Twenty-four–hour L-[1-13C]tyrosine and L-[3,3-2H2]phenylalanine oral tracer studies at generous, intermediate, and low phenylalanine intakes to estimate aromatic amino acid requirements in adults1–3

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ABSTRACT  Daily pattern and rates of whole-body tyrosine oxidation and phenylalanine hydroxylation were determined in young adults (15 men, 1 woman) receiving [13C]tyrosine and [3H]phenylalanine via primed, constant oral infusion and [13C]tyrosine by vein (five subjects also received [3H]leucine simultaneously by vein) continuously for 24 h (12 h fast then 12 h fed). Subjects were given a diet supplying 96.6 (n = 5), 35.6 (the proposed requirement; n = 5), and 18.5 mg phenylalanine · kg−1 · d−1 (n = 6) based on an otherwise adequate l-amino acid mixture for 6 d before the 24-h tracer study began. (Each diet was low in tyrosine: 6.79 mg · kg−1 · d−1.) Our hypothesis was that subjects would be in tyrosine equilibrium, positive balance, or both, at the 96.6- and 35.6-mg intakes and in distinctly negative balance at the 18.5-mg intake. The diurnal pattern in phenylalanine and tyrosine kinetics was dependent on the intake and, presumably, on the adequacy of dietary phenylalanine. Whole-body tyrosine balances, determined from rates of phenylalanine hydroxylation and tyrosine input and oxidation were negative (0.05 < P < 0.1 from zero balance) with the low (18.5 mg) phenylalanine intake [total aromatic amino acid (AAA) intake: 25.3 mg · kg−1 · d−1] but at equilibrium (P > 0.05 from zero balance) with the two higher phenylalanine intakes. Whole-body AAA balance (AAA intake − tyrosine oxidation) was negative (P < 0.05 from zero balance) with the low intake, at equilibrium with the intermediate intake, and apparently distinctly positive (P < 0.05) with the generous intake. Despite model limitations, as discussed, these findings lend further support for a proposed, tentative value for a total mean requirement of 39 mg AAA · kg−1 · d−1. Am J Clin Nutr 1998;67:640–59.

KEY WORDS  Indispensable amino acids, requirements, tyrosine oxidation, phenylalanine hydroxylation, tracer-infusion studies, diurnal rhythm, stable isotopes, tyrosine balance, aromatic amino acid balance, splanchnic uptake, humans, adults

INTRODUCTION  We used both short- (<8 h) and long-term (24 h) 13C-labeled amino acid tracer-infusion protocols to reassess the quantitative requirements for specific indispensable amino acids in healthy, young adults (1–3). Requirement is defined in this study as the minimum intake of the test amino acid necessary to just balance the daily rate of irreversible oxidation of that amino acid in healthy subjects who had been given an otherwise adequate, experimental diet for 6 d before the tracer studies began. Relatively recently, an extensive series of studies were conducted with leucine as the test amino acid that included three separate investigations of the 24-h pattern of whole-body leucine kinetics and oxidation (4–6). Earlier, limited, and short-term tracer-infusion studies were carried out with valine (7), lysine (8), threonine (9), and methionine (10, 11) as test amino acids.

On the basis of these various studies, we concluded that the minimum physiologic requirements for all indispensable amino acids, except perhaps for the sulfur amino acids (methionine plus cystine), are much higher than the current international requirements (1, 3). As suggested by Waterlow (12), our conclusions are most secure for leucine and it remains for an additional series of investigations, involving many, but not necessarily all, of the other indispensable amino acids, to come to a concrete conclusion about the entire indispensable amino acid requirement pattern for adult humans. Thus, we began a reevaluation of the requirements for lysine and the aromatic amino acids (13–15).

The FAO/WHO/UNU’s (16) upper requirement for total aromatic amino acids (phenylalanine and tyrosine) is 14 mg · kg−1 · d−1. However, we predicted the requirement to be ≈39 mg · kg−1 · d−1, considering the calculated obligatory losses of phenylalanine and tyrosine (17). Further, a 24-h [13C]phenylalanine tracer-infusion study in young adults who received 21.9 mg · 

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kg \cdot d^{-1} \) indicated that they were in negative body phenylalanine balance at this low intake (13). At an intake of 100 mg \cdot kg^{-1} \cdot d^{-1}, on the other hand, subjects were estimated to be in distinct positive phenylalanine balance (14), which we attributed to the fact that the \([^{13}C]\)phenylalanine tracer—oxidation model underestimated the true, whole-body rate of phenylalanine oxidation. We expected, therefore, that healthy, young adults receiving a daily intake of 39 mg phenylalanine \cdot kg^{-1} \cdot d^{-1} \) (without tyrosine) would be in approximate whole-body \([^{13}C]\)phenylalanine balance. Our recent communication (15) supports this hypothesis as well as provides additional data indicating a requirement for total aromatic amino acids of \(\approx 30 \) (18) to 39 mg \cdot kg^{-1} \cdot d^{-1}. Finally, Rose et al (19) proposed that a safe intake of phenylalanine (twice the highest determined requirement for any individual) would be \(\approx 31 \) mg \cdot kg^{-1} \cdot d^{-1} \) and that optimal intakes “may be quite high.”

Our previous interpretation of the isotopic data generated from the 24-h kinetic studies of whole-body \([^{13}C]\)phenylalanine metabolism (13–15) led us to the interpretation that a metabolic channeling was involved in the conversion of phenylalanine to tyrosine and its subsequent oxidation. Indeed, there is a growing body of evidence that points to metabolic channeling as an important feature in nutrient utilization and metabolic regulation (20). Because this appeared to be the case for our \([^{13}C]\)phenylalanine tracer—derived estimate of aromatic amino acid oxidation and that the quantitative importance of this regulatory feature of nutrient metabolism might vary with the nutritional input and status of subjects, we considered it important to conduct a comparable series of dietary studies using a 24-h continuous tracer protocol with \([^{13}C]\)tyrosine as the tracer.

Our purpose was to explore the oxidation of tyrosine, compared with phenylalanine hydroxylation and oxidation, which were based on earlier plasma \([^{13}C]\)phenylalanine and \([^{13}C]\)tyrosine abundances, at similar dietary intakes of phenylalanine and tyrosine (13–15). Furthermore, we were interested in obtaining additional data on whole-body aromatic amino acid kinetics that also might be used to strengthen the validity of our tentative estimated requirement for aromatic amino acids (1–3, 17), which greatly exceed those proposed by the FAO/WHO/UNU (16).

**SUBJECTS AND METHODS**

**Subjects**

Sixteen young adult (15 men, 1 woman) volunteers were recruited from the population of students at the Massachusetts Institute of Technology (MIT) and from within the community of the Boston-Cambridge region. Subjects were aged 18–28 y, had a body weight of 62–83 kg, and had a height of 1.67–1.90 m. Their mean body mass index (in kg/m²) was 23.4. The subjects were studied as outpatients at the Clinical Research Center (CRC) and as inpatients at the Medical Department of MIT (24-h study). They were judged to be in good health as determined by their medical history, a clinical examination, as well as a profile included a complete blood cell count, standard blood chemistry tests, and urinalysis. Criteria for exclusion from the study were cigarette smoking and the ingestion of more than five alcoholic drinks per week or more than six cups of caffeinated beverages daily.

Daily energy intake was estimated to be sufficient to maintain body weight throughout the study and was evaluated individually by a diettian through a dietary history and assessment of the subjects’ usual level of physical activity. The energy intake of the subjects was \(\approx 188 \) kJ \cdot kg^{-1} \cdot d^{-1} \) (45 kcal \cdot kg^{-1} \cdot d^{-1}). Subjects were asked to adhere to their usual levels of activity but to not participate in competitive or particularly strenuous exercise. Energy intake was decreased to 155–159 kJ \cdot kg^{-1} \cdot d^{-1} \) (37–38 kcal \cdot kg^{-1} \cdot d^{-1}) on day 7 because of the decline in physical activity on the 24-h tracer day.

The design and aims of the study, as well as the potential risks involved, were explained to each subject. Written consent was obtained in agreement with the protocol, which was approved by the MIT Committee on the Use of Humans as Experimental Subjects and the MIT CRC Advisory Committee. The subjects were admitted to the MIT Medical Department on the last day of the dietary study period for the 24-h, stable-isotope-tracer study. All subjects remained healthy throughout the study period and received financial compensation for their participation.

**Diet and experimental design**

All subjects received a diet, based on an l-amino acid mixture, designed to maintain their weight and to supply 1.0 g protein (N \cdot kg^{-1} \cdot d^{-1} \) for 6 d (Table 1). Besides the l-amino acid mixture, the major portion of the daily energy requirement was provided by wheat-starch cookies, as described in detail previously (4). The nonprotein energy sources were \(\approx 40\% \) fat and 60% carbohydrate. The latter was supplied as beet sugar and wheat starch to give a low abundance of \(^{13}\)C in the diet and a steady background of \(^{13}\)C enrichment from exogenous sources in the breath during the 24-h study period (4). The phenylalanine intake supplied by the amino acid mixture was 78.06 mg \cdot kg^{-1} \cdot d^{-1} \) (n = 5), 17.06 mg \cdot kg^{-1} \cdot d^{-1} \) (n = 5), or 0 mg \cdot kg^{-1} \cdot d^{-1} \) (n = 6), all without tyrosine. In addition, on each diet day 18.53 mg l-phenylalanine \cdot kg^{-1} \cdot d^{-1} \) and 6.79 mg l-tyrosine \cdot kg^{-1} \cdot d^{-1} \) were added to the diet to compensate for the \(^{13}\)C- and \(^{2}\)H_{2}-labeled amino acid tracers given subsequently during the 24-h infusion day. Hence, the total aromatic amino acid intake for the three groups of subjects was 103.3 (generous), 42.4 (intermediate; the proposed requirement), and 25.3 (low) mg \cdot kg^{-1} \cdot d^{-1}. The other nutrients were given in their appropriate amounts as reported previously (4).

The subjects consumed their daily diet as three isoenergetic, isonitrogenous meals at 0800, 1200, and 1800 during the first 5 d and at 0800, 1200, and 1500 on day 6. Vital signs and body weight were monitored every morning before the first meal of the day. After 6 d, a 24-h stable-isotope-tracer study was carried out in each subject beginning at 1800 and continuing until day 7 at 1800. Oral doses hourly of [1-\(^{13}\)C]tyrosine and [3,3-\(^{2}\)H_{2}]phenylalanine were given to all subjects with an intravenous infusion of l-[ring-\(^{2}\)H_{2}]tyrosine. In addition, five of the subjects in the low-phenylalanine group also received [5,5,5-\(^{2}\)H_{3}]leucine intravenously, as described below.

**Twenty-four-hour tracer protocol**

A primed, constant, intravenous, tracer-infusion protocol was used for administration of labeled amino acids given by vein. All 16 subjects were also given labeled amino acids orally every hour, dissolved in distilled water. The details and rationale for the design of the 24-h study were essentially identical to those of our previous 24-h \([^{13}C]\)leucine and \([^{13}C]\)phenylalanine tracer studies (4, 13, 15). All subjects received a known, primed oral dose of l-[1-\(^{13}\)C]tyrosine (99 atom%) \((\approx 1.06 \) \(\mu\)mol \cdot kg^{-1} \cdot h^{-1}) and [3,3-\(^{2}\)H_{2}]phenylalanine (98 atom%) \((\approx 4.95 \) \(\mu\)mol \cdot kg^{-1} \cdot h^{-1}) with a constant...
TABLE 1
Composition of the L-amino acid mixtures used to provide an intake of 96.6, 35.6, and 18.5 mg phenylalanine·kg⁻¹·d⁻¹

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>96.6 mg Phe·kg⁻¹·d⁻¹</th>
<th>35.6 mg Phe·kg⁻¹·d⁻¹</th>
<th>18.5 mg Phe·kg⁻¹·d⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>15.83</td>
<td>16.31</td>
<td>16.45</td>
</tr>
<tr>
<td>Threonine</td>
<td>47.79</td>
<td>49.24</td>
<td>49.67</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>63.67</td>
<td>65.70</td>
<td>66.27</td>
</tr>
<tr>
<td>Leucine</td>
<td>84.89</td>
<td>87.46</td>
<td>88.22</td>
</tr>
<tr>
<td>Lysine·HCl</td>
<td>76.83</td>
<td>79.16</td>
<td>79.84</td>
</tr>
<tr>
<td>Methionine</td>
<td>30.12</td>
<td>31.03</td>
<td>31.30</td>
</tr>
<tr>
<td>Cystine</td>
<td>22.34</td>
<td>24.45</td>
<td>23.21</td>
</tr>
<tr>
<td>Phenylalanine†</td>
<td>69.23</td>
<td>15.59</td>
<td>0</td>
</tr>
<tr>
<td>Tyrosine†</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Valine</td>
<td>71.30</td>
<td>73.47</td>
<td>74.10</td>
</tr>
<tr>
<td>Histidine·HCl·H₂O</td>
<td>31.12</td>
<td>32.06</td>
<td>32.34</td>
</tr>
<tr>
<td>Arginine·HCl</td>
<td>76.70</td>
<td>79.06</td>
<td>79.72</td>
</tr>
<tr>
<td>Alanine</td>
<td>194.32</td>
<td>200.22</td>
<td>201.95</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>12.11</td>
<td>12.47</td>
<td>12.58</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>29.95</td>
<td>30.80</td>
<td>31.12</td>
</tr>
<tr>
<td>Glycine</td>
<td>50.97</td>
<td>77.85</td>
<td>85.67</td>
</tr>
<tr>
<td>Proline</td>
<td>40.91</td>
<td>42.15</td>
<td>42.51</td>
</tr>
<tr>
<td>Serine</td>
<td>81.82</td>
<td>84.30</td>
<td>85.03</td>
</tr>
</tbody>
</table>

†18.53 mg L-phenylalanine·kg⁻¹·d⁻¹ and 6.79 mg L-tyrosine·kg⁻¹·d⁻¹ were added daily to the three amino acid mixtures, except on the 24-h infusion day to compensate for the labeled amino acids given to the subjects during the 24-h infusion day. 1.09–1.12 g amino acid mixture·kg⁻¹·d⁻¹ was given to each subject.

intravenous infusion of L-[ring-²H₂]tyrosine (98 atom%) (=0.51 μmol·kg⁻¹·h⁻¹); [5,5,5-²H₃]leucine (98 atom%) (=2.70 μmol·kg⁻¹·h⁻¹) was given intravenously to five subjects studied at the low phenylalanine intake. The intravascular tracers were delivered through a 20-gauge, 5-cm catheter placed into an antecubital vein on the nondominant side. All of the tracers were obtained from MassTrace Inc, Woburn, MA. The oral tracers were dissolved in distilled water. The priming oral doses of tracers were given to the subjects ≈15 min before 1800, after the baseline breath and blood samples had been taken. At 1800 the subjects drank their first hourly dose of the tracers and this was repeated at each subsequent hour during the remainder of the 24-h period.

Blood samples (=3.5 mL) were drawn every 0.5 h through a 20-gauge, 3.2-cm catheter placed in a superficial vein of the hand or wrist on the subject’s nondominant side. The hand was placed in a custom-made warming box at 68 °C for ≥10 min before the sampling time to achieve arterialization of the venous blood. The patency of the sampling catheter was maintained by a slow infusion of 0.9% NaCl. Samples of expired air were collected and determinations of total carbon dioxide output made according to the schedule followed previously (4, 13, 14).

Collection and analysis of samples

Indirect calorimetry and isotopic abundances

Measurement of total carbon dioxide production, details of collection and analysis of breath and blood samples for determination of ¹³C abundance in carbon dioxide, and determinations of ¹³C and ²H₂ in plasma tyrosine and phenylalanine, respectively, and [²H₃]tyrosine, [²H₄]tyrosine, and [²H₃]leucine were all performed as described previously (4, 13, 15).

Tracer model for phenylalanine, tyrosine, and leucine kinetics

As in our previous studies of phenylalanine and tyrosine kinetics, we used the tracer model of phenylalanine and tyrosine metabolism first proposed by Clarke and Bier (21), with modifications suggested by Thompson et al (22). The amino acid (phenylalanine, tyrosine, or leucine) flux (Qᵢ, Qᵢᵣ, Qᵢ_, in μmol·kg⁻¹·h⁻¹) was calculated according to standard, steady state isotope-dilution principles:

\[ Q = i(E_i/E_p) \] (1)

where \( i \) is the rate of tracer infusion (μmol·kg⁻¹·h⁻¹) and \( E_i \) and \( E_p \) are the isotopic enrichments of the infusate and plasma tracer amino acid, respectively.

The rate of phenylalanine conversion to tyrosine (Qᵢᵣ, in μmol·kg⁻¹·h⁻¹) was derived as follows:

\[ Q_{ᵢᵣ} = Q_{ᵢ₄}(E_{ᵣ₄}/E_{ᵢ₄}) \] (2)

where \( Q_{ᵢ₄} \) is the tyrosine flux (estimated from the primed, constant, oral intake of [¹³C]tyrosine) and \( E_{ᵣ₄} \) and \( E_{ᵢ₄} \) are the plasma enrichments of [¹³C]tyrosine and [¹³C]phenylalanine, respectively, after oral administration of [²H₄]phenylalanine.

The rate of ¹³CO₂ released by the L-[¹-¹³C]tyrosine tracer and the oxidation rate of tyrosine were calculated as described previously for phenylalanine (15). The estimates were corrected for retention of ¹³CO₂ in the body. On the basis of the results of our 24-h infusion studies with [¹³C]bicarbonate, we used the value of 0.77 for the fasted (postabsorptive state) and 0.85 for the fed state (4).

Leucine flux (Qₚ) was determined, as described above, because it can be used as a standard index of whole-body protein turnover (23), permitting investigators to make a comparison of leucine kinetics of our subjects with those studied in other laboratories. The enrichment of plasma leucine was measured rather than that of α-ketoisocaproate (24) because the latter would have required a separate determination and this was not considered to be essential for the purposes of our study.
Finally, we and others (25–30) have shown, using isotopic methods, that the extent of disappearance of orally derived labeled amino acids within the splanchnic region varies with the specific amino acid and, possibly, with the amount of the amino acid in the diet (31). For these reasons, the first-pass splanchnic uptake of the oral tyrosine was calculated by using the following equation (27, 30):

\[ F = 1 - \left( \frac{Q_{\text{Tyr,IV}}}{Q_{\text{Tyr,IG}}} \right) \]  

where \( F \) is the fraction of tracer taken up during its first pass through the splanchnic tissues; \( Q_{\text{Tyr,IV}} \) is the plasma \([\text{H}_4]\)tyrosine flux from the tracer administered intravenously and \( Q_{\text{Tyr,IG}} \) is the plasma \([\text{C}^{13}]\)tyrosine flux from the tracer administered orally.

**Evaluation of primary data**

**Tyrosine oxidation from measurement of \([\text{C}^{13}]\)CO\(_2\) output**

Tyrosine oxidation was calculated for 48 consecutive 0.5-h intervals as described above; we refer here to the measured appearance of \([\text{C}^{13}]\)CO\(_2\) in expired air and its relation to the plasma \([\text{C}^{13}]\)tyrosine isotope abundance as tyrosine oxidation.

**Phenylalanine hydroxylation**

Phenylalanine hydroxylation was also computed for 48 consecutive 0.5-h intervals. For these intervals, the average isotope abundance of the amino acids (phenylalanine and tyrosine) in plasma collected at the two time points defining that 0.5-h interval was used for calculation purposes.

**Body tyrosine balance**

The major focus of interest in this study was on the daily whole-body tyrosine balance determined by using a \([\text{C}^{13}]\)tyrosine tracer given orally to estimate irreversible, whole-body tyrosine loss. Furthermore, the net input of tyrosine, under the present experimental conditions, into the metabolically active pool was that derived from the hydroxylation of phenylalanine together with the small amount supplied in the form of \([1-\text{C}^{13}]\)- and \([\text{H}_4]\)tyrosine tracers. Hence, body tyrosine balance (\( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \)) was calculated as follows:

\[
\text{Body tyrosine balance} = \text{[phenylalanine hydroxylation plus tyrosine tracer input] – tyrosine oxidation}
\]

where phenylalanine hydroxylation, tyrosine tracer input, and tyrosine oxidation were daily rates computed from summation of the 48 0.5-h intervals over the 24-h tracer period.

**Body aromatic amino acid balance**

Our previous studies indicated that estimations of whole-body phenylalanine balance or of whole-body aromatic amino acid balance, based on calculations of phenylalanine and tyrosine intakes in relation to phenylalanine oxidation, tend to give higher balance values than would be expected for the prevailing experimental dietary conditions, especially at generous intakes of phenylalanine (14). It was of interest, therefore, to calculate body aromatic amino acid balance by using values for phenylalanine intake and then to compare these balance calculations with our primary measure of tyrosine balance, as indicated above. Therefore, aromatic amino acid balance was also determined: total aromatic amino acid input – tyrosine oxidation. It was calculated as follows: aromatic amino acid input (\( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \)) = phenylalanine (diet and tracer) + tyrosine tracer (intravenous and oral), and tyrosine output (\( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \)) was calculated as the sum of the determined tyrosine oxidation rates for the 48 consecutive 0.5-h intervals.

**Statistical methods**

For comparison of means of repeated measurements (fasting and feeding) generated from the same subjects within a diet group, a paired \( t \) test was used. For variables measured in the different groups, within each physiologic state (eg, fasting or feeding), we performed a two-factor analysis of variance (ANOVA) with repeated measures on one factor using subject \( \times \) condition (fasting and feeding) \( \times \) group (dietary phenylalanine intake) design. If a significant condition \( \times \) group interaction was observed, we used an additional one-way repeated-measures ANOVA for each condition (fasting and feeding), followed by pairwise comparisons among means with the Student-Neuman-Keuls test. To compare the mean of a variable with zero in each of the three different groups (eg, 24-h tyrosine or aromatic amino acid balance) a one- or two-tailed (depending on the hypotheses), one-sample \( t \) test was used. Data analyses were performed with SAS software (version 6; SAS Institute, Inc, Cary, NC). An \( \alpha \) level of 0.05 was considered to be significant.

**RESULTS**

As shown in Figure 1, total carbon dioxide production declined during about the first 9 h after the last meal, eaten at 1500 on day 6 (3 h before the beginning of the 24-h isotope-tracer protocol). As expected, with the beginning of the feeding period
at 0600 on day 7, there was a prompt rise in carbon dioxide output, with a relatively steady rate then being maintained to within ∼2.5–3 h after the first small meal. All three diet groups showed a similar 24-h pattern. In contrast, the pattern of $^{13}$C enrichment of the expired carbon dioxide differed among the diet groups and these differences were reflected in the total output of $^{13}$CO$_2$ as shown in Figure 2. The differences were evident during the fed period. For the generous phenylalanine group, $^{13}$CO$_2$ output rose with the beginning of meal ingestion and after ∼3 h the higher rate was maintained for the remainder of this metabolic phase. In contrast, for both the intermediate- and low-phenylalanine groups the output of $^{13}$CO$_2$ did not rise with feeding and then declined significantly after ∼3 h into the fed period, reaching values that were somewhat below the fasting, mean rate of $^{13}$CO$_2$ output by the time that this feeding phase was terminated.

Patterns of change in the isotope abundance of plasma tyrosine after oral administration of [1-$^{13}$C]tyrosine and [2H$_2$]phenylalanine tracers are shown in Figures 3 and 4, respectively. Similarly, the 24-h pattern of plasma [2H$_4$]tyrosine enrichment after a constant intravenous administration of this tracer is depicted in Figure 5. The pattern of enrichment was similar among the diet groups during the fast but diverged with feeding. Thus, there was relatively little change from the fasting value in the generous-phenylalanine group but an evident and similar rise in the intermediate- and low-phenylalanine groups. This indicates a fall in plasma tyrosine flux with ingestion of lower phenylalanine intakes. The 24-h pattern of change in the isotopic enrichment of [2H$_3$]leucine in the five subjects given this tracer was essentially identical to that reported previously (4). Hence, these results are not presented here in detail.

A summary of the isotopic-enrichment data, for the entire 12-h fast and fed periods and for selected times within these two metabolic phases, is given in Table 2 for the orally administered tracers as well as for the [2H$_2$]tyrosine arising from [2H$_2$]phenylalanine. In Table 3, comparable data for both intravenously administered tracers are presented. From these isotopic data, together with those shown in Figures 1–5, the various estimations presented below for whole-body phenylalanine, tyrosine, and leucine kinetics were derived.

The phenylalanine flux, determined with the oral [2H$_2$]tracer, was lower in the feeding than in the fasting period ($P < 0.05$) with the 35.6 and 18.5 mg·kg$^{-1}$·d$^{-1}$ intakes of phenylalanine (Table 4). This contrasted with the small but significant ($P < 0.05$) rise in flux when phenylalanine intake was generous. When the oral [13C]tyrosine tracer and plasma [13C]tyrosine enrichment were used, the calculated tyrosine flux was similar among the three diet groups during the fasting period and declined slightly ($P < 0.05$) with the 96.6- and 35.6-mg intakes but only a tendency for a decline with the 18.5-mg intake. On the other hand, as summarized in Table 5, tyrosine flux declined ($P < 0.05$) with feeding at the two lower phenylalanine intakes when this estimate was derived from the intravenously administered [2H$_4$]tyrosine tracer. Leucine flux (Table 5) increased ($P < 0.05$) with feeding in the five subjects who were given the leucine tracer.

From the plasma isotopic data for [1-$^{13}$C]- and [2H$_2$]tyrosine, estimates were made of the first-pass splanchnic uptake of labeled tyrosine in all three groups. As summarized in Table 6, splanchnic uptake was ∼40% of the tracer dose during the fast.
and from 30% to 50% during the feeding period. However, none of the differences between diet groups were significant.

The rate of tyrosine oxidation throughout the 24-h period for each of the three diet groups is shown in Figure 6. Absolute values for selected periods of the 24-h day are summarized in Table 7. The rate of tyrosine oxidation declined with feeding at the two lower phenylalanine intakes whereas it was maintained with the generous phenylalanine intake. These changes paralleled those seen for [2H₄]tyrosine flux, although the changes in oxidation appeared to be quantitatively more pronounced.

Estimates were also made of the rate of phenylalanine hydroxylation. As shown in Figure 7, and summarized in Table 7, the diet-group differences in the patterns of change with feeding in the rate of phenylalanine hydroxylation were similar to those seen for tyrosine oxidation. The hydroxylation rates were ∼40–50% lower for the 35.6- and 18.5-mg phenylalanine intakes during the fasting and feeding periods when compared with those determined with the 100-mg phenylalanine intake.

From the estimates of phenylalanine hydroxylation and tyrosine oxidation we calculated tyrosine and total aromatic amino acid balances, as described in Methods. As summarized in Table 8, tyrosine balance did not differ significantly from zero (or equilibrium) when phenylalanine intakes were 96.6 and 35.6 mg · kg⁻¹ · d⁻¹ but it appeared to be negative (0.05 < P < 0.1) at the 18.5-mg phenylalanine intake. A generally comparable diet-related pattern in the status of aromatic amino acid balance was found among the three diet groups: balance was not maintained with the 18.5-mg intake (P < 0.05), equilibrium was achieved at the 35.6-mg intake, and a distinctly positive balance (P < 0.05 from zero) was achieved at the generous (96.6 mg) phenylalanine intake. The differences in the absolute values for tyrosine and aromatic amino acid balances found at the generous intake will be addressed in the Discussion.

Finally, as shown in Figure 8, plasma phenylalanine and tyrosine concentrations had 24-h rhythms that were dependent on the dietary intake of phenylalanine. By the end of the 12-h fast period, phenylalanine concentrations were generally similar among the intermediate- and low-phenylalanine-intake groups, but during the earlier 9-h phase of the fast the concentrations in plasma qualitatively reflected the prior dietary intake. This was also generally the case for plasma tyrosine. As the duration of the fast progressed, plasma tyrosine concentrations rose in the 18.5- and 35.6-mg phenylalanine intake groups, reaching concentrations that were lower than those achieved at the generous phenylalanine intake. With feeding, plasma phenylalanine concentrations rose, achieving relatively steady concentrations within 2–4 h, when the meals provided a generous intake of phenylalanine (Figure 8A). In contrast, phenylalanine concentrations fell after the intermediate and low phenylalanine intakes but different plateaus were reached after ∼5–6 h, reflecting the two phenylalanine intakes (Figure 8, B and C). Plasma tyrosine concentrations in the fed state also responded differently to the different phenylalanine-containing, low-tyrosine diets (Figure 8); there was a modest but progressive decline in the concentration of plasma tyrosine when the generous-phenylalanine, low-tyrosine meals were consumed (Figure 8A) and a comparable but more dramatic fall in fed-state plasma tyrosine concentrations in those subjects given the intermediate- and low-phenylalanine diets (Figure 8, B and C). These plasma phenylalanine and tyrosine changes qualitatively reflected the altered [2H₄]tyrosine fluxes, phenylalanine hydroxylation rates,
<table>
<thead>
<tr>
<th>Time and Condition</th>
<th>96.6 mg Phe ( \cdot ) kg(^{-1} \cdot ) d(^{-1} ) ((n = 5))</th>
<th>35.6 mg Phe ( \cdot ) kg(^{-1} \cdot ) d(^{-1} ) ((n = 5))</th>
<th>18.5 mg Phe ( \cdot ) kg(^{-1} \cdot ) d(^{-1} ) ((n = 6))</th>
<th>Mole fraction %(^2)</th>
<th>96.6 mg Phe ( \cdot ) kg(^{-1} \cdot ) d(^{-1} ) ((n = 5))</th>
<th>35.6 mg Phe ( \cdot ) kg(^{-1} \cdot ) d(^{-1} ) ((n = 5))</th>
<th>18.5 mg Phe ( \cdot ) kg(^{-1} \cdot ) d(^{-1} ) ((n = 6))</th>
<th>Mole fraction %(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–720 min</td>
<td>2.41 ± 0.45</td>
<td>2.60 ± 0.36</td>
<td>2.44 ± 0.45</td>
<td>8.16 ± 0.76</td>
<td>9.23 ± 0.77</td>
<td>8.68 ± 0.98</td>
<td>2.54 ± 0.35</td>
<td>2.08 ± 0.35</td>
</tr>
<tr>
<td>Last hour (15th)(^3)</td>
<td>2.37 ± 0.34</td>
<td>2.89 ± 0.91</td>
<td>2.64 ± 0.35</td>
<td>8.44 ± 0.76</td>
<td>10.54 ± 0.76</td>
<td>9.59 ± 1.90</td>
<td>2.99 ± 0.70</td>
<td>2.76 ± 0.58</td>
</tr>
<tr>
<td>12th h(^4)</td>
<td>2.46 ± 1.03</td>
<td>2.94 ± 0.57</td>
<td>2.11 ± 0.49</td>
<td>9.06 ± 1.52</td>
<td>10.22 ± 1.69</td>
<td>9.78 ± 2.82</td>
<td>2.50 ± 0.42</td>
<td>2.40 ± 0.46</td>
</tr>
<tr>
<td>Fed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>720–1440 min</td>
<td>2.68 ± 0.45</td>
<td>3.93 ± 0.80</td>
<td>3.01 ± 0.84</td>
<td>7.34 ± 1.04</td>
<td>10.99 ± 1.60</td>
<td>12.50 ± 1.38</td>
<td>3.27 ± 0.63</td>
<td>2.86 ± 0.48</td>
</tr>
<tr>
<td>5th h</td>
<td>2.70 ± 0.49</td>
<td>3.55 ± 0.79</td>
<td>2.52 ± 0.74</td>
<td>7.40 ± 2.21</td>
<td>10.66 ± 2.97</td>
<td>10.98 ± 2.12</td>
<td>3.40 ± 0.77</td>
<td>2.10 ± 0.55</td>
</tr>
</tbody>
</table>

\(^{1}\) \(\bar{x} \pm \text{SD.}\)

\(^{2}\) Mole fraction above baseline \( \times 100.\)

\(^{3}\) The last hour of the tracer fasting period was 15 h after the last meal.

\(^{4}\) 12 h after the last meal or 9 h into the tracer fasting period.
TABLE 4
Receiving known but varying intakes of phenylalanine in combination with a low tyrosine intake. Therefore, we carried out the present study to narrow this gap in our understanding of the response of whole-body tyrosine metabolism to altered phenylalanine intakes in healthy, young adults. Furthermore, we emphasized previously (14, 15) the desirability of determining the rate of oxidation of tyrosine, with the aid of a [1-13C]tyrosine tracer, in studies of dietary aromatic amino acid requirements. In part, this suggestion emerged from our investigations of different phenylalanine intakes with [1-13C]phenylalanine as a tracer. From the results obtained we proposed a possible metabolic channeling (20) in the process of whole-body phenylalanine and tyrosine catabolism. We considered it important, therefore, to explore this issue by carrying out a similar set of dietary-metabolic experiments using a new combination of tyrosine tracers. The present results both enhance our capacity to describe the quantitative nature of whole-body tyrosine metabolism in healthy, adult subjects and lend additional support for our hypothesis that dietary requirements of aromatic amino acids exceed those proposed currently in 1985 by the FAO/WHO/UNU (16). These latter values provide, in our judgment, a questionable “norm” and, therefore, an unreliable database from which an assessment might be made of the metabolic basis for amino acid needs or of the quantitative effect of disease and trauma on adult human amino acid requirements. An international expert group

and rates of tyrosine oxidation after ingestion of meals supplying the different phenylalanine intakes. These responses suggest that plasma tyrosine concentrations in the fed state, under the present experimental conditions, were determined by the rate of conversion of dietary phenylalanine to tyrosine in the liver (and perhaps the kidney) with release of formed tyrosine into the peripheral circulation. On the other hand, the plasma tyrosine concentration during the postabsorptive period appeared to be affected particularly by the entry of tyrosine into the circulation, derived from breakdown of tissue proteins.

Finally, the rhythm of plasma leucine concentrations was similar among the three diet groups (Figure 8, A, B, and C). The intake of leucine was in excess of that which we proposed as being sufficient to meet requirements (1, 17) and also about six times higher than the FAO/WHO/UNU requirement (16). Furthermore, the pattern of change in plasma leucine concentration was also reflected by that for leucine flux for those five subjects that received the leucine tracer.

DISCUSSION
There are no published studies, to our knowledge, on whole-body kinetics and daily oxidation of tyrosine in human adults receiving known but varying intakes of phenylalanine in combination with a low tyrosine intake. Therefore, we carried out the present study to narrow this gap in our understanding of the response of whole-body tyrosine metabolism to altered phenylalanine intakes in healthy, young adults. Furthermore, we emphasized previously (14, 15) the desirability of determining the rate of oxidation of tyrosine, with the aid of a [1-13C]tyrosine tracer, in studies of dietary aromatic amino acid requirements. In part, this suggestion emerged from our investigations of different phenylalanine intakes with [1-13C]phenylalanine as a tracer. From the results obtained we proposed a possible metabolic channeling (20) in the process of whole-body phenylalanine and tyrosine catabolism. We considered it important, therefore, to explore this issue by carrying out a similar set of dietary-metabolic experiments using a new combination of tyrosine tracers. The present results both enhance our capacity to describe the quantitative nature of whole-body tyrosine metabolism in healthy, adult subjects and lend additional support for our hypothesis that dietary requirements of aromatic amino acids exceed those proposed currently in 1985 by the FAO/WHO/UNU (16). These latter values provide, in our judgment, a questionable “norm” and, therefore, an unreliable database from which an assessment might be made of the metabolic basis for amino acid needs or of the quantitative effect of disease and trauma on adult human amino acid requirements. An international expert group

TABLE 3
Isotopic abundance of [1H6]tyrosine and [1H6]leucine in plasma at specific times throughout the 24-h infusion day at different daily phenylalanine intakes

<table>
<thead>
<tr>
<th>Time and condition</th>
<th>96.6 mg Phe · kg⁻¹ · d⁻¹ (n = 5)</th>
<th>35.6 mg Phe · kg⁻¹ · d⁻¹ (n = 5)</th>
<th>18.5 mg Phe · kg⁻¹ · d⁻¹ (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast 0–720 min</td>
<td>1.87 ± 0.29</td>
<td>1.91 ± 0.24</td>
<td>2.01 ± 0.26</td>
</tr>
<tr>
<td>Last hour (15th)</td>
<td>1.94 ± 0.27</td>
<td>1.84 ± 0.14</td>
<td>1.85 ± 0.33</td>
</tr>
<tr>
<td>12h h¹</td>
<td>2.01 ± 0.37</td>
<td>1.86 ± 0.32</td>
<td>1.98 ± 0.19</td>
</tr>
<tr>
<td>Fed 720–1440 min</td>
<td>1.95 ± 0.18</td>
<td>2.87 ± 0.36</td>
<td>2.90 ± 0.75</td>
</tr>
<tr>
<td>5th h²</td>
<td>1.87 ± 0.28</td>
<td>2.82 ± 0.50</td>
<td>2.40 ± 0.64</td>
</tr>
</tbody>
</table>

¹ Mole fraction above baseline × 100.
² The last hour of the tracer fasting period was 15 h after the last meal.

TABLE 4
Plasma [13C]Tyrosine and [13C]Phenylalanine fluxes for the fed and fast periods and for selected times within these periods at different daily phenylalanine intakes

<table>
<thead>
<tr>
<th>Time and condition</th>
<th>[13C]Tyrosine (oral)</th>
<th>[13C]Phenylalanine (oral)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96.6 mg Phe · kg⁻¹ · d⁻¹ (n = 5)</td>
<td>35.6 mg Phe · kg⁻¹ · d⁻¹ (n = 6)</td>
<td>18.5 mg Phe · kg⁻¹ · d⁻¹ (n = 6)</td>
</tr>
<tr>
<td>0–720 min</td>
<td>49.2 ± 8.7</td>
<td>43.8 ± 8.3</td>
</tr>
<tr>
<td>Last hour (15th)²</td>
<td>47.2 ± 7.0</td>
<td>38.5 ± 8.5</td>
</tr>
<tr>
<td>12h h²</td>
<td>50.3 ± 17.2</td>
<td>36.6 ± 7.3</td>
</tr>
<tr>
<td>Fed 720–1440 min</td>
<td>42.4 ± 6.8</td>
<td>28.9 ± 7.1</td>
</tr>
<tr>
<td>5th h²</td>
<td>41.1 ± 6.3</td>
<td>31.0 ± 8.7</td>
</tr>
</tbody>
</table>

¹ Mole fraction above baseline × 100.
² The last hour of the tracer fasting period was 15 h after the last meal.
also concluded that "...values for amino acid requirements in the 1985 report derived from experiments of Rose and collaborators are no longer acceptable or nutritionally relevant... (32)." Before turning to a discussion of our specific findings, it is desirable to put the present major experiment into context and point out some of its limitations so that the conclusions we draw might be evaluated more easily.

The first major issue we raise concerns the question of whether the 6-d period of adaptation used here was long enough to permit the derivation of amino acid requirements. This issue is legitimately raised because the 24-h pattern of tyrosine oxidation (Figure 6) and of plasma phenylalanine and tyrosine concentrations (Figure 8) showed some asymmetry: values at 1440 min were below those found at the beginning of the 24-h tracer protocol. This appears likely, as discussed below, to be more a reflection of the design of our tracer protocol than an expression of a continuing adaptation to a lower than usual intake of or requirement for dietary phenylalanine.

The following points are relevant to this question of adaptation:

1) On the basis of earlier nitrogen excretion-balance studies carried out in our laboratories (33–35), it is entirely reasonable to propose that a 6-d adjustment period is suitable for purposes of establishing the amino acid needs of well-nourished subjects. Furthermore, relatively short-term experimental dietary studies supplied the data used by the FAO/WHO/UNU (16) to establish adult amino acid requirements for populations worldwide. Although Quevedo et al (37), from the results of their study on the transition of nitrogen balance from a high-protein (292 mg N · kg⁻¹ · d⁻¹) to a lower-protein (124 mg N · kg⁻¹ · d⁻¹) diet, suggest that it takes longer, possibly > 2 wk, for a new nitrogen equilibrium to be reached, their results for nitrogen excretion are confounded by the apparently low energy intake supplied by their experimental diet. Our calculations suggest that it approximated 142 kJ · kg⁻¹ · d⁻¹ (34 kcal · kg⁻¹ · d⁻¹), providing for a physical activity level (total energy expenditure expressed as a multiple of the basal metabolic rate) of ≈1.4. This value is much lower than those of ≥ 1.8 for healthy, young adults (38, 39) and of 1.6–1.8 for free-living, elderly men (40). On this basis it appears that energy intake might have been inadequate and so the mean ± SEM is questionable whether their nitrogen balance data can be used to help resolve the question of the time required to achieve a new steady state of whole-body protein metabolism. Nevertheless, leucine oxidation during fasting and fed states appeared to have reached a constant level within 7 d of the protein intake being lowered in their subjects.

2) A study was carried out in our laboratories to determine the rates of leucine oxidation (41) and phenylalanine hydroxylation (42) 1 and 3 wk after healthy subjects consumed diets containing adequate t-amino acid mixtures patterned after the 1985 FAO/WHO/UNU (16) adult amino acid requirements, the MIT tentative requirements, and whole hen’s egg proteins. The kinetics of whole-body leucine and phenylalanine catabolism during fasting and fed states did not differ between the 1- and 3-wk time periods, again supporting the premise that a 6-d diet adjustment period is suitable for exploring aromatic amino acid kinetics, balances, and requirements in healthy adults. In addition, Zello et al (18) reported that phenylalanine flux and oxidation rates at

### Table 5

<table>
<thead>
<tr>
<th>Time and condition</th>
<th>Plasma [³H]tyrosine</th>
<th>[³H]Tyrosine</th>
<th>[³H]Leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>96.6 mg Phe · kg⁻¹ · d⁻¹</td>
<td>35.6 mg Phe · kg⁻¹ · d⁻¹</td>
<td>18.5 mg Phe · kg⁻¹ · d⁻¹</td>
</tr>
<tr>
<td>Fast</td>
<td>0–720 min</td>
<td>28.8 ± 5.3</td>
<td>28.2 ± 5.1</td>
</tr>
<tr>
<td></td>
<td>Last hour (15th)</td>
<td>27.1 ± 4.6</td>
<td>28.4 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>12th h</td>
<td>26.3 ± 5.5</td>
<td>28.8 ± 6.3</td>
</tr>
<tr>
<td>Fed</td>
<td>720–1440 min</td>
<td>27.4 ± 2.8</td>
<td>19.9 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>5th h</td>
<td>28.1 ± 5.4</td>
<td>18.7 ± 2.8</td>
</tr>
</tbody>
</table>

Note: Only five subjects received [³H]tyrosine and [³H]leucine as intravenous tracers (see Methods).

### Table 6

<table>
<thead>
<tr>
<th>Time and condition</th>
<th>96.6 mg Phe · kg⁻¹ · d⁻¹</th>
<th>35.6 mg Phe · kg⁻¹ · d⁻¹</th>
<th>18.5 mg Phe · kg⁻¹ · d⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–720 min</td>
<td>39 ± 19</td>
<td>35 ± 6</td>
</tr>
<tr>
<td></td>
<td>Last 2 h</td>
<td>45 ± 15</td>
<td>30 ± 19</td>
</tr>
<tr>
<td>Fed</td>
<td>720–1440 min</td>
<td>33 ± 16</td>
<td>29 ± 13</td>
</tr>
<tr>
<td></td>
<td>3rd–5th</td>
<td>31 ± 11</td>
<td>37 ± 14</td>
</tr>
</tbody>
</table>

Note: Standard deviation.
test intakes of phenylalanine ranging from 5 to 60 mg·kg⁻¹·d⁻¹ (in conjunction with a generous tyrosine intake), each given as six meals, were not affected by giving subjects a diet supplying 4.2 or 14 mg phenylalanine·kg⁻¹·d⁻¹ for up to 9 d before their kinetic measurements.

3) It could be argued that even a 3-wk dietary period might not be long enough for a full degree of adaptation, but then this becomes an exceedingly difficult issue to resolve both conceptually and in terms of its implications for the determination of functional state and nutritional requirements. Perhaps, however, it should be asked whether the amino acid requirements of individuals from populations in developing regions of the world—particularly when protein, dietary lysine, or both, are likely to be more limiting—are similar to or different from those for US subjects despite the fact that the current international FAO/WHO/UNU (16) amino acid requirements—based largely on studies conducted in young, adult, American subjects—are recommended for populations worldwide. There has not been any major effort to carry out ¹³C-tracer studies comparable with those described here in subjects outside of North America. An initial study that we completed with our collaborators at St John’s College, Bangalore, India (43), using the indicator amino acid oxidation technique (44) to assess the lysine requirement of healthy Indians whose long-term lysine intake appears to be ~60% of the amount characteristic of the subjects in the present study, does not suggest that these Indian subjects require different intakes for maintenance compared with MIT subjects. Clearly, further studies will have to be conducted to strengthen this initial conclusion.

4) There are few relevant data that can be used to answer the question of whether indispensable amino acid requirements, in particular those of lysine, are similar or different among various population groups who have adapted to different dietary conditions. Studies of obligatory nitrogen losses in US (45–47), Chinese (48), Indian (49), Nigerian (50, 51), and Japanese men (52) have shown that the requirements are remarkably uniform (53). This finding implies similar obligatory amino acid losses, and, by implication, in our opinion, a similarity in the dietary requirements for indispensable amino acids (17), unless the efficiency of retention of specific amino acid at requirement intakes differs among apparently similar subjects in these different population groups. According to the FAO/WHO/UNU (16), nitrogen balance studies have not shown any striking differences in estimates of total protein requirements, in relation to body cell mass, in studies of well-nourished subjects in different countries. Earlier studies by Nicol and Phillips (50, 54) suggesting that Nigerian men of low income adapt to low-protein diets and utilize dietary protein more efficiently than, for example, US students (46), are not appropriate to answer the question posed above. The nitrogen balance results in these studies indicate that the subjects were depleted and that they were undergoing a body protein repletion response to the adequate diet given during the experiments. Later

<table>
<thead>
<tr>
<th>Time and condition</th>
<th>Tyrosine oxidation (μmol·kg⁻¹·h⁻¹)</th>
<th>Phenylalanine hydroxylation (μmol·kg⁻¹·h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>96.6 mg Phe·kg⁻¹·d⁻¹ (n = 5)</td>
<td>18.5 mg Phe·kg⁻¹·d⁻¹ (n = 6)</td>
</tr>
<tr>
<td></td>
<td>35.6 mg Phe·kg⁻¹·d⁻¹ (n = 5)</td>
<td>18.5 mg Phe·kg⁻¹·d⁻¹ (n = 5)</td>
</tr>
<tr>
<td></td>
<td>18.5 mg Phe·kg⁻¹·d⁻¹ (n = 5)</td>
<td>18.5 mg Phe·kg⁻¹·d⁻¹ (n = 6)</td>
</tr>
</tbody>
</table>

/ Table 7

Rates of tyrosine oxidation and phenylalanine hydroxylation for 12-h fast and 12-h fed periods and during specific time intervals for the study at different daily phenylalanine intakes.

/ Figure 6. Rate and pattern of tyrosine oxidation throughout a 24-h period in subjects given 96.6 (A; n = 5), 35.6 (B; n = 5), and 18.5 (C; n = 6) mg phenylalanine·kg⁻¹·d⁻¹, x ± SEM.
FIGURE 7. Rate and pattern of phenylalanine hydroxylation throughout a 24-h period in subjects given 96.6 (A; n = 5), 35.6 (B; n = 5), and 18.5 (C; n = 6) mg phenylalanine · kg⁻¹ · d⁻¹, x ± SEM.

studies in Nigerian adult men (55) indicate that at maintenance nitrogen intakes, the efficiency of dietary protein utilization is essentially the same as that for white and Asian subjects.

5) From our collaborative studies with Masud et al (56) and Tom et al (57), it was concluded that the postabsorptive rate of whole-body leucine oxidation at the end of a 16 ± 2-h period during which nonacidotic, chronic renal failure patients, were fed a very-low-protein diet (0.28 g protein · kg⁻¹ · d⁻¹) plus an amino acid–keto acid supplement was not different from that at baseline. Furthermore, the leucine oxidation rate for these subjects was quite comparable with that which we reported in healthy adults given either 14 or 38 mg leucine · kg⁻¹ · d⁻¹ for 6 d before the measurement of [¹³C]leucine kinetics (5).

6) Finally, inspection of the 24-h pattern of tyrosine oxidation (Figure 6) and of the plasma phenylalanine and tyrosine concentrations (Figure 8) showed that the values obtained at 0 and 1440 min of measurement were not the same, possibly suggesting that adaptation had not been achieved by the end of the 6 d. However, we had not expected a perfectly symmetrical 24-h pattern because the 24-h tracer day began and ended under somewhat different conditions. The reason for giving the last meal of the day at 1500 on days 6 and 7 (instead of at 1800 as on days 1–5) was to permit us to capture within the 24-h period essentially the complete oxidation of phenylalanine and tyrosine that had been given on that day because a delay in the total output of [¹³C]tyrosine oxidation, emerging from the last meal, would be anticipated. We could have given a last meal at 1800 on day 7 while continuing to measure tyrosine oxidation for the next few hours but this was not considered to be a necessary design characteristic. A similar type of 24-h pattern of leucine oxidation and plasma leucine concentration was found in our previous study at intermediate and low leucine intakes (5). Thus, the higher values at the beginning than at the end of the 24-h tracer period are possibly a further reflection of the fact that a large meal was ingested at 1500 on day 6 rather than a small meal as at the end of the 24-h tracer period (day 7) and possibly the effects of the tracer primes. However, the error that may have been introduced, for these reasons, into our estimate of tyrosine oxidation can be estimated not to exceed =5% of the recorded daily tyrosine loss, assuming that during the first 3 h of the 24-h study the tyrosine oxidation rate had equaled that observed for the last 3 h of the 24-h d. In summary, it is reasonable to propose that the present 6-d period of dietary “lead-in” permitted an appropriate adjustment, or adaptation, to the variable phenylalanine intakes. This hypothesis should now be validated further through metabolic studies of varying duration and preferably in comparable population groups in different geographic regions.

A second experimental issue concerns the design of our 24-h tracer-infusion protocol. Specifically, we gave the tyrosine and phenylalanine tracers in equal amounts during both the 12-h fast and 12-fed periods. At the low phenylalanine test intake this meant that a significant fraction of the total daily intake was given during the postabsorptive phase, raising the question about the relative utilization of phenylalanine when given in this way compared with the usual requirement for an intake of the other indispensable amino acids when intakes exceed immediate needs for protein synthesis (58, 59) and that these amino acids can later be used when there is an intake of the other indispensable amino acids (60, 61).

However, the extent to which the input of the tracers, during the 24-h period, affects the rate of their appearance in the plasma cannot be judged easily.
from the present results. The rate of tyrosine oxidation during the later phase of the fast amounted to 10 μmol·kg⁻¹·h⁻¹ for the intermediate intake of phenylalanine (35.6 mg·kg⁻¹·d⁻¹), which seemed to be the appropriate intake to use for evaluation and comparison here. Thus, if we assume that the obligatory loss of aromatic amino acids during the fast period is ≈7 μmol·kg⁻¹·h⁻¹ (62), or more, then it does not appear that the tracers given in this study had a profound effect on the fasting rate of whole-body aromatic amino acid oxidation.

Furthermore, studies by Bergstrom et al (63) on the changes in muscle amino acids in adult subjects after consumption of protein-free and protein-rich meals suggest that it also might be concluded that the tracer doses of tyrosine and phenylalanine given during the fast were retained, at least in part, in tissue free amino acid pools. This effect is also implied in stable-isotope-tracer studies of amino acid oxidation, protein turnover, and calculated nitrogen balance, such as those by Quevedo et al (37) referred to above. Our studies with leucine as a tracer further support this contention, in view of the agreement obtained between measured and leucine-derived estimates of nitrogen excretion (4, 64, 65). This problem of the 24-h distribution of a tracer that is not "massless" has been discussed critically by Fuller and Garlick (66) and they concluded reasonably that this could give rise to an overestimate of the amino acid requirement. Clearly, future studies must resolve this problem of isotope administration during the fasting period and determine the best way to provide the daily test amino acid intake during the 24-h tracer protocol.

The third and final major issue, at this point, relates to the choice of tracers and their routes of administration. We administered the primary L-[1-¹³C]tyrosine tracer orally because we had concluded from a previous experiment with L-[1-¹³C]phenylalanine that a reliable tracer-based estimation of the rate of whole-body phenylalanine oxidation may require oral administration of the labeled phenylalanine. The [³H]phenylalanine tracer was given orally on the premise that the estimate of the whole-body, daily rate of conversion to tyrosine via hydroxylation would similarly be improved when given orally rather than intravenously. [³H]Tyrosine was given by vein so that the first-pass splanchnic disappearance of the absorbed tyrosine tracer could be determined by procedures described previously (31). In this context, it is reasonable to question the extent to which the oral administration of the tyrosine label mimics the hepatic (splanchnic) production and oxidation of tyrosine from phenylalanine. Although there is no gold standard for us to depend on, there was an internal consistency for estimates of rates of tyrosine oxidation and phenylalanine hydroxylation. Thus, tyrosine oxidation exceeded the rate of phenylalanine hydroxylation at the lowest (18.5 mg) phenylalanine intake.

At a zero intake of aromatic amino acids it is to be expected that the rate of tyrosine oxidation, reflecting the loss of aromatic amino acids, would be greater than that for phenylalanine hydroxylation. The ratio of tyrosine oxidation to phenylalanine hydroxylation might be predicted to be ≈1.7, assuming that the molar concentrations of phenylalanine and tyrosine in whole-body mixed proteins are ≈1.7 and 1.1 μmol/g protein N (67, 68), respectively. On the other hand, at the generous phenylalanine intake, in conjunction with a low tyrosine intake, the rates of phenylalanine hydroxylation and tyrosine oxidation (assuming a single homogeneous metabolic pool) would be expected to be about equal. Therefore, the ratio of tyrosine oxidation to phenylalanine hydroxylation would be predicted to rise as the intake of phenylalanine (with low dietary tyrosine) falls below an intake sufficient to maintain body tyrosine balance. We observed this direction of change in the ratios of tyrosine oxidation to phenylalanine hydroxylation in the fasting and fed states as the test intake of phenylalanine was reduced from 96.6 to 18.5 mg·kg⁻¹·d⁻¹. The mean ratio for the generous intake was 1.1 during the fast period; at the 18.5-mg intake when subjects were in negative tyrosine and aromatic amino acid balance, the observed mean ratio of tyrosine oxidation to phenylalanine hydroxylation was 1.79 for the fast state, approximating that expected from the amino acid composition of mixed body proteins (67, 68). However, we should also point out that it is not possible to predict a precise ratio of tyrosine oxidation to phenylalanine hydroxylation during the fast state at the actual test intakes of phenylalanine applied in the present experiment. This is because the ratio would depend, in part, on the efficiency with which the tyrosine derived via phenylalanine hydroxylation was used to meet the tyrosine needs of cells. This efficiency factor, as well as that for the utilization and retention of dietary tyrosine, remains unknown.

On the basis of these considerations, as outlined above, we now turn to the results of this investigation on the kinetics of whole-body tyrosine metabolism that were examined at phenylalanine intakes of 96.6, 35.6, and 18.5 mg·kg⁻¹·d⁻¹ in conjunction with a low tyrosine intake. Our purpose was to compare these results with those from our previous [¹³C]phenylalanine tracer studies that were carried out at essentially the same test amino acid intakes. Hence, we expected that our estimations of tyrosine and aromatic amino acid balances would be in the neutral to positive range at the 96.6- and 35.6-mg intakes but nega-
ative at the 18.5-mg intake. We proposed earlier (1), as supported by results of three recent phenylalanine tracer studies (13–15), that the mean requirement for phenylalanine, when determined in conjunction with a low tyrosine intake, is \(\approx 39 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}\). This value is not fundamentally dissimilar to that of \(\approx 30 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}\), which was proposed by Zello et al. (18) as an approximate requirement for healthy adults. However, both of these newer estimates are substantially higher than the value of 14 mg \(\cdot \text{kg}^{-1} \cdot \text{d}^{-1}\) that was accepted in 1985 by the FAO/WHO/UNU (16). Indeed, as described in the Results section, the balances of that the mean requirement for phenylalanine, when determined?

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· h−1; however, at the higher phenylalanine intake (24.5 μmol · kg−1 · h−1, or 96.6 mg · kg−1 · d−1), the rates were 0.56 and 1.05 μmol · kg−1 · h−1, respectively (Figure A2). These results indicate that there was a lower proportion of ingested [13C]tyrosine tracer entering the nonsplanchnic region or phase of metabolism with the low phenylalanine intake. This low intake would result in a relatively lower enrichment of [13C]tyrosine in the nonsplanchnic pool when compared with a higher phenylalanine intake. Because the calculation of total-body tyrosine oxidation after enteral infusion of [13C]tyrosine is based on its enrichment in the nonsplanchnic pool, such a difference in the relative dilution of [13C]tyrosine enrichment within the splanchnic compared with the nonsplanchnic pool would lead to a relative overestimation of its total oxidation at the low phenylalanine intake, or conversely, a relative underestimation of tyrosine oxidation at a more generous dietary phenylalanine intake.

Using our previously published data for phenylalanine kinetics as determined by tracing the intravenous and intragastric administration of 1-[1-13C]phenylalanine tracers in subjects given high (14) and low (13) phenylalanine intakes, we estimated further the possible fate of ingested phenylalanine within the splanchnic and nonsplanchnic regions in the present study and how this might affect the dilution of the [13C]tyrosine tracer in these regions. Therefore, from the estimates given in Appendix A (Tables A1 and A2 and Figure A2 and Figure A3), at the lower phenylalanine intake the [13C]tyrosine tracer extraction during its first pass in the splanchnic region (eg, 3.5 mg · kg−1 · d−1, or 0.80 μmol · kg−1 · h−1) would be diluted by phenylalanine oxidation via the splanchnic tyrosine pool during the first pass of phenylalanine in the splanchnic region (0.23 μmol · kg−1 · h−1). However, during the nonsplanchnic phase the entry of [13C]tyrosine (3.5 mg · kg−1 · d−1, or 0.80 μmol · kg−1 · h−1) would be further diluted by phenylalanine hydroxylation in the nonsplanchnic region (0.26 μmol · kg−1 · h−1), by phenylalanine oxidation via the tyrosine pool in the nonsplanchnic region (0.19 μmol · kg−1 · h−1), and by phenylalanine hydroxylation in the splanchnic region with the tyrosine formed being subsequently released into the nonsplanchnic region (0.21 μmol · kg−1 · h−1). From these estimates, at the low phenylalanine intake we calculated that the comparative degrees of dilution of [13C]tyrosine tracer in the splanchnic and nonsplanchnic regions were 78% and 55% (Figure A2), giving a relative ratio of nonsplanchnic to splanchnic dilution of [13C]tyrosine tracer of 0.71 (Figure A2). For the higher phenylalanine intake, on the basis of the same approach the latter ratio was estimated to be 0.57 (0.11/0.19; Figure A2). As a consequence of these relative differences, where in the present case the tyrosine intakes with the two diets were the same, there was a tendency to overestimate tyrosine oxidation at the low phenylalanine intake, or conversely, to underestimate tyrosine oxidation at the high phenylalanine intake.

At the intermediate intake, the [13C]-derived tyrosine and aromatic amino acid balances were neutral, as we had anticipated from our previous results with [13C]phenylalanine as tracer (15). This implied that the complex interrelations between tracer route and labeling in the splanchnic region and peripheral circulation possibly converge to give a reasonable approximation of the rates of phenylalanine hydroxylation and tyrosine oxidation when the phenylalanine intake is neither excessive nor restricted compared with the amount required to maintain aromatic amino acid homeostasis. This hypothesis might be tested by using alternative measures of requirements, including, for example, [13C]leucine as a tracer in the indicator amino acid oxidation method (44).

We have now determined rates of whole-body phenylalanine hydroxylation in four separate investigations (13–15), including the present study, using the models originally proposed by Clarke and Bier (21) and Thompson et al (22). These various estimates, determined in healthy adults under controlled but varying dietary conditions, for the fast state, range from ≈16 μmol · kg−1 · h−1 with a generous phenylalanine intake to ≈8–10 μmol · kg−1 · h−1 with low intakes of phenylalanine. In comparison, published rates of postabsorptive phenylalanine hydroxylation for healthy adults range from ≈6 to 11 μmol · kg−1 · h−1 (21, 22, 78) when based on intravenous tracer studies. However, a close comparison of these estimates must be made with caution because of differences in 1) the design of the tracer protocols, including length and route of tracer administration, which we showed here as well as previously (13, 14) to have significant effects on the values derived; 2) the nutritional and metabolic state of the experimental subjects; and 3) the specific choice of isotopomers (42, 79). Nevertheless, it seems reasonable to suggest for healthy, adult subjects postabsorptively, whose diet is typical for that of the US population, that the whole-body rate of phenylalanine hydroxylation might approximate 16–20 μmol · kg−1 · h−1 over the course of a day and about half this rate when phenylalanine intakes are low. This approximation might be compared with the mean of 4.8 μmol · kg−1 · h−1 (range: 0.9–8.4 μmol · kg−1 · h−1) reported by Thompson et al (78) for phenylketonuric subjects postabsorptively who had relaxed their strict diets at the time of the study; the mean intake of their subjects (36 mg · kg−1 · d−1) approximated the present requirement. Also, Kiliani et al (80) reported a value of 5.9 μmol · kg−1 · h−1 for preterm infants during the first 4 d of life before they had received amino acid nutrition and it increased significantly to 11 μmol · kg−1 · h−1 when they received amino acid feeding.

In conclusion, from estimates of tyrosine balance, made by combining determinations of phenylalanine hydroxylation and 13C tracer–derived values for tyrosine oxidation, and estimates of whole-body aromatic amino acid balance it is evident that the 1985 FAO/WHO/UNU (16) upper requirement of 14 mg · kg−1 · d−1 for total aromatic amino acids (phenylalanine plus tyrosine) in healthy adults is too low. We assume that the low, nonnegligible tyrosine intake of 6.8 mg · kg−1 · d−1 had a favorable effect on phenylalanine utilization as well as on tyrosine balance, and so the results of our present and previous studies, together with those from Zello et al (18), suggest a mean aromatic amino acid requirement ranging from ≈30 to ≈40 mg · kg−1 · d−1. Until more extensive data become available it seems prudent, in our judgment, to accept a value of 39 mg phenylalanine plus tyrosine · kg−1 · d−1 (17) for use in nutritional and metabolic considerations of the dietary aromatic amino acid needs of healthy adults.


longer-term amino acid kinetic study with support for the adequacy of the Massachusetts Institute of Technology amino acid requirement pattern. Am J Clin Nutr 1953;58:670–83.


APPENDIX A
Tracing the fate of dietary phenylalanine and tyrosine in the splanchnic (first pass) and nonsplanchnic (second pass) regions in vivo

For purposes of assessing the metabolic fate of dietary phenylalanine (Phe), the following equations are based on a study design in which tracer L-[1-13C]Phe ([13C]Phe) is administered under the same dietary conditions either intragastrically (IG, the same route as dietary intake) or intravenously (IV), but on different occasions. L-[ring 2H4]Tyrosine ([D4]Tyr) is infused IV simultaneously. The metabolic fate of [13C]Phe infused IG represents the fate of dietary phenylalanine.

1) Fraction of total IG infused [13C]Phe tracer metabolically taken up by the splanchnic compartment (first pass):

\[
F_{\text{sp,uptake}} = 1 - \frac{Q_{\text{IG}}}{Q_{\text{IV}}} \tag{A1}
\]

where \(Q_{\text{IV}}\) and \(Q_{\text{IG}}\) are the metabolic fluxes measured during tracer infusions given IV and IG.

\[
Q_{\text{IV}} = i_{\text{IV}}^{13\text{C} \text{Phe}} (E_{\text{p,IV}}^{13\text{C} \text{Phe}} / E_{\text{p,IV}}^{13\text{C} \text{Phe}}) - 1)
\]

\[
Q_{\text{IG}} = i_{\text{IG}}^{13\text{C} \text{Phe}} (E_{\text{p,IG}}^{13\text{C} \text{Phe}} / E_{\text{p,IG}}^{13\text{C} \text{Phe}}) - 1)
\]

and where \(i_{\text{IV}}^{13\text{C} \text{Phe}}\) and \(i_{\text{IG}}^{13\text{C} \text{Phe}}\) are the rates of tracer infusion given IV and IG, \(E_{\text{p,IV}}^{13\text{C} \text{Phe}}\) and \(E_{\text{p,IG}}^{13\text{C} \text{Phe}}\) are the infused enrichments and \(E_{\text{p,IV}}^{13\text{C} \text{Phe}}\) and \(E_{\text{p,IG}}^{13\text{C} \text{Phe}}\) are the plateau plasma enrichments after tracer infusions given IV and IG, respectively. Then the fraction of total IG-infused [13C]Phe tracer metabolically taken up by the nonsplanchnic compartment (second pass) is as follows:

\[
F_{\text{nsp,uptake}} = 1 - F_{\text{sp,uptake}} \tag{A2}
\]

2) Fraction of IG-infused [13C]Phe (hence, the enterally fed phenylalanine) oxidized in the splanchnic (first pass) and nonsplanchnic (second pass) compartments.

Fraction of total IG-infused [13C]Phe tracer oxidized (\(F_{\text{IG,oxid}}\)) is as follows:

\[
F_{\text{IG,oxid}} = \frac{V_{\text{IG}}^{13\text{C} \text{CO}_2}}{i_{\text{IG}}^{13\text{C} \text{Phe}}} \times 0.81 \tag{A3}
\]

where \(V_{\text{IG}}^{13\text{C} \text{CO}_2}\) is the rate of total 13CO2 production in expired air after [13C]Phe was infused IG and 0.81 is the 13CO2 recovery factor.

By the same reasoning, the fraction of total IV-infused [13C]Phe oxidized (\(F_{\text{IV,oxid}}\)) is as follows:

\[
F_{\text{IV,oxid}} = \frac{V_{\text{IV}}^{13\text{C} \text{CO}_2}}{i_{\text{IV}}^{13\text{C} \text{Phe}}} \times 0.81 \tag{A4}
\]

where \(V_{\text{IV}}^{13\text{C} \text{CO}_2}\) is the rate of total 13CO2 production in expired air after IV-infused [13C]Phe.

The fraction of total 13CO2 production contributed by splanchnic (first pass, \(F_{\text{sp,oxid}}\)) and nonsplanchnic (second pass, \(F_{\text{nsp,oxid}}\)) compartments is estimated as follows, where tot is total. Because the rate of IG-infused [13C]Phe appearing in the nonsplanchnic pool is \(i_{\text{IG}}^{13\text{C} \text{Phe}} \times F_{\text{nsp,uptake}} \) (see Eq 2), which is handled in first pass in a manner similar to that for the IV-infused tracer, then the fraction of nonsplanchnic contribution to total [13C]Phe oxidation after the intragastric (\(F_{\text{nsp,oxid}}\)) is as follows:

\[
F_{\text{sp,oxid}} = \frac{i_{\text{IG}}^{13\text{C} \text{Phe}} \times F_{\text{nsp,uptake}} \times F_{\text{IV,oxid}}}{i_{\text{IG}}^{13\text{C} \text{Phe}} \times F_{\text{IV,oxid}}} = F_{\text{nsp,uptake}} \times F_{\text{IV,oxid}} / F_{\text{IG,oxid}} \tag{A5}
\]

Therefore, the fraction of splanchnic (first pass) contribution to total IG-administered [13C]Phe oxidation (\(F_{\text{sp,oxid}}\)) is as follows:

\[
F_{\text{sp,oxid}} = 1 - F_{\text{nsp,oxid}} \tag{A6}
\]

The above approaches are similar to that reported by Matthews et al (1) in calculating the first-pass leucine oxidation in the splanchnic region.

3) Fraction of IG-infused [13C]Phe (hence the ingested Phe) converted to Tyr and subsequently appearing in the nonsplanchnic pool (\(F_{\text{IG,oxid}} \times \text{Tyr} \)) as follows:

\[
F_{\text{IG,oxid}} \times \text{Tyr} = \frac{E_{\text{IG}}^{13\text{C} \text{Tyr}} / i_{\text{IG}}^{13\text{C} \text{Phe}}}{D_{\text{Tyr},\text{IV}} / i_{\text{IV}}^{13\text{C} \text{Phe}}} \tag{A7}
\]

where \(E_{\text{IG}}^{13\text{C} \text{Tyr}}\) is the plateau plasma enrichment of [13C]Tyr arising from the IG-infused [13C]Phe, \(D_{\text{Tyr},\text{IV}} / i_{\text{IV}}^{13\text{C} \text{Phe}}\) is the metabolic flux of tyrosine measured by IV-infused [D4]Tyr.

Fraction of total IV-infused [13C]Phe converted to [13C]Tyr that subsequently appears in the nonsplanchnic pool (\(F_{\text{IV,oxid}} \times \text{Tyr} \)) as follows:

\[
F_{\text{IV,oxid}} \times \text{Tyr} = \frac{E_{\text{IV}}^{13\text{C} \text{Tyr}} / Q_{\text{IV}}^{13\text{C} \text{Phe}}}{D_{\text{Tyr},\text{IV}} / i_{\text{IV}}^{13\text{C} \text{Phe}}} \tag{A8}
\]

where \(E_{\text{IV}}^{13\text{C} \text{Tyr}}\) is the plateau plasma enrichment of [13C]Tyr arising from the IV-infused [13C]Phe.

Fractional contributions made by the splanchnic (first pass, \(F_{\text{sp,oxid}} \times \text{Tyr} \)) and nonsplanchnic (second pass, \(F_{\text{nsp,oxid}} \times \text{Tyr} \)) compartments to the total oxidation of the IG-infused [13C]Phe are as follows:

By reasoning similar to that used for calculating \(F_{\text{nsp,oxid}}\), then

\[
F_{\text{sp,oxid}} \times \text{Tyr} = \frac{i_{\text{IG}}^{13\text{C} \text{Phe}} \times F_{\text{nsp,uptake}} \times F_{\text{IV,oxid}} \times \text{Tyr}}{i_{\text{IG}}^{13\text{C} \text{Phe}} \times F_{\text{IV,oxid}} \times \text{Tyr}} = F_{\text{nsp,uptake}} \times F_{\text{IV,oxid}} \times \text{Tyr} / F_{\text{IG,oxid}} \tag{A9}
\]

and

\[
F_{\text{sp,oxid}} \times \text{Tyr} = 1 - F_{\text{nsp,oxid}} \times \text{Tyr} \tag{A10}
\]

4) Metabolic fate of IG-infused [13C]Phe (hence the dietary Phe) within the splanchnic region.

Fraction of total splanchnic metabolic uptake of Phe that undergoes oxidation within the splanchnic region (\(F_{\text{sp,oxid}}\)) is as follows:

\[
F_{\text{sp,oxid}} = \frac{i_{\text{IG}}^{13\text{C} \text{Phe}} \times F_{\text{sp,oxid}} \times F_{\text{IV,oxid}}}{i_{\text{IG}}^{13\text{C} \text{Phe}} \times F_{\text{IV,oxid}}} = F_{\text{sp,oxid}} \times F_{\text{IV,oxid}} / F_{\text{IG,oxid}} \tag{A11}
\]
Fraction of total splanchnic metabolic uptake of Phe converting to Tyr that subsequently appears in systemic plasma ($F_{\text{sp,Phe}}$) is as follows:

$$F_{\text{sp,Phe}} = \frac{i_{\text{IG}} [\text{13C}]\text{Phe}}{i_{\text{IG}} [\text{13C}]\text{Phe} \times F_{\text{sp,uptake}}}$$

$$= \frac{F_{\text{Phe-Tyr}} \times F_{\text{sp,uptake}}}{i_{\text{IG}} [\text{13C}]\text{Phe}} \quad (A12)$$

5) Metabolic fate of IG-infused [13C]Phe (hence the dietary Phe) in the nonsplanchnic region.

The quantitative information for the major pathways in the nonsplanchnic region can be deduced using the same reasoning as described in section 4 above for the splanchnic region. Therefore, the fraction of total nonsplanchnic metabolic disposal of ingested Phe that undergoes oxidation within the nonsplanchnic region, $F_{\text{nsp,Phe}}$ oxid is as follows:

$$F_{\text{nsp,Phe}} = \frac{i_{\text{IG}} [\text{13C}]\text{Phe}}{i_{\text{IG}} [\text{13C}]\text{Phe} \times F_{\text{nsp,uptake}}}$$

$$= \frac{F_{\text{Phe-oxid}} \times F_{\text{nsp,uptake}}}{i_{\text{IG}} [\text{13C}]\text{Phe}} \quad (A13)$$

6) Metabolic fate of tyrosine.

The metabolic fate of tyrosine in the splanchnic and nonsplanchnic regions can also be measured by the same approach, except that the major pathways of its disposal are via oxidative and nonoxidative pathways, mostly for protein synthesis.

From the equations above and using the data published previously by our group (2, 3), the relative contribution made by the splanchnic compared with nonsplanchnic compartments to the whole-body metabolism of orally administered Phe and Tyr are shown in Table A1 and Figures A2 and A3. The fractions of the extracted Phe entering different metabolic pathways within each region or phase of metabolism (splanchnic and nonsplanchnic, respectively) are shown in Table A2 and Figure A3.
TABLE A1
Proportionate contribution of splanchnic (SP) and nonsplanchnic (NSP) phases to whole-body phenylalanine (Phe) and tyrosine (Tyr) metabolism during the fed state

<table>
<thead>
<tr>
<th>Metabolism</th>
<th>Phe kinetics</th>
<th>Tyr kinetics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Phe</td>
<td>High Phe</td>
</tr>
<tr>
<td>First-pass extraction (SP)</td>
<td>0.50²</td>
<td>0.24²</td>
</tr>
<tr>
<td>Second-pass extraction (NSP)</td>
<td>0.50</td>
<td>0.76</td>
</tr>
<tr>
<td>Fraction of total oxidation in SP</td>
<td>0.65</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>0.35</td>
<td>0.64</td>
</tr>
<tr>
<td>SP</td>
<td>0.62</td>
<td>0.34</td>
</tr>
<tr>
<td>NSP</td>
<td>0.38</td>
<td>0.66</td>
</tr>
</tbody>
</table>

¹ Data taken and calculated from references 1 and 2.
² Data are also shown schematically in Figure A3.
³ Data taken from Table 7 and shown schematically in Figure A2.

TABLE A2
Total metabolism of phenylalanine (Phe) associated with oxidation and with conversion to plasma tyrosine within the splanchnic and nonsplanchnic regions during the fed state ⁴

<table>
<thead>
<tr>
<th>Phe intake</th>
<th>SP</th>
<th>NSP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxidation</td>
<td>To tyrosine</td>
</tr>
<tr>
<td>Low</td>
<td>9.2</td>
<td>8.3</td>
</tr>
<tr>
<td>High</td>
<td>38.9</td>
<td>25.1</td>
</tr>
</tbody>
</table>

⁴ These data are also shown schematically in Figure A3.

Figure A2. Estimates of the metabolic fate of enterally given tyrosine (Tyr) tracer at low and generous dietary intakes. Values are µmol · kg⁻¹ · h⁻¹, except for %. NSP, nonsplanchnic; SP, splanchnic.
REFERENCES

