fractional tracer balance is 15% (i.e., 65–50%) of the dietary intake (130 µmol·kg⁻¹·h⁻¹), so it was on average 21 µmol·kg⁻¹·h⁻¹. This is the equivalent of 5% of the arterial flow of phenylalanine to the portal-drained viscera (410 µmol·kg⁻¹·h⁻¹).

Recent studies with combined intravenous and intragastric tracer infusion in humans (7, 9, 20, 21, 25) have demonstrated considerable first-pass splanchnic utilization of enteral amino acids, and the present study was also designed to quantify the contributions of intestinal and hepatic phenylalanine metabolism to the splanchnic extraction. Although it is possible that the method we and Biolo et al. (7), Cayol et al. (9), Hoerr et al. (20, 21), and Matthews et al. (25) have used to determine the splanchnic extraction of phenylalanine underestimates the true value to the extent that the splanchnic catabolism of arterial phenylalanine is underestimated, the present results suggest that intestinal metabolism accounted for 75% of the total splanchnic metabolism of the intragastric tracer. This estimate is similar to that determined for leucine in dogs (intestine, 85%; liver, 15%; Ref. 36).

There are, unfortunately, few studies that can be compared directly with the present results. The total splanchnic metabolism of phenylalanine (45% of dose)
determined in this study is similar to that found in studies involving dual intravenous/intragastric infusions of labeled phenylalanine in fed humans (58% of dose; Ref. 7) but is higher than that measured in the postabsorptive state (29%; Ref. 25). In general, however, values for first-pass splanchic extraction of enteral phenylalanine are higher than those obtained with leucine and lysine in both dogs and humans (20–30% of dose; Refs. 7, 20, 21, 23, 25, 34, 36). Similarly, in the fed state, the fractional portal mass balance (50% of intake) of phenylalanine obtained in the present and in a previous study (29) is lower than that obtained with leucine in the dog (34, 36) and in the piglet (29). It appears from the limited evidence available that there are differences in the proportion of the enteral supply of different amino acids that are metabolized by the splanchic bed and that less dietary phenylalanine than leucine is available for peripheral tissue protein metabolism.

The processes that are responsible for the substantial first-pass splanchic metabolism of enteral phenylalanine have an important bearing on our understanding of phenylalanine nutrition. This is because dietary phenylalanine incorporated into mucosal protein can be recycled and does not necessarily lead to a loss of phenylalanine from the body as a whole, whereas phenylalanine catabolism in the mucosa clearly is a net loss from the body. In their study of splanchic leucine metabolism in fed dogs, Yu et al. (36) infused the animals intravenously with [13C,15N]leucine and measured the portal and hepatic mass and tracer balances as well as leucine transamination and oxidation. They concluded that protein synthesis (measured as nonoxidative leucine disposal) accounted for 80% and 84% of the leucine metabolized by the gut and liver, respectively. The present results differ from those obtained with leucine. The incorporation of the intragastric tracer into the protein of the proximal small intestinal mucosa accounted for 3% of the total dose of [13C]phenylalanine, and even if the incorporation of the enteral tracer into mucosal protein was distributed evenly throughout the small intestine, mucosal protein synthesis consumed no more than 9% of the dose and accounted for ~25% of total first-pass intestinal phenylalanine metabolism.

It should be pointed out that this may be an underestimate of the quantity of dietary phenylalanine utilized in total mucosal protein synthesis, because label incorporation into secreted protein would not have been measured by the technique that we adopted. Unfortunately, in the present experiment, we collected no data on the labeling of luminal protein and cannot estimate directly the contribution of secretion to total protein synthesis. However, in isotopic studies in older pigs, de Lange et al. (13) estimated that protein secretion into the small intestine was ~25 g protein/kg of dry matter intake. Extrapolating this to our study leads to an estimate of protein secretion of 62 mg protein·kg⁻¹·h⁻¹, the equivalent of 12 µmol phenylalanine·kg⁻¹·h⁻¹. This is 25% of total first-pass phenylalanine utilization, which when added to the estimate derived from the incorporation of tracer into mucosal constitutive protein leads us to conclude that no more than 50% of total first-pass phenylalanine utilization was used for mucosal protein synthesis.

Incorporation of the intragastric tracer phenylalanine into liver protein (85 µmol [13C]phenylalanine/6 h) was substantial. It accounted for 12.9% of the total dose of [13C]phenylalanine and 19% of the total portal delivery of [13C]phenylalanine over the course of the 6-h infusion, a value that is similar to that obtained by Yu et al. (36) for the utilization of portal leucine in hepatic protein synthesis. On the basis of these results, it seems that an important metabolic fate of enteral phenylalanine in the mucosa is catabolism, in addition to protein synthesis, whereas the principal fate of portal phenylalanine in the liver is incorporation into protein. In this context, we found it particularly noteworthy that recent studies (4, 30) have emphasized that intravenous tracer phenylalanine underestimates whole body phenylalanine catabolism and that intragastric tracer phenylalanine infusions are necessary to obtain reliable estimates of whole body phenylalanine metabolism.

**Hepatic Protein Synthesis**

A second objective of this study was to gain further insight into the utilization of amino acids for the synthesis of proteins secreted by the liver. In both humans (9, 27, 28) and piglets (22), it has been found that during infusions of tracer essential amino acids the tracer-to-tracee ratio of apo B-100 and plasma amino acids are remarkably close. This has led to the tentative conclusion that extracellular amino acids may be quantitatively the most important source of substrates for hepatic protein synthesis (9, 28).

In the present study, we used two approaches to expand on these data. First, we compared the tracer-to-tracee ratios of phenylalanine in the hepatic free and apo B-100-bound pools. Second, we compared the relative tracer-to-tracee ratios of the intragastric and intravenous tracers in different free and protein-bound phenylalanine pools. The first approach allowed conclusions about the contribution of extracellular (systemic) phenylalanine to the hepatic protein synthetic precursor pool. The second allowed conclusions about the relative contributions of portal and arterial phenylalanine.

It was clear that the steady-state tracer-to-tracee ratio of both tracers in apo B-100 was between 2- and 2.3-fold higher than the tracer-to-tracee ratio of acid-soluble hepatic free phenylalanine. This suggests strongly that extracellular phenylalanine is channeled toward apo B-100 synthesis. Thus, whereas 35% of the total acid-soluble hepatic pool derived from the uptake of systemic phenylalanine, 75% (intravenous tracer) and 80% (intragastric tracer) of the apo B-100 precursor derived from the extracellular compartment. It is of specific interest that radioisotopic studies carried out 20 years ago (1, 33) showed that in fed rats the specific radioactivity of hepatic [14C]valyl tRNA was 2.1 ± 0.3-fold higher than the bulked hepatic free[14C]valine.
Vidrich et al. (33) proposed that there is a close structural relationship between the inward face of the plasma membrane valine transporter and the amino acid activating enzymes. It can be argued as well that the substantial difference between the labeling of the acid-soluble and protein synthetic precursor pools favors the idea that there may be a separate outward transport system for essential amino acids that have derived from hepatic proteolysis and are contained in the lysosomal compartment.

As expected, the $^{13}$C/$^2$H$_3$ ratio in arterial phenylalanine was lower than that of portal phenylalanine. The $^{13}$C/$^2$H$_3$ ratio of hepatic free phenylalanine was midway between the two extracellular values and was compatible with the conclusion that 70% of the labeled phenylalanine in the liver derived from the portal blood. However, the ratio of $[^{13}$C$_1$/phenylalanine to $[^{3}$H$_3$]/phenylalanine in apo B-100 was similar to that of portal phenylalanine and significantly higher than that of arterial phenylalanine. This suggests that in the fed state, apo B-100 is derived almost entirely from portal phenylalanine, a conclusion that is supported by the observation of Cayol et al. (9) that during an intragastric leucine infusion in fed humans, the tracer-to-tracee ratio of apo B-100 leucine was higher than that of peripheral venous leucine.

The final objective of this study was to quantify albumin and fibrinogen synthesis and to examine the degree to which the assumption that apo B-100 defines the labeling of their precursor held true. As in previous studies in humans (9, 12), protein ingestion was associated with an increase in albumin synthesis but with little or no change in that of fibrinogen. We had originally planned to compare the rates of synthesis of the two proteins as calculated with the two tracers, similar to the approach by Cayol et al. (9), a study that appeared while this work was in progress. The approach was feasible only for fibrinogen, because the tracer-to-tracee ratio of albumin-bound $[^{13}$C$_1$]phenylalanine was too low to allow accurate measurements. With fibrinogen, however, we obtained a trend (27%; P = 0.09) for higher synthesis rates when determined on the basis of the tracer-to-tracee ratio of the intragastric tracer in apo B-100. In Cayol et al. (9), the albumin and fibrinogen synthesis rates derived from intragastric leucine were also 30% higher than those determined with the intravenous leucine. These observations suggest that the channeling of portal phenylalanine into apo B-100 synthesis might be even more accentuated with regard to the precursor pools of other plasma proteins. Whether this holds true for hepatic constitutive proteins remains to be examined, although it should be pointed out that there is previous evidence for microheterogeneity of the hepatic protein synthetic pools (18).

At this stage, the mechanism that underlies the apparent preferential utilization of portal phenylalanine and leucine for hepatic secretory protein synthesis is not clear. It is possible that apo B-100 and fibrinogen are produced specifically by perportal hepatocytes, but we have been unable to find evidence either in favor or against such a proposition. Despite this current lack of mechanistic understanding, it appears that apo B-100 provides a considerably more valid estimate of the isotopic enrichment of the hepatic protein synthetic precursor than does the hepatic free amino acid pool.

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