Threonine requirement of healthy adults, derived with a 24-h indicator amino acid balance technique

Sudhir Borgonha, Meredith M Regan, Seung-Ho Oh, Mervelina Condon, and Vernon R Young

ABSTRACT
Background: Because we question the validity of the 1985 FAO/WHO/UNU upper requirement for threonine of 7 mg·kg⁻¹·d⁻¹, we proposed a tentative mean requirement of 15 mg·kg⁻¹·d⁻¹.
Objective: Our goal was to assess threonine adequacy at 3 test intakes and the consequences of a 6-d compared with a 13-d dietary adaptation phase.
Design: We used a 24-h indicator amino acid balance technique ([1-¹³C]leucine as indicator) to assess the threonine requirement. Fifteen healthy adults were randomly assigned to receive 7, 15, or 46 mg threonine·kg⁻¹·d⁻¹ and were studied after 6 and 13 d of adaptation to the experimental diets. Diets were based on an L-amino acid mixture in which the threonine content was varied. At 1700 on days 6 and 13, a 24-h intravenous [¹³C]leucine tracer infusion protocol was begun to assess leucine oxidation and daily leucine balances.
Results: There was no detectable effect of duration of dietary adaptation in leucine oxidation or balance, but the 24-h leucine oxidation rates differed significantly between the 7-mg intake and each of the 2 higher intakes (P < 0.05). The latter were not significantly different. The 24-h leucine oxidation rate decreased across threonine intakes (P < 0.01 for main effect of diet, independent of infusion day). Leucine oxidation was highly correlated (r = 0.80) between the 2 dietary adaptation phases across all test intakes.
Conclusion: The 1985 FAO/WHO/UNU threonine recommendation is inadequate, and 15 mg·kg⁻¹·d⁻¹ is sufficient to achieve mean indicator (leucine) amino acid balance.

KEY WORDS Threonine requirements, indicator amino acid oxidation, amino acid balance, leucine, healthy adults

INTRODUCTION
The 1973 FAO/WHO Expert Committee (1) proposed an upper threonine requirement of 7 mg·kg⁻¹·d⁻¹ for healthy adults. This was derived from a combination of the highest estimate of the individual requirement to achieve positive nitrogen balance in 1 of 3 men, or 8.7 mg·kg⁻¹·d⁻¹, in the studies by Rose et al (2) and from results of studies in women by Linkswiler et al (3) and Leverton et al (4). The committee presumably chose from these latter findings a value of 375 mg/d to set a requirement of 5.8 mg·kg⁻¹·d⁻¹ for females. The approximate mean of these 2 values is 7 mg, although the precise basis for the committee’s recommendation remains unclear. However, this value was used later by the 1985 FAO/WHO/UNU Expert Consultation (5) to develop the amino acid requirement pattern for healthy adults and remains the official, international threonine requirement for use in adult protein nutrition. A 1991 FAO/WHO Committee (6) proposed the tentative use of the amino acid requirement pattern, including that for threonine, derived from studies in preschool children for purposes of assessing the nutritional quality of dietary protein in adults. If the preschool children’s threonine requirement pattern were a more appropriate estimate of that for adults, the threonine requirement for adults would be ≈25 mg.

We questioned the validity of the international amino acid requirements proposed by the 1973 FAO/WHO Expert Committee and 1985 FAO/WHO/UNU Expert Consultation (7–9). Our initial short-term 1-[¹³C]threonine tracer studies suggest that the mean requirement would be ≈10–20 mg (10). In further consideration of the predicted obligatory oxidative losses of threonine, we proposed a mean threonine requirement of 15 mg (8).

We conducted a further assessment of the threonine requirement in healthy adults, adjusted to 1 of 3 threonine intakes, with the use of an indicator amino acid balance technique and 24-h oxidation, as we described previously (11, 12). Wilson et al (13), using an indicator amino acid balance technique, proposed a mean requirement of 19 mg. In their study, the tracer-infusion protocol was conducted over 4 h in subjects in the fed state who had not undergone a period of dietary adaptation. However, our studies showed that there is a complex diet-dependent, temporal pattern to the rate of oxidation and utilization of the limiting amino acid within a 24-h period (14, 15). Therefore, we considered it desirable to conduct a 24-h indicator amino acid balance study in which we examined the response of amino acid oxidation to different threonine intakes and the daily indicator balance.

1 From the Laboratory of Human Nutrition, Massachusetts Institute of Technology, Cambridge (SB, MMR, S-HO, MC, and VRY), and Chonnam National University, Kwang-JU, Korea (S-HO).
2 Supported by NIH grant DK42101 and Massachusetts Institute of Technology Clinical Research Center core grant RR88.
3 Address reprint requests to VR Young, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Room E17-434, Cambridge, MA 02139. E-mail: vryoung@mit.edu.
Accepted for publication May 3, 2001.

Many of our earlier tracer studies in this series of investigations were carried out after a 6–7-d dietary adaptation phase. To determine whether this adaptation period is adequate, we studied the subjects twice, after 6 and 13 d.

SUBJECTS AND METHODS

Subjects

The 9 men and 6 women who participated in this study were students at the Massachusetts Institute of Technology (MIT) or lived in the Boston-Cambridge area. Their mean (±SD) weight was 64.6 ± 2.3 kg, height was 168.1 ± 6.8 cm, and age was 23.4 ± 2.6 y. All subjects were nonsmokers and in good health, as determined by medical history, physical examination, blood cell count, routine blood biochemical profile, and urinalysis.

Because there is no evidence that the minimum threonine requirement differs between healthy men and women, women of childbearing age were encouraged to volunteer, and the first of the 2 tracer experiments was conducted during the 7–10 d after the onset of menstrual bleeding. Intake of mild doses of contraceptive agents did not exclude women from the study. A negative pregnancy test result was required and was obtained 2–3 d before the study started. The subjects signed a consent form and were paid for their participation. The experimental protocol was approved by the MIT Committee on the Use of Humans as Experimental Subjects and the Scientific Advisory Committee of the MIT Clinical Research Center. The 24-h stable-isotope-tracer protocol required the admission of the subjects as inpatients to the MIT Medical Department.

Diet and experimental design

Subjects were studied over 14 d while consuming 1 of 3 diets (low, adequate, or generous in threonine; ≈7, 15, or 46 mg·kg⁻¹·d⁻¹, respectively) to which they were randomly allocated. The 7-, 15-, and 46-mg groups consisted of 2 men and 3 women, 4 men and 1 woman, and 3 men and 2 women, respectively.

The subjects were fed a weight-maintaining diet based on an L-amino acid mixture in which the threonine content was varied. Daily energy intakes were designed to maintain body weight with the use of diet histories and estimates of basal metabolic rates (5). Intakes were constant and ranged between 2238 and 246 J·kg⁻¹·h⁻¹·d⁻¹. On the days in which the tracer was infused, energy intake was reduced to 70% of usual intake to account for the reduced physical activity during this phase, but amino acids were provided at their previous intake. The subjects were encouraged to maintain their customary levels of physical activity during the dietary period but were asked to refrain from excessive or competitive exercise. The major energy supply was in the form of a sugar-oil formula and protein-free wheat-starch cookies. Nonprotein energy was provided as fat (≈40%) and carbohydrate (≈60%). The main sources of carbohydrate were beet sugar and wheat starch. The diet was described in detail previously (16).

The leucine content of the diet was adequate, supplying ≈40 mg·kg⁻¹·d⁻¹ and was held constant at all threonine intakes (Table 1). The intake of leucine was chosen to be close to a published requirement (14), with the expectation that this would allow a more sensitive measure of a change in leucine use (and, therefore, oxidation) and balance after a change in threonine intake than would a leucine intake considerably higher than the requirement. Subjects resumed consumption of the same diet after the completion of the first 24-h tracer-infusion protocol.

Nitrogen (160 mg·kg⁻¹·d⁻¹) was supplied via the L-amino acid mixture (Table 1). Flavoring agents (Vivonex flavor packets; Norwich Eaton Pharmaceuticals, Norwich, NY) were offered to the subjects to improve the taste of the amino acid mixture. Vitamins, minerals, choline, and fiber were supplied as supplements (16). The total daily food intake was provided as 3 isoenergetic, isonitrogenous meals (at 0800, 1300, and 2000). At least 2 of the 3 meals were consumed at the Clinical Research Center dining room. Each morning, body weight and vital signs were monitored.

Twenty-four-hour tracer-infusion protocol

We performed a primed, constant tracer-infusion study, similar to studies we described previously (16). Intravenous administration was used because we previously validated this approach for estimating the rate of whole-body leucine oxidation (16). Thus, subjects were admitted to the inpatient facility at 1600 on days 6 and 13 of the experiment after consuming their last meal at 1400. Two indwelling catheters were inserted into the nondominant arm of the subjects: one was inserted in the antecubital superficial hand vein in a retrograde manner for blood sampling. The tracer administration began at 1700 on days 6 and 13 and continued for the next 24 h. Baseline blood and breath samples were taken at 1650 and 1655. At 1700 a priming bolus of [¹³C]sodium bicarbonate (0.8 μmol/kg; MassTrace, Woburn, MA) was given, followed by a priming bolus of L-[¹³C]leucine (4.2 μmol/kg; MassTrace) and a constant infusion of L-[¹³C]leucine (2.8 μmol·kg⁻¹·h⁻¹).

Subjects received isonitrogenous meals at 1900 on days 6 and 13 and at 0500 and 1100 on the following days.
Breath and blood samples were taken at half-hourly intervals during the day and at hourly intervals between 0000 and 0500 to allow the subjects a longer rest at the night. Blood samples were collected in chilled, heparin-containing tubes and centrifuged at 4°C for 10 min at 1000 × g; plasma was extracted and stored at −20°C. Breath samples were collected in 15-mL non-silicon-coated tubes and stored at room temperature.

Indirect calorimetry

Total carbon dioxide production (VCO2) and oxygen consumption (VO2) rates were determined with an indirect calorimeter (Deltatrac; SensorMedics, Anaheim, CA) with a ventilated-hood system. Measurements were carried out according to a standardized procedure at intervals throughout the 24-h period (16).

Background enrichment of breath 13CO2 and [13C]bicarbonate recovery

The background 13C enrichment of expired carbon dioxide and the recovery of infused [13C]bicarbonate associated with the 3-meal pattern were measured as described previously (17) under essentially the same dietary conditions and in similar subjects. As described below, the calculations of oxidation took into account the bicarbonate recovery factor and changes in 13CO2 background under present experimental conditions.

Sample analysis

Breath 13CO2 was measured with the use of an isotope ratio mass spectrometer (MAT Delta E; Finnigan, Bremen, Germany) and blood samples were analyzed for [13C]ketosocaproate (KIC), both as described previously (16). Isotopic enrichments were obtained by gas chromatography–mass spectrometry (HP5890 Series II and HP 5988A; Hewlett Packard, Palo Alto, CA). Plasma concentrations of leucine and threonine were measured with the use of HPLC (16).

Evaluation of primary data

Leucine oxidation was computed for consecutive half-hourly or hourly intervals as described previously (16, 17). Briefly,

\[
\text{Leucine oxidation} (\text{mol} \cdot \text{kg}^{-1} \cdot 30 \text{ min}^{-1}) = \frac{\text{13CO}_2 \text{ production} (\text{mol} \cdot \text{kg}^{-1} \cdot 30 \text{ min}^{-1}) \times [\text{13C}]\text{KIC enrichment} (\text{APE/100})}{\text{R}}
\]

where APE is atom percent excess and [13C]KIC enrichment is the average of the 2 enrichments determining the specific half-hourly interval and where

\[
\text{13CO}_2 \text{ Production} (\text{mol} \cdot \text{kg}^{-1} \cdot 30 \text{ min}^{-1}) = \frac{\text{VCO}_2 (\text{mol} \cdot \text{kg}^{-1} \cdot 30 \text{ min}^{-1}) \times \text{13CO}_2 \text{ enrichment} (\text{APE/100})}{\text{R}}
\]

where R is the recovery of 13CO2 computed for each time interval, as previously described (17). Specific correction factors were used for each consecutive half-hourly period. The values ranged from ≈65% to ≈95%, depending on the particular time or meal period involved, as previously described (17).

In addition, within each metabolic state (fasting and fed), VCO2 over the specific time interval when it was not directly measured was derived as the arithmetic average of VCO2 measured just before and after this interval.

Leucine balance

The 24-h leucine balance (input − measured output) was computed as follows:

\[
\text{Input} (\mu\text{mol/kg}) = \text{dietary leucine + intravenous tracer} \quad (3)
\]

\[
\text{Output} (\mu\text{mol/kg}) = \text{sum of determined oxidation for the 48 half-hourly intervals} \quad (4)
\]

Postprandial leucine oxidation was calculated as the sum of half-hourly intervals for the 6 h after the beginning of each meal.

Statistical methods and data evaluation

Data are presented as means ± SDs. Leucine metabolic variables were analyzed by using mixed-models analysis of variance (SAS version 6.12 PROC MIXED; SAS Institute, Cary, NC). For each variable the model included factors for threonine intake (a between-subject factor), infusion day (a within-subject factor), and the interaction. If the interaction was significant, model contrasts were used to make the pairwise comparisons of interest. If the interaction was not significant, it was removed from the model and the main effects were considered alone; for significant main effects, model contrasts were used to compare means without regard to the other factor. To consider the effect of meal-time on leucine oxidation, a model was fit to leucine oxidation (\text{mol} \cdot \text{kg}^{-1} \cdot 6 \text{ h}^{-1}) that assessed the effects of threonine intake, infusion day, meal, and the interactions. Mealtime includes a fourth, “nonmeal,” period composed of the initial 2 unfed hours and a 4-h nighttime period between dinner and breakfast. Finally, we examined possible diet-related differences in plasma threonine and leucine concentrations during the last hour (1380–1440 min) of the 24-h tracer period on day 7; for each variable the mixed-models analysis of variance model included factors for intake, time, and the interaction. A P value of 0.05 indicated statistical significance for all tests of interaction and main effects; P values of all pairwise comparisons were adjusted by using Tukey’s method. All reported P values are two-sided.

RESULTS

The temporal pattern of leucine oxidation for the 24-h period beginning on day 6 for each threonine intake is given in Figure 1. The mean 24-h and 6-h (meal period) rates of leucine oxidation on days 7 and 14 of the diet periods are summarized in Table 2. Twenty-four-hour leucine oxidation did not differ significantly between tracer days but differed significantly among threonine intakes. The 24-h rate of leucine oxidation was significantly higher with the 7-mg threonine intake than with the 15- or 46-mg intakes. However, leucine oxidation was significantly higher during breakfast than during lunch and dinner; oxidation rates during lunch and dinner did not differ significantly. The leucine oxidation rate during the nonmeal period was significantly lower than that during breakfast and dinner at all 3 threonine intakes, but was significantly lower than during lunch only in the 7-mg group.

Downloaded from https://academic.oup.com/ajcn/article-abstract/75/4/698/4689374 by guest on 09 March 2019
Leucine balances on days 7 and 14 of the diet period are given in Table 2. There was a significant effect of intake but no significant interaction with, or main effect of, infusion day. The estimates showed that balance at the 7-mg threonine intake was significantly lower than that at each of the 2 higher threonine intakes. However, balances did not differ between these latter 2 intakes and were apparently positive at 7–8 mg leucine · kg\(^{-1}\) · d\(^{-1}\). n = 5 per group. Bars represent \(\pm\) 1 SD. Times of meals indicated by △.

The pattern of plasma leucine and threonine concentrations throughout the 24-h period over days 6–7 at each of the 3 threonine intakes is depicted in Figure 2. At the generous (46 mg) threonine intake, the plasma concentration was consistently higher than at the 2 lower intakes and it showed a marked meal-dependent pattern. This latter response was greatly diminished at the 15-mg threonine intake and barely detectable at the 7-mg intake. Because threonine and leucine concentrations did not change significantly over the last hour of the 24-h period (Table 3), the mean threonine concentrations on day 7 (μmol/L) did not differ significantly between the 7-mg and 15-mg intakes, but the concentrations at both of these intakes were significantly lower than at 46 mg. The plasma leucine concentrations were significantly higher at the 7-mg intake than at the 46-mg intake. However, the difference in leucine concentrations at this time, which was 6 h after the lunch meal, between the 15- and 46-mg groups was not significant. Similar results were found on the day 14.

**DISCUSSION**

We previously attempted to estimate the minimum physiological requirement for threonine in healthy adults. In an initial series of studies with L-[1-\(^{13}\)C]threonine as tracer (10), we observed that at threonine intakes of \(\leq 20\) mg · kg\(^{-1}\) · d\(^{-1}\) the plasma pool of threonine and its oxidation remained low and relatively constant. We based our proposal for a mean threonine requirement for adults of 15 mg · kg\(^{-1}\) · d\(^{-1}\) on these findings and on the estimated obligatory oxidative losses of threonine (7, 8). This value is much higher than the upper requirement, derived from results of nitrogen balance experiments, of 7 mg · kg\(^{-1}\) · d\(^{-1}\) suggested by the 1973 FAO/WHO Joint Expert Committee (1) and later endorsed by the 1985 FAO/WHO/UNU Expert Consultation (5). The reasons why the international recommendations for most indispensable amino acids may be too low were presented previously (7, 18).

Wilson et al (13) used the indicator amino acid oxidation (IAAO) method to reassess the threonine requirement recommended by the 1973 FAO/WHO Joint Expert Committee (1) and the 1985 FAO/WHO/UNU Expert Consultation (5), ie, 7 mg · kg\(^{-1}\) · d\(^{-1}\). These investigators suggested that because threonine has 2 degradative pathways in mammals (19, 20), the direct oxidation method that we used in 1986 (10) is not as reliable for estimating threonine requirements as is the IAAO technique. However, we used the pattern of change in the rate of threonine oxidation in response to graded intakes of threonine to determine the requirement for this amino acid (10). Furthermore, Darling et al (21) concluded recently that the L-threonine 3-dehydrogenase pathway is a minor pathway of threonine catabolism in human adults. This implies that the threonine dehydrogenase pathway is the dominant route in the catabolism of threonine in men. Nevertheless, the investigation by Wilson et al (13) contributed additional evidence to support the view that the internationally recommended threonine requirement for adults (1, 5) is far too low. Wilson et al infused L-[1-\(^{13}\)C]phenylalanine for 4 h to act as a tracer in fed subjects not adapted to the test diet and concluded that the mean threonine requirement for men is 19.0 mg · kg\(^{-1}\) · d\(^{-1}\). Using values computed from regression equations reported by Hegsted (22) and taking into account an additional nitrogen loss of 0.3 g/d, Millward (23) assumed a threonine requirement of 16 mg · kg\(^{-1}\) · d\(^{-1}\) for adults. Although this estimate is essentially the same as that proposed earlier by Young and Pellett (24), Millward et al (25) concluded that our proposed value was based on a conceptually flawed approach and derived from inadequate data. Therefore, it is interesting to note the general agreement between our earlier estimates and those reached more recently by Wilson et al (13) and Millward (23).

It is likely that the somewhat higher mean estimated requirement proposed by Wilson et al resulted from the differences between their subjects and ours in the immediate metabolic fate of the threonine absorbed from the intestinal lumen. This is suggested because of the quantitatively significant role of the intestinal tissues in amino acid catabolism (26, 27); furthermore, Bertolo et al (28) showed that the threonine requirement of
Leucine intake in the present experiment was 40 mg·kg⁻¹·d⁻¹, which we proposed as a mean requirement for healthy adults (8, 9). When [¹³C]leucine was given intravenously over 24 h while subjects consumed 10 small, isoenergetic, isonitrogenous meals hourly during the fed period, leucine balance was estimated to be −6.2 mg·kg⁻¹·d⁻¹ at an intake of 38 mg leucine·kg⁻¹·d⁻¹ (14) and to be 0.76 mg leucine·kg⁻¹·d⁻¹ at an intake of 89 mg leucine·kg⁻¹·d⁻¹ (16). On the other hand, when leucine was provided in 3 large, discrete meals, balance at the generous intake of 89 mg leucine·kg⁻¹·d⁻¹ was 14 mg·kg⁻¹·d⁻¹ (17).

The finding that our estimates of daily leucine balance were significantly lower than at threonine intakes of 15 and 46 mg·kg⁻¹·d⁻¹ deserves some comment. Leucine intake is relatively high (17). Wilson's IAAO measurement in their study. However, this meal effect is less clear when the sequence of meals could have affected the relation found between the uptake and retention of threonine and other indispensable amino acids in body proteins. This requirement is somewhat, but not substantially, lower than the mean requirement proposed by Wilson et al (13). At the internationally recommended requirement of 7 mg·kg⁻¹·d⁻¹, leucine oxidation was higher and daily leucine balance was lower than at intakes of 15 and 46 mg. Furthermore, because the measures of leucine kinetics did not differ significantly between these 2 higher intakes, it is reasonable to conclude that a mean intake of 15 mg threonine·kg⁻¹·d⁻¹ is sufficient to maximize the uptake and retention of threonine and other indispensable amino acids in body proteins. This requirement is somewhat, but not substantially, lower than the mean requirement proposed by Wilson et al (13).

The finding that our estimates of daily leucine balance were apparently positive (7–8 mg leucine·kg⁻¹·d⁻¹) at threonine intakes of 15 and 46 mg·kg⁻¹·d⁻¹, and significantly lower at a threonine intake of 7 mg·kg⁻¹·d⁻¹ deserves some comment. Leucine intake in the present experiment was 40 mg·kg⁻¹·d⁻¹, which we proposed as a mean requirement for healthy adults (8, 9). When [¹³C]leucine was given intravenously over 24 h while subjects consumed 10 small, isoenergetic, isonitrogenous meals hourly during the fed period, leucine balance was estimated to be −6.2 mg·kg⁻¹·d⁻¹ at an intake of 38 mg leucine·kg⁻¹·d⁻¹ (14) and to be 0.76 mg leucine·kg⁻¹·d⁻¹ at an intake of 89 mg leucine·kg⁻¹·d⁻¹ (16). On the other hand, when leucine was provided in 3 large, discrete meals, balance at the generous intake of 89 mg leucine·kg⁻¹·d⁻¹ was 14 mg·kg⁻¹·d⁻¹ (17). This finding was unexpected after our initial 24-h tracer studies (14, 16), but it was consistent with the measured retention of nitrogen (17).

**TABLE 2**

Summary of leucine oxidation, balance, and flux at 3 threonine intakes after a 6-d and 13-d dietary adaptation phase

<table>
<thead>
<tr>
<th>Variable</th>
<th>7 d (n = 5)</th>
<th>13 d (n = 4)</th>
<th>7 d (n = 5)</th>
<th>13 d (n = 5)</th>
<th>6 d (n = 5)</th>
<th>13 d (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h Leucine oxidation (μmol·kg⁻¹·6 h⁻¹)²</td>
<td>39.7 ± 4.7⁵</td>
<td>39.6 ± 5.5⁵</td>
<td>30.9 ± 5.1</td>
<td>30.8 ± 4.3</td>
<td>29.4 ± 2.9</td>
<td>30.6 ± 4.4</td>
</tr>
<tr>
<td>Leucine oxidation during the meal periods (μmol·kg⁻¹·6 h⁻¹)⁶</td>
<td>77.6 ± 8.7⁵</td>
<td>85.0 ± 14.3⁵</td>
<td>62.5 ± 14.3</td>
<td>65.5 ± 9.8</td>
<td>55.8 ± 6.1</td>
<td>63.5 ± 14.6</td>
</tr>
<tr>
<td>Dinner</td>
<td>86.9 ± 11.3³,⁶</td>
<td>82.6 ± 13.3³,⁶</td>
<td>69.8 ± 9.8³</td>
<td>66.3 ± 13.7³</td>
<td>66.4 ± 5.9³</td>
<td>65.2 ± 10.6³</td>
</tr>
<tr>
<td>Breakfast</td>
<td>73.2 ± 13.0⁶</td>
<td>73.3 ± 10.8⁶</td>
<td>53.9 ± 12.3</td>
<td>53.8 ± 15.4</td>
<td>54.1 ± 5.4</td>
<td>56.0 ± 7.0</td>
</tr>
<tr>
<td>Lunch</td>
<td>65.2 ± 10.8³,⁶</td>
<td>61.7 ± 10.8³,⁶</td>
<td>49.8 ± 8.5³</td>
<td>49.3 ± 6.3³</td>
<td>47.9 ± 7.8³</td>
<td>49.0 ± 11.6³</td>
</tr>
<tr>
<td>Nonmeal</td>
<td>−1.64 ± 4.4²</td>
<td>−0.4 ± 5.6²</td>
<td>7.2 ± 4.9</td>
<td>8.2 ± 4.8</td>
<td>8.0 ± 3.1</td>
<td>7.1 ± 4.4</td>
</tr>
<tr>
<td>Leucine balance (mg·kg⁻¹·d⁻¹)²</td>
<td>95.6 ± 9.8</td>
<td>96.4 ± 14.3</td>
<td>93.9 ± 13.8</td>
<td>98.0 ± 27.3</td>
<td>99.0 ± 9.0</td>
<td>117.3 ± 17.0</td>
</tr>
</tbody>
</table>
| Hourly leucine flux (μmol·kg⁻¹·h⁻¹) | 13.0 | 14.3 | 45% of the mean oral requirement. Another possibility, as we observed previously, is that at moderate intakes the rate of leucine oxidation is higher during the first meal of the day than during subsequent meals (29). This was also the case in the present study, especially in the 7-d tracer protocol. However, this meal effect is less clear when leucine intake is relatively high (17). Wilson’s IAAO measurements were made within the 6-h period after the first meal period of the day; hence, the specific time of measurement in relation to sequence of meals could have affected the relation found between threonine intake and [¹³C]phenylalanine oxidation in their study. The results of the present study support the proposed revisions of the threonine requirement for healthy adults (13, 23, 24). At the internationally recommended requirement of 7 mg·kg⁻¹·d⁻¹, leucine oxidation was higher and daily leucine balance was lower than at intakes of 15 and 46 mg. Furthermore, because the measures of leucine kinetics did not differ significantly between these 2 higher intakes, it is reasonable to conclude that a mean intake of 15 mg threonine·kg⁻¹·d⁻¹ is sufficient to maximize the uptake and retention of threonine and other indispensable amino acids in body proteins. This requirement is somewhat, but not substantially, lower than the mean requirement proposed by Wilson et al (13).

**Figure 2.** Plasma concentrations of leucine (□) and threonine (■) over 24 h of leucine infusion over days 6–7 at 3 threonine intakes: 7 (A), 15 (B), and 46 (C) mg·kg⁻¹·d⁻¹. n = 5 per group. Bars represent ± 1 SD. Times of meals indicated by ▲.
TABLE 3
Concentrations of leucine and threonine in plasma measured twice during the last hour of the 24-h tracer protocol (at 1380 and 1440 min) conducted on day 7 of the dietary period1

<table>
<thead>
<tr>
<th>Threonine intake and time of sample</th>
<th>Threonine2</th>
<th>Leucine3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/L</td>
<td></td>
</tr>
<tr>
<td>7 mg·kg−1·d−1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1380 min</td>
<td>88.2 ± 46.94</td>
<td>135.3 ± 23.35</td>
</tr>
<tr>
<td>1440 min</td>
<td>91.5 ± 44.31</td>
<td>141.8 ± 51.61</td>
</tr>
<tr>
<td>15 mg·kg−1·d−1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1380 min</td>
<td>92.8 ± 21.94</td>
<td>116.8 ± 9.5</td>
</tr>
<tr>
<td>1440 min</td>
<td>95.3 ± 18.54</td>
<td>121.8 ± 8.1</td>
</tr>
<tr>
<td>46 mg·kg−1·d−1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1380 min</td>
<td>234.4 ± 47.6</td>
<td>101.9 ± 9.3</td>
</tr>
<tr>
<td>1440 min</td>
<td>232.2 ± 45.7</td>
<td>103.7 ± 9.9</td>
</tr>
</tbody>
</table>

1 ± SD; n = 5.
2 Significant main effect of intake, P < 0.01 (mixed-models ANOVA).
3 Significant main effect of intake, P < 0.02 (mixed-models ANOVA).
4 Significantly different from values at 46 mg, P < 0.01 (mixed-models ANOVA and Tukey’s method).
5 Significantly different from values at 46 mg, P < 0.05 (mixed-models ANOVA and Tukey’s method).

Leucine balance in the present study was 8.7 mg·kg−1·d−1 after 3 discrete meals daily, providing a leucine intake that was about one-half the generous intake in our previous study (17). Hence, the estimates of leucine balance among these various studies are consistent, and it is conceivable that there was some net retention of amino acids under the conditions of the present study, at least for a limited time. Nevertheless, it is clear from the comparisons between our data and those of others that the internationally recommended threonine requirement is too low and that a more favorable state of nitrogen homeostasis is achieved at an intake of ≥15 mg·kg−1·d−1. The data on plasma threonine and leucine concentrations in the present study support this interpretation when considered with data from our earlier studies (30–32). Also note that there was considerable retention of [13C]phenylalanine (the indicator) in the study by Wilson et al (13). If we assume that the hourly rate of phenylalanine oxidation at the test threonine intakes of 15 and 20 mg·kg−1·d−1 reported by Wilson et al were sustained throughout the day, then their subjects would have a marked positive phenylalanine concentration of 8.7 mg·kg−1·d−1 at a daily phenylalanine intake of 14 mg·kg−1·d−1. A positive [13C]amino acid balance, therefore, does not appear to complicate our interpretation of the oxidation and derived amino acid balance responses in the present study.

Finally, we observed no significant differences in leucine kinetics and balance, nitrogen balance, or plasma threonine and leucine concentrations between days 6–7 and 13–14. Thus, it appears that a dietary adaptation of 6–7 d is sufficient to permit an experimental support. Am J Clin Nutr 1989;50:80–92.


Young VR, Marchini JS. Mechanisms and nutritional significance of metabolic responses to altered intakes of protein and amino acids, 13. Wilson DC, El-Khoury AE, Beaumier L, et al. Threonine requirement for healthy adults is ≥15 mg·kg−1·d−1. Hence, we propose that this value be used in assessments of the nutritional quality of dietary protein for healthy adults until additional evidence warrants a further substantial revision.

REFERENCES
18. Young VR, Marchini JS. Mechanisms and nutritional significance of metabolic responses to altered intakes of protein and amino acids,