Original Article

Protein Requirements in Healthy Adults: A Meta-analysis of Nitrogen Balance Studies*

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Abstract

Objective The goal of this study was to analyze protein requirements in healthy adults through a meta-analysis of nitrogen balance studies.

Methods A comprehensive search for nitrogen balance studies of healthy adults published up to October 2012 was performed, each study were reviewed, and data were abstracted. The studies were first evaluated for heterogeneity. The average protein requirements were analyzed by using the individual data of each included studies. Study site climate, age, sex, and dietary protein source were compared.

Results Data for 348 subjects were gathered from 28 nitrogen balance studies. The natural logarithm of requirement for 348 individuals had a normal distribution with a mean of 4.66. The estimated average requirement was the exponentiation of the mean of the log requirement, 105.64 mg N/kg·d. No significant differences between adult age, source of dietary protein were observed. But there was significant difference between sex and the climate of the study site (P<0.05).

Conclusion The estimated average requirement and recommended nutrient intake of the healthy adult population was 105.64 mg N/kg·d (0.66 g high quality protein/kg·d) and 132.05 mg N/kg·d (0.83 g high quality protein/kg·d), respectively.

Key words: Protein requirement; Nitrogen balance; Estimated average requirement; Recommended nutrient intake

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INTRODUCTION

The classical method for determining proper protein requirements in individuals is by assessing nitrogen balance, which has been viewed by many as theoretically the most satisfactory way of determining the protein requirement. The basic concept is that protein is by far the major nitrogen-containing substance in the body, so that gain or loss of nitrogen from the body can be regarded as synonymous with gain or loss of protein.

In 2000, the Chinese Nutrition Society proposed estimated average requirement (EAR) and recommended nutrient intake (RNI) of 0.92 and 1.16 g/kg·d of Chinese traditional mixed protein for adults, respectively[1]. These recommendations were based on a nitrogen balance study of 16 Chinese adult men in 1984[2]. The 2002 China National Nutrition and Health Survey revealed that only 18.4% of residents

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were meeting or exceeding the recommended RNI[3]. Surprisingly however, the incidence of malnutrition in Chinese adults is very low at only 8.5%[4]. It is our opinion that the current EAR and RNI for protein are overvalued and thus need to be reevaluated.

In 2003, Rand and colleagues performed a meta-analysis of nitrogen balance studies to estimate protein requirements in healthy adults[5]. In 2005, the American Expert Group on Protein Requirements, the European Food Safety Authority (ESFA), and the Joint WHO/FAO/UNU consultation adopted these findings[6-7]. Since a decade has passed since this study, we sought to reapply the meta-analysis of nitrogen balance studies to re-estimate protein requirements in healthy adults. The present study is a necessary addition to the study by Rand et al. because it provides an important update of the study by including studies from the past ten years and includes additional primary articles, it includes heterogeneity test, and it provides important data to adjust recommended dietary protein levels for China and other countries.

METHODS

Definition of Protein Requirement

We defined the protein requirement in healthy adults as the lowest level of dietary protein intake necessary to balance nitrogen loss in healthy persons maintaining energy balance at modest levels of physical activity.

Search Strategy

To identify studies of interest we searched MEDLINE (1966 to October 2012), Embase (1966 to October 2012) and the China Hospital Knowledge Database (CHKD) (1915 to October 2012) using the following search strategy: '(dietary Proteins[mesh] or dietary protein? or protein requirement? or protein allowance or protein intake? or protein need?) and (Nitrogen/metabolism[mesh] or nitrogen balance).' The search was limited to human adult studies but no language restriction. The title and abstract of the studies were then scanned to exclude any that were clearly irrelevant. The remaining articles and relevant cited articles were then thoroughly reviewed.

Selection Criteria

We focused on studies that presented data on nitrogen balance as a function of nitrogen intake among healthy persons. This included studies of individual nitrogen balance for subjects with greater than or equal to three protein intakes. For nitrogen balance studies, linear regression has been established to attain the protein intake levels at nitrogen balance. So analyzing more than three protein intakes will reduce error for establishing linear regression. We excluded studies that examined the response of nitrogen balance to different energy intakes, individual nitrogen balance for subjects with less than three intakes and those studies that measured nitrogen balance as a component of other and often more complex investigations.

Data Extraction

Two reviewers analyzed the publications independently and collected the following information from each study: publication data (first author's last name, year of publication and country of the population studied); climate of the study site; number of subjects; population characteristics (sex and age); protein origins (animal protein, vegetable protein, and mixed protein); nitrogen intake; nitrogen balance and energy intake. Animal protein included milk, meat (beef, fish, etc.), and egg. Vegetable protein included rice, wheat, cottonseed, potato, soy, bean, etc. Mixed diet included animal protein and vegetable protein. Nitrogen balance and nitrogen intake data were uniformly converted into units of mg N/kg·d.

Data Analysis

The calculation of nitrogen balance requires estimation of nitrogen losses through the urine, feces, skin (primarily sweat), and miscellaneous means (hair, tooth brushing, and exhaled ammonia), whereas some studies of nitrogen balance measure only urine and fecal nitrogen losses. Consistent with Rand et al.[5], we adjusted the nitrogen balance data for each study by a constant of 4.8 mg N/kg·d if the study was conducted in a temperate area or a constant of 11 mg N/kg·d if the study was conducted in a tropical area, and used these corrected values for all analyses. Since distribution of individual requirements was skewed and several probable outliers were present, the primary estimation data set was first trimmed (3% trimming, with the 1% highest and 2% lowest values removed) and the natural logarithms taken. This resulting data set, consisting of 348 individual requirements, was normally distributed. The EAR refers to the average daily nutrient intake level estimated to meet the requirement of half the healthy individuals in a particular life stage and gender group. The RNI refers to the average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97% to 98%) healthy individuals in a particular life stage and gender group. In present study, the EAR was equal to the exponentiation of the mean of the log requirement distribution in mg N/kg·d. The RNI was equal to 1.25×EAR[6] .

To test for total variation due to heterogeneity between studies, both the χ^2-based Q statistic test (Cochran's Q statistic) and the I^2 index were used. I^2 values of 25%, 50%, and 75% were used as evidence of low, moderate, and high heterogeneity, respectively[8]. Forest plot, heterogeneity tests, sensitivity analyse, publication bias analysis were performed with the STATA version 11.0 (StataCorp LP, College Station, TX). Normality test, the natural logarithms taken, mean counting, and ANOVA analysis were performed using the Statistical Analysis Systems 9.1 software (SAS Inc., Cary, NC, USA). A value of $P<0.05$ was considered statistically significant.

RESULTS

Study Selection and Study Characteristics

We identified 1 448 records using MEDLINE, 219 using the Ebase database and 98 using the CHKD. However, most abstracts did not specifically address the topic of our analysis and were excluded from full-text review. We reviewed the full text and checked the reference lists for 35 potentially relevant manuscripts. Of these 35, we identified 21 adult protein requirement studies that met our inclusion criteria and contained primary data regarding nitrogen intake and nitrogen balance. Using the references from these studies, an additional seven articles were identified, bringing the total number to 28, all published between 1972 and 2010 (Table 1).

Table 1. Characteristics of the 28 Nitrogen Balance Studies Included in The Meta-analysis

ID	Study	Year	No. of Subjects	Sex*	Climate	Age (y)*	Diet Protein Source
1	Agarwal et al.[9]	1984	11	F+M	Tropical	Y: 25-39	V: rice, wheat
2	Alford et al.[10]	1978	14	F	Temperate	Y: 19-25	V: cottonseed
3	Atinmo et al.[11]	1988	15	M	Tropical	Y: 19-21	M: rice, beef, potato
4	Atinmo et al.[12]	2010	18	M	Tropical	Y: 21-27	M: Mixed
5	Bourges et al.[13]	1982	11	M	Temperate	Y: 15-30	V: corn, bean, wheat+A: milk
6	Calloway et al.[14]	1982	6	F	Temperate	Y: 19-33	A: egg
7	Campbell et al.[15]	2008	42	F+M	Temperate	Y: 21-46 O: 63-81	A: egg
8	Cheng et al.[16]	1978	14	M	Temperate	Y: 23-29 O: 60-73	M: soy, milk, wheat
9	Clark et al.[17]	1972	6	F+M	Temperate	Y: 22-26	M: rice, milk, wheat
10	De Unamuno et al.[18]	1991	8	M	Tropical	O: 60-81	V: rice, bean
11	Dutra et al.[19]	1984	8	M	Tropical	Y: 18-28	V: rice, soy
12	Egana et al.[20]	1992	9	M	Tropical	Y: 18-31	A: egg+V: lupin
13	Egun et al.[21]	1993	12	F	Tropical	Y: 20-32	M: rice, beef, potato
14	Fajardo et al.[22]	1981	14	F+M	Tropical	Y: 21-26	A: chicken+V: rice, soy
15	Huang et al.[23]	1982	15	M	Temperate	Y: 20-29	M: Mixed
16	Hussein et al.[24]	1984	8	F	Tropical	Y: 18-27	M: Mixed
17	Inoue et al.[25]	1981	20	F+M	Temperate	Y: 19-28	V: soy+A: fish+M: soy, fish
18	Istfan et al.[26]	1983	8	M	Temperate	Y: 19-21	V: soy
19	Kaneko et al.[27]	1988	12	F	Temperate	Y: 18-24	M: rice, egg, milk, potato
20	Morse et al.[28]	2001	11	M	Temperate	O: 70-81	M: Mixed
21	Ozalp et al.[29]	1984	11	M	Temperate	Y: 19-26	M: Mixed
22	Scrinshaw et al.[30]	1983	21	F+M	Temperate	Y: 18-23	V: soy+A: milk
23	Thomas et al.[31]	1979	7	F	Temperate	Y: 18-23	V: cottonseed
24	Tontisirin et al.[32]	1981	13	M	Tropical	Y: 19-27	A: egg
25	Uauy et al.[33]	1978	14	F	Temperate	O: 70-84	A: egg
26	Vannucchi et al.[34]	1983	8	M	Tropical	Y: 18-28	V: rice, bean
27	Yanez et al.[35]	1982	15	M	Temperate	Y: 20-31	M: rice, milk, wheat+A: egg
28	Yin et al.[2]	1984	10	M	Temperate	Y: 24-44	M: Mixed

Note. *F: female; M: male; O: old: Y: young.

Data Analysis

Requirement Estimation This resulting data set, consisting of 359 individual requirements from the 28 nitrogen balance studies. Since distribution of individual requirements was skewed and several probable outliers were present, the primary estimation data set was trimmed and the natural logarithms taken. After trimming and converting, this resulting data consisted 348 individual requirements which was normally distributed. Figure 1 shows the mean and 95% CI for the requirement of each nitrogen balance study.

The natural logarithm of requirement (mg N/kg·d) has a normal distribution with a mean of 4.66. Since there was heterogeneity for the meta-analyses of the 28 nitrogen balance studies (P=0.000 of χ^2-based Q statistic test, I^2=66.9%), a Galbraith plot presented there are two studies maybe affect the homogeneity of the study[21,24]. IF excluding the two studies, 26 studies remained that had no heterogeneity for the meta-analyses (P=0.292 of χ^2-based Q statistic test, I^2=11.8%). We take a random-effects model in the sensitivity analyse owing to heterogeneity of the 28 studies. There are no large changes after excluding each study one by one in the sensitivity analyse (Figure 2), indicating that the sensitivity is low, the result is credible. Therefore, we didn't excluding any study. Publication bias was evaluated using the Begg's test and P value is 0.179 (>0.05), so protein requirements are not affected by the publication bias.

The natural logarithm of requirement (mg N/kg·d) of 28 nitrogen balance studies (348 individual requirements) had a mean of 4.66. The EAR is the exponentiation of the mean of the log requirement was 105.64 mg N/kg·d (0.66 gprotein/kg·d). RNI was equal to 1.25×EAR, 132.05 mg N/kg·d (0.83 g protein/kg·d).

Factors Affecting Protein Requirement The results of a comparison of subsets of the data according to climate, age, sex, and diet are shown in Table 2.

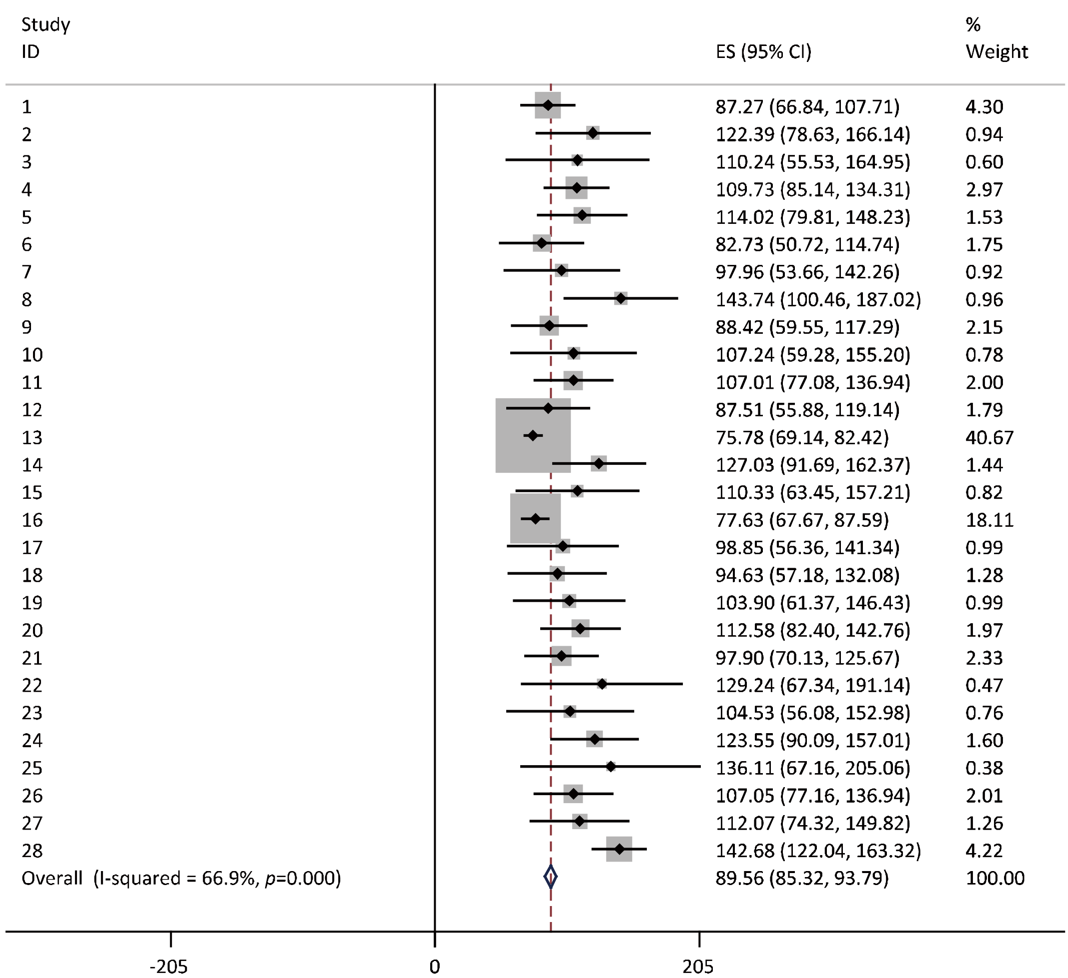

Figure 1. Summary mean and 95% CI for the requirement of each nitrogen balance study.

Climate There was significant difference in the natural logarithm of protein requirement when comparing data from studies conducted in temperate and tropical climates, with resulting values of 108.85 mg N/kg·d and 100.49 mg N/kg·d, respectively.

Age There was no significant difference in the natural logarithm of protein requirement when comparing data from young and old individuals, with resulting values of 104.58 mg N/kg·d and 111.05 mg N/kg·d, respectively. Aged 60 and over were categorized as old.

Sex There was significant difference in the natural logarithm of protein requirement when comparing data from males and females, with resulting values of 108.85 mg N/kg·d and 97.51 mg N/kg·d, respectively.

Diet Individuals who consumed an animal protein source diet and vegetable protein source diet had the lowest and highest protein requirement with resulting values of 101.49 mg N/kg·d and 108.85 mg N/kg·d, respectively. The protein requirement of individuals who ate a mixed diet was 106.7 mg N/kg·d. But there are no significant differences in the natural logarithm of protein requirement among the different diet.

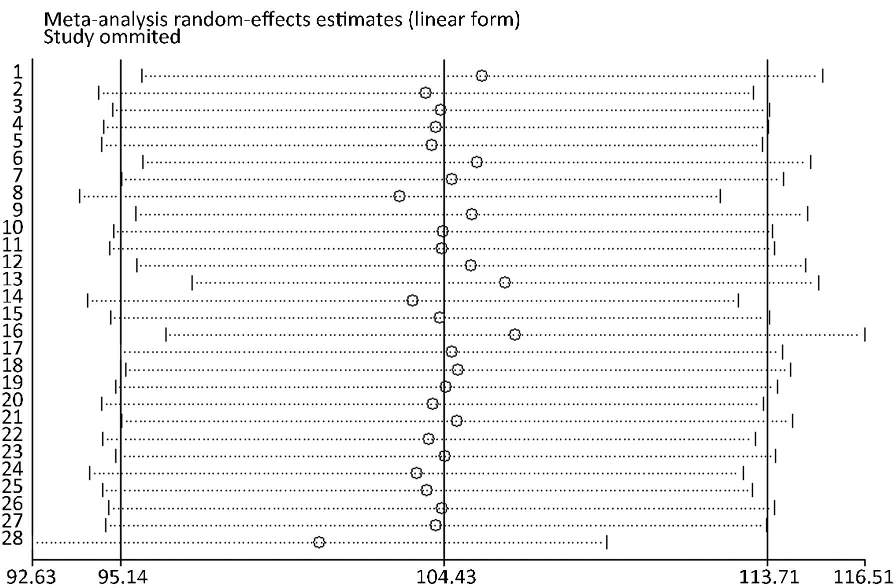

Figure 2. Sensitivity analyse of the 28 nitrogen balance studies.

Table 2. Meta-analysis of Nitrogen Balance in Healthy Adults

Variables		No. of Subjects	*P* Value[a]	Mean[b]	Protein Requirements		
					(mg N/kg·d)	95% CI (mg N/kg·d)	(g/kg·d)
Total		348	0.155	4.66	105.64	103.02-108.32	0.66
Climate	Tropical	126	0.126	4.61[c]	100.48	96.54-104.59	0.63
	Temperate	222	0.153	4.69	108.85	105.64-112.17	0.68
Age	Young	294	0.237	4.65	104.59	102.51-107.77	0.65
	Old	54	0.84	4.71	111.05	103.54-119.10	0.69
Sex	Female	92	0.067	4.58[d]	97.51	92.76-102.51	0.61
	Male	256	0.324	4.69	108.85	105.64-112.17	0.68
Diet	Vegetable	104	0.892	4.69	108.85	104.59-113.30	0.68
	Mixed	144	0.06	4.67	106.70	102.51-111.05	0.67
	Animal	100	0.261	4.62	101.49	96.54-106.70	0.63

Note. [a]: *P* value of W test (Shapiro-Wilk, normality test); [b]: Mean of the natural logarithm of requirement (in mg nitrogen/kg·day); [c]: Significantly different from temperate climate: *P*=0.0041; [d]: Significantly different from male: *P*=0.0002.

DISCUSSION

In 1957, Rose defined the classic approach to study amino acid and protein requirements. This method was based on nitrogen balance, and remains the basis for protein requirement determination to date[36]. We performed a meta-analysis on 348 subjects from 28 separate nitrogen balance studies, and estimated the EAR and RNI of the healthy adult population to be 105.64 mg N/kg·d and 132.05 mg N/kg·d, respectively. In 2007, a joint report from the WHO/FAO/UNU on protein and amino acid requirements indicated that the Protein Digestibility Corrected Amino Acid Score (PDCAAS) could be used to adjust dietary protein intakes to meet requirements. For example, for any diet, recommended intake is equal to a safe level of protein/PDCAAS value of diet[37]. According the 2002 China National Nutrition and Health Survey, we calculated the PDCAAS value of dietary protein for people over the age of eighteen (Table 3). So using 0.8 as the PDCAAS value to adjust dietary protein quality; we suggest the RNI for Chinese adults is 165.06 mg N/kg·d (1.03 g protein/kg·d).

The nitrogen balance technique requires accurate quantification of all routes of intake of nitrogen and all routes of loss. But monitoring all food consumed and quantification of all nitrogen loss from the body is actually very difficult in practice. The loss of nitrogen from the body occurs primarily via the urine and feces, which can be measured accurately. However, losses also occur through the skin and via other miscellaneous ways such as nasal secretions, menstrual losses, or seminal fluid. These losses are always an underestimate, thus overestimating intake and leading to an erroneously positive nitrogen balance[38]. These miscellaneous losses were neglected in many of the published studies using nitrogen balance. In addition, some published studies also neglected the dermal nitrogen losses, mainly from sweat. Therefore, in the present meta-analysis, we accounted for these miscellaneous and dermal nitrogen losses by correcting the nitrogen balance data using a constant of 4.8 mg N/kg·d if the study was conducted in a temperate area or 11 mg N/kg·d if the study was conducted in a tropical area.

Table 3. The PDCAAS Value Of Dietary Protein Quality in China for People Aged over 18 Years by Areas

Age (y)	Rural Areas		Urban Areas	
	Male	Female	Male	Female
18-	0.80	0.79	0.93	0.93
65-	0.80	0.80	0.93	0.93

In the present study, protein requirement conducted in temperate site was higher than the tropical site (*P*>0.05), which is surprising since it is generally believed that dermal nitrogen losses are generally higher in studies conducted in the tropics or during the hot season than in studies conducted in temperate or cold-weather areas. We hypothesize the difference in protein requirements between the temperate and tropic regions can be attributed to the limited data, since one would expect protein requirements for individual living in the tropic regions should tend to be higher.

For age category analysis, older individuals tended to have a higher protein requirement than younger individuals, but the difference was not statistically significant. Similar results were found in a previous meta-analysis[5]. But it is hypothesized that the elderly could not maintain proper nitrogen balance if they intake the same amount of protein as younger individuals, resulting in a higher protein intake requirement (1.0-1.3 g/kg·d)[39]. It is our hypothesis that the controversial findings for the elderly nitrogen balance is that elderly individuals have much more variability within their bodies when compared to young people. These variables are due to a number of changes, including the reduction of lean body mass and an increased level of adipose tissue. Further, the elderly commonly have complications with weakening digestive organ functions, a decline of the endocrine gland secretion and other problems with organ decline. All of these potential variables can have a direct effect on the necessary requirement for protein intake. Therefore, although there was no statistically significant difference between young individuals and old individuals, we suggest older individuals should have higher protein requirement than young people.

There are statistically significant differences in the protein requirements between females and males. Based on our literature search, protein dietary reference intakes (DRIs) between females and males in most countries was similar except Australia and New Zealand[40].

Protein requirements change based on the quality of protein used. For example, higher-quality protein from meat or bean products was found to be utilized more efficiently than lower-quality protein from vegetables. Some experimental diets that we characterized as vegetable diets included soy protein, a source considered to be good protein[19,22,25-26,30]. These original soy studies showed the well-processed soy proteins were equivalent to animal protein. So in present study, there is no significant difference among the source of dietary protein.

The present study has made an update of the Rand study, and the most important aspect is that the present study including more nitrogen balances studies and making a heterogeneity test, sensitivity analyse, publication bias during the meta-analysis.

In conclusion, the results of our meta-analysis suggest the EAR and RNI of the healthy adult population are 105.64 mg N/kg·d and 132.05 mg N/kg·d, respectively. According to the PDCAAS value of Chinese dietary patterns, we suggest the RNI for Chinese adults is 165.06 mg N/kg·d (1.03 g protein/kg·d).

CONTRIBUTION

LI Min participated in the design of the present study, collection and assembly of data, data analysis and interpretation, manuscript writing. SUN Feng participated in the data collection and analysis. PIAO Jian Hua assisted with the data analysis and the drafting of the manuscript. YANG Xiao Guang participated in the design of the present study, data analysis and interpretation, manuscript writing. All of the authors participated in a critical review and in the final approval of the manuscript. None of the authors had a personal or financial conflict of interest.

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