Evidence that protein requirements have been significantly underestimated

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Purpose of review

This review discusses recent evidence that suggests a significant underestimation of protein requirements in adult humans.

Recent findings

Traditionally, total protein requirements for humans have been determined using nitrogen balance. The recent Dietary Reference Intake 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 a meta-analysis of nitrogen balance studies using single linear regression analysis. We reanalyzed existing nitrogen balance studies using two-phase linear regression analysis and obtained mean and safe protein requirements of 0.91 and 0.99 g/kg/day, respectively. The two-phase linear regression analysis is considered more appropriate for biological analysis of dose–response curves. Considering the inherent problems associated with the nitrogen balance method, we developed an alternative method, the indicator amino acid oxidation technique, to determine protein requirements The mean and population-safe requirements in adult men were determined to be 0.93 and 1.2 g/kg/day and are 41 and 50%, respectively, higher than the current Dietary Reference Intakes recommendations.

Summary

The indicator amino acid oxidation-based requirement values of 0.93 and 1.2 g protein/kg/day and the reanalysis of existing nitrogen balance studies are significantly higher than current recommendations. Therefore, there is an urgent need to reassess recommendations for protein intake in adult humans.

Keywords

estimated average requirement, humans, indicator amino acid oxidation, nitrogen balance, protein requirements

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Introduction

The current recommendations for protein requirements in adult humans are based on the recent Dietary Reference Intakes (DRIs) published in 2005 [1]. The mean (estimated average requirement, EAR) and population-safe (recommended dietary allowance, RDA) recommendations for good quality protein were set at 0.66 and 0.8 g/kg/day, respectively. These recommendations were based on a meta-analysis of nitrogen balance studies [2], in which protein requirements were estimated by fitting a linear regression analysis model to the data and measuring zero nitrogen balance as the criterion of nutritional adequacy. However, the physiological response relationship between nitrogen intake and balance is not linear due to a decreased efficiency of protein utilization as zero balance is approached [3,4]. Because the physiological response relationship is curvilinear, a two-phase linear regression model [5] or a smooth nonlinear model [4,5] has been proposed to be a more realistic biological analysis to determine protein requirements. The latter two models were, however, not adopted in the current DRI report [1] because it was perceived that more data points on each individual were needed than were available in published studies.

The most recent international dietary protein recommendation for healthy adults, proposed by the Food and Agricultural Organization (FAO) 2007 report [6], is also based on the same meta-analysis [2]. The corresponding author of the present study was a member of the Panel on DRIs for macronutrients [1] and a member of the FAO 2007, that is, Joint WHO/FAO/United Nations University (UNU) Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition [6], and disagreed with the single linear regression analysis of the nitrogen balance data. To examine the results of two-phase linear regression analysis, we reanalyzed the previously published nitrogen balance data [7••]. Furthermore, considering the inherent methodological concerns relating to the nitrogen balance technique [8–14], there is a real need for a valid alternative method to assess protein requirements. Hence, we applied the stable isotope-based indicator amino acid oxidation (IAAO) method to determine protein requirements in adult humans [7••]. This review will briefly summarize the results from our reevaluation of adult human protein requirements and highlight the urgent need to reassess current recommendations for protein requirements.

Limitations of nitrogen balance studies

Nitrogen balance is the difference between nitrogen intake and the amount excreted in urine, feces, and miscellaneous losses such as sweat, hair, nails, and secretions [1]. The nitrogen balance studies [1,8–14] have numerous shortcomings due to various practical and data interpretation limitations, and these have been extensively reviewed and discussed. Briefly, the balance technique tends to overestimate nitrogen intake and underestimate nitrogen excretion. The net result leads to an overly positive balance and therefore an underestimation of the requirement [10]. Nitrogen balance studies in adults consistently display positive balances, with considerable apparent retention of nitrogen, which is biologically implausible. The miscellaneous losses of nitrogen are inherently difficult to measure and vary by almost two-fold due to environmental conditions (temperate versus tropical climate) [1]. Long periods (5–7 days) of adaptation to test intakes are required for the conduct of nitrogen balance studies because the method requires the equilibration of the slow changing and large body urea pool [10]. Furthermore, there are significant confounding effects on the measurement of zero nitrogen balance due to altered dietary energy intake levels [14]. Data analysis and interpretation limitations arise primarily due to the fact that the efficiency of protein utilization decreases near zero balance [3]. With increasing nitrogen intakes, the nitrogen response curve is nonlinear. Earlier balance studies had test intakes at or near zero balance, and the intercept is usually determined by linear interpolation, which also leads to an underestimation of the true balance [4]. In spite of these various limitations, nitrogen balance remains the primary method for identifying protein needs, mainly because there is no validated or accepted alternative method.

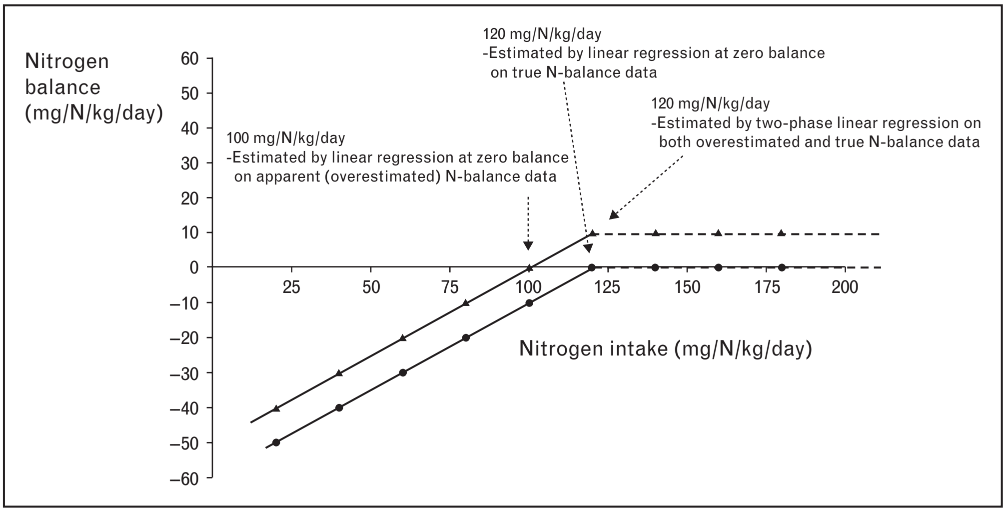
Reanalysis of nitrogen balance studies

Rand *et al.* [2] conducted a thorough and careful meta-analysis of published nitrogen balance studies to suggest protein intake recommendations. The analysis included 19 studies in which individuals were tested at three intakes for periods of 10–14 days, with urinary and fecal nitrogen collection during the final 5 days. The selected studies had test intakes around the expected requirements. Single linear regression analysis was utilized to identify the intercept at zero balance, although the authors acknowledged that the nitrogen intake response curve is not linear [2].

Biologically, higher intakes of protein in adults do not result in further protein accretion. Therefore, at some point, the slope of nitrogen balance versus protein intake must equal zero, further supporting the idea that simple linear regression analysis is not appropriate. We argue that the breakpoint (protein requirement) determined by the two-phase linear regression is more reliable because it remains the same for both true and apparent (overestimated) nitrogen balance values. Figure 1 illustrates this argument by showing the effect on the estimate of protein requirements using both statistical methods on hypothetical data representing either true nitrogen balance or a 10% overestimation of nitrogen balance values (apparent nitrogen balance). As shown in the figure, a 10% overestimation of nitrogen balance resulted in a 20% underestimation of protein requirement when linear regression analysis was applied to determine the zero balance value. On the contrary, similar protein requirement values were obtained when two-phase linear regression analysis was applied to both the true and apparent nitrogen balance values. Furthermore, the application of both linear and two-phase regression analysis on true nitrogen values yields the same estimates of protein requirements. However, as the true nitrogen balance values are very difficult to obtain and are seldom accurately measured, it is prudent to apply two-phase linear regression analysis on nitrogen balance data.

On the basis of the above argument, we applied a two-phase linear regression analysis to 28 nitrogen balance studies [7••], including the 19 studies used by Rand *et al.* [2], for estimation of the current EAR and RDA using linear regression analysis. Our reanalysis included studies, which fed individuals' intakes above the expected requirements, which allowed us to partition the data for two-phase linear regression analysis (one line with ascending slope and one line with minimal or no slope). Our selection criteria for the studies included use of repeated measures within the same individual (minimum number of three levels per individual), adaptation of individuals to each level of intake for at least 6 days, and use of standard nitrogen balance techniques (at least 3 days of balance and inclusion of urine and feces in excretion measurements) [7••]. Data for nitrogen intakes, nitrogen balances, number of individuals, and the type of protein source used were collected from these studies. Data were uniformly converted into units of mg N/kg/day

Figure 1 Hypothetical pattern of response in nitrogen balance observed due to increasing intake of protein



A hypothetical example of relationship between various protein intake levels and the nitrogen balances (true and apparent, 10% overestimated). Application of linear regression analysis on both true and overestimated nitrogen balance values resulted in nitrogen requirement of 100 and 120 mg/kg/day, respectively (0.63 and 0.75 g/kg/day protein, respectively). Application of two-phase linear regression analysis on both true and overestimated nitrogen balance values resulted in nitrogen requirement of 120 g/kg/day. Application of linear regression analysis underestimated nitrogen requirements by 20% when the nitrogen balance values were overestimated by 10%. Adapted with permission [7••].

and corrected for miscellaneous nitrogen losses [1]. The two-phase linear regression crossover statistical model selects for the minimum residual standard error in a stepwise partitioning of data points between two regression lines. The first regression line has a slope and the second line is horizontal with minimal or no slope [15]. In our experience, this statistical model is best suited to objectively determine nutrient requirements, provided multiple test intakes are studied. The model also allows the calculation of 95% confidence intervals (CIs), which provides a population-safe requirement estimate as well. Our view is supported by other researchers, especially in animal nutrition [16,17], as determination of exact nutrient requirements has significant implications in optimizing feed costs. Application of the two-phase linear regression model to the nitrogen balance data from 28 studies resulted in the estimation of a breakpoint (mean, EAR) of 0.91 g/kg/day protein and an upper 95% CI (population safe, equivalent to RDA) of 0.99 g/kg/day [7••] (Table 1). These values are significantly higher than the current mean and population-safe protein recommendations of 0.66 and 0.8 g/kg/day, respectively.

Table 1 Comparison of protein requirement estimates in adult humans

	EAR	RDA
	g/kg/day	
DRI 2005 [1]/FAO 2007 [6]	0.66	0.8
Reanalysis of nitrogen balance studies (two-phase linear regression analysis) [7••]	0.91	1.0
IAAO [7••]	0.93	1.2

DRI, Dietary Reference Intakes; EAR, estimated average requirement; FAO, Food and Agricultural Organization; IAAO, indicator amino acid oxidation; RDA, recommended dietary allowance.

Application of indicator amino acid oxidation to determine protein requirements

The above-described reanalysis of balance data highlighted the need to develop and validate alternative methods to determine protein requirements. A little earlier, Ball and Bayley [18] had developed a novel method to determine protein requirements in growing pigs using the IAAO method. The minimally invasive IAAO method, developed for humans in our laboratory, is based on the concept that when one indispensable amino acid (IDAA) is deficient in the diet, then all other amino acids, including the indicator amino acid (another IDAA, usually L-[1-^{13}C]phenylalanine), will be oxidized [5]. With increasing intake of the limiting amino acid (or total protein), 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 at which 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 two-phase linear regression analysis indicates the mean or EAR of the limiting amino acid or total protein [5,19–21]. The IAAO method has earlier been successfully applied to identify amino acid requirements in adult humans by us [22,23] and others [24,25], and recently in children

[26,27] and neonates [28•,29•]. The IAAO method has several advantages in determining requirements (for amino acids and protein) when compared with the other available methods and has been reviewed recently [5,19,20].

Due to the various advantages of the method [5,20], we examined the total protein requirement in adult humans using the IAAO method [7••], as previously successfully applied in pigs [18]. Eight young adult humans participated in seven studies each, in which they received graded intakes of protein ranging from 0.1 to 1.8 g/kg/day, and indicator amino acid (L-[1-^{13}C]phenylalanine) oxidation was measured on each day. The diets provided energy at 1.5× resting energy expenditure, with 33% of energy from fat and variable energy from carbohydrate (48–66%) and protein (1–19%). The intake of phenylalanine (indicator amino acid) was maintained at a constant value, with excess tyrosine, to ensure that with increasing intakes of total nitrogen, the indicator amino acid is partitioned between oxidation and protein synthesis. With increasing nitrogen intakes, oxidation of phenylalanine decreased until a breakpoint was reached (between an intake of 0.9 and 1.2 g/kg/day). There was no further decrease in phenylalanine oxidation with increasing nitrogen intake, suggesting no further incorporation into protein. Application of the two-phase linear regression analysis to the data identified a breakpoint (mean requirement) and the upper 95% CI (population-safe requirement). The mean and population-safe intakes were determined to be 0.93 and 1.2 g/kg/day and are 41 and 50%, respectively, higher than the current DRI recommendations [7••]. The IAAO-based protein requirement values and the reanalysis of preexisting nitrogen balance studies using two-phase linear regression analysis support each other. The new values are significantly higher than current recommendations, and therefore, there is an urgent need to reassess recommendations for protein intake in adult humans.

Supporting evidence for increasing protein intake recommendations

The current DRI [1] and FAO [13] recommendations of 0.65 and 0.8 g protein/kg/day of mean and population-safe intakes, respectively, are tentative, as there are no long-term studies to suggest that these values would maintain nitrogen balance along with lean body mass, muscle mass, serum protein levels, immunity and functional capacity. Previously, a series of long-term balance studies [30–32] showed that an intake of proposed safe allowance of 0.57 g egg protein (FAO 1973 recommendations) resulted in negative nitrogen balance, loss of lean body mass, and deteriorating serum protein and transferase values unless additional energy or nonessential nitrogen was provided. Recently, Jackson *et al.* [33] determined the effect of a proposed safe protein intake (0.75 g/kg/day) on erythrocyte glutathione synthesis rate in young men. The result of that study showed that the erythrocyte glutathione synthesis rate was significantly lower ($P < 0.05$) on days 3 and 10 of the diet, with proposed safe protein intake level (0.75 g/kg/day) as compared with baseline values on a habitual protein intake (~1.13 g/kg/day) [34]. The authors of that study suggested reduced antioxidant capacity and possibly increased susceptibility to oxidation stress that occurred at a protein intake level of 0.75 g/kg/day. This raises the concern that the safe level of 0.75 g/kg/day of protein intake may not be either adequate or safe.

To examine whether consumption of protein intake below the habitual intakes caused alterations in metabolic functions, Jackson *et al.* [35] studied adult humans fed 0.6 g/kg/day for 7 days and observed a decrease in whole body protein turnover, along with a significant reduction in albumin synthesis and nutrient transport proteins. There was also a simultaneous increase in synthesis of fibrinogen and haptoglobin, acute-phase proteins, indicative of a low-grade stress response. In a follow-up study [36], healthy adults consuming 0.75 g protein/kg/day (safe intake) still had a slower rate of hepatic transport protein synthesis. These studies suggest that the current recommendations for protein intake in adults are not satisfactory and could result in loss of metabolic capacity.

Validation and future applications of the indicator amino acid oxidation method to determine protein requirements

The IAAO method has been criticized for the minimal length of adaptation to test intakes prior to the study day [1]. We recently determined whether adaptation for 8 h (current minimal invasive IAAO model), 3 days, or 7 days significantly affected phenylalanine oxidation due to a range of lysine intakes (deficient to excess) in healthy young men [37•]. $F^{13}CO_2$ (label phenylalanine tracer oxidation), which is the primary variable used to determine the breakpoint estimate, was not significantly affected due to 8 h, 3 days, or 7 days of adaptation to any of the test lysine intakes. This provides evidence that our short-term adapted IAAO model is sufficient to determine amino acid and protein requirements. Recently, we also showed in healthy young adults that the breakpoint for IAAO measured using $F^{13}CO_2$ was very similar to the breakpoint for phenylalanine hydroxylation measured using apolipoprotein B-100, a hepatic export protein which is synthesized from intrahepatocyte amino acids [38••]. This provides evidence that measurements in breath are representative of the intracellular enrichment of phenylalanine at the site of protein synthesis in the liver. Therefore, sampling of breath, which is relatively easy to obtain and noninvasive, is sufficient to measure sensitive changes in protein synthesis due to altered test intakes.

The application of IAAO method to determine protein requirements is novel and raises the possibility of studying protein requirements during critical stages in the life cycle, in which identification of protein needs is critical. We have recently completed studying protein requirements in healthy school-age children using the IAAO method [39]. Preliminary results suggest a significant underestimation of the requirements for protein intake in children of age 6–10-years-old. There are only a few nitrogen balance studies conducted in children of school-age, and the current DRI recommendations are therefore based on a factorial calculation [1]. There is also increasing evidence that inadequate protein intake in elderly populations might be detrimental [40]. Current recommendations for protein in elderly people are set the same as in healthy adults [1] and could be a significant underestimate as well. Future studies need to be conducted to identify protein needs in vulnerable populations to optimize protein nutrition.

Conclusion

The current recommendations for protein intakes in adults are primarily based on the reanalysis of existing nitrogen balance studies [1,12]. The nitrogen balance technique has inherent methodological limitations, which lead to an underestimation of the requirement estimate. Furthermore, the application of a single linear regression analysis to identify zero nitrogen balance is not appropriate because the nitrogen intake response relationship is not linear. On the basis of these concerns, we reanalyzed published nitrogen balance studies using two-phase linear regression analysis. We also applied the IAAO method to determine total protein requirements in adults. The mean and population-safe intakes based on the reanalysis were determined to be 0.91 and 1.0 g protein/kg/day and 0.93 and 1.2 g/kg/day, respectively, based on the IAAO method. These new values are approximately 40% higher than the current recommendations, and therefore, there is an urgent need to reassess recommendations for protein intake in adult humans.

Acknowledgement

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Papers of particular interest, published within the annual period of review, have been highlighted as:
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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 114).

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