Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults[1–3]

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ABSTRACT
Background: The most recent international dietary protein recommendations for healthy adults are those developed and proposed by the 1985 FAO/WHO/UNU Joint Expert Consultation.
Objective: The objective was to analyze available nitrogen balance data to establish new recommendations for the protein required by healthy adults.
Design: Data were gathered from published nitrogen balance studies that had as their primary objective either the estimation of basal or maintenance requirements or the testing of the adequacy of specific nitrogen intakes in healthy adults. These data were synthesized to characterize the distribution of individual protein requirements; the effects of climate of the study site, adult age, sex, and dietary protein source on individual requirements; and the midpoint of and the variability between the protein requirements of healthy persons.
Results: Data for 235 individual subjects, each studied at ≥ 3 test protein intakes, were gathered from 19 studies. The median estimated average requirement (EAR) of nitrogen from these data was 105 mg $N \cdot kg^{-1} \cdot d^{-1}$. Individual requirements were found to fit a log-normal distribution. The median EAR was estimated as the median of this distribution, 105 mg $N \cdot kg^{-1} \cdot d^{-1}$, whereas the 97.5th percentile (the recommended dietary allowance; RDA) was estimated from the distribution of the log of the requirement (after correction of the total observed variability to remove within-individual variability) as 132 mg $N \cdot kg^{-1} \cdot d^{-1}$. No significant differences between the climate of the study site, adult age class, sex, or source of dietary protein were observed, although there was an indication that women might have a lower requirement than do men.
Conclusion: This meta-analysis provides new recommendations for dietary reference values, ie, an EAR (median) and RDA (97.5th percentile) for healthy adults of 105 and 132 mg $N \cdot kg^{-1} \cdot d^{-1}$ (0.65 and 0.83 g good-quality protein $\cdot\ kg^{-1} \cdot d^{-1}$), respectively. *Am J Clin Nutr* 2003;77:109–27.

KEY WORDS Protein requirements, nitrogen balance, elderly, protein source, protein quality, obligatory nitrogen losses, models, recommended dietary allowance, RDA, estimated average requirement, EAR

INTRODUCTION

Assessment of the nutritional status of individuals or populations and the planning of adequate diets requires information on the amount of dietary protein that is necessary to maintain a condition of good health. This article examines the published literature relevant to this problem and explicitly examines 3 aspects of this problem: *1*) the average protein intake that is consistent with good health on the basis of body nitrogen balance; *2*) the influence of age and sex, climate of the study site, and the source of dietary protein on the estimated individual need for protein; and *3*) the variability in the protein need between individuals.

History of protein requirements

In the latter half of the 19th century, dietary protein standards for adults were based on estimates of dietary protein intake (1). Foremost among the various proponents of this approach were Carl Voit in Germany and Wilbur O Atwater in the United States (1). On the basis of his dietary survey, carried out in Munich in 1880, Voit recommended a dietary protein intake of 118 g/d for an adult human of average weight doing moderate muscular work. Similar standards were proposed by other investigators, according to McCay (2), which reflect "a general consensus of opinion that the protein element should amount to over 100 g a day, and the energy value should be over 3000 calories." However, by the turn of the 20th century, these standards were being questioned by Chittenden (3), among others, who used the nitrogen balance technique to explore whether relatively low intakes of protein would be sufficient to maintain nitrogen equilibrium. From 3 series of studies, Chittenden (3) concluded that "one-half of the 118 g of protein food called for daily by the ordinary standards is quite sufficient to meet all the real physiologic need of the body, certainly under the ordinary conditions of life; and with most individuals, especially persons not leading an active out-of-door life, even smaller amounts of will suffice" (3).

Dietary protein standards continued to be set by individuals until national and international committees were organized to make recommendations about protein and energy intakes. Munro

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(4) reviewed early committee deliberations, and Scrimshaw (5) provided a historical and personal perspective on the committee approach to establishing protein requirement estimates and allowances. Hence, we do not repeat this detail but we make particular reference to the United Nations (UN) committees and recommendations for protein requirements that began in 1955 (6). The most recent statement, which appears in the report of the 1981 FAO/WHO/UNU Expert Consultation on Energy and Protein Requirements, was published in 1985 (7). To derive the protein requirement of healthy young adults, the 1981 Expert Consultation reviewed evidence from both short-term and longer-term nitrogen balance studies and calculated the mean requirement reported from each study in these 2 data sets. After rounding off the average requirements for proteins of high quality—such as meat, milk, eggs, and fish—the adult mean protein requirement was set at 0.6 $g \cdot kg^{-1} \cdot d^{-1}$.

Additionally, the 1981 Expert Consultation concluded from studies of obligatory nitrogen losses and short-term nitrogen balance studies that there was no evidence to suggest that a distinction could be made between men and women. The Consultation also reviewed 4 reports of nitrogen balance studies in the elderly, the results of which were not entirely consistent, and stated "This figure is higher than for young adults in relation to lean body mass, because it is an accepted fact that protein utilization is less efficient in the elderly." The validity of this statement has been challenged (8) and we will revisit this issue in this article.

The variation in requirements among apparently similar subjects was estimated by taking the mean of the CV from the various short-term balance studies used to estimate the mean protein requirement. This was found to be 16.2%, and the assumption that this variation was equally due to both between- and within-subject variation led to an estimated CV for the requirement of 12.5%.

Motivation and goals of this paper

In 1994 a committee of the International Dietary Energy Consultative Group (9, 10) reevaluated the 1985 FAO/WHO/UNU (7) protein recommendations for infants and children and recommended that a meta-analysis of all available nitrogen balance data for adults be carried out before making new recommendations for adults. More recently, the current authors were requested by the FAO/WHO/UNU to prepare an article on the protein requirements of healthy adults for a working group meeting on protein and amino acid requirements that was held in Rome, 1–5 July 2001. The present article is based on that working paper and additionally incorporates suggestions, insights, and proposed analyses that arose from that meeting and from more recent thinking, discussions, and analyses.

Definition of adult protein requirement

For the present purpose we defined the protein requirement in healthy adults as the continuing intake of dietary protein that is sufficient to achieve body nitrogen equilibrium (zero balance) in an initially healthy person of acceptable body composition at energy balance and under conditions of moderate physical activity and as determined after a brief period of adjustment to a change in test protein intake. Although the nitrogen balance technique has serious shortcomings, as discussed in the 1985 FAO/WHO/UNU report (7) and in many other articles—Hegsted (11), Waterlow (12), Young (13), Millward (14), and Manatt and Garcia (15)—this method remains the primary approach for determining protein requirement in adults, in large part because there is no validated or accepted alternative.

We used this definition of measured body nitrogen equilibrium (zero nitrogen balance) as the criterion of nutritional adequacy, despite the fact that positive nitrogen balances are frequently measured in mature adults at nitrogen intakes that are clearly generous, even after correction for dermal and other miscellaneous nitrogen losses. Such positive nitrogen balances obviously cannot be sustained in adults and they might be due to consistent technical errors of overestimating nitrogen intake or underestimating nitrogen losses. In this case there might be some justification for using an agreed on, specific positive nitrogen balance value as the criterion of protein intake adequacy. However, we do not believe, given the present data, that a sufficient case for any particular level of positive balance can be made; therefore, we choose zero nitrogen balance as the criterion for determining the protein requirement. Our reasoning, in brief, is as follows:

1) In short-term nitrogen balance studies, as the data we examined show, there are numerous instances of both positive and negative nitrogen balances for the range of nitrogen intakes from ≈50 to 160 $mg\ N \cdot kg^{-1} \cdot d^{-1}$. At higher nitrogen intakes, positive balances are frequently observed (11), but such intakes fall well above a likely minimum physiologic level.

2) Short-term nitrogen balance studies are confounded by the uncertain effects of variations in dietary energy intake relative to energy expenditure and balance. For example, an error of ≈10% in the intake of energy that is judged by a diet history or from prediction equations (7) to be sufficient to balance total energy expenditure can affect the final nitrogen balance estimate of an individual subject by as much as 6 $mg\ N \cdot kg^{-1} \cdot d^{-1}$ (16). Despite a meticulous effort to match energy intake to total expenditure, such errors are likely, as experienced in our own studies (17, 18).

3) Positive nitrogen balance over the short term may also reflect actual nitrogen retention, as indicated, for example, by a positive relation between cumulative nitrogen balance and the change in body weight in one long-term experimental nitrogen balance study (19). We appreciate that there is generally little relation between the degree of nitrogen retention or loss and body weight change in short-term investigations.

4) In many long-term studies in healthy adults receiving 0.8 $g\ N \cdot kg^{-1} \cdot d^{-1}$ from good-quality soy protein, balances were not significantly different from zero (19, 20). For example, Oddoye and Margen (21) reported that in 5 of 6 healthy men given 12 g N/d, nitrogen balances were not significantly different from zero. However, at a much higher intake of 36 g N/d, all subjects apparently retained nitrogen. This intake is well beyond that relevant to establishing a minimum physiologic requirement for protein. Nevertheless, their findings might suggest the possible activation of an additional or unmeasured route of nitrogen loss; such a pathway would lead to elimination of molecular nitrogen (22, 23). In our opinion, this is a possibility (24) and there may also be an increase in nitrate and nitrite excretion (25), which usually represents a relatively small rate of nitrogen loss but is not included by the analytic methods commonly used to assess nitrogen losses via urine and feces.

In summary, it is our judgment that the nitrogen balance evidence generally supports the use of a zero nitrogen balance—where appropriate estimates of dermal and miscellaneous losses are included in the determination after a suitable adaptation period (≈5 d) (26) to a new test protein intake—as a suitable criterion for the adequacy of protein intake. Although arguments might be developed that would lead to the choice of alternative positive nitrogen balance values as the criterion, we find it difficult to justify any specific alternative nitrogen balance criterion in view of the current data.

Study selection

We identified published papers that report nitrogen balance data, starting with those cited in the 1985 FAO/WHO/UNU report (7) and those that reported on the studies conducted under the auspices of the United Nations University specifically for this purpose (27, 28). Additionally, we conducted an electronic bibliographic search (MEDLINE; National Library of Medicine, Bethesda, MD) for more recent literature and solicited input and advice from our professional colleagues. We focused on studies that presented data on nitrogen balance as a function of nitrogen intake among healthy persons and excluded studies that examined the response of nitrogen balance to different energy intakes and those studies that measured nitrogen balance as a component of other and often more complex investigations. The resultant papers were examined for their relevance to our goals, on the basis of the primary intent of the study and their inclusion of individual data, and were divided into 3 major types of studies: estimation, obligatory, and test (**Table 1**).

Estimation studies

These 27 studies, involving 411 subjects, were explicitly designed to estimate the protein requirement by studying many different nitrogen intakes near purported requirements. These studies had similar protocols (28): each test intake was given for 10–14 d, and the urinary and fecal nitrogen excretion data from the last 5 d were used to represent the response to that intake. Because we defined the requirement in terms of the individual and because there is a need to estimate within-individual variability, we based our analysis on 19 of these studies ($n = 237 - 2$ subjects who were dropped from the study—*see* below). These studies presented individual balance data for subjects studied at ≥ 3 intakes, and we defined them as the primary estimation studies. The other 8 estimation studies ($n = 174$ subjects), which presented either grouped data only or data from different persons at different intakes, are designated as secondary estimation studies.

Obligatory studies

These 14 studies of 273 subjects measured endogenous or obligatory nitrogen losses after providing subjects very low amounts of dietary protein. The data from these studies are summarized and compared with analyses of the estimation studies.

Test studies

These 17 studies of 320 individuals were designed to measure the nitrogen balance at 1 or 2 specific nitrogen intakes. The data from these studies were analyzed independently of the data from the estimation studies, because they represent experiments that were not designed to estimate the requirement and usually involved longer experimental periods with individuals consuming a single nitrogen intake.

Data extraction

Nitrogen intake, nitrogen balance, age, sex, diet, and climate of the study site were extracted from these studies, and nitrogen balance and nitrogen intake data were uniformly converted into units of mg $N \cdot kg$ body $wt^{-1} \cdot d^{-1}$ and with zero dermal and miscellaneous nitrogen losses. As indicated in Table 1, these adult subjects were classified by sex and age: young (≤ 55 y, although most data were from subjects in their twenties and thirties) or old (> 55 y, although most subjects were > 65 y). The experimental diets were classified as "animal" (animal sources provided > 90% of total protein), "vegetable" (vegetable sources provided > 90% of total protein), or "mixed" (combination of animal and vegetable sources). The climate under which the study was conducted was classified as temperate or tropic. Of the subjects in the primary estimation studies, 2 were excluded because balance data were determined at only 2 intakes. Studies that involved both men and women, or young and old subjects, or included 2 or more separate diet patterns were divided into substudies of consistent experiments.

Data analysis

All analyses were performed by using SPSS version 10 (SPSS Inc, Chicago) and followed standard statistical procedures (84, 85).

Dermal and miscellaneous nitrogen losses

The calculation of nitrogen balance requires estimation of nitrogen losses through the urine, feces, skin (primarily sweat), and miscellaneous means (eg, hair, tooth brushing, and exhaled ammonia), whereas most studies of nitrogen balance measure only urine and fecal nitrogen losses. Thus, we examined 12 studies that measured some aspect of dermal and miscellaneous losses (**Table 2**). Although most of these studies measured dermal (mainly sweat) losses only, the study by Calloway et al (86) provides an estimate of the full range of dermal and miscellaneous losses. Therefore, we derived a miscellaneous nitrogen loss of 1.77 mg $N \cdot kg^{-1} \cdot d^{-1}$ from Calloway et al's study and used this value for those studies that did not provide a specific estimate of miscellaneous nitrogen losses. Because Calloway et al presented only grouped data on miscellaneous losses, 1.77 mg N was derived by dividing the total daily value of 115 mg N by the then current reference weight for men weighing 65 kg, which agrees with the average of the weights of the men that they studied.

As shown in **Figure 1**, dermal losses differed significantly depending on the climate under which the study was conducted; therefore, we analyzed the data from the 2 climatic regions separately. A summary of the average dermal and miscellaneous nitrogen losses obtained in each study [excluding those individual data points reported by Calloway et al (86) for nitrogen intakes > 700 $mg \cdot kg^{-1} \cdot d^{-1}$] are shown in Table 2. This analysis suggests constant mean (± SD) dermal plus miscellaneous losses of 5.0 ± 0.9 mg $N \cdot kg^{-1} \cdot d^{-1}$ for studies conducted in temperate regions and 11.1 ± 2.3 mg $N \cdot kg^{-1} \cdot d^{-1}$ for studies conducted in tropical regions ($P < 0.001$, Mann-Whitney U test).

Because it has often been reported that dermal nitrogen losses are directly related to nitrogen intake (45, 86, 88, 91, 92), we alternatively used analysis of covariance to estimate separate regression equations for the response of dermal (plus miscellaneous) nitrogen losses as a function of nitrogen intake within each of the 2 climatic regions. We found a significant ($P = 0.006$) response curve for the temperate studies only:

$$\text{Losses } (\text{mg N} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}) = 4.00 + 0.0057 \times \text{nitrogen intake} \quad (1)$$

At nitrogen intakes near the requirement (≈100 mg $N \cdot kg^{-1} \cdot d^{-1}$), this equation predicts dermal and miscellaneous losses of 4.6 mg $N \cdot kg^{-1} \cdot d^{-1}$.

On the basis of these analyses, we adjusted the nitrogen balance data for each study by *1*) the measured dermal losses, if available (with the use of additional 1.77 mg N for miscellaneous

TABLE 1
Nitrogen balance studies used to estimate the protein requirements of healthy adults

Reference	Study type[1]	No. of individual studies	Sex	Climate	Age[2]	Diet source[3]
					y	
Agarwal et al, 1984 (29)	Estimation (Mu, I)	11	M + F	Tropical	Y: 25–39	V: rice, wheat
Atinmo et al, 1985 (30)	Obligatory (I)	15	M	Tropical	Y: 19–39	—
Atinmo et al, 1988 (31)	Test (I)	12	M	Tropical	Y: 23–29	Mx: beef, rice
Atinmo et al, 1988 (32)	Estimation (Mu, I)	15	M	Tropical	Y: 19–21	Mx: beef, rice
Bodwell et al, 1979 (33)	Obligatory (I)	24	M + F	Temperate	Y: 19–52	—
Bourges and Lopez-Castro, 1982 (34)	Estimation (Mu, I)	11	M	Temperate	Y: 20s	A: milk + V: corn, beans
Bourges et al, 1984 (35)	Test (I)	20	M	Temperate	Y: 19–25	V: corn, beans
Bricker and Smith, 1951 (36)	Obligatory (I)	25	F	Temperate	Y: 19–26	—
Calloway and Margen, 1971 (37)	Obligatory (Mu, G)	13	M	Temperate	Y: 21–37	—
Campbell et al, 1994 (38)	Test (I)	12	M + F	Temperate	O: 56–80	Mx: milk, egg, vegetable
Castaneda et al, 1995 (39)	Test (I)	12	F	Temperate	O: 66–79	Mx: milk, vegetable
Cheng et al, 1978 (40)	Estimation (Mu, I) + test (I)	14	M	Temperate	Y: 23–29 O: 60–72	Mx: milk, wheat, soy
Clark et al, 1972 (41)	Estimation (Mu, I)	6	M + F	Temperate	Y: 22–26	Mx: milk, wheat, rice
Dutra et al, 1981 (42)	Test (I)	14	M	Tropical	Y: 17–26	Mx: rice, beans, meat, milk
Dutra and Vannucchi, 1984 (43)	Estimation (Mu, I)	9	M	Tropical	Y: 18–28	V: rice, beans
Egana et al, 1992 (44)	Estimation (Mu, I)	14	M	Tropical	Y: 18–31	A: egg + V: lupin
Egun et al, 1993 (45)	Estimation (Mu, I)	12	F	Tropical	Y: 21–32	Mx: rice, wheat, beef
Egun et al, 1993 (46)	Test (Mu, I)	11	F	Tropical	Y: 21–30	Mx: rice, wheat, beef
Fajardo et al, 1981 (47)	Estimation (Mu, I)	14	M + F	Tropical	Y: 21–26	Mx: meat, wheat, potatoes + V: rice, beans, potatoes
Gersovitz et al, 1982 (48)	Test (I)	15	M + F	Temperate	O: 70–99	A: egg
Huang et al, 1972 (49)	Obligatory (I)	50	M	Tropical	Y: 20–32	—
Huang and Lin, 1982 (50)	Estimation (Mu, G)	41	M	Tropical	Y: 20–29	A: egg + Mx: mixed
Hussein, 1984 (51)	Estimation (Mu, I)	8	F	Tropical	Y: 18–27	Mx: mixed
Inoue et al, 1973 (52)	Estimation (S, I)	25	M	Temperate	Y: 20–27	A: egg + V: rice
Inoue et al, 1974 (53)	Obligatory (I)	9	M	Temperate	Y: young	—
Inoue et al, 1981 (54)	Estimation (Mu, I)	20	M	Temperate	Y: 19–28	A: fish + V: soy + Mx: fish, soy
Istfan et al, 1983 (20)	Test (I)	6	M	Temperate	Y: 18–21	V: soy
Istfan et al, 1983 (55)	Estimation (Mu, I)	8	M	Temperate	Y: 18–26	V: soy
Kaneko and Koike, 1985 (56)	Estimation (S, I)	15	F	Temperate	Y: 18–22	A: egg
Kaneko et al, 1988 (57)	Estimation (Mu, I)	12	F	Temperate	Y: 18–24	Mx: mixed
Komatsu et al, 1983 (58)	Estimation (S, G)	28	M	Temperate	Y: 19–30	A: amino acids (egg)
Nicol and Phillips, 1976 (59)	Obligatory (I)	9	M	Temperate	Y: 21–30	—
Nicol and Phillips, 1976 (60)	Test (I)	17	M	Temperate	Y: 21–30	V: rice
Oddoye and Margen, 1979 (21)	Test (I)	12	M	Temperate	Y: 23–30	A: egg + Mx: egg, soy
Ozalp et al, 1984 (61)	Estimation (Mu, I)	11	M	Temperate	Y: 19–26	Mx: Wheat, yogurt
Ozalp et al, 1984 (62)	Test (I)	15	M	Temperate	Y: 19–28	Mx: wheat, yogurt
Ozalp et al, 1984 (63)	Test (I)	49	M	Temperate	Y: 19–30	Mx: wheat, yogurt
Scrimshaw et al, 1972 (64)	Obligatory (G)	83	M	Temperate	Y: 18–26	—
Scrimshaw et al, 1976 (65)	Obligatory (I)	11	F	Temperate	O: 67–91	—
Scrimshaw et al, 1983 (66)	Estimation (Mu, I)	22	M	Temperate	Y: 18–23	A: milk + V: soy
Thomas et al, 1979 (67)	Estimation (Mu, I)	7	F	Temperate	Y: 18–23	V: cottonseed
Tontisirin et al, 1981 (68)	Estimation (Mu, I)	13	M	Tropical	Y: 19–27	A: egg
Tontisirin et al, 1981 (69)	Obligatory (I)	4	M	Tropical	Y: 21–25	—
Tontisirin et al, 1984 (70)	Test (Mu, I)	12	M	Tropical	Y: 19–26	Mx: rice, fish
Uauy et al, 1978 (71)	Estimation (Mu, I)	14	M + F	Temperate	O: 68–84	A: egg
Uauy et al, 1978 (72)	Obligatory (G)	8	M	Temperate	O: 68–72	—
Uauy et al, 1982 (73)	Obligatory (I)	8	M	Temperate	Y: 24–31	—
Uauy et al, 1984 (74)	Test (I)	53	M	Temperate	Y: 18–19	Mx: wheat, rice, milk
Wayler et al, 1983 (75)	Estimation (Mu, G)	21	M	Temperate	Y: 18–26	A: beef + A: milk + Mx: beef, soy
Xuecun et al, 1984 (76)	Test (I)	6	M	Temperate	Y: 24–41	Mx: rice, wheat, pork, egg
Xuecun et al, 1984 (76)	Estimation (Mu, G)	10	M	Temperate	Y: 24–44	Mx: rice, wheat, pork, egg
Yanez et al, 1982 (77)	Estimation (Mu, I)	15	M	Temperate	Y: 20–31	Mx: wheat, milk + A: egg
Yanez and Uauy, 1984 (78)	Test (I)	8	M	Temperate	Y: 19–33	Mx: wheat, rice, milk
Young and Scrimshaw, 1968 (79)	Obligatory (I)	8	M	Temperate	Y: 17–22	—

(Continued)

TABLE 1 (*Continued*)

Reference	Study type[1]	No. of individual studies	Sex	Climate	Age[2]	Diet source[3]
					y	
Young et al, 1973 (80)	Estimation (Mu, G)	19	M	Temperate	Y: 18–28	A: egg
Young et al, 1975 (81)	Estimation (Mu, G)	15	M	Temperate	Y: 18–24	A: beef + V: wheat
Young et al, 1984 (82)	Test (I)	32	M	Temperate	Y: 20s	A: egg + V: soy
Young et al, 1984 (82)	Estimation (Mu, I)	15	M	Temperate	Y: 20s	A: egg + V: soy
Zanni et al, 1979 (83)	Estimation (Mu, 2 levels, G) + obligatory (G)	6	M	Temperate	O: 63–77	A: egg white

[1] Mu, multiple intakes per individual; S, single intake per individual; I, individual data published; G, grouped data published.
[2] Y, young; O, old.
[3] A, 90% animal; V, 90% vegetable; Mx, mixed.

losses, where not explicitly measured); *2*) a constant of 4.8 mg $N \cdot kg^{-1} \cdot d^{-1}$ (the average of expected dermal plus miscellaneous losses at intakes near the requirement by the 2 models) if the study was conducted in a temperate area; or *3*) a constant 11 mg $N \cdot kg^{-1} \cdot d^{-1}$ if the study was conducted in a tropical area. These corrected values were used for all analyses.

Individual requirements

Requirement, as defined above, is for the individual, and therefore, we derived our estimates from those studies (primary estimation studies) that provided sufficient individual data to estimate individual requirements (individuals studied at ≥3 intakes). Because these studies rarely provided a nitrogen intake that resulted in exactly zero nitrogen balance for any individual, it is necessary to interpolate between given intakes to estimate individual nitrogen requirements, and we chose to use linear interpolations. We used regression to estimate the coefficients of each individual's linear interpolation and solved for the estimated requirement (93). Because of the few data points for each individual (ranging from 3 to 6), we did not consider the statistical properties of the fits (indeed many of the lines are not statistically significant). We used them only to derive the estimate of the intercept.

We appreciate that the biological relation between nitrogen intake and nitrogen retention cannot be linear over the full range from very low, submaintenance to very high, supramaintenance intakes (80). Indeed, many alternative models might be used to describe nutrient dose-response relations, for example, the saturation kinetics model (94), the Michaelis-Menton type model (95), and the biphase linear model (96–98). However, the primary estimation studies gathered nitrogen balance data at intakes relatively close to those that might be expected to produce zero balance. In this range, with these few data points, there was no support for the use of a nonlinear interpolation scheme for the population response (**Figure 2**).

Estimated average requirement for the population

We combined individual estimated requirements to produce the estimated average requirement (EAR) for the population in 2 different ways; both were estimates of the median of the requirements, ie, that intake sufficient to meet the requirement of 50% of healthy individuals. First, the median of the entire sample of individual subjects from the primary estimation studies was calculated (weighting all individual subjects equally) and, second, the median of each distinct substudy (as described above, if a study examined 2 different diets, sexes, or ages, the study was split into consistent substudies) were calculated, and the median of these substudy medians was used as the EAR (weighting each substudy equally). The median was chosen for the population estimate because individual requirements are not normally distributed, either overall or within studies, as shown graphically in **Figure 3**. Individual requirements are significantly skewed and kurtotic and are characterized by the presence of more than expected very large or very small requirements. In this case, the mean is not a robust estimate of the center of the population. Although it could perhaps be suggested that these extreme values represent outliers and that their data be eliminated (by trimming as we do below for variability), we chose to include all the data to estimate the midpoint.

TABLE 2
Average dermal and miscellaneous nitrogen losses in healthy adults[1]

Study	No. of individual subjects	Nitrogen intake	Dermal and miscellaneous nitrogen losses	
			Tropical	Temperate
		*mg·kg^−1^·d^−1^*	*mg·kg^−1^·d^−1^*	
Atinmo et al, 1985 (30)	8	16	9.23	—
Atinmo et al, 1988 (31)	12	122	12.17	—
Calloway et al, 1971 (86)	6–35	1–369	—	4.13
Egun and Atinmo, 1993 (45)	12	49–102	8.14	—
Egun and Atinmo, 1993 (46)	11	96	11.47	—
Forslund et al, 1998 (87)	6, 8	160, 400	—	3.75
Huang and Lin, 1981 (88)				
Egg diet	15	72–121	8.97	—
Mixed diet	15	56–72	10.34	—
Huang et al, 1975 (89)				
Cool temperature	4	114	—	5.57
High temperature	4	110	13.87	—
Inoue et al, 1984 (90)				
Winter	5	192	14.47	—
Summer	3	192	—	5.37
Kaneko et al, 1988 (57)	4, 19	80,192	—	5.21
Oddoye and Margen, 1979 (21)	6	166, 491	—	6.46
Zanni et al, 1979 (83)	6	0	—	4.42
$\bar{x}$	—	—	11.1	5.0
SD	—	—	2.3	0.9

[1] Where miscellaneous nitrogen losses were not measured, the value of 1.77 $mg \cdot kg^{-1} \cdot d^{-1}$ was added to the reported dermal losses.

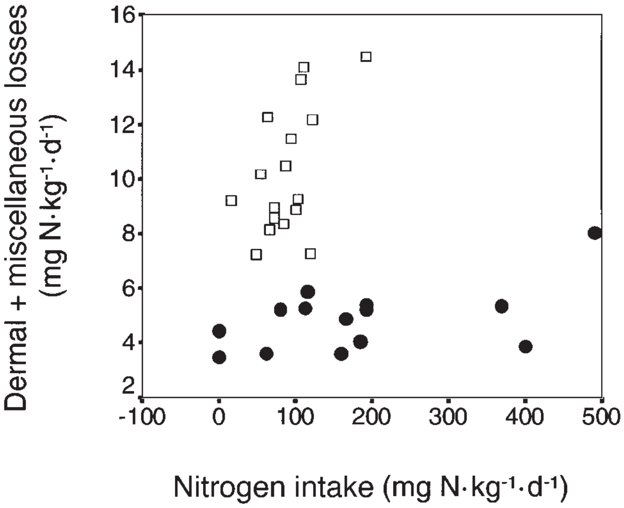

FIGURE 1. Relation between dermal + miscellaneous nitrogen losses and nitrogen intake in healthy adults. Each point represents responses to individual nitrogen intakes in studies conducted under tropical (□) or temperate (●) conditions.

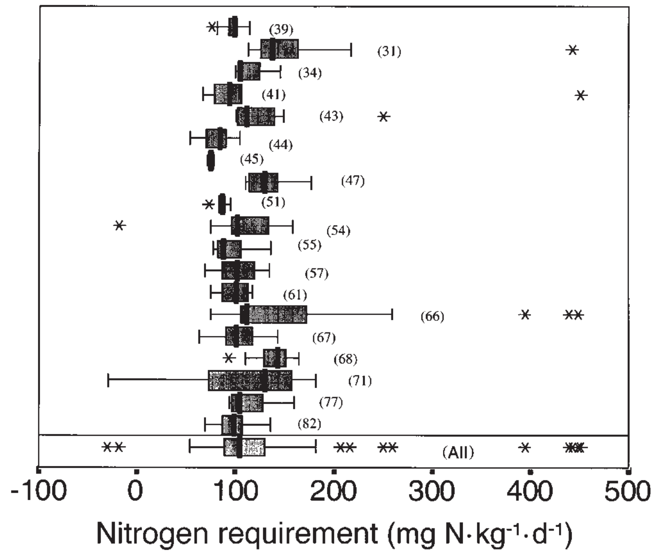

FIGURE 3. Box plots of the distribution of the nitrogen requirements of all healthy adults in the primary estimation studies. For each study, an individual box plot shows the requirement median (heavy vertical line), the 25th to 75th percentiles (the box itself), the range of the data (the "whiskers"), and potential outliers (*).

Our use of the term EAR for the median is consistent with the most recent US Food and Nutrition Board (Institute of Medicine) definition of the EAR (99).

Additional nitrogen balance studies

Because the secondary estimation studies cannot be used to estimate individual requirements, a single requirement for each substudy was estimated as the intersection with zero balance of the single linear regression of nitrogen balance (corrected for dermal and miscellaneous losses) on nitrogen intake for that substudy.

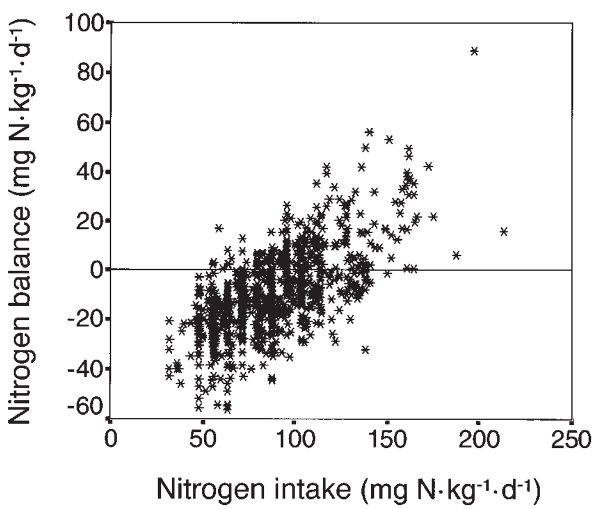

FIGURE 2. Relation between individual nitrogen balances, corrected for dermal and miscellaneous losses, and nitrogen intake in healthy adults. Individual data from all of the estimation studies examined are represented. Each point represents an individual subject's observed response to a specific intake; many subjects are represented several times.

These 13 estimates were compared with the substudy medians calculated from the primary estimation studies.

Nitrogen balance data (corrected for nitrogen intake and dermal and miscellaneous nitrogen losses) from individuals given very-low-protein diets (obligatory studies) were examined and compared with the intercepts obtained with the use of data from estimation studies. Averages and normal statistics were used because these balances were normally distributed.

The data from the test studies were also examined independently. Several different approaches were used:

1) Substudy nitrogen balance was linearly regressed on nitrogen intake:

$$\text{Average nitrogen balance} = \alpha + \beta \times \text{average nitrogen intake} \tag{2}$$

2) The probability of each substudy's means being negative, as characterized by substudy *z* scores, was linearly regressed on nitrogen intake:

$$\text{Substudy } z \text{ scores (average nitrogen balance)} = \alpha + \beta \times \text{average nitrogen intake} \tag{3}$$

3) Whether each substudy's mean nitrogen balance was positive or negative was logistically regressed on nitrogen intake:

$$\text{Probability (nitrogen intake is adequate)} = 1/[1 + \exp(-\alpha - \beta \times \text{average nitrogen intake})] \tag{4}$$

These 3 models successively reduce the amount of information that is assumed in the study data. For each substudy, the nitrogen intake that produces zero balance for Equation *2* or the intake that has a 50% probability of positive balance for Equations *3* and *4* is calculated from the estimated regression equation.

Potentially important influential factors

The effect of age, sex, diet, and climate on nitrogen requirement was assessed with the use of nonparametric tests (Mann-Whitney *U*

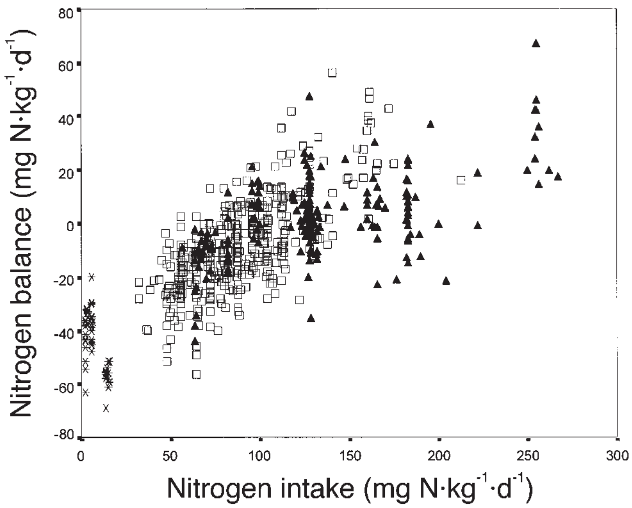

FIGURE 4. Relation between individual nitrogen balances, corrected for dermal and miscellaneous losses, and nitrogen intake in healthy adults. A randomly selected 40% of all the individual data from the estimation (□), obligatory (*), and test (▲) studies that were examined are represented. Each point represents an individual's observed response to a specific intake; many individuals are represented several times.

test for comparing 2 groups and the Kruskal-Wallis test for comparing 3 groups) because these data are not normally distributed. Separate analyses were carried out for all of the individual requirement estimates (primary estimation studies) and for the substudy medians.

Requirement variability

Estimation of the variability between individual subjects must deal with 2 problems with the data. First, the distribution of estimated requirements is skewed and several probable outliers are present (eg, subjects with a negative efficiency of utilization). Therefore, the primary estimation data set was first trimmed (5% trimming, with the 2.5% highest and 2.5% lowest values removed) and the natural logarithms taken. This resulting data set, consisting of 225 individual requirements, was normally distributed. Second, the observed variability in the data arises from 3 different sources: *1*) variability between studies, because each study was conducted at a unique place and time and often by investigators who used slightly different experimental methods; *2*) variability within individuals, because of experimental error and temporal variability; and *3*) variability between individuals, the ultimate variability estimate of interest. Analysis of variance was used to partition the total observed variability into these 3 sources with the use of transformed data. Because there were few replicate estimates of requirements measured in the same subjects, this was accomplished in 2 steps. First, analysis of variance was used to partition total variability into between-study variability and within-study variability. Separately, 20 subjects in 4 of the primary estimation studies (34, 44, 54, 77) were studied twice and the data from these subjects were analyzed with analysis of variance to estimate the ratio of between-individual to within-individual variability. This ratio was applied to the within-study variability as estimated from the full (trimmed) database to obtain an estimate of the between-individual variability. This ratio was used, with the estimate of the median, to calculate an estimate of the 97.5% point of the log requirement distribution, and this point was transformed back into the original units (by exponentiation) to estimate the 97.5% point of the population requirement.

Alternative fits to the aggregate of nitrogen balance data

As described above, our estimate of the population protein requirement is based on a combination of the results of individual estimates of requirements, calculated from linear regressions for those subjects with different intakes within a limited range. Many alternative methods of statistical analyses exist (97, 100–102), the most common of which fits all available data points without linking the subjects or restricting the range of intake. To contrast our findings with these alternative approaches, we also fitted all 1593 individual data points (all obligatory, estimation, and test data for individuals, with many individuals contributing several points) to the linear (default model, with minimal assumptions), log [as suggested by Hegsted (102)], asymptotic exponential [as used by Rand and Young (100)], and biphase linear [as used by Zello et al (96)] models:

Linear model:

$$\text{Nitrogen balance} = \alpha + \beta \times \text{nitrogen intake} \qquad (5)$$

Log model:

$$\text{Nitrogen balance} = \alpha + \beta \times \log(\text{nitrogen intake}) \qquad (6)$$

Asymptotic exponential model:

$$\text{Nitrogen balance} = \alpha - \exp(\beta - \gamma \times \text{nitrogen intake}) \qquad (7)$$

Biphase linear model:

$$\text{Nitrogen balance} = \alpha + \beta \times \text{nitrogen intake}$$
(for nitrogen intakes below γ, the breakpoint)
$$\text{Nitrogen balance} = \Delta \text{ (for nitrogen intake above } \gamma) \qquad (8)$$

The actual nonlinear fitting program used was SPSS (version 10.1; SPSS Inc, Chicago), with Levenberg-Marquardt estimation; several starting points were used to ensure a stable solution. An estimate of population requirement was calculated as the intersection of each of these fitted equations with the line of zero balance. In addition, the breakpoint of the biphase linear model was calculated, both in terms of nitrogen intake and nitrogen balance. Note that these estimates of population requirement are neither means nor medians. Moreover, this approach gives only an SE of the requirement estimate itself (the uncertainty of the estimate of requirement) rather than an estimate of how variable the requirement is between individuals.

RESULTS

The relations between nitrogen intake and nitrogen balance for all of the subjects for whom we had individual data (n = 1593, representing all the individual data from the estimation, obligatory, and test studies) are shown in **Figure 4**. This plot shows 2 important points. First, although the overall impression is that the nitrogen balance response to increasing nitrogen intake is nonlinear, as is theoretically expected and discussed above, when the type of study from which each of these data points resulted was identified, it was observed that the apparent nonlinearity was largely due to the mixture of study

TABLE 3
Medians of slope, intercept, and nitrogen requirement for estimation substudies

Reference	No. of individual studies	Climate	Age[1]	Sex	Diet	Efficiency (slope)	Zero intake intercept	Median nitrogen requirement	Estimation study type
							$mg\ N \cdot kg^{-1} \cdot d^{-1}$	$mg\ N \cdot kg^{-1} \cdot d^{-1}$	
Agarwal et al, 1984 (29)	6	Tropical	Y	M	Vegetable	0.63	−55.05	92.26	Primary
Agarwal et al, 1984 (29)	5	Tropical	Y	F	Vegetable	0.55	−54	103.5	Primary
Atinmo et al, 1988 (31)	15	Tropical	Y	M	Mixed	0.26	−38.32	137.6	Primary
Bourges et al, 1982 (34)	8	Temperate	Y	M	Vegetable	0.71	−80.8	113.6	Primary
Bourges et al, 1982 (34)	3	Temperate	Y	M	Animal	0.47	−48.1	101.7	Primary
Clark et al, 1972 (41)	5	Temperate	Y	M	Mixed	0.31	−32.4	94.47	Primary
Clark et al, 1972 (41)	1	Temperate	Y	F	Mixed	0.19	−12.7	66.84	Primary
Dutra and Vanucchi, 1984 (43)	9	Tropical	Y	M	Mixed	0.8	−85.3	111.1	Primary
Egana et al, 1992 (44)	8	Temperate	Y	M	Vegetable	0.5	−45.9	89.87	Primary
Egana et al, 1992 (44)	6	Temperate	Y	M	Animal	0.62	−35.2	75.78	Primary
Egun et al, 1993 (45)	12	Tropical	Y	F	Mixed	0.49	−36.74	75.19	Primary
Fajardo et al, 1981 (47)	5	Tropical	Y	M	Mixed	0.47	−66.9	143.3	Primary
Fajardo et al, 1981 (47)	2	Tropical	Y	F	Mixed	0.49	−54.5	130.1	Primary
Fajardo et al, 1981 (47)	7	Tropical	Y	M	Vegetable	0.84	−93.3	111.6	Primary
Hussein, 1984 (51)	8	Tropical	Y	F	Mixed	0.74	−64.45	86.78	Primary
Inoue et al, 1981 (54)	5	Temperate	Y	M	Vegetable	0.32	−41.8	146.2	Primary
Inoue et al, 1981 (54)	7	Temperate	Y	M	Animal	0.35	−34.9	100.5	Primary
Inoue et al, 1981 (54)	8	Temperate	Y	M	Mixed	0.42	−41.1	99.35	Primary
Istfan et al, 1983 (55)	8	Temperate	Y	M	Vegetable	0.51	−44.15	87.73	Primary
Kaneko et al, 1988 (57)	12	Temperate	Y	F	Mixed	0.44	−46	102.6	Primary
Ozalp et al, 1984 (61)	11	Temperate	Y	M	Mixed	0.55	−53.3	100.3	Primary
Scrimshaw et al, 1983 (66)	8	Temperate	Y	M	Vegetable	0.32	−41.15	118.3	Primary
Scrimshaw et al, 1983 (66)	6	Temperate	Y	M	Animal	0.53	−58.85	108.4	Primary
Scrimshaw et al, 1983 (66)	8	Temperate	Y	M	Vegetable	0.28	−28.7	134.8	Primary
Thomas et al, 1979 (67)	7	Temperate	Y	F	Vegetable	0.33	−29.1	101	Primary
Tontisirin et al, 1981 (68)	13	Tropical	Y	M	Animal	0.5	−54.4	143.9	Primary
Uauy et al, 1978 (71)	7	Temperate	O	M	Animal	0.28	−35.4	73.36	Primary
Uauy et al, 1978 (71)	7	Temperate	O	F	Animal	0.33	−37.9	148.4	Primary
Yanez et al, 1982 (77)	8	Temperate	Y	M	Animal	0.61	−58.15	96.47	Primary
Yanez et al, 1982 (77)	7	Temperate	Y	M	Mixed	0.77	−85	127.8	Primary
Young et al, 1984 (82)	8	Temperate	Y	M	Vegetable	0.5	−55.25	101.3	Primary
Young et al, 1984 (82)	7	Temperate	Y	M	Animal	0.5	−49.7	76.82	Primary
Inoue et al, 1973 (52)	11	Temperate	Y	M	Animal	0.41	−41.7	101.7	Secondary
Inoue et al, 1973 (52)	14	Temperate	Y	M	Vegetable	0.27	−36.8	137.1	Secondary
Huang and Lin, 1982 (50)	21	Tropical	Y	M	Animal	0.53	−53.25	101.2	Secondary
Huang and Lin, 1982 (50)	20	Tropical	Y	M	Mixed	0.41	−53.26	131.2	Secondary
Kaneko et al, 1985 (57)	15	Temperate	Y	F	Animal	0.26	–39.2	153.1	Secondary
Komatsu et al, 1983 (58)	28	Temperate	Y	M	Animal	0.14	−20.46	123.7	Secondary
Wayler et al, 1983 (75)	7	Temperate	Y	M	Animal	0.45	−52.45	117.3	Secondary
Wayler et al, 1983 (75)	7	Temperate	Y	M	Mixed	0.48	−50.48	104.7	Secondary
Wayler et al, 1983 (75)	7	Temperate	Y	M	Animal	0.41	−36.93	90.73	Secondary
Xuecun et al, 1984 (76)	10	Temperate	Y	M	Mixed	0.33	−47	142.4	Secondary
Young et al, 1973 (80)	19	Temperate	Y	M	Animal	0.39	−28.6	73.33	Secondary
Young et al, 1975 (81)	7	Temperate	Y	M	Animal	0.51	−41.9	82.16	Secondary
Young et al, 1975 (81)	8	Temperate	Y	M	Vegetable	0.27	−33.8	125.2	Secondary

[1] Y, young; O, old.

designs. Data from the estimation studies alone (Figure 2) show a linear response over the relatively narrow range studied (as do the test studies alone, although they are more variable and have a smaller slope). This shows the necessity of restricting this meta-analysis to the response of healthy subjects to short-term intakes of various nitrogen intakes (estimation studies) rather than using both the estimation and the test data combined. Second, there was a high level of variability in the nitrogen balance response data, with some subjects being in positive balance with intakes as low as 50 mg and others in negative balance at intakes > 250 mg $N \cdot kg^{-1} \cdot d^{-1}$. As discussed above, this variability arose from 3 major sources: *1*) differences between studies, *2*) differences between subjects, and *3*) differences in subjects from day to day. The separation of the amount of variability that is contributed from each of these sources and the specific estimation of the variability in nitrogen requirements for zero balance between individuals is an essential part of the current analysis.

Requirement estimation

The medians of the slopes, intercepts, and nitrogen requirements determined from each of the primary substudies for the estimation data are shown in **Table 3**. All data were corrected for consistent miscellaneous and dermal losses, and each published

TABLE 4
Estimation of the nitrogen requirement in healthy adults

Data source and factor	No. of points	Median slope	Median intercept	Median requirement
			mg N·kg⁻¹·d⁻¹	*mg N·kg⁻¹·d⁻¹*
Individual subjects				
All factors (95% CI)	235	0.47 (0.44, 0.50)	−48.1 (−51, −45)	104.6 (101, 110)
Climate				
Temperate	154	0.45	−45.3	102.8
Tropical	81	0.50	−51.9[1]	113.3[2]
Age				
Young	221	0.48	−49.4	103.9
Old	14	0.31[3]	−36.7[4]	130.5
Sex				
Male	181	0.46	−49.4	109.3
Female	54	0.47	−43.1	91.4[5]
Diet				
Animal	64	0.46	−48.8	104.0
Vegetable	77	0.47	−49.4	106.7
Mixed	94	0.48	−46.6	104.2
Primary substudies				
All factors (95% CI)	32	0.49 (0.42, 0.53)	−47.1 (−54, −41)	101.5 (96, 112)
Climate				
Temperate	22	0.45	−43.0	100.8
Tropical	10	0.52	−54.8[6]	111.3
Age				
Young	30	0.50	−48.9	101.5
Old	2	0.31	−36.7	110.9
Sex				
Male	24	0.50	−48.9	101.5
Female	8	0.46	−42.0	101.8
Diet				
Animal	9	0.50	−48.1	100.5
Vegetable	11	0.50	−45.9	103.5
Mixed	12	0.48	−49.7	101.5

[1,2,6] Significantly different from temperate: [1] $P = 0.011$, [2] $P = 0.047$, [6] $P = 0.02$.
[3,4] Significantly different from young: [3] $P = 0.003$, [4] $P = 0.025$.
[5] Significantly different from men, $P < 0.001$.

study was subdivided, where relevant, into substudies—each of which consisted of data from subjects of the same age class, or sex or with the same major dietary protein source.

As summarized in **Table 4**, the median nitrogen requirement of the 235 individual subjects in the primary estimation studies was 104.6 mg $N \cdot kg^{-1} \cdot d^{-1}$, with a median slope of 0.47 and a median intercept at zero intake of −48 mg $N \cdot kg^{-1} \cdot d^{-1}$. Approximate 95% CIs for these medians were 101 and 110 mg $N \cdot kg^{-1} \cdot d^{-1}$ for the requirement, 0.44 and 0.50 for the slope, and −51 and −45 mg $N \cdot kg^{-1} \cdot d^{-1}$ for the intercept. Note that these 95% CIs indicate how precisely these medians are known and only indirectly represent the population variability. The analysis of the medians of the 32 substudies gave essentially the same values, 101.5 mg $N \cdot kg^{-1} \cdot d^{-1}$ for the requirement, 0.49 for the slope, and −47 mg $N \cdot kg^{-1} \cdot d^{-1}$ for the intercept. Because the medians of the substudies are based on fewer data points (32 substudy medians compared with 235 individual requirements), their CIs were larger.

Factors affecting requirement

The results of a comparison of subsets of the data according to climate, age, sex, and diet are also shown in Table 4.

Climate

For climate, a marginally significant ($P < 0.047$) difference appeared for requirements derived from individual data, reflecting differences in intercept. This difference in nitrogen balance was ≈10 mg $N \cdot kg^{-1} \cdot d^{-1}$. When the analysis was conducted on primary estimation substudies, only the differences between intercepts remained.

Age

For age category, there was a difference of almost 27 mg $N \cdot kg^{-1} \cdot d^{-1}$ in the median requirement; however, the difference was not statistically significant. Although the young tended to have a lower median requirement than did the old, their values were much more variable and more positively skewed than were those for the older group (**Figure 5**). Note that there is only one study that reported individual data on requirements of older subjects (2 substudies of men and women) (71) and that the analysis of substudy medians showed no statistical significance for the effect of group age.

Sex

On the basis of the primary estimation studies, men appeared to have a statistically significantly different ($P < 0.001$) median requirement (higher by almost 20 mg $N \cdot kg^{-1} \cdot d^{-1}$) than

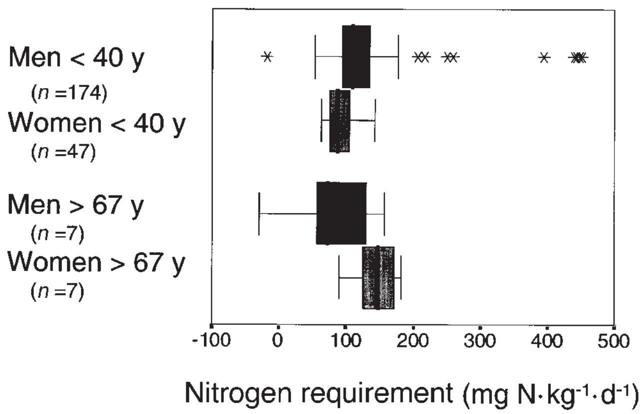

FIGURE 5. Distribution of nitrogen requirements by age and sex group in healthy adults. Individual box plots show the requirement median (heavy vertical line), the 25th to 75th percentiles (the box itself), the range of the data (the "whiskers"), and potential outliers (*).

did women. 95% CIs for these estimates were 103.6 and 113.5 for the men and 84.7 and 103.5 for the women. The significance of this difference disappeared when the substudy medians were compared; individual data highlighting the variability and skewness of the data for men are shown in Figure 5.

Diet

The major source of dietary protein was found to have an insignificant effect on the median requirement, slope, and intercept. This lack of effect of major source of dietary protein was also seen when data were analyzed by substudy.

A comparison of the nitrogen responses between the 13 secondary estimation substudies and the 32 primary estimation substudies is shown in **Table 5**. None of the secondary estimates differed significantly from the estimates based on the primary substudies, although the secondary estimates were consistently higher, and the slopes and intercepts were consistently lower than were the equivalent primary estimates.

Nitrogen losses and balance for low protein intakes

The nitrogen balance results for 14 published investigations (15 substudies) that were designed to estimate obligatory urinary and metabolic fecal nitrogen losses are shown in **Table 6**. These include 10 separate studies in young men, 2 in young women, and 2 in older adults (1 in men and 1 in both men and women). Overall, the mean nitrogen balance at near zero nitrogen intake was −47, well within the value predicted from the estimation data. Analyses of these data showed no significant difference by age category ($P = 0.14$), but women had a significantly lower (by 15 mg $N \cdot kg^{-1} \cdot d^{-1}$) value than did men (−48.8 and −35.4 mg $N \cdot kg^{-1} \cdot d^{-1}$, respectively, $P = 0.005$). Note that there were only 3 studies in women and that individual data were limited.

Test data

The results from the test studies are summarized in **Table 7** and are shown in **Figure 6**. These nitrogen balance studies were conducted to determine whether a single test intake was capable of maintaining nitrogen balance in healthy subjects. Because these studies followed a protocol that differed from that of the studies designed for estimating the requirement (usually involving a single intake given over an extended experimental period), they could not be directly compared with the results from the estimation studies. An estimate of the nitrogen requirement was obtained from these data in 3 different ways.

Linear fitting

The approach of regressing the substudy nitrogen balances on nitrogen intakes and calculating the intersection of this estimated response line with zero balance gave a requirement of 104.2 mg $N \cdot kg^{-1} \cdot d^{-1}$.

TABLE 5
Comparison of nitrogen responses between primary and secondary estimation studies[1]

	No. of substudies		Median of substudy median slopes		Median of substudy median intercepts		Median of substudy median requirements	
	Primary estimation studies	Secondary estimation studies	Primary estimation studies	Secondary estimation studies	Primary estimation studies	Secondary estimation studies	Primary estimation studies	Secondary estimation studies
					mg N·kg⁻¹·d⁻¹		*mg N·kg⁻¹·d⁻¹*	
All	32	13	0.49	0.41	−47.1	−41.7	101.5	117.3
Climate								
Temperate	22	11	0.45	0.39	−43.0	−39.2	100.8	117.3
Tropical	10	2	0.52	0.47	−54.8	−53.3	111.3	116.2
Age								
Young	30	13	0.50	0.41	−48.9	−41.9	101.5	117.3
Old	2	0	0.31	—	−36.7	—	110.9	—
Sex								
Male	24	12	0.50	0.41	−48.9	−41.8	101.5	111.0
Female	8	1	0.46	0.26	−42.0	−39.2	101.8	153.1
Diet								
Animal	9	8	0.50	0.41	−48.1	−40.5	100.5	101.2
Vegetable	11	2	0.50	0.27	−45.9	−35.3	103.5	131.2
Mixed	12	3	0.48	0.41	−49.7	−50.5	101.5	131.2

[1] There were no significant differences between the primary and secondary studies.

TABLE 6
Summary of data for obligatory nitrogen losses in healthy adults

	Sex	Age[1]	No. of subjects	Nitrogen intake	Urinary nitrogen	Fecal nitrogen	Nitrogen balance[2]
				$mg \cdot kg^{-1} \cdot d^{-1}$	$mg \cdot kg^{-1} \cdot d^{-1}$	$mg \cdot kg^{-1} \cdot d^{-1}$	$mg \cdot kg^{-1} \cdot d^{-1}$
Individual substudies							
Atinmo et al, 1985 (30)	M	Y	15	14.8	44.8	20.2	−59.43 ± 5.7
Bodwell et al, 1979 (33)	F	Y	11	1.8	30.7	7.7	−41.4 ± 6.1
Bodwell et al, 1979 (33)	M	Y	13	1.8	30.9	8.8	−42.7 ± 6.8
Bricker and Smith, 1951 (36)	F	Y	25	3	25.2	8.7	−35.7 ± 4.1
Calloway and Margen, 1971 (37)	M	Y	13	0	38	14	−55.59 ± 7.6
Huang et al, 1972 (49)	M	Y	50	5	33.4	13.1	−52.5 ± 5.3
Inoue et al, 1974 (53)	M	Y	9	2	33.3	12.7	−52 ± 3.7
Nicol and Phillips, 1976 (59)	M	Y	9	14.7	34	23	−53.3 ± 6.4
Scrimshaw et al, 1972 (64)	M	Y	83	11	37.2	8.8	−39.8 ± 6
Scrimshaw et al, 1976 (65)	F	O	11	10	24.4	9.8	−29 ± 6.3
Tontisirin et al, 1981 (69)	M	Y	4	0	34.9	12.6	−58.5 ± 4.2
Uauy et al, 1978 (72)	M	O	8	0	34.5	12.2	−51.5 ± 11.2
Uauy et al, 1982 (73)	M	Y	8	6.7	36.2	16.1	−50.4 ± 9.9
Young and Scrimshaw, 1968 (79)	M	Y	8	6	36.6	9	−44.4 ± 3.2
Zanni et al, 1979 (83)	M	O	6	0.9	27.3	9.5	−40.32 ± 3.4
Substudy comparisons							
12 substudies in males	—	—	—	—	—	—	−50.0 ± 6.6
3 substudies in females	—	—	—	—	—	—	−35.4 ± 5.6[3]
12 substudies in the young	—	—	—	—	—	—	−48.8 ± 6.0
3 substudies in the old	—	—	—	—	—	—	−40.3 ± 7.7
All 15 substudies	—	—	—	—	—	—	−47.1 ± 6.4

[1] Y, young; O, old.
[2] $\bar{x}$ ± SD.
[3] Significantly different from the 12 substudies in males, $P = 0.005$.

Probability fitting

The use of the means and SDs of each substudy to calculate a probability of adequacy for each substudy and fitting these to a normal distribution gave an estimate of 113.2 mg $N \cdot kg^{-1} \cdot d^{-1}$ for the intake that has a 50% probability of being adequate.

Logistic fitting

The classification of each substudy intake as being adequate or inadequate on the basis of whether it produced and average nitrogen balance that was positive and then regressing it on nitrogen intake produced an estimate of 102.1 mg $N \cdot kg^{-1} \cdot d^{-1}$ for the nitrogen intake that has a 50% probability of being adequate.

Estimation of requirement variability

Trimming (5%) and log transformation of individual requirement estimates produced a normally distributed variable, the variability of which was partitioned between the 3 sources of variability (between studies, between individuals, and within individuals). As shown in **Table 8**, this was accomplished in 2 steps. First, we split the variability of the requirement estimates of the (trimmed) primary database as follows:

$$\text{Total observed variability} = \text{between-study variability} + \text{within-study variability} \qquad (9)$$

This showed that, in these data, 40% of the observed variance could be ascribed to the differences between studies, whereas 60% of the variances could be ascribed to the variance within studies. Second, we examined the 20 replicated individuals and found that two-thirds of the within-study variance was represented within individual variance (temporal or experimental error), whereas one-third represented true between-individual variance.

Combining these steps gave an estimate of 0.12 for the between-individual SD of the log requirement. Because log requirements are normally distributed, the 97.5th percentile was calculated as the log median plus 1.96 times this SD. Exponentiation of this value gave 132 as the estimate of the 97.5th percentile of the population distribution of requirement. This is an estimate of the RDA (99).

Although a meaningful SD cannot be calculated for the requirement because of its skewness, for comparative purposes, one-half of the difference between the estimated 16th and 84th percentiles (which would contain those individual within 1 SD of the mean for a normal distribution) was calculated as 12.5, with a comparative CV of ≈12%.

Fitting of alternative models

The results of fitting different models to the totality of all the individual data points from the estimation, obligatory, and test studies combined (n = 1593) and solving for a requirement estimate were as follows: linear model (intake for zero balance = 122.5 mg $N \cdot kg^{-1} \cdot d^{-1}$; $R^2 = 0.54$), logarithmic model (intake for zero balance = 135.2 mg $N \cdot kg^{-1} \cdot d^{-1}$; $R^2 = 0.45$), asymptotic growth exponential model (intake for zero balance = 116.8 mg $N \cdot kg^{-1} \cdot d^{-1}$; asymptote = 42.4 mg $N \cdot kg^{-1} \cdot d^{-1}$; $R^2 = 0.57$), and linear biphase (intake for zero balance = 108.3 mg $N \cdot kg^{-1} \cdot d^{-1}$; break point = 125.5 mg N intake $\cdot kg^{-1} \cdot d^{-1}$; asymptote = 7.2 mg N balance $\cdot kg^{-1} \cdot d^{-1}$; $R^2 = 0.55$).

TABLE 7
Summary of test subtudies in healthy adults

Reference	Protein source	Sex	Age[1]	No. of individual studies	Nitrogen intake[2]	Nitrogen balance[2]
					mg · kg⁻¹ · d⁻¹	*mg · kg⁻¹ · d⁻¹*
Atinmo et al, 1988 (31)	Mixed	M	Y	12	122.8 ± 11.6	6.47 ± 8.61
Bourges et al, 1984 (35)	Vegetable	M	Y	20	164.7 ± 1.35	−0.84 ± 18.94
Campbell et al, 1994 (38)	Mixed	F	O	1	253.8	13.3
Campbell et al, 1994 (38)	Mixed	M	O	5	257.8 ± 7.03	17.46 ± 2.13
Campbell et al, 1994 (38)	Mixed	F	O	3	124 ± 4.51	2.73 ± 5.24
Campbell et al, 1994 (38)	Mixed	M	O	3	130 ± 7.51	−5.47 ± 9.93
Castaneda et al, 1995 (39)	Mixed	F	O	6	72.82 ± 9.44	−2 ± 3.36
Castaneda et al, 1995 (39)	Mixed	F	O	6	148.9 ± 20	4.88 ± 6.09
Cheng et al, 1978 (40)	Mixed	M	Y	7	254.5 ± 1.06	43.83 ± 10.04
Cheng et al, 1978 (40)	Mixed	M	O	7	254.4 ± 0.37	46.27 ± 19.27
Dutra et al, 1981 (42)	Vegetable	M	Y	14	182.8 ± 21.7	−1.33 ± 13.58
Egun and Atinmo, 1993 (46)	Mixed	F	Y	11	96.5 ± 9.4	5.8 ± 8.52
Gersovitz et al, 1982 (48)	Animal	F	O	8	128.8 ± 2.22	−2.2 ± 7.81
Gersovitz et al, 1982 (48)	Animal	M	O	7	129.7 ± 3.7	0.6 ± 9
Istfan et al, 1983 (20)	Vegetable	M	Y	6	128 ± 0	8.9 ± 3.55
Nicol and Phillips, 1976 (60)	Vegetable	M	Y	17	71 ± 3.46	−9 ± 4.74
Oddoye and Margen, 1979 (21)	Animal	M	Y	6	161.1 ± 11.4	−4.78 ± 3.88
Oddoye and Margen, 1979 (21)	Mixed	M	Y	6	480.9 ± 34.1	18 ± 2.54
Ozalp et al, 1984 (63)	Mixed	M	Y	49	98.19 ± 2.93	3.94 ± 11.46
Ozalp et al, 1984 (63)	Mixed	M	Y	15	125.2 ± 0.6	5.6 ± 16.9
Tontisirin et al, 1984 (70)	Mixed	M	Y	12	207.8 ± 25	−13.5 ± 21.2
Tontisirin et al, 1984 (70)	Mixed	M	Y	53	182.8 ± 0.81	6.01 ± 11.74
Xuecun et al, 1984 (76)	Mixed	M	Y	6	150.2 ± 2.46	5.45 ± 3.84
Yanez and Uauy, 1984 (78)	Mixed	M	Y	8	160 ± 0	9.93 ± 4.92
Young et al, 1984 (82)	Animal	M	Y	8	128 ± 0	5.6 ± 13.34
Young et al, 1984 (82)	Vegetable	M	Y	8	128 ± 0	1.8 ± 9.51
Young et al, 1984 (82)	Animal	M	Y	8	82 ± 0	−6.8 ± 12.9
Atinmo et al, 1988 (32)	Vegetable	M	Y	8	82 ± 0	−12.8 ± 11.32

[1] Y, young; O, old.
[2] $\bar{x}$ ± SD.

These R^2 values were not statistically significantly different, and their moderate magnitude indicates a great influence of individual variability. Note that all these models, when fitted to this data set, gave higher estimated nitrogen requirements than did the primary analysis.

DISCUSSION

Protein requirement

On the basis of the analysis of the nitrogen balance responses of 235 subjects in 19 separate studies, we estimated the median protein requirement (EAR) of the healthy adult population as 105 mg N · kg^{-1} · d^{-1} (0.65 g good-quality protein · kg^{-1} · d^{-1}). These data further provide an estimate of the RDA, or safe practical allowance (7), that would be expected to meet the requirements of most (97.5%) of the healthy adult population (7, 99), ie, 132 mg N · kg^{-1} · d^{-1} (0.83 g good-quality protein · kg^{-1} · d^{-1}). These values apply to everyone in these healthy populations because our analyses failed to provide compelling evidence to recommend different requirements for adult age groups, sex, or diet groups (*see* below); however, note that the data do not provide sufficient power to detect more than very major differences.

Dermal and miscellaneous nitrogen losses

The 1981 FAO/WHO/UNU Expert Consultation (7) chose to use an allowance of 8 mg N · kg^{-1} · d^{-1} for dermal and miscellaneous losses for adults, without detailed justification. Millward and Roberts (8), in their review of the literature, concluded that a value of ≤5 mg N · kg^{-1} · d^{-1} would be more appropriate. Studies in infants (103) and preadolescent children (91, 92) suggest that dermal or sweat nitrogen losses are ≈10 mg · kg^{-1} · d^{-1}, or more, and that they vary with the nitrogen intake (91, 92, 104). Because the rate of nitrogen loss via the skin varies significantly, as does body surface area, we chose to use only data from adults to estimate a suitable allowance for dermal and miscellaneous losses. Calloway et al (86) conducted meticulous studies of dermal (mainly sweat) and miscellaneous nitrogen losses in healthy adults, and they also observed that dermal nitrogen loss varied with nitrogen intake. In addition, these investigators found that there was a reasonably constant additional nitrogen loss (miscellaneous nitrogen losses via nails, hair, and tooth brushing) of ≈115 mg N/d or 1.77 mg N · kg^{-1} · d^{-1}.

Our examination of the available data indicates that dermal nitrogen losses, which would be mainly due to urea and other nitrogen constituents, were consistently higher in those studies conducted in the tropics or during the hot season than in

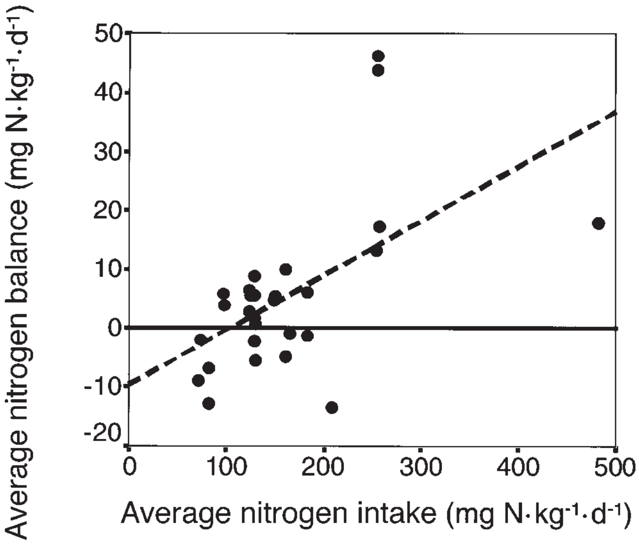

FIGURE 6. Relation in the substudies between average nitrogen balances, corrected for dermal and miscellaneous losses, and average nitrogen intakes in the test studies in healthy adults. Shown is the regression line of nitrogen balance on nitrogen intake (nitrogen balance = −9.7 + 0.093 × nitrogen intake), which intersects the zero balance line at 104.2 mg $N \cdot kg^{-1} \cdot d^{-1}$.

studies conducted in temperate or cold-weather regions. Further analysis of these data showed a significant linear relation between nitrogen intake and dermal nitrogen loss in the studies conducted in the temperate region but not in the studies conducted in the tropics. Because of the few studies available and the consistency of the results that were in the requirement range of nitrogen intakes, we estimated dermal plus miscellaneous losses as a constant 11 and 4.8 mg $N \cdot kg^{-1} \cdot d^{-1}$ for the studies conducted in tropical and temperate regions, respectively, and corrected all our data accordingly. We could not assess whether differences in ethnic groups or genotypes contributed to this difference.

It is of interest to note that the difference in the median nitrogen balance predicted for zero intake from individual response curves that was observed between the temperate- and tropic-region studies (the intercepts in Table 4) was approximately the same as the difference in dermal plus miscellaneous nitrogen losses (6.6 compared with 6.2 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively). Therefore, it seems that the higher nitrogen losses from the skin in subjects studied in the tropics were not necessarily compensated for by a lower output of nitrogen via urine. However, given the small differences and inherent variation in the data, we regard this as only reasonable speculation.

Efficiency of nitrogen utilization

Our analyses indicate that the efficiency of nitrogen utilization for retention is ≈50% in healthy adults. No differences were found between the results when the data were grouped by sex, diet, or climate. However, we did find a significantly lower efficiency for older individuals (31% compared with 48% for young adults, $P = 0.003$); this same magnitude of difference was found in the analysis of substudy medians, although the difference was not significant ($P = 0.18$). Because of the apparent interaction between age and sex in our data, the extreme variability of the younger men (Figure 5), and the fact that the lower values for the elderly came from a single study (71), we did not accept these results as conclusive. Nevertheless, they do suggest a possible age difference in nitrogen utilization that needs to be explored further and that may be related to the apparently higher first-pass splanchnic extraction of dietary leucine (105) and phenylalanine (106) in healthy elderly subjects than in younger adults.

Obligatory nitrogen losses

The linear extrapolation of the relation between the nitrogen balance response and a zero nitrogen intake gives a median negative nitrogen balance of 48 mg $N \cdot kg^{-1} \cdot d^{-1}$ (Table 4). This compares with the observed mean negative nitrogen balance of 47 mg $N \cdot kg^{-1} \cdot d^{-1}$ from the data of the 14 studies designed to quantify obligatory nitrogen losses (Table 6). This comparison suggests (as shown in Figure 4) that the relation between nitrogen balance and nitrogen intake in the submaintenance-to-maintenance range of protein intake is not significantly nonlinear.

The 3 substudies of obligatory nitrogen losses in women showed lower losses in women than in men (−35 and −50 mg N, $P = 0.005$). However, because a significant difference was not found in the intercepts from the primary data analysis (−49 for men and −43 for women, $P = 0.23$), we did not conclude that obligatory nitrogen losses differed significantly between men and women.

Test data results

The 17 studies that were designed to test specific nitrogen intakes for adequacy (Table 7) are consistent with the nitrogen requirement estimate based on the primary estimation studies. Beyond this consistency, the most striking feature of these test studies was their great variability (Figure 6), which suggests that there are important factors relating to nitrogen balance that have not been captured in the short-term nitrogen balance studies that we examined.

Implications and comparisons with previous work on protein requirements in adults

Estimation of requirements of the population

The 1981 FAO/WHO/UNU Expert Consultation (7) relied on the results of essentially 9 direct, short-term and 4 longer-term nitrogen

TABLE 8
Components of variance of the natural logarithm of the nitrogen requirement of healthy adults[1]

Analysis	Total observed variance	Variance between studies	Variance within studies	Variance within individuals	Variance between individuals
ANOVA of all subjects, 5% trim (*n* = 225)	0.0686	0.0277	0.0430	—	—
ANOVA of replicate subjects (*n* = 20)	—	—	0.0543	0.0369	0.0173

[1] The variance is the SD squared.

balance studies to derive the protein requirement of young men. The aggregated data suggest a mean protein requirement (after adjustment for 8 mg $N \cdot kg^{-1} \cdot d^{-1}$ miscellaneous nitrogen losses) of 0.63 $g \cdot kg^{-1} \cdot d^{-1}$ when subjects were studied with single sources of high-quality protein. Eight additional nitrogen balance studies with subjects given usual mixed diets were also summarized, and the mean requirement from these studies was 0.75 g protein $\cdot kg^{-1} \cdot d^{-1}$. Although the longer-term nitrogen balance studies were not specifically designed to estimate the requirement for long-term nitrogen maintenance, the Expert Consultation interpreted these data as indicating that a value of 0.58 g protein $\cdot kg^{-1} \cdot d^{-1}$ would be a sufficient mean intake for long-term nitrogen balance maintenance. From these short- and long-term balance studies, the Consultation used the mean requirements derived from the 2 data sets and, after rounding off, the average requirement for proteins of high quality, such as meat, milk, eggs, and fish, was set at 0.6 g. protein $\cdot kg^{-1} \cdot d^{-1}$.

The variation in requirements among apparently similar subjects was estimated initially by taking the mean of the CVs from the short-term balance studies. This was found to be 16.2%, and it was assumed that this variation was contributed equally by between- and within-subject variation. Hence, the true CV in requirements was calculated to be 12.5%. Consequently, a value of 25% (2 SD) above the average physiologic requirement (0.6 g protein $\cdot kg^{-1} \cdot d^{-1}$) was expected to be sufficient to meet the needs of nearly all individuals (97.5%) within a target population. This amount of good-quality protein (0.75 $g \cdot kg^{-1} \cdot d^{-1}$) was then taken to be the safe protein intake.

The present EAR for protein requirements in healthy adults is based on a considerably larger data set, with the requirement estimate being 0.65 g protein, ≈10% higher than the value of 0.6 g proposed in the 1985 FAO/WHO/UNU report (7). The present estimated RDA, 0.83 g protein, is also ≈10% higher than the 0.75 g that was recommended in 1985. Although the distribution in EARs was found to be log normally distributed, a comparative CV can be calculated as 12%.

Subgroup requirements

Sex. From studies of obligatory nitrogen losses and short-term nitrogen balance studies, the 1981 Expert Consultation (7) concluded that there was no firm evidence to suggest that a distinction could be made between men and women when setting the safe protein intake. Accordingly, the safe intake of good-quality, highly digestible protein was set at 0.75 $g \cdot kg^{-1} \cdot d^{-1}$ for both sexes.

The present analysis does not add total clarity to this issue of sex and protein requirements. Analysis of the primary estimation studies indicated a highly significantly ($P < 0.001$) lower requirement in women than in men (91 compared with 109 mg $N \cdot kg^{-1} \cdot d^{-1}$). However, the closeness of the 95% CIs for these estimates, 84.7 and 103.5 for women and 103.6 and 113.5 for men, suggests that caution be used in the interpretation. Moreover, when the medians of the primary estimation studies were compared, the difference in requirements between the men and the women disappeared.

Note that the difference observed might be expected simply on a body-composition basis, because women in general have greater BMIs than do men and about a 20% higher fat-free mass than do men. We believe that these results are especially significant because they raise the fundamental question as to what is the most appropriate method of expressing the protein requirement, namely on the basis of absolute body weight or lean body mass.

Elderly. The 1981 Expert Consultation (7) had available for review 4 nitrogen balance studies in the elderly, the results of which were not entirely consistent. The Consultation concluded that the safe intake of protein should not be < 0.75 $g \cdot kg^{-1} \cdot d^{-1}$ for older adults and the elderly. They stated that, "This figure is higher than that for young adults in relation to lean body mass, because it is an accepted fact that protein utilization is less efficient in the elderly" (7). The validity of this statement has been challenged by Millward and Roberts (8) and, whereas our analysis of the primary estimation studies did indicate a lower efficiency of nitrogen utilization ($P = 0.003$), then the estimated higher requirement of 130 mg $N \cdot kg^{-1} \cdot d^{-1}$ was not significantly different from the value for young adults. This was due, at least in part, to the high variation seen in the studies of younger men. No significant differences in either the efficiency of nitrogen retention or the requirement between older and younger subjects was observed when the substudy medians were examined.

There have been many reviews of these and related nitrogen balance data by others. Thus, Kurpad and Vaz (107) estimated from published nitrogen balance studies that the mean protein requirement was ≈0.8 $g \cdot kg^{-1} \cdot d^{-1}$. Using data from 6 nitrogen balance studies, Campbell and Evans (108) concluded that the mean protein requirement of elderly men and women may be ≥ 1 g protein $\cdot kg^{-1} \cdot d^{-1}$. Millward and Roberts (8) drew the conclusions that there was no change with age in the protein requirement per kg body weight and that there were no studies that unequivocally showed that a requirement would be higher than that defined by the FAO/WHO/UNU (7), namely 0.6 $g \cdot kg^{-1} \cdot d^{-1}$, or 0.75 $g \cdot kg^{-1} \cdot d^{-1}$, as a safe practical allowance. Essentially the same nitrogen balance data were used for the assessment in all 3 of these earlier reviews.

From our analysis, we could not conclude with confidence that healthy elderly subjects require a different and higher protein intake for maintenance of nitrogen equilibrium than do healthy young adults. There are many qualifiers to this statement. First and foremost, the data are limited and somewhat contradictory. Second, energy requirements generally decline with progressive aging; therefore, an adequate dietary protein-energy ratio would clearly be higher for the elderly. Third, factors such as infection, trauma, and disease tend to lower the efficiency of nitrogen utilization and retention and are more common in the elderly than in the young.

The conclusions to be drawn from the current analyses are that, whereas there is a suggestion that nonpregnant, nonlactating women may have a somewhat lower requirement than do men and that the healthy elderly may have a somewhat higher requirement, there is not enough evidence to make different recommendations. It is noted, however, that public health policy considerations could justify different recommendations for these various groups.

Major dietary protein source. The analysis of the primary estimation studies did not show a significant difference between studies classified according to whether the dietary protein was predominantly from animal, vegetable, or mixed-protein sources. In most cases, the experimental diets that we characterized as vegetable included complementary mixtures of vegetable proteins, such as corn and beans (34) and rice and beans (43) or good-quality soy protein (54, 55, 66, 82). These original soy studies showed clearly that the well-processed soy proteins were equivalent to animal protein, whereas wheat proteins were used with lower efficiency than were animal protein (beef) (81). Similar studies that compared rice and egg proteins (52), wheat gluten and egg proteins (53), and lupin and egg proteins (44) also showed significant differences in protein utilization between these plant- and animal-protein sources. Our aggregate analyses of all available studies obscured these results, adding to the comparative studies many studies that assessed single-protein sources. This indicates the conservative nature of our analysis and, moreover, underscores a conclusion that we drew previously (109), ie, that whereas lysine is likely to be the most limiting of the indispensable amino acids in diets based predominantly on cereal proteins, especially wheat, the risk of lysine inadequacy is substantially reduced by the inclusion of relatively modest amounts of animal or vegetable proteins, such as those from legumes and oil seeds or, where appropriate, through lysine fortification of cereal flour.

Statistical procedures involved

Our meta-analysis of the available nitrogen balance data rests on several key issues, which are outlined below.

We defined the nitrogen requirement as the nitrogen intake that would achieve zero nitrogen balance (corrected for dermal and miscellaneous losses) within an individual. At present, this is the best available approach, and it should be stressed that our analyses and recommendation are based on this criterion of protein adequacy.

For our primary analysis we selected studies that *1*) had as their primary goal the estimation of the nitrogen requirement to minimize potential confounding; *2*) published individual data on the response of each individual studied to ≥ 3 nitrogen intakes, so that both individual nitrogen requirements and within-individual variability could be estimated; and *3*) tested nitrogen intakes in a restricted range (from ≈50 to 200 mg N/kg body wt), where linear interpolation is valid.

We used linear regression to interpolate for individual nitrogen requirements. Whereas a biologically more realistic equation is certainly the best approach for modeling protein utilization in adults for whom data exist covering a wide range of intakes, the data that we used clearly do not justify anything but linear interpolation. In this relatively narrow range of intake data in the adult, the response curve is best approximated by a linear equation, and our estimate of the nitrogen requirement for each individual was obtained by fitting a straight line through their data (corrected for dermal and miscellaneous losses) and calculating the intersection of this line with zero balance.

We used variance components analysis to partition the total observed variability into between-individual and within-individual variability, working with the natural logarithms of individual requirements. The data that would permit us to directly disentangle the different sources of variability were not available; therefore, we examined components of the variability by using different data sets and then synthesized the results.

We analyzed the primary estimation data, weighted both by individual subjects and by substudy, to contrast the influence of the studies and of individual subjects on the summary values obtained. In general, the results are consistent; the major exception was the sex difference. These parallel analyses showed complexities of the data and raise questions about the potential confounding of our results because of the characteristics of the individual studies. Unfortunately, the data needed to resolve these questions do not exist.

Finally, we compared our results of estimating the requirements from data for individual subjects with estimates of requirements obtained by an overall approach of fitting all available nitrogen balance data. This comparison showed that, in addition to not providing an estimate of population variability, the latter approach, in general, tends to overestimate the requirement, irrespective of the model used.

Limitations, caveats, and other factors of importance

Confounding in the data

The identification of and testing for possible physiologic and dietary effects on protein requirements is hampered by the uncertainty of the potential confounding of the effects of these factors by other experimental conditions. As mentioned above in the discussion of the effect of dietary protein source, most studies included only a single level of the factors evaluated (eg, a single sex or age group) so that a paired analyses that would control for these factors could not be conducted. Thus, in general, we were conservative in our interpretation of the data.

Energy

It is known that nitrogen metabolism and nitrogen balance are particularly sensitive to variations in energy intake. We previously analyzed nitrogen balance data to examine the effect of energy intake on nitrogen balance and concluded that about one-third of the variation in nitrogen balance among individuals could be explained by a variation in energy intake relative to requirement (16). We did not examine the relation between energy intake and balance in the present study; in fact, we chose nitrogen balance studies in which the energy intake was intended to match energy expenditure. Despite the fact that the experimental diets and protocols were designed to achieve energy balance or equilibrium it is likely that many subjects either received somewhat higher or lower intakes than this. One result of this is that some of the variation that we have ascribed as being between individuals may well be due to the fact that the subjects were not at a similar state of energy balance or equilibrium.

Physical activity

There may be effects of physical activity per se, in addition to the question of energy balance, on protein metabolism (110–112) and, therefore, on dietary nitrogen utilization and body nitrogen balance. This possible effect may depend on the physical condition and state of training of the individual subject (113), and this could influence the status of nitrogen balance in any given experiment and the variability within and among experiments, which were not controlled for these effects.

Duration of nitrogen balance studies

We used nitrogen balance data obtained after relatively brief periods of adjustment to the experimental diets. Although there is a reasonably strong argument to be made that nitrogen requirements in adults can be judged from experimental diet periods with a duration of < 2 wk (26, 114), some investigators concluded or implied that experiments of a much longer duration are necessary to achieve a new steady state in whole-body nitrogen metabolism (115). The results obtained from the present test studies and our earlier studies (26, 114) would argue against this latter view. Furthermore, an argument was also made that there is a distinct and quantitatively important cyclic variation in nitrogen over periods of days or weeks that could seriously complicate the use of short-term nitrogen balance data for purposes of setting requirement estimates (116). Again, our earlier studies do not support this contention (117). Nevertheless, in the final analysis, a more detailed examination of the relation between length of diet period and status of nitrogen balance will be necessary before it is possible to predict accurately the longer-term consequences for function and health maintenance of the requirement intakes obtained from the short-term nitrogen balance studies.

Future work necessary

More nitrogen balance data for the subgroups examined herein would be useful, especially in terms of defining the extent and ultimately the factors that account for individual reproducibility. However, the most important task for research is an exploration of new approaches and criteria for defining the protein requirements that are not dependent on the results of short-term nitrogen balance experiments. We agree with Munro (4), who concluded in 1985 that "Measurements of safe levels of protein intake by zero balance (adults)…have achieved their potential and this approach is unlikely to yield significant further revisions of requirements." Maintenance of a measured nitrogen balance does not necessarily imply an equivalent maintenance of nitrogen balance or of protein and amino acid function in all organs and tissues. Other measures of both organ and whole-body protein adequacy are needed and the fields of molecular biotechnology, proteomics, and metabolomics need to be exploited in the search for new paradigms for nutrient requirement studies in general (118) as human nutrition expands into more challenging interdisciplinary research.

We owe a great deal of thanks to our mentor, Nevin S Scrimshaw, whose efforts and vision helped to generate much of the nitrogen balance data used for the present meta-analysis. We thank Peter Garlick, Paul Pencharz, and Peter Reeds for many stimulating discussions and suggestions and greatly appreciate the sound advice from George Beaton during the preparation and review of this manuscript.

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