Branched-Chain Amino Acid Requirements in Healthy Adult Human Subjects\textsuperscript{1–4}

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ABSTRACT There is now an expanding body of evidence to recommend, in the case of adult humans, the use of revised indispensable amino acid requirement values; these are \textasciitilde2 to 3 times higher than the current international recommendations. The earlier methodologies for determining amino acid requirements, based on nitrogen balance, were criticized because of their design and the associated high energy intakes. The 1985 World Health Organization/Food & Agriculture Organization/United Nations University requirement for leucine has been demonstrated to be too low by short- and long-term (24-h) tracer-derived estimates of leucine oxidation and balance. The best values for leucine requirements come from 24-h direct amino acid oxidation (DAAO) and direct amino acid balance (DAAB) studies. Finally, we also collated all available data from studies on fed-state leucine oxidation with an adequate dietary adaptation period to assess the inflection on the leucine oxidation–leucine intake curve. The mean requirements for leucine, valine, and isoleucine are likely to be 40, 17–25, and 19 mg kg\textsuperscript{-1} d\textsuperscript{-1}, respectively. This adds up to a total of \textasciitilde84 mg kg\textsuperscript{-1} d\textsuperscript{-1}, which is much lower than the lowest estimate of the total BCAA requirement of \textasciitilde110 mg kg\textsuperscript{-1} d\textsuperscript{-1} made by the short-term indicator amino acid oxidation (IAAO) method, which determined the BCAA requirement from the pattern of oxidation of an indicator amino acid (phenylalanine) at different levels of BCAA intake. An additional estimate of the leucine requirement was also made by a meta-analysis of all available 24-h DAAO/DAAB data from different studies. This resulted in a higher value for the leucine requirement than that obtained by the specific studies that utilized the 24-h DAAO/DAAB approach; however, even adding this value to the total BCAA requirement does not account for the difference in the total BCAA requirement estimates and the summed individual BCAA estimates. J. Nutr. 136: 256S–263S, 2006.

KEY WORDS: • BCAA requirement • direct amino acid oxidation • direct amino acid balance • indicator amino acid oxidation • indicator amino acid balance

There is now an expanding body of evidence to recommend, in the case of adult humans, the use of revised indispensable amino acid (IAA)\textsuperscript{6} requirement values (1); these are \textasciitilde2 to 3 times higher than the current international recommendations (2). The earlier methodologies for determining amino acid requirements based on nitrogen balance were criticized because of their design and the associated high energy intake (3). A further problem was related to the exclusion of the miscellaneous nitrogen losses when calculating nitrogen balance (see below). Young et al. (4) proposed a new set of amino acid requirement values calculated from estimates of obligatory nitrogen loss (ONL), based on the pattern of IAA in tissue protein mobilized to provide for the ONL. The use of tracer methodologies in the next phase of studies (e.g., 5,6) also suggested that the daily requirement for BCAAs was greater than the 1985 World Health Organization (WHO)/Food & Agricultural Organization (FAO)/United Nations University (UNU) value (2). Particularly for leucine, the short-term tracer balance findings were then confirmed in longer term 24-h tracer balance studies in western subjects (7,8) and Indian subjects (9), where leucine equilibrium was achieved at a leucine intake of 40 mg kg\textsuperscript{-1} d\textsuperscript{-1}, but not at an intake of 14 mg kg\textsuperscript{-1} d\textsuperscript{-1}. Estimates of the total BCAA requirement have also been made by a tracer technique used in an indicator amino acid oxidation
(IAAO) paradigm (10), as described below, and these estimates of the BCAA requirement, when portioned into the constituent amino acids, also suggest higher requirements than those in the 1985 WHO/FAO/UNU report. A recently convened Expert Committee of the WHO/FAO/UNU (11) has also reviewed the evidence available from recent tracer studies and concluded that BCAA requirements are higher than set out in the earlier 1985 WHO/FAO/UNU report, as discussed below.

The requirement for leucine

From the earlier nitrogen balance studies (12) and studies in women (13), it was concluded that the mean requirement for leucine approximated 10 mg·kg⁻¹·d⁻¹ or less (Table 1). An important difficulty in accurate measurement of nitrogen balance is determining the integumental and other minor or unmeasured routes (miscellaneous) of nitrogen loss; these were not measured in the earlier nitrogen balance studies. A reanalysis of nitrogen balance studies in adults, using a correction factor of 0.5 g of nitrogen as the daily integumental loss, increased the derived leucine requirement values severalfold (14); however, a similar reassessment, using an assumed ±0.3-g nitrogen daily miscellaneous loss gave generally lower values for amino acid requirements; for leucine, this was 26 mg·kg⁻¹·d⁻¹ (15). It is not possible to state the magnitude of the miscellaneous nitrogen loss with any confidence; therefore, the nitrogen balance method does not provide a result for the leucine requirement with any certainty. The nitrogen balance is also highly sensitive to changes in energy intake and, at high energy intake, could account for an underestimation of the requirement. These problems have been reviewed earlier with reference to the earlier nitrogen balance studies (3,4,15,16). An alternative theoretical paradigm of IAAO requirements was proposed in 1989 (4) based on an estimate of the minimal obligatory nitrogen loss (ONL) and the intake of IAA necessary to balance this loss, predicted from the composition of mixed body proteins,

with the assumption that the efficiency of absorption of the IAA was ~70%. Based on these considerations, the leucine requirement was calculated to be nearly 40 mg·kg⁻¹·d⁻¹ (Table 1; 17,18).

Tracer approaches to the measurement of leucine requirements have been used more recently in terms of measuring the intake of leucine required to achieve leucine balance. When the tracer amino acid used is the same as the amino acid being evaluated, the technique is called the direct amino acid oxidation (DAAO) or the direct amino acid balance (DAAB) method. The potential advantage of the DAAO/DAAB technique is that the rate of oxidation of the dietary amino acid of interest is directly estimated; it is then possible to evaluate both the pattern of change in the oxidation rate and the body balance of the amino acid under study. In this model, the inflection or breakpoint on the 24-h leucine oxidation–leucine intake or the 24-h leucine balance–leucine intake curve is determined to be the requirement level of leucine because it is presumed that leucine oxidation would be at its lowest plateau value at leucine intake below the requirement level, and that leucine oxidation would progressively rise with rising leucine intake above the requirement level. For leucine balance, calculated as the difference between intake and oxidation, the balance would plateau at a value of zero at leucine intake above requirement, whereas it would fall progressively at leucine intake below requirement (Fig. 1). Particularly for leucine, this method is attractive because a more precise determination of the rate of leucine oxidation (and balance) can be made because the isotopic enrichment of the precursor pool directly supplying the substrate for oxidation is known (19). This method of determining the breakpoint requires at least four, and preferably five or more, test intakes that ideally are chosen to have two or more values on either side of the putative requirement value. The breakpoint is derived by fitting a two-phase linear regression model for 24-h leucine oxidation (or balance) versus leucine intake; the intercept and slope of one line segment and the intercept of the second line segment are estimated, and the slope of the second line segment is restricted to zero. The model is constrained such that the two line segments intersect at the unknown breakpoint; the breakpoint parameter is estimated as −1 times the ratio of the difference between intercepts divided

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FIGURE 1 Different patterns of responses of tracer oxidation or balance to graded intakes of test amino acid. The lines represent kinetic responses to graded intakes of test amino acid. The point of inflection of each line represents the requirement for the test amino acid. Both IAAO and DAAB would be expected to plateau at a zero balance. For IAAO, either whole-body indicator amino acid oxidation, or a surrogate in the form of the proportion of tracer oxidized, can be used. DAAO, short-term or 24-h direct amino acid oxidation; DAAB, 24-h direct amino acid balance; IAAO, short-term fed-state or 24-h indicator amino acid balance; IAAAB, 24-h indicator amino acid balance.
by the difference between slopes. Ordinary least-squares estimation is used if all measurements are from independent subjects or, in the case of repeated measurements among subjects, random effects or mixed-model regressions are fit. The 95% confidence interval (CI) for the breakpoint is calculated using Fieller’s theorem; likelihood ratio or bootstrapped CIs may be used instead.

The DAAO/DAAB method can be used to measure leucine balance through short-term measurements usually for 3 h in the fed state (5). To estimate the daily amino acid balance, assumptions have to be made about the rates of amino acid oxidation during the remaining 9 h of the fed period and also during the 12-h postabsorptive period. These assumptions have been described (5), but a precise estimate of daily balance would ideally require the use of a 24-h tracer protocol because the rate of amino acid oxidation during the fed period could vary throughout the 12-h period of measurement making extrapolations difficult (8). Therefore, the DAAB technique as applied for leucine requirements has more recently been extended to 24-h tracer balance studies (7–9,17) and has been validated against nitrogen balance (7). A possible criticism of the 24-h DAAO/DAAB technique is that the labeled leucine is not given in massless amounts and then forms a nontrivial part of the leucine intake. In a 24-h protocol that is divided into a 12-h fasting and a 12-h fed state, one-half the tracer leucine intake is infused in the fasting state. Because no other nutrients are administered in the 12-h fasting state, it is possible that the tracer leucine administered could be oxidized during this period. This could be due to the effect of a mass action of the free leucine or due to the direct effect of leucine on branched-chain keto-acid dehydrogenase (20,21). However, in the fasting state, a regression of leucine oxidation against different amounts of tracer leucine intake found a significant relation with a slope of 0.38 (22), suggesting that about one-third of the leucine infused during the fasting state is oxidized, whereas the rest is retained in free leucine pools in the body. Another possible criticism of the 24-h DAAB studies (9,17) is that they were essentially designed to measure leucine requirement by a zero balance intercept on the leucine intake–balance curve because the leucine intake levels did not go beyond the putative requirement level of 40 mg · kg⁻¹ · d⁻¹. In doing so, these studies did not assess leucine balance at leucine intakes above the putative requirement value of 40 mg · kg⁻¹ · d⁻¹ and the data could not be subjected to a breakpoint analysis as described above.

Earlier tracer studies involving the short-term DAAB technique suggested that the mean requirement fell in the range of 20–40 mg · kg⁻¹ · d⁻¹ (5; Table 1) and these data, when reanalyzed through a breakpoint analysis, yielded a value of 25 mg · kg⁻¹ · d⁻¹ (23). The 24-h DAAB tracer studies by El-Khoury (7,8) confirmed that the WHO/FAO/UNU (2) requirement of 14 mg · kg⁻¹ · d⁻¹ leucine was too low and their results gave a requirement value of ~38 mg · kg⁻¹ · d⁻¹, thereby also supporting the prediction of a leucine requirement of ~40 mg · kg⁻¹ · d⁻¹. More recently, Kurpad et al. (9,17), using four test intake levels of leucine and the 24-h DAAB approach, have estimated the mean leucine requirement to be 37 and 40 mg · kg⁻¹ · d⁻¹ in well-nourished and undernourished Indian subjects, respectively (Table 1).

Another approach that has been used to measure the leucine requirement is the IAAO technique (24; Fig. 1). Thus, the requirement for an IAA (e.g., lysine) is determined from the pattern or rate of change in oxidation of another (indicator) amino acid (e.g., ¹³C-phenylalanine). This method, which is deployed in the fed state over short periods of time, also uses the measurement of a surrogate for whole-body IAAO by measuring the proportion of tracer oxidized (F¹³CO₂). There are a number of advantages to this short-term IAAO approach, including 1) the possibility of carrying out a relatively large number of relatively short-term tracer studies within the same subject; 2) problems arising from changes in pool sizes and kinetics that might affect the behavior of a direct tracer are largely avoided when an indicator amino acid tracer is used; and 3) there is no a priori reason to determine the actual rate of IAAO because the pattern of release of the ¹³C label in expired air can be assessed through a breakpoint analysis on the intake–oxidation response curve. This pattern of ¹³C appearance, which represents the proportion of tracer oxidized (F¹³CO₂) should, in theory, parallel that for the absolute oxidation rate of the indicator according to the concept on which this approach is based. Based on the determination of the total BCAA requirement by the short-term IAAO technique, Riazi et al. (10) derived a leucine requirement value of ~47–55 mg · kg⁻¹ · d⁻¹ based on the proportion of leucine in an egg protein–based amino acid mix that was fed to the study subjects. The range of requirement values in this study was because different values within this range were derived depending on which outcome measure of indicator amino acid equilibrium was used (F¹³CO₂, phenylalanine oxidation, or balance). These values could have been overestimated by ~10% (18) and therefore the range of values of leucine requirement derived from the short-term IAAO measurement of the total BCAA requirement is probably from 42–50 mg · kg⁻¹ · d⁻¹. These are still higher than estimates from the 24-h DAAO/DAAB studies and, as has been pointed out above, one criticism of the latter studies is that they did not assess leucine balance at leucine intake above the putative requirement value of 40 mg · kg⁻¹ · d⁻¹. An argument against this is that, in an indicator amino acid balance (IAAB) paradigm, the breakpoint should ideally occur at a zero leucine balance and therefore the regression estimate of the zero balance intercept should be identical to the breakpoint estimate.

**Leucine requirement from a meta-analysis of 24-h leucine balance studies**

We attempted a meta-analysis of the leucine requirement by 24-h DAAO/DAAB evidence by collecting data on those studies in which there was a defined 1-wk period of experimental dietary intake in human adult subjects with adequate levels of energy and nitrogen intake, and in which there were adequate levels of intake of all amino acids (adequacy defined by the requirement pattern that was accepted by the recent WHO/FAO/UNU Expert Committee on Protein and Amino Acid Requirements; 11). Therefore, the 24-h leucine balance data were collated from all of the 24-h leucine balance studies conducted in the US (7,8) and India (9,25–29) on well-nourished healthy young adult male subjects. A total of eight studies were therefore identified that had the requisite 24-h leucine balance data at otherwise adequate energy, IAA, and protein intakes. In these studies, leucine balance was measured at different intakes; collating these yielded leucine balance data at leucine intakes of ~14, 22, 30, 40, 50, 90, and 105 mg · kg⁻¹ · d⁻¹ (Fig. 2, filled circles). Because the study at the highest leucine intake (26; Fig. 2, open square) yielded balances that were far too positive for probable reasons discussed below, the data from this study were deleted from the database from which the breakpoint on the leucine balance–leucine intake curve was calculated. The breakpoint estimate for the leucine requirement was determined to be 47.6 mg · kg⁻¹ · d⁻¹ with lower and upper 95% CI of 42.2 and 55.5 mg · kg⁻¹ · d⁻¹, respectively. This breakpoint occurred at a slightly positive balance of 1.7 mg · kg⁻¹ · d⁻¹, whereas the zero intercept value (leucine intake value at which...
the leucine balance was zero on the ascending slope line) was 44.4 mg·kg⁻¹·d⁻¹. It is not clear as to which estimate (breakpoint or zero balance) of the leucine requirement is correct. Ideally, they would be expected to be the same and the small positive balance at the breakpoint may have been due to technical reasons; it is possible that the calculation of leucine balance from intake and oxidation is not completely accurate because the tracer-based estimate of oxidation does not capture all the leucine loss from the body. For example, there may be some intestinal loss of metabolic leucine, which would not be captured by the tracer model. In contrast, if the positive balances were due to subjects accreting protein, it is unlikely that this small anabolic gain over a week could be assessed by anthropometric measures such as body weight or even body composition. The subjects in these studies did not gain weight and, in the Indian studies, actually lost ~0.2–0.3 kg at the end of a week on the experimental diet. This was attributed to the loss of body glycogen and associated water through the measurement of substrate balances (17).

The leucine requirement estimate from the 24-h balance studies is higher than, but within the CI limits, of the value of 37.3 mg·kg⁻¹·d⁻¹ (95% CI = 32.50 mg·kg⁻¹·d⁻¹) that we have estimated earlier in well-nourished subjects (9) and well within the range suggested by Riazi et al. (10,18). The 95% CI of all these estimates overlap, implying no significant difference between them. The relation of the leucine balance–intake curve also shows that, over a range of leucine intakes from 40 to ~90 mg·kg⁻¹·d⁻¹, the balances are essentially neutral. Above this value, we have observed positive leucine balances (26), which appear to be a model-related problem linked perhaps to the first-pass splanchnic oxidation of leucine, which may not have been fully quantified by the intravenously administered tracer at such high leucine intakes. Positive leucine balances of a similar magnitude were observed in another study at a leucine intake of ~90 mg·kg⁻¹·d⁻¹ and, in this case, may have been related to the consumption of three bolus meals instead of the usual multiple small-meal plateau consumption that was followed in all of the other 24-h studies (30).

Interestingly, adding the leucine balance data of undernourished Indian subjects (17) into the database (Fig 2, open triangles) and then estimating the breakpoint gave a higher estimate for the leucine requirement than when only the data on the well-nourished subjects were subjected to breakpoint analysis. The breakpoint for the leucine requirement occurred at a leucine intake of 50.2 mg·kg⁻¹·d⁻¹, with 95% CI of 43.1 and 58.1 mg·kg⁻¹·d⁻¹. The zero balance intercept also occurred at a higher value of 45.6 mg·kg⁻¹·d⁻¹, and this was higher than the leucine requirement of 39 mg·kg⁻¹·d⁻¹ (95% CI = 32.56 mg·kg⁻¹·d⁻¹) measured in chronically undernourished Indian subjects by the 24-h DAAB method, using a zero balance intercept instead of a breakpoint (17). Two points are noteworthy here: first, the slope of the 24-h leucine balance response to graded leucine intakes was shallower with the combined dataset because the mean balances tended to be less negative in the undernourished subjects, particularly at lower leucine intakes, and it is possible that the shallower slope of the leucine balance response influenced the breakpoint and zero intercept analysis of the data, such that a higher estimate was obtained. Second, the higher leucine requirement estimate with the mixed undernourished and well-nourished database is reminiscent of a higher lysine requirement in undernourished subjects measured by the 24-h IAAO/IAAB technique (31), although, in this case, it should be emphasized that the estimate was from a mixed group. Certainly, the undernourished subjects could accrete protein under ideal dietary (and activity) conditions as experienced during the dietary adaptation period, where adequate energy, nitrogen, and micronutrients were provided because their muscle mass (as a proportion of body weight) was lower than that of well-nourished subjects (17).

In addition to the 24-h tracer studies, we also attempted an assessment of the breakpoint on the 12-h fed leucine oxidation vs. leucine intake curve derived from several short- and long-term studies. To do so, we collated data from 24-h (as above) and short-term fed-state leucine oxidation studies (5,32–37). In the short-term studies, the reported leucine oxidation was extrapolated to a 12-h value and the leucine intake was taken as the total from the diet and the projected 24-h tracer intake. We chose to use the 24-h tracer intake because this would allow for the 24-h studies (9,25–29) and the short-term studies to be used in the same dataset. Where necessary, these studies were corrected for the use of the enrichment of plasma leucine as the precursor pool instead of keto-isocaproic acid (5,35,36) by the use of a factor of 0.8 (19). A total of 16 short- and long-term studies employing different leucine intake levels in each study were therefore identified for this analysis, for a total of 47 observations. The data from the studies are presented in Figure 3; it was not possible to achieve a breakpoint estimate because these did not converge and the relation was best described by a straight-line equation. However, in Figure 3 it might be seen that, from an intake of ~40 mg·kg⁻¹·d⁻¹, there was a more obvious increase in leucine oxidation.

Overall, it would appear that the leucine requirement is certainly higher than the value suggested in the 1985 WHO/FAO/UNU report (2). It is likely to be in the region of 40 mg·kg⁻¹·d⁻¹, measured by specific studies to determine the leucine requirement, although indirect estimates from the short-term IAAO technique and by a meta-analysis of available
The requirement for valine

The earlier nitrogen balance studies on the valine needs in healthy adults (38,39) suggested requirements in the region of 10 mg · kg⁻¹ · d⁻¹ for valine (Table 2; 40); therefore, the 1985 WHO/FAO/UNU (2) daily requirement for valine was set at 10 mg · kg⁻¹ · d⁻¹. As referred to earlier, the nitrogen balance technique has several disadvantages and reanalyses of the nitrogen balance data by Hegsted (14) and Millward (15) gave valine requirement estimates of 17 and 14 mg · kg⁻¹ · d⁻¹, respectively. Based on the ONL model (see above), Young et al. (4) suggested a value of 24 mg · kg⁻¹ · d⁻¹ for the daily valine requirement. Because the BCAAs share common enzymes in their oxidative pathways, the valine requirement can also be theoretically estimated by an assumed proportionality with the leucine requirement based on the amino acid composition of body protein (41); such a procedure yields a value of 26 mg · kg⁻¹ · d⁻¹ when a value of 40 mg · kg⁻¹ · d⁻¹ is used for the leucine requirement (9,18). The response of plasma valine concentrations to different levels of valine intake has also suggested a requirement value of 20 mg · kg⁻¹ · d⁻¹ (6).

Tracer studies, using ¹³C-labeled valine to determine the valine oxidation rate (DAAO) method over the short term, at different valine intakes in an egg protein–based amino acid mix, have suggested a valine requirement of ~16 mg · kg⁻¹ · d⁻¹ or greater (6). However, there are potential problems with the short-term DAAO approach, as discussed above. The IAAO method has been employed in short-term measurements to measure the total BCAA requirement (10), where the total requirements of these amino acids was found to range from 122 to 144 mg · kg⁻¹ · d⁻¹ depending on the proportion of valine (22.5%) in the egg protein–amino acid mix pattern used in this study (10). A later paper from the same group (24), which also suggested that valine may be the limiting BCAA in an egg protein–based mixture, also suggested that the requirement values for the BCAAs may have been overestimated by ~10%; if so, the tentative valine requirement derived from these studies would be in the range of 25 to 29 mg · kg⁻¹ · d⁻¹.

The disadvantages of the IAAO method as usually conducted are that it has been based essentially on a short-term fed-state model without any significant prior adaptation of the subjects to the test diets. To circumvent some of the limitations of the short-term IAAO technique, a 24-h IAAO/IAAB approach has been developed (25; Fig 1) and applied in ¹³C-leucine tracer studies of the lysine, threonine, and methionine requirement of adult Indian subjects (25–29) and in studies of the threonine requirement in US adults (42). The approach is similar in concept to that of the IAAO technique, but is based on a 24-h IAAO–daily balance protocol using leucine (for which the kinetics are well described) as the indicator amino acid. It has the advantage of providing a direct estimate of 24-h IAAO and IAAB. The disadvantage of the 24-h IAAO and IAAB approaches relates to the complexity of the 24-h tracer study and possibly the rather stringent demands and restraints that it places on the experimental subject.

A recent 24-h IAAO/IAAB study, using phenylalanine as the indicator amino acid, in well-nourished young adult Indians determined the valine requirement to be 17 mg · kg⁻¹ · d⁻¹, with 95% CI of 12 to 28 mg · kg⁻¹ · d⁻¹ (40). This study assessed phenylalanine balances at a zero tyrosine intake, using a protocol that had earlier obtained fairly neutral phenylalanine balances at an intake of 39 mg · kg⁻¹ · d⁻¹ phenylalanine on a tyrosine-free diet (43). Because the labeled tyrosine formed from the hydrolyzation of labeled phenylalanine tracer is partitioned into oxidation and protein synthesis, this partitioning could be variable depending on the needs of protein synthesis. In this respect, the provision of a tyrosine-free diet does not reduce the conversion of phenylalanine into tyrosine and could add an additional variability to determination of the breakpoint estimate from estimates of phenylalanine oxidation.
It is not possible to predict whether this would have changed the estimate of the valine requirement in this study, but it can also be assumed that the net phenylalanine oxidation and balance under a defined and constant set of conditions would effectively serve as an indicator of valine equilibrium. In addition, this study also measured the valine requirement by the short-term IAAO tracer technique by measuring the fraction of tracer oxidized, similar to the method used in the short-term IAAO method. Using this latter approach gave a numerically higher requirement value of 20 mg kg\(^{-1}\) d\(^{-1}\), with 95% CI of 12 to >35 mg kg\(^{-1}\) d\(^{-1}\). Given the large CIs, it is quite likely that an expensive study involving a very large number of subjects would be required to actually determine whether there was a significant difference between the 24-h and the short-term methods.

The valine requirement in the 24-h IAAO/IAAB study was similar to that suggested from earlier DAAO studies at different valine intakes (6). However, this estimate was lower than that predicted from the total BCAA requirement by the short-term IAAO technique (10), or by the obligatory amino acid losses (OAAL) prediction (4). It is not possible to state with any certainty what the reason for this discrepancy is. One possibility is that the short-term IAAO study (10) determined the total BCAA requirement and from that predicted the valine requirement based on a similar proportionality in the amino acid composition of the fed protein. A similar higher valine requirement value is predicted from the OAAL method based on the same principle (4), and it may be that the prediction of the valine requirement based on an assumed proportionality of the BCAA in body protein is unwarranted. A second possibility is that the short-term IAAO technique is conducted on relatively unadapted subjects compared with the 24-h IAAB technique. Whereas studies on pigs suggest that the 1-d experimental dietary period in the short-term IAAO technique may be adequate for adaptation to the diet (44), it is still not clear whether the cause for the difference between these two estimates of the valine requirement is related to the adaptation period to the experimental diet. For example, if a dietary effect of the habitual diet persisted into the experimental period, then this would, in the simplest interpretation, push the requirement estimate upward, assuming that the habitual dietary intake had generous amounts of the test amino acid. A third possibility is the composition of the intake of the other BCAAs during the adaptation period of the 24-h IAAO/IAAB study. In this case, the leucine intake was held at the maintenance requirement value of 40 mg kg\(^{-1}\) d\(^{-1}\). This is not a safe intake that would meet the needs of 95% of the population and it is conceivable that this intake may have been suboptimal for some of the subjects in this study and hence might have been responsible for a lower valine requirement. However, as discussed below, it has been shown that varying leucine intake in the range supplied by normal diets (40 to 80 mg kg\(^{-1}\) d\(^{-1}\) had no effect on valine oxidation (45) and hence it is possible that the concern about the level of leucine intake is unwarranted.

Overall, the valine requirement measured by the IAAB technique (which might be considered to be the best estimate available) of 17 mg kg\(^{-1}\) d\(^{-1}\) is lower than what is expected from proportionality with the leucine requirement, based on the composition of mixed-body proteins. It is also lower than the indirect estimate obtained from total BCAA requirement estimates by the IAAO method (10,18).

The requirement for isoleucine

Based on recalculated data from nitrogen balance studies (12, 46; Table 3), it is possible that the mean requirement for

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<td>Hegsted (14)</td>
<td>28</td>
<td>Reanalysis of nitrogen balance</td>
</tr>
<tr>
<td>Millward (15)</td>
<td>18</td>
<td>Reanalysis of nitrogen balance</td>
</tr>
<tr>
<td>OAAL</td>
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<tr>
<td>Young et al. (4)</td>
<td>23</td>
<td>Based on composition of mixed body protein</td>
</tr>
<tr>
<td>Proportionality to leucine requirement of 40 mg kg(^{-1}) d(^{-1})</td>
<td>19</td>
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</tr>
</tbody>
</table>

**TABLE 3 Isoleucine requirements (mg kg\(^{-1}\) d\(^{-1}\)) measured using different approaches**
In these two experiments based on different principles, the valine requirement estimate was very similar even though the amount of leucine supplied in the diet varied 2-fold. Therefore, it appears that there may be no regulatory effect of dietary leucine on valine catabolism within the physiological range of leucine intake.

**Summary**

The mean requirement for leucine, valine, and isoleucine is likely to be 40, 17–20, and 19 mg·kg⁻¹·d⁻¹, respectively. This adds up to a total of ~79 mg·kg⁻¹·d⁻¹, which is much lower than the lowest estimate of the total BCAA requirement made by the short-term IAAO method (10,18). The estimate of the leucine requirement by meta-analysis gave a higher value for the leucine requirement than that obtained by the 24-h DAAO/DAAB approach, but even adding this value to the total BCAA requirement does not account for the difference in the total BCAA requirement estimates and the summed individual BCAA estimates. An unexplored variable that may account for this difference is the length of the adaptation period for which the experimental diet is consumed because this differs by ~5 d between the short-term IAAO and the 24-h IAAO/IAAB or 24-h DAAO/DAAB methods. Within the range of normal dietary intake, it is also unlikely that each of the BCAs exerts a significant influence on the others in terms of their requirements.

**LITERATURE CITED**


44. Moehn S, Bertolo RFP, Pencharz PB, Ball RO. Indicator amino acid oxidation responds rapidly to changes in lysine or protein intake in growing and adult pigs. J Nutr. 2004;134:836–41.


