

Individual amino acid requirements in humans: an update

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Purpose of review

To discuss recent amino acid requirement studies in adult humans and school-age children, primarily determined using the indicator amino acid oxidation technique.

Recent findings

Using the minimally invasive indicator amino acid oxidation model, requirements for most indispensable amino acids have been defined in adult humans. The estimates are comparable to the values obtained using the more elaborate 24-h indicator amino acid oxidation and balance model. The less-invasive indicator amino acid oxidation model has also been successfully applied to define requirements in healthy school-age children and children with disease. A recent adaptation of the indicator amino acid oxidation method to determine protein requirements in adult humans resulted in mean and safe values of 0.93 and 1.2 g protein/kg/day, respectively. These estimates are 40–50% higher than current recommendations and suggest an urgent need to reassess recommendations for protein intake in humans.

Summary

In summary, indicator amino acid oxidation is a robust technique, and has resulted in the definition of amino acid and protein requirements in adult humans and children. A wider application of the technique in other vulnerable populations across life stages and in other diseases is now possible.

Keywords

amino acids, children, humans, indicator amino acid oxidation, requirements

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Introduction

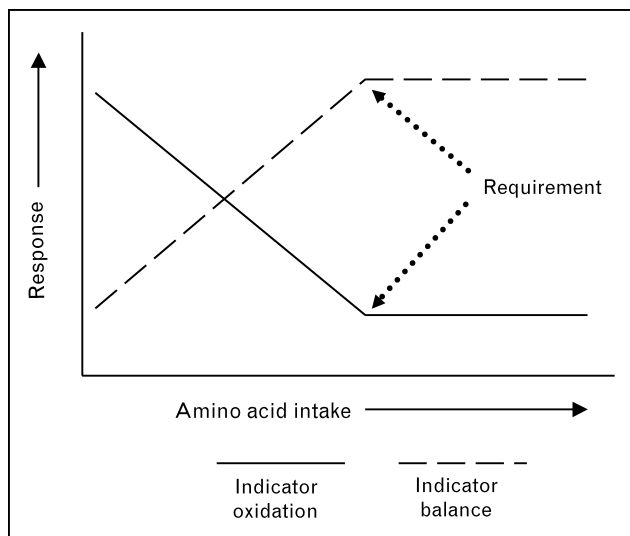
The study of amino acid requirements in humans has been an active area of research for the past two decades with the introduction of stable isotope-based carbon oxidation methods [1]. This is a major advancement from earlier when nitrogen balance was the primary method of choice to determine amino acid and protein requirements. The nitrogen balance technique has many limitations due to overestimation of nitrogen intake and an underestimation of nitrogen excretion. The net result leads to an overly positive balance and therefore underestimation of the requirement [2]. Furthermore, the nitrogen balance method requires adaptation of the body urea pool to the test amino acid intake – this usually takes up to 7 days [1] and therefore is not always suitable for application in vulnerable populations, such as children. Methods based on carbon oxidation, on the other hand, have proven more rapid and sensitive to changes in amino acid intakes, and have resulted in identification of requirement values much higher than nitrogen balance-based methods. Detailed reviews exist for discussions on the different methods for determination of amino acid requirements, the advantages and disadvantages

of the various methods, and requirement values [1,3–6].

Recently, the Institute of Medicine, Food and Nutrition Board published the Dietary Reference Intakes (DRIs) for macronutrients, including protein and amino acids [2]. Requirement estimates for adult humans were recommended based on a summary of studies based on carbon oxidation methods. Two methods were accepted as appropriate for the determination of amino acid requirements – indicator amino acid oxidation (IAAO) method, and the 24-h IAAO/indicator amino acid balance (IAAB) method [2]. The response pattern differs based on the outcome parameter being measured (Fig. 1). Both methods to determine amino acid requirements involve feeding a range of test amino acid intakes and the measurement of a dose response in the oxidation of an indicator amino acid [1].

Since the publication of the DRI report a number of studies have been reported on amino acid requirements primarily using the IAAO method, including requirement estimates in children. This review will summarize adult human amino acid requirements and discuss recent

Figure 1 Pattern of response observed due to increasing intake of a deficient amino acid by using the indicator amino acid oxidation (IAAO) and indicator amino acid balance (IAAB) method



IAAO. With increasing intake of the limiting amino acid, oxidation of the indicator amino acid will continue to decrease, reflecting increasing incorporation into protein. Once the requirement is met for the limiting amino acid, there will be no further change in the oxidation of the indicator amino acid. The inflection point is referred to as the 'breakpoint' or requirement. The breakpoint identified with the use of biphasic linear regression analysis indicates the mean or estimated average requirement of the limiting (test) amino acid. **Twenty-four-hour IAAB.** With increasing intake of the limiting amino acid there will be a progressive increase in indicator balance reflecting amino acid retention. Once the requirement is reached there will be no further change and indicator balance will achieve a plateau. The inflection point identifies the 'breakpoint' or requirement.

developments in requirement estimates for children of school-age.

Indicator amino acid oxidation

The IAAO method is based on the concept that when one indispensable amino acid is deficient in the diet, then all other amino acids including the indicator amino acid (another indispensable amino acid, usually L-[1-¹³C]phenylalanine) will be oxidized [3]. With increasing intake of the limiting amino acid, oxidation of the indicator amino acid will continue to decrease, reflecting increasing incorporation into protein (Fig. 1). Once the requirement is met for the limiting amino acid, there will be no further change in the oxidation of the indicator amino acid. The inflection point where the oxidation of the indicator amino acid stops decreasing and reaches a plateau is referred to as the 'breakpoint'. The breakpoint identified with the use of biphasic linear regression analysis indicates the mean or estimated average requirement of the limiting (test) amino acid [1].

The IAAO technique was initially applied in young pigs using [¹⁴C]phenylalanine to determine histidine [7] and tryptophan [8] requirements. Zello *et al.* [9] adapted the IAAO method in adult humans to determine the lysine requirement using intravenous infusion of L-[1-¹³C]phenylalanine, and collection of breath and blood for measurement of ¹³CO₂ and plasma phenylalanine enrichment, respectively. In order to make the IAAO protocol less invasive, Bross *et al.* [10] validated the IAAO protocol with hourly oral isotope doses and sampling of urine to measure isotopic enrichment. To test whether the route of isotope infusion has an impact on the determination of the 'breakpoint' estimate, Kriengsinyos *et al.* [11] applied the IAAO method to determine the lysine requirement in subjects infused intravenously or orally with L-[1-¹³C]phenylalanine. Identical requirement estimates of 35 mg/kg/day for lysine were determined through both routes of isotope infusion. Therefore, the current minimally invasive IAAO model involves a 2-day protein intake adaptation followed by study day adaptation (8 h) to the specific test amino acid intake. On the study day 8-hourly meals are given with oral hourly doses of L-[1-¹³C]phenylalanine given during the last 4 h of the study. Breath and urine samples are collected every half hour during the last 2.5 h of the study [11]. This less-invasive model allows repeated measures within the same subject, and therefore all subjects undergo IAAO studies at all test intakes and thereby it has been possible to define individual requirements for amino acids [1].

Adult human amino acid requirements have been determined for most indispensable amino acids using the IAAO method, including lysine [9,11], tryptophan [12], threonine [13], tyrosine [14], methionine (no cysteine) [15], minimum methionine (with cysteine) [16,17], total branched-chain amino acids (leucine + isoleucine + valine) [18] and total aromatic amino acids (phenylalanine, with no tyrosine) [19,20,21] (Table 1). Most of the studies mentioned above have been conducted in male subjects, with the exception of the tryptophan study [12], where women were studied during the follicular phase of the menstrual cycle. In order to examine whether the different phases of the menstrual cycle affect amino acid requirement, Kriengsinyos *et al.* [22] examined lysine requirements in healthy women using the IAAO method during both the follicular and luteal phases. The lysine requirement during the follicular phase was determined to be 35 mg/kg/day, which is identical to the requirement estimate in adult males [11]. Conversely, during the luteal phase the requirement was determined to be 37.7 mg/kg/day and significantly higher when compared to the follicular phase. The increased requirement may be due to increased amino acid obligatory losses during the luteal phase, as it was observed that total plasma amino acid concentrations,

Table 1 Indispensable amino acid requirements (mg/kg/day) in adult humans

Amino acid	Indicator amino acid oxidation-based requirement estimate [9,11–16,17*,18,19,20*,21,22]	Twenty-four-hour indicator amino acid oxidation/balance-based requirement estimate [23–29]	Dietary Reference Intakes [2]	Food and Agriculture Organization/World Health Organization/United Nations University [30]
Histidine	–	–	11	10
Isoleucine	42	–	15	20
Leucine	55	40	34	39
Lysine	35	31	31	30
Methionine (with no cysteine)	12.6	15	15	15
Phenylalanine (with no tyrosine)	42	37	27	25
Threonine	19	15	16	15
Tryptophan	4	–	4	4
Valine	47	20	19	26
Total branched-chain amino acids ^a	144	–	–	–

^a Indicator amino acid oxidation-based requirements for isoleucine, leucine and valine are derived from total branched-chain amino acid requirements and the branched-chain amino acid proportion in egg protein.

including plasma lysine, were increased 71–83% relative to the follicular phase. The current DRI recommendation for a lysine requirement of 31 mg/kg/day is based on studies conducted in men [2] and it may not meet the lysine requirements for women, especially during the luteal phase of the menstrual cycle.

The 24-h indicator amino acid oxidation and balance method

Kurpad *et al.* [23] applied the IAAO method with modifications to include test amino acid intake adaptation for 6 days followed by measurement of an indicator amino acid oxidation (usually [1-¹³C]leucine), during a 24-h period (12-h fasted and 12-h fed). This method referred to as the 24-h IAAO also allows the calculation of the 24-h IAAB, which is derived by subtracting leucine oxidation from leucine intake. This model uses intravenous isotope infusion for 24 h, and involves collection of breath and blood samples for measurement of plasma enrichment of the isotope; the protocol is demanding and more invasive for the subjects. Requirements have been defined for lysine [23], threonine [24], methionine (with no cysteine) [25], valine [26] and phenylalanine [27] in healthy, well-nourished Indians (Table 1). The 24-h IAAO/IAAB model was also applied to chronically undernourished Indian men and it was found that the lysine requirement was increased by around 50%. A later study showed that the increased requirement could have been because of intestinal parasites, and subjects treated for intestinal parasites had similar lysine requirements as well-nourished Indian men [28]. Similar requirement estimates were obtained for methionine (with no cysteine) [29] in undernourished men when compared to healthy, well-nourished men. These elaborate and expensive studies have provided valuable information with respect to the application of amino acid requirement values globally because these studies were the first to show that the amino acid requirement values are similar in populations

living in developing countries and are comparable to estimates obtained in healthy subjects living in the Western World.

Comparison of requirement estimates for adult humans

Over the past several years adult human requirements for several amino acids have been determined with the use of both the IAAO method and the 24-h IAAO/IAAB method. Comparisons can be made for lysine [11,23], threonine [13,24], methionine (with no cysteine) [15,25] and phenylalanine (with no tyrosine) [20*,27] (Table 2). There appears to be no systematic effect on the requirement estimate due to the method used for determination of amino acid requirements (Table 2). The requirements for lysine and threonine determined by the IAAO method are 11 and 21%, respectively, higher than the requirements determined by the 24-h IAAO/IAAB method. Conversely, the requirement for maximum methionine (no cysteine) determined by the IAAO method is 16% lower than the requirement derived by the 24-h IAAO/IAAB method.

The fundamental difference between the two methods has been the period of adaptation and measurements during fed/fasted conditions. Earlier, in growing and adult pigs we showed that there was no influence of days of adaptation to different test protein and lysine intakes on indicator amino acid oxidation [31]. Furthermore, recently we also observed that L-[1-¹³C]phenylalanine oxidation measured as $F^{13}\text{CO}_2$, which is the primary variable used for determination of the breakpoint, is not significantly affected by 8 h, 3 days or 7 days of adaptation to a wide range of lysine intakes in healthy young men [32]. With respect to measurements during fed and fasted conditions, there is evidence from the 24-h IAAO/IAAB studies that the requirements for threonine or lysine derived from 12-h fed and 24-h oxidation

Table 2 Comparison of amino acid requirements in adult humans determined by indicator oxidation and indicator balance studies^{a,b}

Amino acid	Reference	Method applied	Adaptation period (h)	Breakpoint estimate (mg/kg/day)
Lysine	[11]	IAAO	8	35
	[23]	IAAO	12	31
	[23]	IAAB	24	31
Threonine	[13]	IAAO	8	19
	[24]	IAAO	12	15
	[24]	IAAB	24	15
Methionine ^c	[15]	IAAO	8	12.6
	[25]	IAAB	24	15
Phenylalanine ^d	[20 [•]]	IAAO	8	42
	[27]	IAAO	12	36
	[27]	IAAB	24	37

IAAO, indicator amino acid oxidation; IAAB, indicator amino acid balance.

^aIAAO: studies conducted following 2-day adaptation to protein intake and study day (8-h fed) adaptation to test amino acid intake.

^bIAAB: studies conducted following 6-day adaptation to test amino acid intake, and study day 12-h fasted + 12-h fed measurements.

^cMethionine (with no cysteine).

^dPhenylalanine (with no tyrosine).

measurements do not differ (Table 2). Virtually identical threonine requirements of 15 mg/kg/day and lysine requirements of 31 mg/kg/day are obtained by using either 12-h fed IAAO measurements or 24-h IAAO/IAAB measurements.

The 24-h IAAO/IAAB model is more demanding, time consuming and expensive to conduct. Not all subjects are studied at all levels and the number of test amino acid intakes is usually four to six around the expected breakpoint. Conversely, the IAAO model is minimally invasive and relatively less demanding, more rapid, and therefore more suitable for application in vulnerable populations and in disease [4]. Furthermore, each subject is studied at all test intakes with the number of test intakes being six or seven, ranging from deficient to excess, thereby allowing the determination of individual amino acid requirements, as well as a statistically stronger estimate of the requirement [1].

Individual amino acid requirements in children

Determination of amino acid and protein requirements in infants and children has been particularly difficult due to the fact that feeding a wide range of amino acid intakes, especially deficient intakes, for prolonged periods of time is not practical. Previous nitrogen balance studies in school-age children (6–10 years) [33[•]] were considered insufficient to recommend specific dietary intakes for

amino acids in children [2] and a factorial approach was utilized to recommend intakes.

Healthy children

In the past 2 years, since the DRIs were published, the minimally invasive IAAO model has been applied to determine the requirements for total branched-chain amino acids [34], methionine (with no cysteine) [35], minimum methionine [36] and lysine [37[•]] in healthy school-age children (Table 3). Earlier, the mean total branched-chain amino acid requirement in children was determined to be 147 mg/kg/day [34], similar to the requirement in adults of 144 mg/kg/day [18]. The mean total sulfur amino acid requirement in children was determined to be 12.9 mg/kg/day [35] compared to 12.6 mg/kg/day [15] in adults. The minimum methionine requirements in children was found to be 5.8 mg/kg/day [36] compared to 4.5 mg/kg/day [16] in adults. Similarly, the lysine requirement in children was determined to be 35 mg/kg/day [37[•]] when compared to the adult lysine requirement of 35–37 mg/kg/day [9,11].

Similar requirement estimates for children and adults suggest that the estimated requirement values reflect predominantly the maintenance requirement and do not account for all the growth needs for children of this age group. Children of this age group (8–10 years) are much slower growing (0.03%/day) when compared to other children in significant age ranges such as neonates or adolescents who grow more rapidly. During the IAAO

Table 3 Indispensable amino acid requirements (mg/kg/day) in healthy school-age children

Amino acid	Indicator amino acid oxidation-based requirement estimate	Dietary Reference Intakes [2]
Total branched chain amino acids ^a	147 (157) ^b	81
Methionine (with no cysteine)	12.9 (16) ^b	18
Methionine (with cysteine)	5.8	–
Lysine	35 (41) ^b	37

^aIsoleucine + leucine + valine.

^bRequirement values within parentheses reflect addition of growth component to experimentally determined value; refer text for details.

8-h study protocol the estimate of amino acid requirement does include the requirement for both maintenance and growth. As the maintenance component is the predominant part of most indispensable amino acid requirements [2] we predict that addition of the calculated growth component to the estimated average requirement would ensure proper growth in these children.

The growth component is estimated from the rate of protein deposition for different age groups [38,39], the amino acid composition of whole-body protein [40] and the incremental efficiency of protein utilization [2]. For example, the lysine required for growth in 9 to 13-year-old children is calculated as the product of rate of protein deposition (49 mg/kg/day) and lysine composition of the whole body (0.073%) which is divided by 0.58, the efficiency of dietary protein utilization, to yield a value of 6.1 mg/kg/day [37]. Similarly, the amino acid requirements for growth in school-age children for total BCAA is 10 mg/kg/day and for total sulfur amino acids (methionine with no cysteine) is 3 mg/kg/day. Until future studies are conducted in children with more long-term outcome variables, it is prudent to apply the growth component to the experimentally determined requirement value in children and adolescents.

These recent IAAO studies in healthy school-age children are the first to use stable isotope techniques for the determination of amino acid requirements in children and have clearly established that the factorial method of calculating requirements is indeed valid in healthy children.

Application of indicator amino acid oxidation to determine protein requirements

Total protein requirements for humans have been traditionally determined using nitrogen balance. The recent DRI recommendations for mean and population safe intakes of 0.66 and 0.8 g/kg/day, respectively of high-quality protein in adult humans are based on meta-analysis of nitrogen balance studies using linear regression analysis [2]. Considering the inherent problems associated with the nitrogen balance method, we hypothesized that the protein requirements are under estimated. Therefore, we examined the total protein requirement in adult humans using the IAAO method [41]. Graded intakes of protein ranging from 0.1 to 1.8 g/kg/day were fed and indicator amino acid (L-[1-¹³C]phenylalanine) oxidation was measured. The mean and population safe intakes were determined to be 0.93 and 1.24 g/kg/day, and are 41 and 50%, respectively, higher than the current DRIs. We also reanalyzed previous nitrogen balance studies using biphasic linear regression analysis, and obtained mean and safe protein requirements of 0.91 and 0.99 g/kg/day, respectively [41]. The fundamental basis for the reanalysis was

that linear regression analysis of nitrogen balance data is not appropriate, because there is a decreased efficiency of protein utilization as zero balance is approached. Biphasic linear regression analysis is considered more appropriate for biological analysis of dose-response curves [2]. In summary, the IAAO-based requirement values of 0.93 and 1.24 g protein/kg/day and the reanalysis of preexisting nitrogen balance studies are significantly higher than current recommendations, and therefore there is an urgent need to reassess recommendations for protein intake in adult humans.

Conclusion

Individual amino acid requirements have been systematically defined in adult humans using the IAAO model. The requirement estimates are comparable to the values derived using the 24-h IAAO/IAAB model. Due to the minimal invasive nature of the IAAO method it has been possible to define amino acid requirements in healthy school-age children and in children with certain metabolic disorders [1,4]. The way is now open to examine requirements in other vulnerable populations, especially pregnant and lactating women, the elderly, and in disease. The recent adaptation of the IAAO method to determine protein requirements in adult humans is of importance because the results suggest that current protein intake recommendations are underestimated by around 40% and therefore reassessment of the recommendations is necessary.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 84–85).

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