Continuous 24-h L-[1-13C]phenylalanine and L-[3,3-2H2]tyrosine oral-tracer studies at an intermediate phenylalanine intake to estimate requirements in adults1–3

Anibal Basile-Filho, Antoine E El-Khoury, Louis Beaumier, San W Yang, and Vernon R Young

ABSTRACT The daily rates of whole-body phenylalanine oxidation (phe-ox) and hydroxylation (phe-OH) were determined in young men (n = 10) receiving [13C]phenylalanine and [2H2]tyrosine via primed constant oral infusion (four also received simultaneously [1H6]tyrosine and [1H5]leucine via primed constant intravenous infusions) continuously for 24 h (first 12 h fast and then 12 h fed). The subjects were given a diet supplying a proposed requirement phenylalanine intake (six subjects: 39 mg phenylalanine kg⁻¹ d⁻¹ without tyrosine; four subjects: 36 mg phenylalanine plus 6.8 mg tyrosine), based on an otherwise adequate t-amino acid mixture for 6 d before the tracer study. Our hypothesis was that the subjects would be in approximate body phenylalanine equilibrium at these intakes. Estimates of the daily rate of phe-ox were 26.9 ± 7.5 mg kg⁻¹ d⁻¹ (17.2 ± 5.2 and 9.7 ± 3.2 mg kg⁻¹ d⁻¹ during the 12-h fast and fed periods, respectively), and for phe-OH they were 32.1 ± 11.9 mg kg⁻¹ d⁻¹ (21.7 ± 10.5 and 10.4 ± 2.5 mg kg⁻¹ d⁻¹ during the 12-h fast and fed periods, respectively). The daily phenylalanine balance was approximately neutral (P > 0.05) when based on phe-ox or phe-OH (+4.73 ± 7.6 mg kg⁻¹ d⁻¹). In comparison with recent, comparable 24-h tracer studies at deficient (22 mg kg⁻¹ d⁻¹) and generous (100 mg kg⁻¹ d⁻¹) phenylalanine intakes, these results support the hypothesis that phenylalanine intake of 39 mg kg⁻¹ d⁻¹ (without significant tyrosine) approximates the mean requirement in healthy adults. This contrasts with the upper requirement value of 14 mg kg⁻¹ d⁻¹ for the total of the aromatic amino acids proposed in 1985 by FAO/WHO/UNU. Am J Clin Nutr 1997;65:473–88.

KEY WORDS Indispensable amino acid requirements, phenylalanine oxidation, phenylalanine hydroxylation, diurnal rhythm, plasma concentration, stable isotopes, phenylalanine balance, tyrosine flux

INTRODUCTION

We (1-4) applied 13C-labeled amino acid tracer infusion protocols to reassess the quantitative requirements for specific indispensable amino acids in healthy young adults (5, 6). An extensive series of studies has been conducted with leucine as the test amino acid, including three separate investigations of the 24-h pattern of whole-body leucine kinetics and oxidation (1, 2, 7).

Recently, we began a reevaluation of the requirements for phenylalanine (3, 4). The 1985 FAO/WHO/UNU (8) upper requirement for the total aromatic amino acids (phenylalanine and tyrosine) is 14 mg kg⁻¹ d⁻¹ in healthy adults. However, we predicted the requirement to be ≈39 mg kg⁻¹ d⁻¹, based on considerations of the estimated obligatory losses of phenylalanine (5, 9, 10). A 24-h [13C]phenylalanine tracer experiment in young adults, who received a diet supplying 21 mg kg⁻¹ d⁻¹, indicated that they were in negative body phenylalanine balance at this intake, which was somewhat higher than the FAO/WHO/UNU (8) upper requirement. At a generous phenylalanine intake (100 mg kg⁻¹ d⁻¹), daily phenylalanine balance was determined to be positive, by ≈7% of intake when based on an estimate of the rate of phenylalanine hydroxylation or ≈20% of intake when derived from the estimated rate of [13C]phenylalanine oxidation (4). Thus, our tracer model probably underestimates the whole-body rate of phenylalanine loss and so at the low phenylalanine intake it is likely that the actual body phenylalanine balance was somewhat more negative than we had estimated it to be (3). There is, of course, no “gold standard” method for determination of body amino acid balance in healthy human adults. However, we anticipated that body phenylalanine balance at an intermediate, or approximate requirement, when based on [13C]phenylalanine oxidation, would be estimated to be slightly positive. Furthermore, this was to be expected from the studies by Zello et al (11) in which a [13C]phenylalanine tracer was used to measure phenylalanine oxidation during single test meals providing graded intakes of phenylalanine. These investigators concluded that the FAO/WHO/UNU upper requirement for the

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3 Address reprint requests to VR Young, MIT, 77 Massachusetts Avenue, Room E17-434, Cambridge, MA 02139. E-mail: vryoung@mit.edu. Received July 11, 1996. Accepted for publication September 23, 1996.
total aromatic amino acids is too low and they proposed a requirement of 30 mg \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \). Hence, their estimate is consistent with our position (5, 10, 12) regarding the likely significant underestimation of the physiologic needs for most indispensable amino acids, as judged by FAO/WHO/UNU from earlier nitrogen-balance studies (13, 14).

In this paper we present our findings for the kinetics of whole-body phenylalanine metabolism in healthy subjects given a diet supplying either 39 mg phenylalanine without tyrosine or 35.5 mg phenylalanine \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \) and 6.8 mg tyrosine \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \). The tracers were given orally because our recent studies (3, 4) showed that the intravenous route of tracer administration led to a substantial underestimation of the daily rate of phenylalanine catabolism. The present results are compared with those obtained both at lower (3) and higher (4) test intakes of phenylalanine.

SUBJECTS AND METHODS

Subjects

Ten young adult male volunteers were recruited from the student population of the Massachusetts Institute of Technology (MIT) and the community of the Boston-Cambridge region (Table 1). The subjects were studied as outpatients of the Clinical Research Center (CRC) at the Medical Department of MIT. The subjects were judged to be in good health as determined by medical history and clinical examination, as well as by a laboratory profile, which included a complete blood cell count, standard blood chemistry, and urinalysis. Criteria for exclusion from the study were cigarette smoking and ingestion of more than five alcoholic drinks per week and more than six cups of caffeinated beverages daily.

The daily energy intake was estimated to be sufficient to maintain body weight throughout the study and was evaluated by a dietician through a dietary history and assessment of the subject’s usual level of physical activity. The mean energy intake for these subjects was 188 kJ (\( \approx \) 45 kcal) \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \); they were asked to maintain their usual levels of activity, without participating in competitive sports or particularly strenuous periods of exercise.

The design and aims of the study, as well as potential risks involved, were explained to each subject. Written consent was obtained from each subject as 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 for the last day of the dietary study period for the 24-h stable-isotope tracer study protocol. All remained healthy throughout the study period and they received financial compensation for their participation in the study.

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 g protein \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \) for 6 d (Table 2). Besides the l-amino acid mix, the major portion of the energy requirement was provided by wheat-starch cookies as described in detail previously (1). 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 \(^{13}\)C abundance in the diet and a steady background of \(^{13}\)C enrichment in the breath during the 24-h study period (1). For the six subjects who were studied with oral \(^{13}\)Cphenylalanine and \(^{2}\)H\(_2\)tyrosine tracers, the phenylalanine intake provided by the l-amino acid mixture diet was 17.1 mg \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \), without tyrosine. In addition, on each day, except during the 24-h tracer infusion day, 21.9 mg l-phenylalanine \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \) was added to allow for the tracer given during the 24-h infusion day. In the remaining four subjects who were given oral \(^{13}\)Cphenylalanine and \(^{2}\)H\(_2\)tyrosine and intravenous \(^{2}\)H\(_2\)tyrosine and \(^{2}\)H\(_3\)leucine the l-amino acid mixture supplied 17.1 mg phenylalanine \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \) and then the diet was supplemented with an additional 18.6 mg phenylalanine and 6.8 mg tyrosine \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \). On the day of the 24-h tracer study, the \(^{13}\)Cphenylalanine tracer supply was the equivalent of 18.5 mg \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \) and \(^{2}\)H\(_2\)tyrosine and \(^{2}\)H\(_2\)tyrosine provided 6.8 mg \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \). Hence, total aromatic amino acid intake (diet plus tracer) for the six subjects who did not receive \(^{2}\)H\(_2\)tyrosine intravenously was 44.5 mg \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \), and for the four subjects given

### Table 1

<table>
<thead>
<tr>
<th>Tracer route and subject</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>Height (m)</th>
<th>BMI (kg/m(^2))</th>
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<tbody>
<tr>
<td>Oral tracer(^1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>65.8</td>
<td>1.79</td>
<td>20.51</td>
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<td>2</td>
<td>21</td>
<td>60.6</td>
<td>1.72</td>
<td>20.48</td>
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<td>3</td>
<td>25</td>
<td>66.8</td>
<td>1.74</td>
<td>22.06</td>
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<tr>
<td>4</td>
<td>20</td>
<td>70.4</td>
<td>1.78</td>
<td>22.22</td>
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<tr>
<td>5</td>
<td>22</td>
<td>78.0</td>
<td>1.86</td>
<td>22.55</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>81.6</td>
<td>1.82</td>
<td>24.42</td>
</tr>
<tr>
<td>Oral and intravenous tracer(^2)</td>
<td>20</td>
<td>66.1</td>
<td>1.72</td>
<td>22.16</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>80.5</td>
<td>1.76</td>
<td>25.84</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>63.0</td>
<td>1.68</td>
<td>22.27</td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>75.9</td>
<td>1.83</td>
<td>22.54</td>
</tr>
<tr>
<td>( \bar{x} \pm SD)</td>
<td>21.9 ± 1.7</td>
<td>70.5 ± 7.9</td>
<td>1.77 ± 0.05</td>
<td>22.5 ± 1.6</td>
</tr>
</tbody>
</table>

\(^1\) These subjects received oral \(^{13}\)Cphenylalanine and \(^{2}\)H\(_2\)tyrosine.

\(^2\) These subjects received both oral \(^{13}\)Cphenylalanine and \(^{2}\)H\(_2\)tyrosine and intravenous \(^{2}\)H\(_2\)tyrosine and \(^{2}\)H\(_3\)leucine.
TABLE 2
Composition of the L-α-amino acid mixture used to provide an intake of ≈39 mg phenylalanine · kg⁻¹ · d⁻¹

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Quantity</th>
<th>g/kg mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td></td>
<td>16.31</td>
</tr>
<tr>
<td>Threonine</td>
<td></td>
<td>49.24</td>
</tr>
<tr>
<td>Isoleucine</td>
<td></td>
<td>65.70</td>
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<tr>
<td>Leucine</td>
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<tr>
<td>Lysine HCl</td>
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<td>79.16</td>
</tr>
<tr>
<td>Methionine</td>
<td></td>
<td>31.03</td>
</tr>
<tr>
<td>Cystine</td>
<td></td>
<td>24.45</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td></td>
<td>15.59</td>
</tr>
<tr>
<td>Tyrosine</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Valine</td>
<td></td>
<td>73.47</td>
</tr>
<tr>
<td>Histidine HCl</td>
<td></td>
<td>32.06</td>
</tr>
<tr>
<td>Arginine HCl</td>
<td></td>
<td>79.06</td>
</tr>
<tr>
<td>Alanine</td>
<td></td>
<td>200.22</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td></td>
<td>12.47</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td></td>
<td>30.80</td>
</tr>
<tr>
<td>Glycine</td>
<td></td>
<td>77.85</td>
</tr>
<tr>
<td>Proline</td>
<td></td>
<td>42.15</td>
</tr>
<tr>
<td>Serine</td>
<td></td>
<td>84.30</td>
</tr>
</tbody>
</table>

¹ For the six subjects who did not receive [²H₅]tyrosine, the amino acid mixture supplied 17.1 mg L-phenylalanine · kg⁻¹ · d⁻¹. An additional 21 mg L-phenylalanine · kg⁻¹ · d⁻¹ was added except on the 24-h infusion day.
² For the four subjects who did receive [²H₅]tyrosine, the amino acid mixture supplied 17.1 mg L-phenylalanine · kg⁻¹ · d⁻¹. Also, 18.58 mg L-phenylalanine · kg⁻¹ · d⁻¹ and 6.79 mg L-tyrosine · kg⁻¹ · d⁻¹ were added daily to the amino acid mixture, except on the 24-hour infusion day, to compensate for the labeled amino acids given to the subjects.

[²H₅]tyrosine it was 42.5 mg · kg⁻¹ · d⁻¹ during the 24-h tracer study. The other essential nutrients were given in appropriate amounts, as reported previously (1).

The subjects consumed their daily diet as three isoenergetic, isonitrogenous meals (at 0800, 1200, and 1800) during the 6 d preceding the 24-h tracer study. Vital signs and body weight were monitored every morning before the first meal. After 6 d of the experimental diet, a 24-h stable-isotope tracer study was carried out in each subject beginning on day 6 at 1800 and continuing until day 7 at 1800. Constant oral infusions of [1,13C]phenylalanine and [3,3,2H₂]tyrosine were given to all subjects. In addition to the oral tracers, four of the subjects received L-[ring-²H₅]tyrosine and [5,5,5,2H₃]leucine via a constant intravenous infusion, as described below.

Twenty-four-hour tracer protocol

A primed, constant intravenous-tracer-infusion protocol was combined with the oral-tracer protocol in 4 of the 10 subjects. All 10 subjects were given labeled amino acids dissolved in distilled water orally every hour. The details and rationale for the design of the 24-h study were described previously (1). Briefly, subjects received a primed, quasi-constant oral dose of L-[1,13C]phenylalanine (99 atom percent) and [3,3-²H₂]tyrosine (99 atom percent) and four subjects also received L-[ring-²H₅]tyrosine (98 atom percent) and [5,5,5,²H₃]leucine (98 atom percent 2.7 μmol · kg⁻¹ · h⁻¹) intravascularly through a 20-gauge, 5-cm catheter placed into an antecubital vein on the subject’s nondominant side. The tracers were purchased from MassTrace, Inc, Woburn, MA. The priming doses and constant infusion rates of the tracers, including the prime with NaH¹³CO₃, were aimed to be as in our previous studies (1–4). The measured mean rates were 4.8, 1.07, and 0.53 μmol · kg⁻¹ · h⁻¹ for [¹³C]phenylalanine (n = 10), [²H₅]tyrosine (n = 10), and [²H₅]tyrosine (n = 4), respectively. The oral tracers were dissolved in distilled water and priming doses 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 during each successive hour for the remainder of the 24-h period.

Blood samples (≈3.5 mL) were drawn every one-half hour 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 ≈15 min before the sampling time to achieve arterIALIZATION of venous blood. The sampling catheter was kept patent slow by infusion of 0.9% NaCl.

Collection and analysis of samples

Indirect calorimetry

Carbon dioxide production (VCO₂) and oxygen consumption (VO₂) rates were measured with an indirect calorimeter (DeltaTrac; SensorMedics, Anaheim, CA), as described previously (1). Measurements were made according to our standardized procedure every hour throughout the 24-h period (1). During the night, three measurements were considered to be sufficient to determine the average sleeping metabolic rate. This technique provides a dependable determination of VCO₂ over the 24-h day (1).

Breath collection and ¹³CO₂

Breath samples were collected every 30 min after three baseline samples were taken, one each at 40, 25, and 15 min before the tracers were given. The method of collection and analysis of ¹³CO₂ by isotope-ratio mass spectrometry was described previously (1).

Blood collection and ¹⁴CO₂ enrichment

Blood samples were drawn at 30-min intervals between 0000 and 0600, and three baseline samples were taken, one each 40, 25, and 15 min before the administration of the tracers. Two milliliters of blood were used for ¹³CO₂ analysis, as described previously (1). The precision of the ¹³CO₂ quality control samples, run seven times over a period of months with the study samples, was 0.014%.

Plasma samples

At each consecutive, half-hourly interval throughout the 24-h study period and 40, 25, and 15 min before the tracers were given, ≈3.5 mL blood was drawn into sodium heparin-coated glass tubes and centrifuged at 4 °C for 15 min at 1200 × g. Plasma was kept at −20 °C until analyzed. Plasma phenylalanine and tyrosine concentrations, as well as infusate concentrations, were analyzed by HPLC (model 334; Beckman, Palo Alto, CA) with an ion-exchange chromatographic method involving postcolumn derivatization with o-phthalaldehyde and quantitation with the aid of a
fluorescence detector. All isotopic measurements of plasma aromatic amino acids and of leucine were carried out with a Hewlett-Packard gas chromatograph (HP 5890 series II; Palo Alto, CA) coupled to an HP 5988A quadrupole mass spectrometer and an HP RTE-6 data system as described previously (1, 3).

Tracer model for phenylalanine, tyrosine, and leucine kinetics

Amino acid fluxes. We used the tracer model of phenylalanine and tyrosine metabolism described by Clarke and Bier (15) with modifications proposed by Tompson et al (16). The amino acid (phenylalanine or tyrosine) flux ($Q_p$ or $Q_t$: $\mu$mol $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$) was calculated according to standard, steady state isotope-dilution principles, as follows, in essentially the same way as described previously (17):

$$Q = i(E/E_p)$$

where, $i$ is the rate of tracer infusion ($\mu$mol $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$) and $E$ and $E_p$ are the isotope enrichments of the infused and plasma amino acid, respectively.

Leucine flux was also calculated as described previously (17, 18). We and others (19) use this parameter as an index of the status of whole-body protein turnover. For the present purpose, the enrichment of plasma leucine was measured rather than that of $\alpha$-ketoisocaproate, for reasons discussed earlier (4). Further, there is no generally accepted, intracellular-derived, plasma metabolite for purposes of estimating the intracellular phenylalanine and tyrosine enrichments. However, Reeds et al (20), from apolipoprotein B-100 isotope-labeling studies, suggested the ratio of plasma to hepatic free phenylalanine enrichment to be $\approx 1.2$ and 1.0 for fed and fasted states, respectively. This implies that the plasma $[1-^{13}$C$]$phenylalanine pool, after an oral administration of the tracer, might provide a reasonable index of the enrichment of the liver free phenylalanine that is undergoing terminal oxidation.

FIGURE 1. Plasma isotopic abundance of $[\text{1}^{13}$C$]$phenylalanine, $[\text{3}^{13}$H$]$tyrosine, $[\text{4}^{13}$C$]$tyrosine, $[\text{5}^{13}$H$]$tyrosine, and $[\text{6}^{13}$H$]$leucine at various times during the 24-h day. "Fast" refers to the 720-min period of tracer infusion while subjects consumed no food. The actual length of the "fast" was 900 min because the last meal was eaten 3 h before the beginning of tracer infusion. As indicated, the last of the 10 small meals was given 1260 min into the 24-h tracer-infusion period.
Splanchnic uptake of tyrosine. The present study involved oral administration of phenylalanine and tyrosine tracers and we (21–25) and others (26, 27) showed, using isotopic methods, that the uptake of orally administered labeled amino acids within the splanchnic region varies substantially according to the specific amino acid and, perhaps, the dietary intake. Hence, the first-pass splanchnic uptake of the oral tyrosine tracer was calculated in four subjects by using the following equation (24):

\[ F = 1 - \left( \frac{Q_{iv}}{Q_{ng}} \right) \]  

(2)

where \( F \) is the fraction of tracer taken up during its first-pass through the splanchnic tissues. \( Q_{iv} \) is the plasma tyrosine flux from the administered \( [^{13} \text{C}] \) tyrosine tracer given intravenously and \( Q_{ng} \) is the plasma flux derived from the \( [^{2} \text{H}] \) tyrosine tracer that was given orally.

Evaluation of the primary data

Phenylalanine oxidation from \( 13\text{C} \)O2

Phenylalanine oxidation was calculated for consecutive half-hourly intervals. Before the plateau in plasma \( [^{13} \text{C}] \) phenylalanine enrichment was reached, during the first 120 min of the 24-h infusion, phenylalanine oxidation for these initial time periods was assumed to be equal to the first half-hourly interval of the plateau period, which began 120 min after the tracers were first given. Hence, phenylalanine oxidation was measured directly for 44 of the total 48 half-hourly intervals. The rate of \( 13\text{C} \)O2 released by the \( [1-^{13} \text{C}] \) phenylalanine tracer and oxidation rate of the amino acid were calculated as described previously (3, 4). These were corrected for the retention of \( 13\text{C} \)O2 in the body, based on results of our previous 24-h infusion studies with \( \text{NaH}^{13}\text{C} \)O2; we used a value of 0.77 for the fast state and 0.85 for the fed state (1).

Phenylalanine hydroxylation

The rate of conversion of phenylalanine to tyrosine (\( Q_{P \rightarrow T} \); \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \)) was derived as follows:

\[ Q_{P \rightarrow T} = \frac{Q_{t} (E_{t} - E_{p})}{E_{t}} \]  

(3)

where \( Q_{t} \) is the tyrosine flux estimated from the primed constant oral infusion of \( [^{2} \text{H}] \) tyrosine with a correction for the splanchnic first-pass disappearance of the oral tracer, as described previously (3, 4), and \( E_{t} \) and \( E_{p} \) are the plasma enrichments of \( [^{1-13} \text{C}] \) tyrosine and \( [1-^{13} \text{C}] \) phenylalanine, respectively.

Phenylalanine hydroxylation was computed for each consecutive half-hourly interval. As for oxidation, we assumed that the rate during the first 2 h of the 24-h tracer infusion period was the same as that during the 5th half hour of this period. For every half-hour interval, phenylalanine hydroxylation was calculated as shown, in equation 3, where \( Q_{t} \) is the tyrosine flux computed for each half hour and based on the average isotope abundance of the amino acid from the two time points defining that half-hour interval.

Phenylalanine balance

“Predicted” 24-h phenylalanine balance (phenylalanine input − predicted output) was computed from the \( 13\text{C} \)O2 phenylalanine oxidation or hydroxylation data as follows:

Input (\( \mu \text{mol/kg} \)) = dietary phenylalanine + tracer phenylalanine

Predicted output (\( \mu \text{mol/kg} \)) = (oxidation or hydroxylation during the selected fast hour × 12) + (oxidation or hydroxylation during the selected feeding hour × 12)

“Measured” 24-h phenylalanine balance (phenylalanine input − measured output) was calculated as follows:

Input (\( \mu \text{mol/kg} \)) = dietary phenylalanine + tracer phenylalanine

Output (\( \mu \text{mol/kg} \)) = sum of the measured oxidation or hydroxylation for the 48 half-hour intervals.

In our two recent studies in subjects who had consumed either a low (3) or a generous (4) phenylalanine intake, we calculated balance both with and without taking into account the intake of tyrosine, because of a possible sparing effect on the phenylalanine requirement (28, 29). This was done because the tyrosine tracer given amounted to a potentially physiologically significant intake over the course of the 24-h study. This was necessary to determine tyrosine flux and the rate of phenylalanine hydroxylation at the very low dietary tyrosine intake given in this experiment. Therefore, we have assumed a reasonable tyrosine-sparing effect on phenylalanine oxidation of 70% (13, 28, 29) for the present “intermediate” level of phenylalanine intake (39 mg · kg\(^{-1} \) · d\(^{-1}\)). Hence, the physiologically effective phenylalanine intake (for estimation of phenylalanine balance from \( [^{13} \text{C}] \) phenylalanine loss) was calculated as follows:

Corrected phenylalanine intake (\( \mu \text{mol/kg} \))

= phenylalanine intake − (tyrosine intake × 0.7)

where phenylalanine intake is the total intake of phenylalanine including tracer and for the tyrosine intake it is the total amount of tracer given during the 24-h tracer study.

Statistical methods

The results were summarized and analyzed with the aid of a Macintosh 6100/60 Power PC computer (Apple Computer, Inc, Cupertino, CA) by using the Data Analysis ToolPak included in the program EXCEL (version 5.0; Microsoft Co, Redmond, WA). To compare directly values derived by measurement with those obtained by the indirect (predicted) approach (ie, those derived by extrapolation), and to evaluate the differences between fasting and feeding with respect to phenylalanine oxidation and hydroxylation and the fluxes and tracer abundances, the data were analyzed by using the two-tailed paired t test. To determine whether phenylalanine balance was different from zero, we used a two-tailed one-sample t test. A \( P \) value ≤ 0.05 was considered to be significant.

RESULTS

Isotopic concentrations in plasma

The plasma isotopic abundances during the 24-h period for the tracers after their oral ([\( ^{13} \text{C} \)] phenylalanine, [\( ^{2} \text{H} \)] tyrosine) or intravenous ([\( ^{2} \text{H} \)] tyrosine and [\( ^{13} \text{C} \) leucine] administration, together with the resulting \( [^{13} \text{C}] \) tyrosine abundance, are shown
in Figure 1. These data are also summarized in Table 3 for specific periods during the 24-h day.

**Plasma amino acid fluxes**

A summary of values for \[^{13}\text{C}\]phenylalanine, \[^{2}\text{H}_4\]tyrosine, \[^{2}\text{H}_4\]tyrosine, and \[^{2}\text{H}_4\]leucine fluxes during the entire 12-h fasting and 12-h fed periods is presented in Table 4. The tyrosine fluxes based on the oral tracer are both uncorrected and corrected for the first-pass disappearance of the oral tracers. Thus, the tyrosine fluxes derived from the \[^{2}\text{H}_4\]tyrosine tracer given by the intravenous route are markedly lower than the uncorrected fluxes calculated from the measured isotopic abundance of plasma tyrosine after the oral administration of \[^{2}\text{H}_4\]tyrosine.

Fluxes for two specific times during the fast (12th and 15th hour) and one for the fed period (5th hour) are also presented in Table 4 because we used these times, as discussed below, to predict daily phenylalanine hydroxylation. A more comprehensive picture of changes in these fluxes at various times throughout the 24-h period is given in Figure 2. Despite the fluctuations in the phenylalanine and tyrosine (\[^{2}\text{H}_2\]) fluxes during the 12-h fast, due perhaps to the uneven delivery of the oral tracer into the metabolic pools, the fluxes were significantly reduced (P < 0.01) with feeding at this test intake of total aromatic amino acid. The flux derived from the \[^{2}\text{H}_4\]tyrosine tracer (given intravenously) showed a similar behavior. In contrast, the plasma leucine flux increased with feeding (Table 4; Figure 3), as anticipated, because of the generous intake of this amino acid. This higher flux was maintained throughout the fed period until the meals were terminated, at which time it declined again. Thus, the mean (± SD) first-pass uptakes of the \[^{2}\text{H}_4\]tyrosine tracer during the fast and fed states were 52 ± 9% and 44 ± 13%, respectively.

**Carbon dioxide output, \(^{13}\text{C}\) abundance, and phenylalanine oxidation**

The patterns of \(\text{VCO}_2\), \(^{13}\text{CO}_2\) enrichment, and \(^{13}\text{CO}_2\) excretion throughout the 24-h tracer period for all subjects are shown in Figures 3, 4, and 5, respectively. From these data and those for plasma \(^{13}\text{C}\)phenylalanine abundance (Figure 1) we determined the rate and pattern of whole-body phenylalanine oxidation during the 24-h day (Figure 6). Absolute values for phenylalanine oxidation for specific time periods within the 24-h day are shown in Table 5. It can be seen from these findings that at this intermediate phenylalanine intake (35–39 mg·kg\(^{-1}\)·d\(^{-1}\)), its rate of oxidation increased, but only briefly, with the initiation of small meals. With continued feeding, the rate of phenylalanine oxidation declined dramatically, falling to a value

---

**Table 3**
The isotopic abundance of plasma free phenylalanine, tyrosine, and leucine in plasma at specific times throughout the 24-h infusion day

<table>
<thead>
<tr>
<th>Time and condition</th>
<th>[^{13}\text{C}]Phenylalanine (n = 10)</th>
<th>[^{2}\text{H}_4]Tyrosine (n = 10)</th>
<th>[^{13}\text{C}]Tyrosine (n = 10)</th>
<th>[^{2}\text{H}_4]Tyrosine (n = 4)</th>
<th>[^{2}\text{H}_4]Leucine (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast (0–720 min)</td>
<td>7.48 ± 1.43</td>
<td>2.08 ± 0.56</td>
<td>2.49 ± 0.27</td>
<td>2.14 ± 0.44</td>
<td>3.21 ± 0.32</td>
</tr>
<tr>
<td>Last hour (15th)</td>
<td>8.59 ± 4.20</td>
<td>2.14 ± 0.95</td>
<td>2.60 ± 0.85</td>
<td>2.08 ± 0.55</td>
<td>3.32 ± 0.46</td>
</tr>
<tr>
<td>12th hour</td>
<td>7.45 ± 2.10</td>
<td>1.95 ± 0.93</td>
<td>1.95 ± 1.02</td>
<td>2.02 ± 0.45</td>
<td>3.44 ± 0.54</td>
</tr>
<tr>
<td>Fed (720–1440 min)</td>
<td>9.73 ± 1.56</td>
<td>3.19 ± 0.55</td>
<td>2.25 ± 0.61</td>
<td>3.23 ± 0.95</td>
<td>2.46 ± 0.25</td>
</tr>
<tr>
<td>5th hour</td>
<td>10.45 ± 2.84</td>
<td>3.05 ± 0.76</td>
<td>2.40 ± 0.55</td>
<td>2.95 ± 0.67</td>
<td>2.41 ± 0.38</td>
</tr>
</tbody>
</table>

1 \(±\) SD.
2 Given as intravenous tracer (see Subjects and Methods).
3 Mole fraction above baseline \(×\) 100.
4 0.56 h after the last meal, or 0 h into the tracer fasting period.

---

**Table 4**
Plasma phenylalanine, tyrosine, and leucine fluxes for the fed and fast periods and for selected times within these periods

<table>
<thead>
<tr>
<th>Time and condition</th>
<th>[^{13}\text{C}]Phenylalanine (n = 10)</th>
<th>[^{2}\text{H}_4]Tyrosine(^{2}) (n = 10)</th>
<th>[^{2}\text{H}_4]Tyrosine(^{2}) (n = 4)</th>
<th>[^{2}\text{H}_4]Leucine(^{2}) (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast (0–720 min)</td>
<td>71.4 ± 16.4</td>
<td>32.4 ± 10.8</td>
<td>67.5 ± 22.5</td>
<td>25.3 ± 4.8</td>
</tr>
<tr>
<td>Last hour (15th)</td>
<td>70.3 ± 40.2</td>
<td>28.6 ± 13.1</td>
<td>59.6 ± 27.3</td>
<td>26.3 ± 6.8</td>
</tr>
<tr>
<td>12th hour</td>
<td>66.9 ± 22.0</td>
<td>31.8 ± 16.5</td>
<td>66.4 ± 34.4</td>
<td>26.7 ± 5.5</td>
</tr>
<tr>
<td>Fed (720–1440 min)</td>
<td>50.1 ± 7.4(^{2})</td>
<td>21.9 ± 4.2(^{2})</td>
<td>41.7 ± 7.3</td>
<td>18.3 ± 5.0(^{2})</td>
</tr>
<tr>
<td>5th hour</td>
<td>46.3 ± 10.4</td>
<td>20.3 ± 4.3</td>
<td>36.2 ± 7.6</td>
<td>18.6 ± 4.2</td>
</tr>
</tbody>
</table>

1 \(±\) SD.
2 Corrected for the first-pass splanchnic uptake of tracer (see Methods).
3 Given as intravenous tracer (see Subjects and Methods).
4 Fluxes were significantly different from fast, \(P < 0.01\).
significantly below the fasting oxidation rate within \(\approx 4\text{-}5\) h after the small meals had begun. A new and lower rate of phenylalanine oxidation was achieved by the seventh hour of feeding (Figure 6).

**Phenylalanine hydroxylation**

The 24-h pattern and rate of phenylalanine hydroxylation were each derived (Figure 7, Table 5) from the amount and pattern of isotope abundance, in plasma, of the tyrosine isotopologs that were shown in Figure 1, together with isotope abundance data for plasma phenylalanine. The picture that emerges is similar to that for phenylalanine oxidation (Figure 6 compared with Figure 7) but the individual variation was large, especially during the fast period. Again, an uneven delivery of the two oral tracers to the metabolic pools and perhaps temporal differences in the relative states of phenylalanine and tyrosine metabolism may account for these observations. The relations between phenylalanine and tyrosine fluxes and measured phenylalanine oxidation and hydroxylation rates are summarized in Table 6. For the fast period, the oxidation and hydroxylation rates accounted for \(\approx 12\% \) and \(15\%\), respectively, of the phenylalanine flux.

**Plasma amino acids**

Plasma free phenylalanine, tyrosine, and leucine concentrations were measured at intervals throughout the 24-h tracer study. The results are presented in Figure 8. There was an increase in the plasma leucine concentration that was accompanied by an increase in the leucine flux (Figure 2). With feeding, there was a slow, progressive decline in the plasma phenylalanine and tyrosine concentrations and in both cases this reflected the change, but in less dramatic terms, in the phenylalanine oxidation and hydroxylation rates (compare Figures 6 and 8).

**Phenylalanine balance**

From measured 24-h estimates of the phenylalanine oxidation and hydroxylation rates (which did not differ; \(P > 0.5\)), we calculated daily, whole-body phenylalanine balance. These results are shown in Table 7. The daily mean balances of phenylalanine based on phenylalanine oxidation tended to be slightly positive and they were essentially at equilibrium when based on phenylalanine hydroxylation. However, the balance differences between the two tracer-derived estimates were not significant \((P = 0.2)\) and the variation in balance among these subjects was relatively large but we do not have a satisfactory explanation for this.

**DISCUSSION**

The present study was carried out to complement and extend our recent findings on the 24-h pattern of plasma phenylalanine and tyrosine fluxes and rates of phenylalanine oxidation and hydroxylation (3, 4). These previous studies explored quantitative aspects of metabolism of phenylalanine, as evaluated with intravenous and oral tracers, in subjects receiving either a generous \((100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1})\) or low \((21.9 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1})\) phenylalanine intake. The latter intake is somewhat higher than the upper requirement range for total aromatic amino acids (phenylalanine plus tyrosine) proposed by the FAO/WHO/UNU (8). We found from the 24-h estimates of phenylalanine oxidation that body phenylalanine balance was negative at the low phenylalanine intake whereas balance was positive for the generous intake of phenylalanine. However, as discussed previously (4) it seemed likely that we had somewhat underestimated the 24-h rate of phenylalanine loss at this generous intake.

These previous studies supported our view that the FAO/WHO/UNU (8) estimated requirement for the total aromatic
FIGURE 3. Rate and pattern of expired carbon dioxide throughout the 24-h d for subjects receiving the labeled tracers. Results are presented for each 30-min period during the study. "Fast" refers to the 720-min period of tracer infusion while subjects consumed no food. The actual length of the "fast" was 900 min because the last meal was eaten 3 h before the beginning of tracer infusion. As indicated, the last of the 10 small meals was given 1260 min into the 24-h tracer-infusion period.

FIGURE 4. $^{13}$C abundance of expired carbon dioxide for each consecutive 30-min period of the 24-h d in subjects receiving $[^{13}\text{C}]$phenylalanine as an oral tracer. "Fast" refers to the 720-min period of tracer infusion while subjects consumed no food. The actual length of the "fast" was 900 min because the last meal was eaten 3 h before the beginning of tracer infusion. As indicated, the last of the 10 small meals was given 1260 min into the 24-h tracer-infusion period. APE: atoms percent excess.
these ranges that are newer and higher than FAO/WHO/UNU requirement estimations.

In our separate studies with leucine (1, 2, 7) and phenylalanine (3, 4), the pattern of amino acid oxidation throughout the 24-h day was markedly affected by the amino acid intake; at deficient intakes of either amino acid there was a marked decline in the rate of amino acid oxidation as feeding progressed, reaching very low rates of oxidation after \( \approx 6 \) h into the 12-h fed period. In contrast, generous intakes of either leucine or phenylalanine resulted in prompt and

FIGURE 5. Total output (for each consecutive 30-min interval) of \( ^{13} \)CO\(_2\) in expired air of subjects receiving \( ^{13} \)C-phenylalanine as an oral tracer. “Fast” refers to the 720-min period of tracer infusion while subjects consumed no food. The actual length of the “fast” was 900 min because the last meal was eaten 3 h before the beginning of tracer infusion. As indicated, the last of the 10 small meals was given 1260 min into the 24-h tracer-infusion period.

FIGURE 6. Rate and pattern of phenylalanine oxidation for consecutive 30-min periods throughout the 24-h d in subjects who received \( ^{13} \)C-phenylalanine as an oral tracer. “Fast” refers to the 720-min period of tracer infusion while subjects consumed no food. The actual length of the “fast” was 900 min because the last meal was eaten 3 h before the beginning of tracer infusion. As indicated, the last of the 10 small meals was given 1260 min into the 24-h tracer-infusion period.
sustained increases in their oxidation rates over the fed period of the 24-h day. In view of the profound decline in phenylalanine oxidation with continued feeding with a diet deficient in phenylalanine, we were interested in determining the prandial response when phenylalanine intake was designed to approximate the requirement intake. Because our previous phenylalanine study indicated that it would be best to give the phenylalanine tracer by the oral route, it was not felt necessary that the present experiment include a comparison with data generated via an intravenous $[1^{13}C]phenylalanine tracer protocol. Indeed, the 24-h pattern of $[1^{13}C]phenylalanine oxidation was the same for oral and intravenous phenylalanine tracers (3, 4), although the estimated, absolute rates of amino acid oxidation were higher when derived from the oral tracer studies.

The 24-h patterns for the phenylalanine and tyrosine fluxes in the present study were qualitatively similar to those observed at the low, or deficient, phenylalanine intake (3), although the decline in the phenylalanine flux with feeding appears not to be as large as that seen earlier at the low intake, 21.9 mg phenylalanine · kg$^{-1}$ · d$^{-1}$. However, the tyrosine flux, especially, as determined with the intravenous $[3^{1}H]tyrosine tracer, appears to have shown similar absolute declines with ingestion of very low and requirement intakes of phenylalanine. In contrast, the tyrosine flux remained constant when meals provided a generous amount of phenylalanine. It seems likely that the fall in tyrosine flux at requirement and/or deficient intakes of phenylalanine, given in the absence of a significant source of dietary tyrosine, reflects a limiting availability of tyrosine. It will be important in future studies to make direct estimates of tyrosine oxidation and utilization with the aid of a $[1^{13}C]tyrosine probe to investigate this nutritional/metabolic issue in more detail.

The estimated rates of phenylalanine oxidation and hydroxylation were comparable and after 12-h of fasting they approximated 7.7 μmol · kg$^{-1}$ · h$^{-1}$. With feeding, phenylalanine oxidation and hydroxylation decreased steadily reaching, after 8 h, a value $\approx$40% of the rate measured at the termination of the fasting period. In this context, note that the present estimates of the rate of phenylalanine oxidation used the plasma $[1^{13}C]phenylalanine enrichment values for the purposes of calculation. Although the results of the 48-h intravenous $\tau$-[ring-$1^{13}C_6]phenylalanine tracer studies by Reeds et al (20) showed that the $^{13}C$-labeling of phenylalanine in very-low-density li-

### TABLE 5
Rates of phenylalanine oxidation and hydroxylation for 12-h fast and 12-h fed periods and during specific time intervals for the study $^4$

<table>
<thead>
<tr>
<th>Time and condition</th>
<th>Oxidation $\mu$mol · kg$^{-1}$ · h$^{-1}$</th>
<th>Hydroxylation $\mu$mol · kg$^{-1}$ · h$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0–720 min)</td>
<td>8.71 ± 2.64</td>
<td>10.95 ± 5.30</td>
</tr>
<tr>
<td>Last hour (15th)</td>
<td>10.21 ± 4.56</td>
<td>11.67 ± 11.23</td>
</tr>
<tr>
<td>12th hour</td>
<td>7.50 ± 2.87</td>
<td>7.91 ± 4.24</td>
</tr>
<tr>
<td>Fed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(720–1440 min)</td>
<td>4.90 ± 1.61</td>
<td>5.24 ± 1.29</td>
</tr>
<tr>
<td>3rd hour</td>
<td>6.98 ± 2.77</td>
<td>6.71 ± 1.73</td>
</tr>
<tr>
<td>5th hour</td>
<td>5.07 ± 2.21</td>
<td>4.47 ± 1.61</td>
</tr>
<tr>
<td>8th hour</td>
<td>2.72 ± 1.05</td>
<td>3.21 ± 1.29</td>
</tr>
</tbody>
</table>

$^4$ x ± SD; n = 10.

![FIGURE 7](https://academic.oup.com/ajcn/article-abstract/65/2/473/4655357)
Table 6
Relation between phenylalanine (phe) oxidation and hydroxylation rates and the fluxes of phenylalanine and tyrosine (tyr) at specific time intervals during the 24-h period.

<table>
<thead>
<tr>
<th>Time and condition</th>
<th>Oxidation ratio with</th>
<th>Hydroxylation ratio with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phe flux</td>
<td>Tyr flux</td>
</tr>
<tr>
<td>Fast</td>
<td>0.12 ± 0.03</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>(0-720 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Last hour (15th)</td>
<td>0.16 ± 0.05</td>
<td>0.18 ± 0.06</td>
</tr>
<tr>
<td>12th hour</td>
<td>0.11 ± 0.02</td>
<td>0.13 ± 0.06</td>
</tr>
<tr>
<td>Fed</td>
<td>0.09 ± 0.02</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td>(720-1440 min)</td>
<td>0.10 ± 0.03</td>
<td>0.14 ± 0.08</td>
</tr>
<tr>
<td>5th hour</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*F ± SD; n = 10.

*2 The ratio of phe oxidation to tyr flux was calculated by using uncorrected flux.

The hydroxylation of phenylalanine and subsequent release of a $^{13}$C label via oxidation of tyrosine is known nor are the effects on these relations of dietary factors and of the nutritional status of the subject. Further, whether a comparable set of ratios might be obtained with a prolonged oral infusion of isotopic tracer and in comparison with the 48-h intravenous tracer infusion protocol used in their (30) earlier study also remains to be determined. Finally, the data of Berthold et al (30) suggest that we may have actually underestimated the rate of phenylalanine oxidation because the peak isotopic enrichment of plasma phenylalanine was higher than that of the phenylalanine in apolipoprotein B-100. Hence, it seems reasonable to use the plasma free phenylalanine enrichment as the basis.

Figure 8. Pattern of change in plasma free phenylalanine, tyrosine, and leucine concentrations during the 24-h in subjects receiving 39 mg phenylalanine·kg$^{-1}$·d$^{-1}$, with $^{13}$C-phenylalanine as an oral tracer. “Fast” refers to the 720-min period of tracer infusion while subjects consumed no food. The actual length of the “fast” was 900 min because the last meal was eaten 3 h before the beginning of tracer infusion. As indicated, the last of the 10 small meals was given 1260 min into the 24-h tracer-infusion period.
TABLE 7
Estimates of phenylalanine balance, based on direct 24-h measurement of phenylalanine oxidation (phe-ox) and hydroxylation (phe-OH) rates

<table>
<thead>
<tr>
<th>Subject</th>
<th>Phe-ox</th>
<th>Phe-OH</th>
<th>Phe-ox</th>
<th>Phe-OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg phenylalanine ( \cdot ) kg(^{-1} \cdot ) d(^{-1} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not adjusted for tyrosine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for tyrosine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+8.69</td>
<td>+12.38</td>
<td>+4.68</td>
<td>+8.38</td>
</tr>
<tr>
<td>2</td>
<td>+11.91</td>
<td>+9.31</td>
<td>+7.89</td>
<td>+5.30</td>
</tr>
<tr>
<td>3</td>
<td>+4.03</td>
<td>-7.79</td>
<td>-0.04</td>
<td>-11.86</td>
</tr>
<tr>
<td>4</td>
<td>+3.89</td>
<td>+3.46</td>
<td>-0.19</td>
<td>-0.62</td>
</tr>
<tr>
<td>5</td>
<td>+17.09</td>
<td>+14.84</td>
<td>+13.17</td>
<td>+10.93</td>
</tr>
<tr>
<td>6</td>
<td>+4.23</td>
<td>-5.30</td>
<td>+0.32</td>
<td>-9.21</td>
</tr>
<tr>
<td>7( )</td>
<td>-3.13</td>
<td>+10.29</td>
<td>-8.24</td>
<td>+5.18</td>
</tr>
<tr>
<td>8( )</td>
<td>+22.31</td>
<td>+17.92</td>
<td>+17.02</td>
<td>+12.62</td>
</tr>
<tr>
<td>9( )</td>
<td>+14.20</td>
<td>+8.61</td>
<td>+8.84</td>
<td>+3.25</td>
</tr>
<tr>
<td>10( )</td>
<td>+8.45</td>
<td>-23.20</td>
<td>+3.56</td>
<td>-28.10</td>
</tr>
<tr>
<td>( )</td>
<td>+9.17</td>
<td>+4.05</td>
<td>+4.70</td>
<td>-0.41</td>
</tr>
<tr>
<td>SD</td>
<td>7.44</td>
<td>12.63</td>
<td>7.34</td>
<td>12.60</td>
</tr>
</tbody>
</table>

\( \) These subjects received both \( ^{2}H_{4} \)tyrosine and \( ^{2}H_{4} \)leucine intravenously.

for estimation of whole-body phenylalanine oxidation, after administration of an oral tracer of this amino acid.

For purposes of comparison with our previous results, which were obtained at both higher and lower phenylalanine intakes, a combined summary of the data obtained from all three studies is presented in Table 8. Thus, for the fasted state, rates of phenylalanine oxidation and hydroxylation were similar for the low- and present-requirement phenylalanine intake, whereas they tended to be higher for the generous phenylalanine intake (11). For the fed state, these rates responded to the differing content of dietary phenylalanine. When the oxidation rates at

the 8-h point of feeding were compared it was apparent that there was a higher rate of change in oxidation rate per unit of change in phenylalanine intake between the requirement and generous intakes than between the requirement and deficient intakes of the amino acid. This response is a characteristic finding from many studies that have investigated the relation between the intake of a specific indispensable amino acid, ranging from low submaintenance amounts to intakes well in excess of requirements, and its oxidation rate (31, 32). Hence, a reasonable interpretation of the present results, in comparison with those obtained previously (3, 4; Table 8), is that phenylalanine intakes between 21.9 and \( \approx \)39 mg kg\(^{-1} \cdot \) d\(^{-1} \) fall within the near-maintenance to submaintenance range rather than in the supramaintenance or excess range of intake, which would be the case if the FAO/WHO/UNU (8) value of 14 mg kg\(^{-1} \cdot \) d\(^{-1} \) accurately represented the upper level of the minimum requirements in healthy adults. The decline in plasma phenylalanine concentration and the progressive fall in tyrosine concentration with ingestion of the small, frequent meals supports this view. A reduction in the plasma concentration of an indispensable amino acid, with ingestion of protein-containing meals, generally indicates that the intake of the amino acid is limiting, or deficient, depending on the degree of change during the prandial period (33, 34). In the present case, the fall in phenylalanine was not as dramatic or, by the end of the fed phase, as great as we observed earlier in subjects consuming an inadequate amount, 21 mg phenylalanine kg\(^{-1} \cdot \) d\(^{-1} \) (3). At the generous intake of phenylalanine (100 mg kg\(^{-1} \cdot \) d\(^{-1} \)), without tyrosine, meal ingestion was associated with a prompt rise in the plasma phenylalanine concentration above that achieved at the end of the fast period and it was maintained at a relatively constant higher concentration while the fed period continued (4). There was, however, a measurable, but perhaps less extensive, decline in the tyrosine concentration at this higher phenylalanine intake, as was the case in the present study. Using a line of reasoning similar to that above, for the plasma tyrosine responses, our findings also indicated that tyrosine was also limiting and this is entirely consistent with our understanding of the biochemistry of mammalian aromatic amino acid metabolism (35).

The estimates of phenylalanine balance obtained in this and our previous studies support the foregoing reasoning. Taking into consideration the fact that the tyrosine tracer was not massless and, therefore, could well have spared some loss of phenylalanine (3, 4), the phenylalanine balance was close to equilibrium \( (P > 0.5) \). We have argued (3, 4) that the present tracer model leads to some underestimation in the rate of body phenylalanine loss and from the present results it is entirely reasonable to interpret these phenylalanine balance and kinetic data as indicative of a phenylalanine intake that is close to the mean physiologic requirement for healthy adults. We suggest, therefore, that our proposed requirement value for total aromatic amino acids (39 mg kg\(^{-1} \cdot \) d\(^{-1} \) (9, 10) be accepted, in the interim, until definitive new data become available that would support a major change in this figure. The studies by Zello et al (11) add further justification for this proposal.

There are a number of important issues that remain to be resolved, but given the complexity and expense of these 24-h metabolic-tracer studies, it will not be easy to reach a solution on these various issues immediately. The first re-
lates to the relatively high degree of variation in the bal-
ances among the various subjects and the extent to which
metabolic and biological factors, as compared with experi-
mental errors, account for this variation. However, and in
agreement with the suggestion made by Beaton (36), it is
important first to make the best estimate of the mean re-
quirement for a nutrient before a comprehensive and quan-
titative inquiry of the biological variability among individ-
uals in their requirements is undertaken. Second, we remain
intrigued by the metabolic significance of the changes in
plasma tyrosine concentrations and fluxes in response to
very-low-tyrosine diets supplying graded amounts ofphe-
nylalanine. Precisely how these variable responses of
plasma phenylalanine and tyrosine concentrations to differ-
ent phenylalanine intakes reflect the cellular adequacy and
functional outcome of total aromatic amino acid intake
remains to be investigated. In this context, a follow-up series
of 24-h tracer studies using L-[1-14C]tyrosine as the probe,
which we hope will provide us with an estimate of total ar-
comatous aromatic acid loss, are now underway in our labora-
tories. Finally, it will be important to explore the quantita-
tive effect of dietary tyrosine on the minimum requirement
for phenylalanine. Because of problems related to the pre-
cise conduct and interpretation of nitrogen-balance studies
(13, 29), which serve as the basis for current estimates of the
quantitative effect of tyrosine on phenylalanine losses (3, 4,
10), it would be prudent to question whether tyrosine does
indeed spare the phenylalanine requirement by 70–75% as
proposed earlier by Rose and Wixom (29) and then accepted
by a US national committee (14).

We thank the MIT CRC nursing, dietary, and laboratory staff for their help in the conduct of these studies. The technical assistance of Mike Kenneway, Sukanya Date, Ambalini Selvaraj, Alan Atkinson, and Ray A Gleason is appreciated. The willingness and dedication of the subjects who volunteered for these studies is gratefully acknowledged.

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