The daily phenylalanine requirement of healthy Indian adults1–4

Anura V Kurpad, Meredith M Regan, Tony DS Raj, Vidya N Rao, Justin Gnanou, and Vernon R Young

ABSTRACT

Background: The daily requirement for phenylalanine is not known with certainty. Earlier 24-h tracer studies have suggested that the requirement is between 30 and 40 mg · kg⁻¹ · d⁻¹ (1), but the true requirement may be higher than this value. Earlier nitrogen balance studies have suggested that the mean requirement for phenylalanine is in the range of 13 to 20 mg · kg⁻¹ · d⁻¹ (2). The findings from these 24-h IAAB studies were consistent with short-term, fed-state DAAO studies (3), which concluded that the total aromatic amino acid requirement should be 30 mg · kg⁻¹ · d⁻¹. All these studies suggest that the total mean aromatic amino acid requirement is in the range of 30 to 40 mg · kg⁻¹ · d⁻¹.

The daily requirement of indispensable amino acids can also be determined through the measurement of the oxidation and balance of another amino acid, whose kinetics are well characterized, such that a response curve to graded intakes of the test substance (in this case, phenylalanine) can be obtained. This type of tracer study, called the indicator amino acid oxidation (IAAO)/indicator amino acid balance (IAAB) method, offers significant advantages over the DAAO/DAAB method (9). We have refined the IAAO technique to include a 6-d adaptation period to the experimental diet, a 24-h measurement of IAAO and IAAB, and the use of leucine as the indicator amino acid. Using this modified method, we assessed the requirements of lysine, methionine, valine, and threonine in adult Indians (10–14). This approach might be reasonably considered to be the best method currently available to measure amino acid requirements in adults.

There are no 24-h IAAO/IAAB studies at multiple aromatic amino acid intakes that would allow for the determination of a point estimate of the daily requirement. There are also concerns that the applicability of estimates of amino acid requirements obtained with the use of Western subjects may not be representative of global human amino acid requirement patterns. Therefore, this study was designed to assess the phenylalanine requirement in healthy, young Indian men with the use of a 6-d dietary adaptation period, the 24-h IAAO/IAAB approach, and [¹³C]leucine as the indicator amino acid.

INTRODUCTION

The daily adult aromatic amino acid requirement is set at 14 mg · kg⁻¹ · d⁻¹ (1), but the true requirement may be higher than this value. Earlier nitrogen balance studies have suggested that the mean requirement for phenylalanine is in the range of 13 to 20 mg · kg⁻¹ · d⁻¹ (2–4). These studies were conducted in the absence of dietary tyrosine. In the absence of tyrosine, the minimum phenylalanine requirement would be that intake just sufficient to meet the metabolic needs for these 2 aromatic acids. Tracer-based studies of the oxidation of phenylalanine or tyrosine have also been carried out at different intakes of phenylalanine in the absence of dietary tyrosine. With the use of an oral tracer of [¹³C]phenylalanine to measure the oxidation of tyrosine over a 24-h period (also called the direct amino acid oxidation (DAAO) or direct amino acid balance (DAAB) method), the daily phenylalanine balance was determined to be negative in all subjects when the phenylalanine intake was 22 mg · kg⁻¹ · d⁻¹ (5), but were in approximate neutral phenylalanine balance when the phenylalanine intake was 39 mg · kg⁻¹ · d⁻¹ (6). Studies using [¹³C]tyrosine have shown that tyrosine and total aromatic amino acid balances were negative at an aromatic amino acid intake of 23.5 mg · kg⁻¹ · d⁻¹ but were at equilibrium at an intake of 42.3 mg · kg⁻¹ · d⁻¹ (7). The findings from these 24-h DAAB studies were consistent with short-term, fed-state DAAO studies (8), which concluded that the total aromatic amino acid requirement should be 30 mg · kg⁻¹ · d⁻¹. All these studies suggest that the total mean aromatic amino acid requirement is in the range of 30 to 40 mg · kg⁻¹ · d⁻¹.

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The energy requirement was calculated to be $1.6 \times$ basal metabolic rate (BMR) during the days of feeding and $1.35 \times$ BMR on day 7. For each subject, energy intake was titrated against energy expenditure, which was estimated by performing daily time and motion studies during their stay in the metabolic ward. The subjects were encouraged to maintain their customary levels of physical activity but were asked to refrain from excessive or competitive exercise. The test phenylalanine intakes during the respective diet periods were 19, 23, 27, 31, 35, 38, 43, and 47 mg·kg$^{-1}$·d$^{-1}$ (Table 2). The 2 phenylalanine intakes that each subject was given were distributed around a putative requirement intake of 35 mg·kg$^{-1}$·d$^{-1}$. The daily dietary intake of the subjects was provided in 3 isoenergetic, isonitrogenous meals at 0800, 1300, and 2000, except on days 6 and 7 (see below).

### 24-h Tracer-infusion protocol and sample collection
A primed 24-h intravenous $[1^3C] $leucine approach was used, with the protocol of indirect calorimetry, and blood and breath sampling was as previously described (9–12). Briefly, $1-[1^3C] $leucine (99.3 atom%; MassTrace, Woburn, MA) was given as a primed constant intravenous infusion, at a known rate of $2.8 \mu$mol·kg$^{-1}$·h$^{-1}$ (the prime was $4.2 \mu$mol/kg), into an antecubital vein. The bicarbonate pool was primed with 0.8 $\mu$mol/kg of $[1^3C] $sodium bicarbonate (99.9 atom%; MassTrace). The tracer administration began at 1700 on day 6, with subjects having consumed their last meal of that day at 1500, and lasted until 1800 on day 7. Thus, the tracer infusion was given for 25 h, although only the data from the last 24 h were used to calculate daily leucine oxidation and balance. Blood and breath samples were collected every half hour, except during sleep, when the samples were collected every hour. On the day of the infusion, the subjects received, at hourly intervals, 10 isoenergetic, isonitrogenous small meals beginning at 0600 on day 7 and lasted until and including 1500 (which together were equivalent to the 24-h dietary intake for that day). A similar feeding pattern was imposed on the subjects on day 6 as well, so that the feeding pattern on the infusion day was not different from the pattern on the previous day.

### Subjects and Methods

#### Subjects
Thirty-two healthy men participated in this experiment. The subjects were weighed to the nearest 0.1 kg, and their height was measured to the nearest 0.1 cm. The logarithm of the sum of 4 skinfold thicknesses (biceps, triceps, subcapsular, and suprailiac) was used in age- and sex-specific equations (15) to obtain an estimate of body density, from which percentage body fat and fat-free mass (FFM) were determined (16; Table 1). The purpose of the study and the potential risks involved were explained to each subject, and the Human Ethical Review Board of St John’s Medical College approved the research protocol.

#### Diet and experimental design
Groups of 8 subjects were each randomly assigned to 2 separate 6-d experimental diet periods, at which time they received a weight-maintaining diet based on a $L$-amino acid mixture, as previously described (9–13). The energy requirement was calculated to be $1.6 \times$ basal metabolic rate (BMR) during the days of feeding and $1.35 \times$ BMR on day 7. For each subject, energy intake was titrated against energy expenditure, which was estimated by performing daily time and motion studies during their stay in the metabolic ward. The subjects were encouraged to maintain their customary levels of physical activity but were asked to refrain from excessive or competitive exercise. The test phenylalanine intakes during the respective diet periods were 19, 23, 27, 31, 35, 38, 43, and 47 mg·kg$^{-1}$·d$^{-1}$ (Table 2). The 2 phenylalanine intakes that each subject was given were distributed around a putative requirement intake of 35 mg·kg$^{-1}$·d$^{-1}$. The daily dietary intake of the subjects was provided in 3 isoenergetic, isonitrogenous meals at 0800, 1300, and 2000, except on days 6 and 7 (see below).

### Table 1

**Characteristics of well-nourished Indian men studied for their phenylalanine requirements**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value ($n = 32$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>21.8 ± 2.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.9 ± 5.7</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>21.5 ± 1.6</td>
</tr>
<tr>
<td>MUAC (cm)</td>
<td>26.9 ± 1.8</td>
</tr>
<tr>
<td>Percentage body fat (%)</td>
<td>14.9 ± 4.1</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>32.6 ± 4.9</td>
</tr>
</tbody>
</table>

All values are $\bar{x} \pm$ SD. MUAC, Midupper arm circumference; FFM, fat-free mass.

### Table 2

**Composition of amino acid mixtures used to supply 8 phenylalanine intakes per day**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>19</th>
<th>23</th>
<th>27</th>
<th>31</th>
<th>35</th>
<th>38</th>
<th>43</th>
<th>47</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L$-Threonine</td>
<td>50.32</td>
<td>50.21</td>
<td>50.12</td>
<td>50.01</td>
<td>49.91</td>
<td>49.84</td>
<td>49.71</td>
<td>49.61</td>
</tr>
<tr>
<td>$L$-Isoleucine</td>
<td>67.14</td>
<td>67.00</td>
<td>66.87</td>
<td>66.73</td>
<td>66.60</td>
<td>66.49</td>
<td>66.32</td>
<td>66.19</td>
</tr>
<tr>
<td>$L$-Lysine·HCl</td>
<td>89.37</td>
<td>89.19</td>
<td>89.01</td>
<td>88.83</td>
<td>88.65</td>
<td>88.52</td>
<td>88.29</td>
<td>88.11</td>
</tr>
<tr>
<td>$L$-Methionine</td>
<td>31.71</td>
<td>31.65</td>
<td>31.58</td>
<td>31.52</td>
<td>31.46</td>
<td>31.41</td>
<td>31.33</td>
<td>31.26</td>
</tr>
<tr>
<td>$L$-Cystine</td>
<td>23.52</td>
<td>23.47</td>
<td>23.42</td>
<td>23.37</td>
<td>23.33</td>
<td>23.29</td>
<td>23.23</td>
<td>23.18</td>
</tr>
<tr>
<td>$L$-Phenylalanine$^d$</td>
<td>17.89</td>
<td>21.61</td>
<td>25.32</td>
<td>29.00</td>
<td>32.68</td>
<td>35.43</td>
<td>40.00</td>
<td>43.62</td>
</tr>
<tr>
<td>$L$-Tyrosine</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>$L$-Valine</td>
<td>75.07</td>
<td>74.92</td>
<td>74.77</td>
<td>74.62</td>
<td>74.47</td>
<td>74.35</td>
<td>74.16</td>
<td>74.01</td>
</tr>
<tr>
<td>$L$-Histidine·HCl</td>
<td>32.77</td>
<td>32.70</td>
<td>32.63</td>
<td>32.57</td>
<td>32.50</td>
<td>32.45</td>
<td>32.37</td>
<td>32.30</td>
</tr>
<tr>
<td>$L$-Arginine·HCl</td>
<td>80.76</td>
<td>80.59</td>
<td>80.43</td>
<td>80.27</td>
<td>80.11</td>
<td>79.98</td>
<td>79.78</td>
<td>79.62</td>
</tr>
<tr>
<td>$L$-Alanine</td>
<td>204.59</td>
<td>204.17</td>
<td>203.77</td>
<td>203.35</td>
<td>202.94</td>
<td>202.63</td>
<td>202.11</td>
<td>201.70</td>
</tr>
<tr>
<td>$L$-Aspartic acid</td>
<td>12.75</td>
<td>12.72</td>
<td>12.70</td>
<td>12.67</td>
<td>12.65</td>
<td>12.63</td>
<td>12.59</td>
<td>12.57</td>
</tr>
<tr>
<td>$L$-Glutamic acid</td>
<td>31.53</td>
<td>31.46</td>
<td>31.40</td>
<td>31.34</td>
<td>31.37</td>
<td>31.32</td>
<td>31.15</td>
<td>31.08</td>
</tr>
<tr>
<td>Glycine</td>
<td>107.95</td>
<td>106.01</td>
<td>104.03</td>
<td>102.12</td>
<td>100.21</td>
<td>98.79</td>
<td>96.44</td>
<td>94.54</td>
</tr>
<tr>
<td>L-Proline</td>
<td>43.07</td>
<td>42.98</td>
<td>42.90</td>
<td>42.81</td>
<td>42.72</td>
<td>42.66</td>
<td>42.55</td>
<td>42.46</td>
</tr>
<tr>
<td>L-Serine</td>
<td>86.14</td>
<td>85.97</td>
<td>85.80</td>
<td>85.62</td>
<td>85.45</td>
<td>85.32</td>
<td>85.10</td>
<td>84.93</td>
</tr>
</tbody>
</table>

$^d$ $9.62 \text{ mg leucine} \times \text{kg}^{-1} \times \text{d}^{-1}$ was added to each mix every day, except on the infusion day, when this amount of leucine was infused as tracer.

$^2$ $1.078 \text{ g mixture} \times \text{kg}^{-1} \times \text{d}^{-1}$ was given to subjects, which provided $160 \text{ mg N} \times \text{kg}^{-1} \times \text{d}^{-1}$.
Breath samples were analyzed for $^{13}$CO$_2$ enrichment by isotope ratio mass spectrometry (Europe Scientific Ltd, Crewe, United Kingdom), and blood samples were analyzed for $^{13}$C enrichments of plasma α-ketoisocaproic acid (KIC) by gas chromatography–mass spectrometry (Varian, Palo Alto, CA), as previously described (17).

**Leucine oxidation and balance calculations**

Leucine oxidation (μmol·kg$^{-1}$·30 min$^{-1}$) was computed for consecutive half-hourly intervals as described previously (18).

Leucine oxidation (μmol·kg$^{-1}$·30 min$^{-1}$) = $^{13}$CO$_2$ production/[1$^{13}$C]KIC enrichment (1)

where $^{13}$CO$_2$ production (μmol·kg$^{-1}$·30 min$^{-1}$) = VCO$_2$ (carbon dioxide production rate, in μmol·kg$^{-1}$·30 min$^{-1}$) × $^{13}$CO$_2$ enrichment (atom% excess/100) × 1/recovery of $^{13}$CO$_2$.

Leucine balance (input - measured output) was computed as follows:

Leucine balance (mg·kg$^{-1}$·d$^{-1}$) = input (dietary leucine + intravenous tracer) - output (sum of leucine oxidation measured at 30-min intervals) (2)

**Statistical methods and data evaluation**

Data are presented as means ± SDs. Weight change and leucine flux were analyzed by using mixed-models analysis of variance. The model for weight change over the 6-d experimental diet periods included a factor for diet period. The model for 12-h leucine flux included diet period, metabolic phase (fasted compared with fed), phenylalanine intake, and the interaction of intake and metabolic phase. For the relations between phenylalanine intake, and the interaction of intake and metabolic phase. For the relations between phenylalanine intake, and the interaction of intake and metabolic phase. For the relations between phenylalanine intake, and the interaction of intake and metabolic phase. For the relations between phenylalanine intake, and the interaction of intake and metabolic phase.

**RESULTS**

**Anthropometric measures**

The anthropometric measures of the subjects were similar to those of the subjects from studies in our previous series (10–13). During the 6-d experimental diet periods, the subjects experienced a small but statistically significant ($P < 0.001$) weight loss of $-0.40$ ± 0.41 kg on average across diet periods. There was no significant difference in weight loss between diet periods ($P = 0.83$).

**Leucine oxidation and breakpoint analysis**

Leucine oxidation and balance at the 8 phenylalanine intakes are shown in Table 3. Two-phase linear regression models were fit to the phenylalanine intake – 24-h IAAO (leucine) and phenylalanine intake – 12-h fed leucine oxidation relations; the

**TABLE 3**

Summary of leucine oxidation, balance, and flux at 8 phenylalanine intakes in well-nourished Indian men

<table>
<thead>
<tr>
<th>Phenylalanine index</th>
<th>19</th>
<th>23</th>
<th>27</th>
<th>31</th>
<th>35</th>
<th>38</th>
<th>43</th>
<th>47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidation (mg phenylalanine · kg$^{-1}$· d$^{-1}$)$^2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-h Fasted</td>
<td>23.5 ± 4.2</td>
<td>21.4 ± 3.2</td>
<td>20.7 ± 3.0</td>
<td>19.7 ± 4.4</td>
<td>21.2 ± 1.5</td>
<td>18.7 ± 2.4</td>
<td>19.0 ± 1.9</td>
<td>19.5 ± 2.9</td>
</tr>
<tr>
<td>12-h Fed</td>
<td>26.0 ± 4.5</td>
<td>23.4 ± 3.3</td>
<td>22.8 ± 4.4</td>
<td>23.3 ± 4.1</td>
<td>20.9 ± 5.4</td>
<td>20.0 ± 4.1</td>
<td>23.0 ± 1.9</td>
<td>20.0 ± 2.1</td>
</tr>
<tr>
<td>Total, 24 h</td>
<td>49.5 ± 8.5</td>
<td>44.8 ± 5.7</td>
<td>43.5 ± 4.7</td>
<td>43.0 ± 8.2</td>
<td>42.1 ± 5.1</td>
<td>38.7 ± 5.8</td>
<td>42.0 ± 2.3</td>
<td>39.6 ± 4.1</td>
</tr>
<tr>
<td>Total intake</td>
<td>40.2 ± 0.9</td>
<td>39.8 ± 1.0</td>
<td>39.7 ± 1.0</td>
<td>40.1 ± 1.1</td>
<td>40.2 ± 0.5</td>
<td>39.8 ± 1.1</td>
<td>40.5 ± 1.7</td>
<td>40.3 ± 1.5</td>
</tr>
<tr>
<td>24-h Balance$^3$</td>
<td>-9.3 ± 8.6</td>
<td>-4.9 ± 4.9</td>
<td>-3.8 ± 5.0</td>
<td>-2.9 ± 8.7</td>
<td>-2.0 ± 5.0</td>
<td>1.2 ± 6.1</td>
<td>-1.5 ± 1.9</td>
<td>0.8 ± 3.9</td>
</tr>
<tr>
<td>Flux (μmol·kg$^{-1}$·30 min$^{-1}$)$^4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-h Fasted</td>
<td>51.1 ± 9.9</td>
<td>50.3 ± 9.8</td>
<td>49.9 ± 9.7</td>
<td>48.3 ± 6.7</td>
<td>50.3 ± 4.7</td>
<td>44.8 ± 9.0</td>
<td>48.2 ± 11.4</td>
<td>45.7 ± 5.5</td>
</tr>
<tr>
<td>12-h Fed</td>
<td>51.5 ± 9.0</td>
<td>48.4 ± 7.3</td>
<td>47.3 ± 6.1</td>
<td>47.9 ± 6.0</td>
<td>49.3 ± 4.6</td>
<td>44.4 ± 6.3</td>
<td>48.5 ± 11.3</td>
<td>45.7 ± 7.6</td>
</tr>
<tr>
<td>Total, 24 h</td>
<td>51.3 ± 9.4</td>
<td>49.3 ± 8.3</td>
<td>48.6 ± 7.6</td>
<td>48.1 ± 6.3</td>
<td>49.8 ± 4.5</td>
<td>44.6 ± 7.6</td>
<td>48.3 ± 11.4</td>
<td>45.7 ± 6.5</td>
</tr>
</tbody>
</table>

$^1$ All values are $\bar{x}$ ± SD. n = 8 observations for all phenylalanine intakes.

$^2$ Within the 2-phase linear random-effects regression model, 24-h leucine oxidation at phenylalanine intakes of 19, 23, and 27 mg was significantly greater than oxidation above the breakpoint ($P < 0.05$ for all), but not at phenylalanine intakes of 31 and 35 mg; 12-h fed leucine oxidation at phenylalanine intakes of 19, 23, and 27 mg was also significantly greater than oxidation above the breakpoint ($P < 0.01$ for all), but not at phenylalanine intakes of 31 and 35 mg.

$^3$ 24-h Balance = intake − oxidation. Within the 2-phase linear random-effects regression model, leucine balance was significantly lower than zero balance at phenylalanine intakes of 19, 23, 27, and 31 mg ($P < 0.01$ for all), but was not significantly different from zero balance at phenylalanine intakes of 35 mg or intakes above the breakpoint. Leucine balance at phenylalanine intakes of 19, 23, 27, and 31 mg was also significantly lower than balance above the breakpoint ($P < 0.05$ for all), but not at phenylalanine intakes of 35 mg.

$^4$ There was no effect of phenylalanine intake on leucine flux, and flux did not differ significantly between the fasted and fed phases ($P = 0.16$; mixed-models ANOVA).
results are summarized in Table 4. For 24-h IAAO, there was no evidence of a nonzero slope in the second line segment and thus the slope was restricted to be zero. The breakpoint estimated from this model was 37 mg · kg\(^{-1}\) · d\(^{-1}\); the 95% CI ranged from 31 to >47 mg · kg\(^{-1}\) · d\(^{-1}\), which indicated that 24-h IAAO (leucine) decreased linearly until a phenylalanine intake of 37 mg · kg\(^{-1}\) · d\(^{-1}\), above which 24-h leucine oxidation was estimated as 40 ± 1.1 mg · kg\(^{-1}\) · d\(^{-1}\) at all higher phenylalanine intakes. Within this model, 24-h leucine oxidation at phenylalanine intakes of 19, 23, and 27 mg were also significantly higher than oxidation above the breakpoint (\(P < 0.05\) for each), but were not significantly different from oxidation above the breakpoint at phenylalanine intakes of 31 and 35 mg.

The results for 12-h fed leucine oxidation were similar to those for 24-h oxidation. There was no evidence of a nonzero slope in the second line segment and, thus, the slope was restricted to be zero. The breakpoint was estimated as 36 mg · kg\(^{-1}\) · d\(^{-1}\); the 95% CI ranged from 28 to >47 mg · kg\(^{-1}\) · d\(^{-1}\), which indicated that 12-h fed leucine oxidation decreased linearly until a phenylalanine intake of 36 mg · kg\(^{-1}\) · d\(^{-1}\), above which it was estimated as 21 ± 0.8 mg · kg\(^{-1}\) · d\(^{-1}\) at all higher phenylalanine intakes. Within this model, 12-h fed leucine oxidation at phenylalanine intakes of 19, 23, and 27 mg was also significantly higher than oxidation above the breakpoint (\(P < 0.01\) for each), but was not significantly different from oxidation above the breakpoint at phenylalanine intakes of 31 and 35 mg.

### Leucine balance and breakpoint analysis

The results of fitting a 2-phase linear regression model to the phenylalanine intake–leucine balance relation are summarized in Table 4 and depicted in Figure 1 along with the mean oxidation rates measured at each phenylalanine intake. There was no evidence of a nonzero slope in the second line segment and, thus, the slope was restricted to be zero. The breakpoint estimated from this model was 38 mg · kg\(^{-1}\) · d\(^{-1}\); the 95% CI ranged from 31 to >47 mg · kg\(^{-1}\) · d\(^{-1}\), which indicated that daily IAAB (leucine) increased linearly until a phenylalanine intake of 38 mg · kg\(^{-1}\) · d\(^{-1}\), above which leucine balance was estimated as 0.07 ± 1.1 mg · kg\(^{-1}\) · d\(^{-1}\) at all higher phenylalanine intakes (Figure 1). Within this model, leucine balance was significantly lower than zero balance at phenylalanine intakes of 19, 23, 27, and 31 mg · kg\(^{-1}\) · d\(^{-1}\) (\(P < 0.01\) for each), but was not significantly different from zero balance at the 35-mg phenylalanine intakes or intakes above the breakpoint. Leucine balance at phenylalanine intakes of 19, 23, 27, and 31 mg were also significantly lower than balance above the breakpoint (\(P < 0.05\) for each), but was not significantly different from balance above the breakpoint at the 35-mg phenylalanine intake.

### Leucine flux

There was no effect of phenylalanine intake on leucine flux, and flux did not differ significantly between the fasted and fed phases (Table 3; \(P = 0.16\)).
DISCUSSION

The findings in the present study add to the earlier tracer-derived balance data that we generated using the diet-adapted, 24-h direct or IAAO/IAAB paradigm to quantify adult amino acid requirements (9–13, 17, 18, 21) in South Asian (Indian) and American subjects. The present finding of a mean phenylalanine requirement of 38 mg · kg⁻¹ · d⁻¹ in the absence of dietary tyrosine also generally confirms and extends the findings from the earlier 24-h and short-term tracer DAAB studies (5–8). The requirement of 38 mg · kg⁻¹ · d⁻¹ is higher than the value of 20 mg · kg⁻¹ · d⁻¹ suggested by recalculations of nitrogen balance data (3) to include miscellaneous nitrogen losses (22), but is similar to the value of ≈37 mg · kg⁻¹ · d⁻¹ proposed by the obligatory amino acid loss (OAAL) method, which is based on a total aromatic OAAL of 26 mg · kg⁻¹ · d⁻¹ and a utilization efficiency of 70% (23).

Tracer-based studies have shown that the 1985 FAO/WHO/UNU (1) requirement for phenylalanine is too low (24), based on the determination of negative phenylalanine balances at the FAO intake and positive balances at more generous intakes based on an egg-protein pattern and the MIT pattern of intake (which was based on the OAAL method). In that short-term study, phenylalanine disposal was measured by the hydroxylation rate with extraptations to a 24-h balance (24). The 24-h IAAB–derived phenylalanine requirement estimate of 38 mg · kg⁻¹ · d⁻¹ found in the present study is also within the range suggested by the tracer 24-h DAAB studies (5–7), but is slightly higher than the short-term DAAO-based value of 30 mg · kg⁻¹ · d⁻¹ (8). The 24-h DAAB studies used an oral tracer of [¹³C]phenylalanine, given over a 24-h period (5, 6, 25), to determine daily phenylalanine balance. The latter was negative in the entire group of 7 subjects when phenylalanine intake was 22 mg · kg⁻¹ · d⁻¹ (5). Subsequent studies with an oral tracer of [¹³C]phenylalanine and [³H]tyrosine indicated that subjects were in approximate neutral body phenylalanine balance when given a daily intake of 39 mg · kg⁻¹ · d⁻¹ (6) or even in positive balance at a generous phenylalanine intake of 100 mg · kg⁻¹ · d⁻¹ (25). The latter study also suggested that phenylalanine oxidation was underestimated when phenylalanine was used as the tracer and suggested that a better estimate could be obtained from [¹³C]tyrosine infusions (25). These [¹³C]tyrosine-based 24-h experiments also showed that tyrosine and total aromatic amino acid balances were negative at an aromatic amino acid intake of 25.3 mg · kg⁻¹ · d⁻¹ and were at equilibrium at an intake of 42.4 mg · kg⁻¹ · d⁻¹ with low intakes of tyrosine (7). Thus, it would appear from tracer data that the total mean aromatic amino acid requirement is in excess of 20 mg · kg⁻¹ · d⁻¹ (range: ≈30–40 mg · kg⁻¹ · d⁻¹).

The short-term [¹³C]phenylalanine DAAO tracer study (fed state) by Zello et al (8) was carried out with 2 different dietary phenylalanine intakes, during a dietary adaptation period and at different incident phenylalanine intakes during the tracer experiment, in the presence of generous amounts of tyrosine (40 mg · kg⁻¹ · d⁻¹). The breakpoint on the phenylalanine oxidation – intake curve occurred at ≈9 mg · kg⁻¹ · d⁻¹ and was not different between the 2 dietary phenylalanine adaptation intakes, even though the amount of phenylalanine oxidized in the fed state was different at the 2 dietary phenylalanine intakes during the dietary adaptation period. On the basis of the assumption that tyrosine can supply two-thirds of the aromatic amino acid requirement, these investigators proposed a total aromatic amino acid requirement of 30 mg · kg⁻¹ · d⁻¹ (8). These investigators also suggested that the lower estimate in their studies than in the 24-h DAAB studies (6, 7, 25) was due to the provision of aromatic amino acids together, which would have resulted in a greater efficiency of utilization than if a tyrosine-free or minimal tyrosine diet were given (26). Other reasons for a possibly low phenylalanine requirement could be the generous dietary intake of tyrosine, which would result in relations between the aromatic amino acids that may not be reflective of natural diets. Furthermore, variations in prior protein intakes between 0.8 and 20 mg · kg⁻¹ · d⁻¹ may also affect amino acid kinetics (27), because of the up- or down-regulation of oxidative enzymes. The effect of this on phenylalanine oxidation in the fasted state (not measured in fed state IAAO experiments) and, hence, the effect on 24-h balance, is unknown but could lead to differences in the pattern of response and the breakpoint estimate of the phenylalanine requirement. Finally, phenylalanine oxidation was much lower than expected (8), ≈2.4 mg phenylalanine · kg⁻¹ · 12 h⁻¹ in the fed period. It is possible that the oxidation rates and their pattern with increasing phenylalanine intakes might have been different if the plasma tyrosine enrichment values had been used to estimate the phenylalanine oxidation rate (25). However, it might equally be suggested that this was a systematic error such that the entire oxidation-intake curve would be shifted upward, with no change in the breakpoint estimate.

An estimate of the total aromatic amino acid requirement is also available from a short-term IAAO study of the tyrosine requirement (26). In this experiment, the effects of increasing tyrosine intakes on the oxidation of [¹³C]tyrosine as an indicator was studied at a constant phenylalanine intake of 9 mg · kg⁻¹ · d⁻¹; this intake was based on the DAAO experiment described above (8). A mean breakpoint in the lysine oxidation-tyrosine intake response curve was obtained at an intake of 6 mg tyrosine · kg⁻¹ · d⁻¹. On the basis of these data, it was concluded that the aromatic amino acid requirement was 15 mg · kg⁻¹ · d⁻¹, although the phenylalanine intake in this study may have been limiting on the basis of the estimated phenylalanine requirement derived from an earlier study in the presence of a generous tyrosine intake (8). The suboptimal phenylalanine intake could therefore have restricted the utilization of tyrosine and lowered the apparent combined requirement.

The estimated aromatic amino acid requirement in Indian subjects found in the present study is important because estimates of amino acid requirements obtained in healthy, well-nourished Western subjects may not be globally representative, particularly in those populations living under environmental stressors such as chronic or subclinical infection, parasitic infestation, or pollution. For example, in an earlier investigation in chronically undernourished men from poor and unsanitary environments, we found a 50% higher daily requirement of lysine (28, 29). Tyrosine has been shown to be a significant component of the positive acute phase proteins synthesized by the liver. These proteins participate in host-defense mechanisms, and their rates of synthesis increase manyfold under infective stress (30). Therefore, although there may be adaptive reductions in amino acid requirements when habitual diets are low in protein, amino acids, or both, there may also be increased requirements because of other factors such as chronic but subclinical immunostimulation (31).

The small average weight loss experienced by the subjects (<0.5 kg in 1 wk) is unlikely to be due to lean tissue loss. A
similar finding was seen in our earlier studies (11, 29) and was linked to an observed negative carbohydrate balance and water loss related to glycogen breakdown (32). This pattern of substrate oxidation with the study diet appears to have been due to the subjects’ high habitual carbohydrate intake; the experimental diet provided less carbohydrate because of constraints on how much wheat starch and beet sugar could be reasonably included daily in an experimental diet. In summary, the present investigation of 24-h [13C]leucine tracer indicator kinetics in well-nourished Indian subjects, who were studied with 8 test intakes of phenylalanine as part of a tyrosine-free diet, indicates that the international mean requirement for total aromatic amino acids should be ~38 mg · kg⁻¹ · d⁻¹.

AVK was involved in the study design, data collection, sample and data analysis, and writing of the manuscript. VNR and TDLS were involved in the data collection and analysis. JG was involved in the sample analyses. VRY was involved in the study design, MMR was involved in the study design, data analysis, and writing of the manuscript. The authors had no conflicts of interest.

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