Aromatic amino acid requirements in healthy men measured by indicator amino acid oxidation

Jean W-C Hsu, Laksiri A Goonawardene, Mahroukh Rafii, Ronald O Ball, and Paul B Pencharz

ABSTRACT

Background: In the current literature, no agreement exists on estimates for aromatic amino acid (phenylalanine plus tyrosine) requirements as measured by stable-isotope techniques.

Objective: The goal of the present study was to determine the phenylalanine requirement in healthy men who were fed a diet without tyrosine by using the indicator amino acid oxidation method.

Design: Five healthy men were assigned to receive in random order diets devoid of tyrosine and with 8 graded intakes of phenylalanine (5, 10, 15, 25, 35, 45, 60, and 70 mg · kg⁻¹ · d⁻¹). The phenylalanine requirement was measured by the rate of ¹³CO₂ release (F¹³CO₂) from L-[¹³C]lysine oxidation.

Results: The graded intakes of phenylalanine had no effect on lysine flux, as required for this method. The phenylalanine (ie, total aromatic amino acid) requirement, in the absence of tyrosine, was estimated to be 48 mg · kg⁻¹ · d⁻¹ by applying a two-phase linear regression crossover model to the F¹³CO₂ data.

Conclusions: In the absence of tyrosine, the mean phenylalanine requirement is higher than the current FAO/WHO/UNU (1985) and Dietary Reference Intake (2002) recommendations. Am J Clin Nutr 2006;83:82–8.

KEY WORDS Amino acid requirements, aromatic amino acids, indicator amino acid oxidation, phenylalanine, tyrosine

INTRODUCTION

The current FAO/WHO/UNU (1) recommended intakes for adults of the indispensable amino acids, which have a basis on nitrogen balance studies, have been questioned because they appear to significantly underestimate intake requirements, as measured by other methods (2). The FAO/WHO/UNU recommendation of aromatic amino acid intake (ie, phenylalanine and tyrosine) for adults is 14 mg · kg⁻¹ · d⁻¹ (1). Recently, stable-isotope techniques have been applied to measure the requirements of the indispensable amino acids with different approaches and theoretical concepts. The estimated mean requirement obtained by Young’s group at the Massachusetts Institute of Technology (MIT) with the use of the 24-h direct amino acid oxidation balance approach was 9 mg · kg⁻¹ · d⁻¹ (6). The estimated minimal phenylalanine requirement obtained by the direct amino acid oxidation method in the presence of excess tyrosine was 9 mg · kg⁻¹ · d⁻¹ (5). Zello et al (5) suggested that the aromatic amino acid requirement should be 30 mg · kg⁻¹ · d⁻¹, based on the assumption that tyrosine can supply up to two-thirds of the aromatic amino acid requirement. However, the estimated tyrosine requirement of healthy men who received 9 mg phenylalanine · kg⁻¹ · d⁻¹ was 6 mg · kg⁻¹ · d⁻¹, as measured with the indicator amino acid oxidation (IAAO) technique, which suggested an average requirement for aromatic amino acid intake of 15 mg · kg⁻¹ · d⁻¹ (6).

Hence, the mean estimates for aromatic amino acid requirements range from 15 to 39 mg · kg⁻¹ · d⁻¹ in the literature; this range represents the widest disagreement between estimates for the indispensable amino acids. In the new Dietary Reference Intakes, the estimated average requirement for phenylalanine + tyrosine was calculated to be 27 mg · kg⁻¹ · d⁻¹ (7), an average that was based on 2 studies (4, 6). It is necessary to resolve this controversy by applying a different experimental approach. A minimum phenylalanine dietary concentration, even in the absence of tyrosine, supported both growth in weanling rats (8) and positive nitrogen balances in adults (9). These findings suggested that the total tyrosine requirement could be obtained from phenylalanine intake. Hence, supplementing only phenylalanine should provide an estimate of the total aromatic amino acid requirements. Therefore, the purpose of the present study was to use phenylalanine supplementation without tyrosine to determine aromatic amino acid requirements with the IAAO method, with the use of [¹³C]lysine as the indicator.

SUBJECTS AND METHODS

Subjects

Five healthy men participated in the study on an outpatient basis at the Clinical Investigation Unit of the Hospital for Sick Children (JW-CH, MR, and PBP); and the Department of Agricultural, Food, and Nutritional Sciences, University of Alberta, Edmonton, Canada (LAG and ROB). Reprints not available. Address correspondence to PB Pencharz, Division of Gastroenterology and Nutrition, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8. E-mail: paul.pencharz@sickkids.ca.

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1 From the Departments of Nutritional Sciences (JW-CH, ROB, and PBP) and Paediatrics (PBP), University of Toronto, Toronto, Canada; The Research Institute, The Hospital for Sick Children, Toronto, Canada (JW-CH, MR, and PBP); and the Department of Agricultural, Food, and Nutritional Sciences, University of Alberta, Edmonton, Canada (LAG and ROB).
2 Presented in part in abstract form at Experimental Biology 2003, San Diego, CA, April 10-15. [Hsu JW-C, Ball RO, and Pencharz PB. Total aromatic amino acid requirements in healthy adult males determined by indicator amino acid oxidation FASEB J 2003;17:450.7(abstr).]
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4 Reprints not available. Address correspondence to PB Pencharz, Division of Gastroenterology and Nutrition, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8. E-mail: paul.pencharz@sickkids.ca.

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Children in Toronto, Canada. The subjects’ characteristics are described in Table 1. Subjects were excluded from the study if they had a history of an inborn error of metabolism, had a chronic disease, had an endocrine disorder, had an unusual dietary practice, had recently lost weight, or were receiving pharmacologic treatment. The purpose of the study and the potential risks involved were explained in detail to each subject, and written consent was obtained from all subjects. All procedures used in the study were approved by the Research Ethics Board of the Hospital for Sick Children. All men received financial compensation for their participation.

Experimental design

Each subject received in random order 8 graded intakes of phenylalanine (5, 10, 15, 25, 35, 45, 60, and 70 mg · kg⁻¹ · d⁻¹) on the study day (1 subject did not receive a phenylalanine intake of 70 mg · kg⁻¹ · d⁻¹). Before the study, multiple skinfold thicknesses (triceps, biceps, subscapular, and suprailiac) were measured, and bioelectrical impedance analysis was performed. Lean body mass was calculated from reactance and resistance (10), which were measured by bioelectrical impedance analysis (BIA model 101A; RJL Systems, Detroit, MI). Fat-free mass was calculated from body mass index multiplied by an activity factor (10). Each meal represented one-twelfth the daily intake. The protein content was provided at a concentration of 1 g · kg⁻¹ · d⁻¹, which was given as a crystalline l-amino acid mixture that was based on the amino acid profile of egg protein. The lysine intake on the study day was fixed at a concentration of 45 mg · kg⁻¹ · d⁻¹ to ensure that lysine intake was more than adequate for all subjects (7). Alanine was given in varying amounts to keep the meals isonitrogenous at different phenylalanine intakes.

Tracer protocol

The isotopic labeled tracers that were used in the studies were NaH¹³CO₃ and L-[¹-¹³C]lysine · 2HCl; both had a 99% enrichment (Cambridge Isotope Laboratories, Woburn, MA). The isotopic and optical purity of L-[¹-¹³C]lysine was verified by the manufacturer of the isotopes with the use of chemical ionization gas chromatography-mass spectrometry and nuclear magnetic resonance. The enrichment of the L-[¹-¹³C]lysine tracer was reconfirmed by liquid chromatography mass spectrometry of the butanol derivative. The measured fractional molar abundance of L-[¹-¹³C]lysine was 98.3%. These values were used in the calculation of lysine flux. The oral tracer study started after a 4 h enteral intake adaptation period. Primed doses of NaH¹³CO₃ (2.023 μmol/kg) and L-[¹-¹³C]lysine (16.305 μmol/kg) were given with the fifth meal. In addition, the L-[¹-¹³C]lysine isotope (8.153 μmol/kg) was given at hourly meals starting with the fifth meal for the following 4 h.

Sample collection

Breath and blood samples were collected on each IAAO study day. Three baseline breath and blood samples were collected 45, 30, and 15 min before the tracer protocol began. Because an isotopic plateau in carbon dioxide and plasma can be reached within 2 h after initiating an isotope infusion (14), expired breath ¹³CO₂ and blood samples were collected every 30 min, beginning 2.5 h after the start of the tracer protocol. The same pattern was also seen in the present study, and the slope of the plateau enrichment in breath and urine samples was not significantly different from zero. The CVs were 0.03–0.15% and 1–10% between the 4 values of breath enrichment and plasma enrichment, respectively. Breath samples were collected in disposable Haldane-Priestley tubes (Venoject; Terumo Medical, Elkton, MD) with a collection mechanism that allows the removal of dead-air space. The breath samples were stored at room temperature until the ¹³C enrichment was analyzed. The arterialized

### TABLE 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>29.4 ± 4.7 (26–37)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.5 ± 10.6 (60–84)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.1 ± 3.0 (169–177)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9 ± 3.2 (20–28)</td>
</tr>
<tr>
<td>Lean body mass (kg)²</td>
<td>54.5 ± 4.8 (49–59)</td>
</tr>
<tr>
<td>Fat-free mass (kg)³</td>
<td>56.8 ± 7.6 (49–66)</td>
</tr>
<tr>
<td>Resting metabolic rate (kcal/d)⁴</td>
<td>1746.2 ± 119.7 (1593–1911)</td>
</tr>
<tr>
<td>Energy intake (kcal/d)⁵</td>
<td>2968.5 ± 203.5 (2861–3249)</td>
</tr>
</tbody>
</table>

¹ Values are x ± SD; range in parentheses, n = 5.
² Determined from bioelectrical impedance analysis (10).
³ Calculated from multiple skinfold-thickness measurements (11).
⁴ Measured with continuous, computerized, open-circuit indirect calorimetry.
⁵ Calculated from resting metabolic rate multiplied by an activity factor of 1.7.
blood samples were collected in heparinized tubes (15), and plasma was separated by centrifuging at 1500 × g for 10 min at 4 °C. The plasma samples were stored at −20 °C until the 13C enrichment and concentrations were analyzed.

Analytic procedures

Expired 13CO2 enrichment was measured by a continuous-flow isotope ratio mass spectrometry (CF-IRMS20/20; PDZ Europa, Cheshire, United Kingdom) and was expressed as atom percentage excess against a reference standard of compressed CO2 gas. Plasma L-[1-13C]lysine enrichment was measured by a triple quadrupole mass spectrometer API 4000 (Applied Biosystems-MDS SCIEX, Concord, Canada) operated in positive ionization mode with a TurbolonSpray (Applied Biosystems-MDS SCIEX) ionization probe source (operated at 5800 V and 600 °C), which was coupled to an Agilent 1100 HPLC system (Agilent Technologies Canada Inc, Mississauga, Canada). For deproteination, 25 μL plasma samples were mixed with 200 μL methanol and centrifuged at 9000 × g for 5 min at room temperature. The supernatant fluids were transferred to derivatizing tubes and dried under nitrogen at 45 °C. The dried room temperature. The supernatant fluids were transferred to resulting from the fragmentation of the protonated [M+H]+ molecule. A solution of pure butylated L-lysine (200 pg/μL) was infused into the mass spectrometer, and the declustering potential was optimized to maximize the intensity of the [M+H]+ precursor (parent) ion (m/z: 203 for the enriched fraction and 204 for the enriched fraction). The collision energy was then adjusted to optimize the signal for the most abundant product (daughter) ion (m/z: 84) with the use of nitrogen as the collision gas. The individual components were separated with a Waters Xterra MS C18 3.5 μm, 2.1 × 150 mm column (Waters Corp, Milford, MA) at 50 °C and were eluted in 5 min with a binary liquid chromatography gradient (10–50% aqueous acetonitrile containing 0.025% formic acid and 0.05% trifluoroacetic acid). The retention time was 1.9 min.

Plasma free amino acid concentrations were measured by HPLC analysis. 200 μL plasma and 80 μL 0.25 mmol norleucine/L, as the internal standard, were extracted with a cation exchange column (Dowex 50 W-X8, 100–200 mesh H+ form; Bio-Rad Laboratories, Hercules, CA). Plasma phenylalanine, tyrosine, and lysine were derivatized with phenylisothiocyanate (adapted from Picotag; Waters Corp) (16–18), and their phenylisothiocyanate derivatives were separated and analyzed against an 18 amino acid standard mix (Sigma, St Louis, MO) with the use of a reverse-phase column (C18, 2.9 mm × 300 mm Pico Tag column; Waters Corp) and HPLC (Dionex Summit HPLC System, Dionex, Sunnyvale, CA; operated under HPLC pump model P580A LPG and UV-VIS 170S). The areas under the peaks were integrated with CHROMELEON software (version 6.2; Dionex, Oakville, Canada).

Estimation of isotope kinetics

Whole-body lysine flux was calculated from the dilution of the isotope in the body amino acid pool at isotopic steady state (19):

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

where \( Q \) is the rate of lysine flux (in μmol · kg⁻¹ · h⁻¹), \( i \) is the isotope infusion rate (in μmol · kg⁻¹ · h⁻¹), and \( E_i \) and \( E_p \) are the enrichments, as mole fractions, of the infused isotope (ie, molecules percent excess) and of plasma lysine, respectively, at the isotopic plateau.

The rate of lysine oxidation was calculated with the following equation (19):

\[ O = F^{13}CO_2(1/E_p - 1/E_i) \times 100 \]

where \( O \) represents lysine oxidation (in μmol · kg⁻¹ · h⁻¹) and \( F^{13}CO_2 \) represents the rate of 13CO2 released by lysine tracer oxidation (in μmol · kg⁻¹ · h⁻¹). \( F^{13}CO_2 \) was calculated with the following equation:

\[ F^{13}CO_2 = (FCO_2 \times ECO_2 \times 44.6 \times 60)/(W \times 0.82 \times 100) \]

where \( FCO_2 \) is the carbon dioxide production rate (in mL/min), \( ECO_2 \) is the enrichment in expired breath at isotopic steady state (ie, atom percent excess), the constants 44.6 μmol/mL and 60 min/h were used to convert \( FCO_2 \) to μmol/h, \( W \) is the weight (in kg) of the subject, the factor 0.82 is the correction for the carbon dioxide that is retained in the body because of bicarbonate fixation (20), and the factor 100 changes atom percent excess to a fraction.

Statistical analysis

Estimates of the mean aromatic amino acid intake were derived by breakpoint analysis of the rate of release of 13CO2 (ie, \( F^{13}CO_2 \)) data with the use of a two-phase linear regression crossover model, as described previously (21). The breakpoint was calculated by using the mixed and regression procedure of SAS (22), and the slope of the second line was not significantly different from zero. An analysis of variance was used to measure the effects of phenylalanine intakes on \( F^{13}CO_2 \) lysine flux, lysine oxidation, and plasma phenylalanine, tyrosine, and lysine concentrations with the use of the mixed procedure of SAS (22). The subject was included as a random effect in the model, and the \( P \) value was adjusted to the Tukey’s test for multiple comparisons. All statistical analyses were performed using SAS (version 8.2, SAS Institute, Cary, NC); differences were considered significant at \( P < 0.05 \).

RESULTS

The characteristics and energy intakes of the men who participated in the study are outlined in Table 1. Weight did not change over the experimental period. Plasma phenylalanine and tyrosine concentrations, which were measured after 8 h of eating, increased with increasing phenylalanine intakes (\( P < 0.01 \)) (Figure 1). However, it was not possible to estimate the aromatic amino acid requirement from plasma phenylalanine or tyrosine concentrations. Phenylalanine intakes did not significantly affect plasma lysine concentrations (\( P > 0.05 \)). The mean (±SD) lysine
concentration from all phenylalanine intakes was 193.1 ± 59.1 μmol/L.

Phenylalanine intake did not significantly affect lysine flux (P = 0.46) (Table 2). The mean enrichment of 13CO2 production rate from L-[1-13C]lysine oxidation was affected by phenylalanine intakes (P = 0.02) (Table 2). The statistical results of the best-fit two-phase linear regression crossover models are tabulated in Table 3. Means of the SE of the model, SE of regression coefficients, correlation variation, square of the coefficient (r2), and P values were similar in these 2 best-fitted models. Therefore, the lower and upper estimates of aromatic amino acid requirements were 44 and 52 mg · kg·d−1, respectively, with an average of 48 mg · kg·d−1 (Figure 2).

**DISCUSSION**

On the basis of these 2 best-fitted, but equal, models for breakpoint analysis in the present study, we believe that the mean aromatic amino acid requirement of the healthy men was between 44 and 52 mg · kg·d−1. Because of the controversy in the current literature regarding aromatic amino acid requirements, the estimated average requirement for phenylalanine plus tyrosine of 27 mg · kg−1 · d−1 in the Dietary Reference Intake report (7) was derived by averaging the large range of estimates, which ranged from 15.1 to 39 mg · kg−1 · d−1. However, based on the maintenance protein requirement intake of 0.66 g · kg−1 · d−1, the total aromatic amino acid requirement for adults was estimated to be 48 mg · kg−1 · d−1 (7). The estimated requirement in the present study was within the range of these 2 values.

The mean requirement in the present study was higher than the estimated mean requirement obtained by Young’s group at MIT, who used 24-h direct amino acid oxidation balance studies, of 39 mg · kg−1 · d−1 (3, 4). Young’s group at MIT examined aromatic amino acid metabolism at low, intermediate, and generous phenylalanine intakes by using 24-h direct amino acid oxidation balance techniques in a series of studies (3, 4, 23, 24). These studies, which were based on phenylalanine hydroxylation and oxidation, suggested that phenylalanine intake at 18.5 or

![Figure 1](https://academic.oup.com/ajcn/article-abstract/83/1/82/4649621/180464901)  
**Figure 1.** Mean (±SD) plasma phenylalanine and tyrosine concentrations in healthy men, measured after 8 h of feeding. Mean values for the same amino acid with different superscript letters are significantly different, P < 0.05 (ANOVA).

![Figure 2](https://academic.oup.com/ajcn/article-abstract/83/1/82/4649621/180464901)  
**Figure 2.** Effect of phenylalanine intake on production of 13CO2 from L-[1-13C]lysine oxidation (F13CO2). All observations (n = 39) and all subjects (n = 5) are shown. The dotted vertical lines represent the lower and upper estimates that were based on the 2 best-fit but equal models. The phenylalanine intake between the 2 regression lines represents the mean total aromatic amino acid requirement of 44–52 mg · kg−1 · d−1, with an average of 48 mg · kg−1 · d−1.

**TABLE 2**

Effect of phenylalanine intake on lysine flux and the rate of 13CO2 release (F13CO2) in the study subjects

<table>
<thead>
<tr>
<th>Phenylalanine intake (mg · kg−1 · d−1)</th>
<th>Flux μmol · kg−1 · h−1</th>
<th>F13CO2 μmol · kg−1 · d−1</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>126.0 ± 26.1</td>
<td>1.566 ± 0.302a</td>
</tr>
<tr>
<td>10</td>
<td>123.0 ± 21.9</td>
<td>1.443 ± 0.255ab</td>
</tr>
<tr>
<td>15</td>
<td>127.3 ± 29.3</td>
<td>1.399 ± 0.308ab</td>
</tr>
<tr>
<td>25</td>
<td>142.9 ± 26.8</td>
<td>1.365 ± 0.224ab</td>
</tr>
<tr>
<td>35</td>
<td>120.8 ± 23.9</td>
<td>1.245 ± 0.269ab</td>
</tr>
<tr>
<td>45</td>
<td>138.8 ± 31.0</td>
<td>1.216 ± 0.222ab</td>
</tr>
<tr>
<td>60</td>
<td>119.2 ± 21.4</td>
<td>1.121 ± 0.127ab</td>
</tr>
<tr>
<td>70</td>
<td>110.7 ± 20.6</td>
<td>1.154 ± 0.151ab</td>
</tr>
</tbody>
</table>

All values are least-square ± SD. n = 5, except for phenylalanine intake of 70 (n = 4). Means within columns with different superscript letters are significantly different, P < 0.05. Phenylalanine intake did not significantly affect lysine flux (P = 0.46) but significantly affected F13CO2 (P = 0.02) (ANOVA).

**TABLE 3**

Comparison of the best-fit models to estimate the breakpoint in the relation between F13CO2 and phenylalanine intake

<table>
<thead>
<tr>
<th>Model</th>
<th>MSE</th>
<th>SE of regression</th>
<th>CV</th>
<th>r2</th>
<th>P</th>
<th>Breakpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 vs 3</td>
<td>0.052</td>
<td>0.108</td>
<td>17.298</td>
<td>0.290</td>
<td>0.002</td>
<td>43.73</td>
</tr>
<tr>
<td>6 vs 2</td>
<td>0.052</td>
<td>0.109</td>
<td>17.240</td>
<td>0.295</td>
<td>0.002</td>
<td>51.71</td>
</tr>
</tbody>
</table>

1 MSE, mean of standard error.  
2 Choose is the parameter used to measure the equation of the individual regression lines (choose = 1 for observations before the breakpoint and choose = 0 for observations after the breakpoint).  
3 Breakpoint is the mean requirement estimate, ie, where the 2 lines of the model intersect.  
4 Five phenylalanine intakes on one line and 3 phenylalanine intakes on the other line.  
5 Six phenylalanine intakes on one line and 2 phenylalanine intakes on the other line.
21.9 mg · kg\(^{-1}\) · d\(^{-1}\) resulted in a negative balance of phenylalanine. On the other hand, persons who received intermediate or generous phenylalanine intakes (39, 96.6, or 100 mg · kg\(^{-1}\) · d\(^{-1}\)) had a positive phenylalanine balance. However, the rate of phenylalanine hydroxylation might be overestimated with the 24-h direct amino acid oxidation balance method during the fasting state (25). Another limitation of this method was the large variability in phenylalanine hydroxylation between the subjects in the fed state at different intakes of phenylalanine and tyrosine (25). For example, the hydroxylation rate estimated by Sanchez et al (24) was 3.20 μmol · kg\(^{-1}\) · h\(^{-1}\) in the fed state at a phenylalanine intake of 21.9 mg · kg\(^{-1}\) · d\(^{-1}\) without tyrosine, whereas the rate estimated by Basile-Filho et al (4) was 8.54 μmol · kg\(^{-1}\) · h\(^{-1}\) with a diet containing 18.5 mg phenylalanine · kg\(^{-1}\) · d\(^{-1}\) and 6.79 mg tyrosine · kg\(^{-1}\) · d\(^{-1}\).

One of the approaches used to measure the requirement for individual amino acids is the plasma amino acid response. Theoretically, the circulating concentration of an indispensable amino acid is not sensitive to intake of that amino acid when the intake is low; however, when the intake increases above the requirement for that amino acid, the plasma concentration will increase (7). In the present study, a two-phase response was not observed in either phenylalanine or tyrosine plasma concentrations with increasing phenylalanine intake. Young et al (26) concluded that it was difficult to measure a breakpoint in the plasma concentration of an indispensable amino acid because the circulating amino acid concentration is not necessarily bilinear. Hence, it was not unexpected that plasma phenylalanine or tyrosine concentrations could not be used to determine the aromatic amino acid requirements.

The estimated tyrosine requirement of healthy men who received a phenylalanine intake of 9 mg · kg\(^{-1}\) · d\(^{-1}\) and was measured by using the IAAO technique was 6 mg · kg\(^{-1}\) · d\(^{-1}\), which resulted in an estimated average requirement and a safe total aromatic amino acid intake of 15 and 21 mg · kg\(^{-1}\) · d\(^{-1}\), respectively (6). Young (27) criticized the design of Roberts et al (6) and argued that the mean requirement for the aromatic amino acids should be higher than 15 mg · kg\(^{-1}\) · d\(^{-1}\) because of the tyrosine-sparing effect. However, the main reason for such a low requirement outcome was not because of the tyrosine-sparing effect but because of the low phenylalanine intake. The phenylalanine requirement for children with classic phenylketonuria (PKU), as measured with the IAAO technique, was 14 mg · kg\(^{-1}\) · d\(^{-1}\) (28). In patients with classic PKU, the ability to oxidizephenylalanine is either negligible or small (29, 30); their dietary phenylalanine requirements are lower than those of healthy children by the amount of phenylalanine lost via obligatory oxidation (31). Hence, the phenylalanine requirement in PKU patients should represent the intake of phenylalanine needed for protein synthesis. Obligatory oxidation of phenylalanine was estimated to be ≈26% of the phenylalanine requirement in men (5); thus, the obligatory loss was ≈4 mg · kg\(^{-1}\) · d\(^{-1}\). The phenylalanine requirement, based on the values from children with classic PKU, can therefore be predicted to be 18 mg · kg\(^{-1}\) · d\(^{-1}\); thus, the phenylalanine intake of 9 mg · kg\(^{-1}\) · d\(^{-1}\) (6) was lower than the requirement. Hence, in Roberts et al (6), only 6 mg tyrosine · kg\(^{-1}\) · d\(^{-1}\) was required to maximize protein synthesis when phenylalanine intake was at a deficient level of 9 mg · kg\(^{-1}\) · d\(^{-1}\). The ratio of phenylalanine to tyrosine requirements that was calculated by Roberts et al (6) was 60:40, which is close to their ratio in body tissue of 55:45 (32). In animals (33–37) and healthy humans (38), the dietary requirements for phenylalanine and tyrosine are usually close to their ratio in mixed tissue protein. The study by Roberts et al (6) did not estimate the true total aromatic amino acid requirement because insufficient phenylalanine was provided; however, the study provided strong evidence that the ratio of phenylalanine to tyrosine for optimal protein synthesis is similar to that of body tissue. We suggest that the optimal intakes for phenylalanine and tyrosine remain in this ratio until the phenylalanine requirement for protein synthesis is reached. Applying this point of view to the IAAO method supports our position of using excess tyrosine, because it will ensure that phenylalanine intake does not become limiting and that the phenylalanine oxidation response is sensitive to a change in the test amino acid intake and, thus, to protein synthesis (31).

The acceptable indicator amino acids for the IAAO method are phenylalanine and lysine. Lysine is not as good an indicator as is phenylalanine (given an excess of tyrosine in the diet) because lysine has a larger and more variable pool size in body tissue and more steps are involved in its catabolic pathway before the carboxyl carbon is oxidized (39). In the piglet model, however, a lysine tracer was validated in the measurement of tryptophan requirement by comparison with a phenylalanine tracer (40); there was no difference in the tryptophan requirements estimated with the 2 indicators. Lysine has also been used with the IAAO method to measure the requirements for tyrosine in healthy men (6) and to measure the phenylalanine or tyrosine requirements in children with classic PKU (28, 41). Thus, we are confident that lysine is an acceptable indicator amino acid.

Concerns about the IAAO approach include the duration of adaptation to the test level. Could a short (4 h) adaptation to the test level result in an overestimation of the requirement (42)? In fact, no systematic effect can be found in the literature. For example, the lysine and threonine requirements measured with IAAO (43, 44) were 17% and 26% higher, respectively, than with the 24-h indicator amino acid balance method (45, 46). Conversely, the maximum methionine requirement was 16% lower (47, 48). In vitro, the uptake of phenylalanine reached a plateau within 1 h in liver slices (49), which implies that only a short period of time is required for adaptation to the conversion of phenylalanine to tyrosine. In our earlier study of the effect of tyrosine intake on the rate of phenylalanine hydroxylation (25), the hydroxylation rate was estimated to be 5.9 μmol · kg\(^{-1}\) · h\(^{-1}\) at intakes of 14 mg phenylalanine · kg\(^{-1}\) · d\(^{-1}\) plus 6 mg tyrosine · kg\(^{-1}\) · d\(^{-1}\), which was close to the hydroxylation rate (8.5 μmol · kg\(^{-1}\) · h\(^{-1}\)) calculated in the fed state with 24-h direct amino acid oxidation at intakes of 18.5 mg phenylalanine · kg\(^{-1}\) · d\(^{-1}\) plus 6.8 mg tyrosine · kg\(^{-1}\) · d\(^{-1}\) (4). Thus, the higher aromatic amino acid requirements that were measured in the present study were probably not due to the short period of adaptation.

In conclusion, the mean requirement for total aromatic amino acid intake by healthy men was between 44 and 52 mg · kg\(^{-1}\) · d\(^{-1}\). The mean estimate (48 mg · kg\(^{-1}\) · d\(^{-1}\)) was higher than the requirements estimated by Young’s group at MIT (3, 4) and was also higher than the current FAO/WHO/UNU (1985) and Dietary Reference Intake (2002) recommendations.

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JW-CH was involved in the study design, data collection, sample and data analyses, and writing of the manuscript. LAG was involved in the sample analyses and writing of manuscript. MR was involved in the study design, data analysis, and writing of the manuscript. The authors had no conflicts of interest.

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