Dietary protein requirements of younger and older adults[1–3]

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ABSTRACT

Background: For older men and women, the Estimated Average Requirement (EAR) and Recommended Dietary Allowance (RDA) for protein are not known with confidence. Data from the limited research studies available suggest that the EAR and RDA might be greater than the assumed 0.66 and 0.80 g protein·kg body wt^{-1}·d^{-1}, respectively.

Objective: This study assessed the effect of age on the EAR and RDA for protein.

Design: Twenty-three younger (age: 21–46 y; 11 men, 12 women) and 19 older (age: 63–81 y; 8 men, 11 women) persons completed three 18-d trials with protein intakes of 0.50, 0.75, and 1.00 g protein · kg body wt^{-1}·d^{-1}. Nitrogen balance was determined by using data from total nitrogen analyses of duplicate food composites and complete urine and feces collections from days 14 to 17 of each trial. Each subject's protein requirement was estimated by using linear regression of protein intake and nitrogen balance data from all 3 trials and inverse prediction.

Results: The mean (± SD) protein requirement was not different between the younger and older subjects: 0.61 ± 0.14 compared with 0.58 ± 0.12 g protein·kg body wt^{-1}·d^{-1}. On the basis of individual requirement estimates from the younger and older subjects combined (2.5% trimming from each tail and variation estimated by the bootstrap), an adequate protein allowance for these subjects was calculated to be 0.85 ± 0.21 g protein·kg body wt^{-1}·d^{-1}.

Conclusions: These short-term nitrogen balance results suggest that the requirement for total dietary protein is not different for healthy older adults than for younger adults and that the allowance estimate does not differ statistically from the RDA. *Am J Clin Nutr* 2008; 88:1322–9.

INTRODUCTION

Aging is associated with various metabolic and physiologic changes that may contribute to alter dietary protein requirements for older adults. These changes may include progressive changes in body composition (especially the loss of muscle mass due to sarcopenia), declines in physical activity, physical functional capacity, and total food intake and increased frequency of disease (1). The scientific foundation of the Dietary Reference Intakes for protein of adults in the United States and Canada rests primarily on data from shorter-term (2 to 3 wk) nitrogen balance studies (2, 3). The Estimated Average Requirement (EAR) of 0.66 g protein · kg body wt^{-1} · d^{-1} and the Recommended Dietary Allowance (RDA) of 0.80 g protein·kg body wt^{-1} · d^{-1} are deemed the same for all apparently healthy men and women age ≥19 y. Very limited nitrogen balance data obtained from older persons were available to support the conclusion, which

was made without great confidence, that age does not affect the need for dietary protein (3). Consensus with regard to the protein needs of older persons is lacking, with some research (4–6) and interpretive reviews (3, 7, 8) supporting the adequacy of the RDA and some research (9–13) and interpretive reviews (10, 14), which suggests that the RDA should be higher. The 2002/2005 Panel on Macronutrients from the Food and Nutrition Board of the Institute of Medicine, working in cooperation with Canadian scientists (2), affirmed the need for more data to evaluate the dietary protein requirements of older persons.

Numerous factors have weakened the results and conclusions of past nitrogen balance studies, for example, only studying men or women, not studying younger adults as a control group, inadequate periods of dietary control to establish metabolic steady state at a given protein intake, using subjects who have undocumented or uncontrolled medical conditions that could affect physiologic and metabolic status, inadequate or excessive energy intakes, incomplete collections of urine and feces, and not accounting for miscellaneous nitrogen losses. These issues can be addressed by using an accepted nitrogen balance protocol (15).

The purpose of this study was to assess the effect of age on the EAR and adequate allowance for dietary protein using a nitrogen balance protocol. On the basis of previous research and on interpretations of published nitrogen balance data (10), we hypothesized that the EAR and adequate allowance for protein would be higher in older than in younger adults.

SUBJECTS AND METHODS

Subjects

Fifty-eight individuals were recruited to participate in this study via community postings and advertisements in local newspapers. The recruits included 13 younger men (YM; 12 whites and 1 Asian), 21 younger women (YW; 15 whites and 6 African Americans), 12 older men (OM; all whites), and 12 older women (OW; all whites). All of these subjects successfully completed a

prestudy evaluation that included a written medical history, routine clinical blood and urine chemistries, and a resting-state electrocardiogram. These evaluations documented that each subject had a clinically normal serum albumin concentration, no diabetes mellitus, and clinically normal heart, liver, kidney, and thyroid function. Oral and written explanations of the study purpose and procedures were provided, and each subject signed an informed consent document. Each subject received monetary reimbursement for their participation. The study protocol, advertisements, and informed consent documents were approved by the Institutional Review Boards of the University of Arkansas for Medical Sciences and Purdue University.

Forty-eight persons completed the protocol (12 YM, 15 YW, 10 OM, and 11 OW). Data from 6 subjects (1 YM, 3 YW, and 2 OM) were not usable because of probable prostate cancer (1 OM) and noncompliance with the dietary control (other 5 subjects). Thus, data from 42 subjects were included in the analyses: 11 YM (10 whites and 1 Asian), 12 YW (10 whites and 2 African Americans), 8 OM (all whites), and 11 OW (all whites).

Experimental design

Each subject completed in random order three 18-d periods (trials) of strict dietary control, with a minimum of 1 wk of unrestricted habitual food intake between trials. The study was conducted on an outpatient basis, and only a few of the OW resided at the University of Arkansas for Medical Sciences General Clinical Research Center to decrease the burdens of transporting food supplies and urine and feces collections. All of the YW began each trial 5–7 d after the onset of their menstrual cycle. The procedures and testing were the same for each trial, except for the macronutrient distribution of the diets provided. The 3 trials were identified by the amount of protein that each subject was provided to consume: lower protein (LPro; 0.50 g protein·kg body wt^{-1}·d^{-1}, 63% of the RDA), medium protein (MPro; 0.75 g protein·kg body wt^{-1}·d^{-1}, 94% of the RDA), and higher protein (HPro; 1.00 g protein·kg body wt^{-1}·d^{-1}, 125% of the RDA). Testing of all OW and one-third of the YW was performed at the University of Arkansas for Medical Sciences between November 1998 and June 2000. Testing of the remaining YW and all YM and OM was performed at Purdue University between March 2001 and May 2003.

Diet

The subject's meals were provided by using a 3-d rotation of menus. Each daily menu was customized to provide sufficient total energy to meet the individual subject's energy requirement. Each subject's energy requirement was estimated to equal their resting energy expenditure [predicted from a sex-specific Harris-Benedict equation (16)] times 1.70 (all women and most men) up to 2.6 (>1.7 for one older man and a few YM) to account for the energy expenditure of physical activity. On the first day of each trial, the subjects were provided a very-low-protein diet (<0.2 g protein·kg body wt^{-1}·d^{-1}) that was used to quicken the metabolic adjustments to the subsequent protein intakes (15). The menus used from days 2 to 18 of each of the 3 trials included highly digestible, animal-based proteins from egg (LPro, 1.9% of total protein; MPro, 3.0%; HPro, 4.2%) and dairy (LPro, 12.0%; MPro, 29.3%; HPro, 26.6%) sources, but were void of meats because the high protein contents of muscle-containing foods make them difficult to incorporate into lower-protein menus. The

nonprotein energy content of each menu within a trial, and among the 3 trials, was maintained at 65% carbohydrate and 35% fat.

The subjects were regularly counseled to completely consume all of the foods and beverages provided to them and to not consume any nonprotocol food items. All weekday morning meals were consumed under supervision at our dining facility, and lunch, dinner, and weekend meals were packaged and taken home. Each subject agreed to scrape and rinse all utensils, dishes, and glassware with water and to consume the rinsings. Starting 1 wk before the first trial and continuing until the end of the third trial, including the periods between trials, the subjects were instructed to not ingest any self-administered vitamin and/or mineral supplements and to not consume alcohol. One multivitamin and multimineral supplement tablet (Advanced Formula Centrum; Wyeth Consume Healthcare, Madison, NJ) was provided to the subjects daily. Ad libitum water intake was allowed, encouraged, and documented (17). The energy and macronutrient contents of the menus were calculated by using Nutritionist Pro computer software (version 1.5, First Databank Inc, San Bruno, CA).

Body composition

Fasting-state nude body weight (total weight minus robe weight) was measured each weekday during the 3 trials with a digital platform scale (model 15S or ES200L; Ohaus Corporation, Pine Brook, NJ). Although body weight was measured repeatedly throughout each trial, the 6-d lead-in (trial days 1–6) to nitrogen balance assessments was not sufficient to accurately establish whether a person is weight stable (especially when they are adjusting to a new diet that could alter bowel habits, fluid balance, etc). Our experiences suggest that accurate assessments of weight stability take several weeks (average: 20 d) (18). Therefore, we made the decision to carefully monitor body weights, but to not adjust energy intakes during each trial.

Standing height without shoes was measured with a wall-mounted stadiometer. Body mass index was calculated as weight divided by height squared (kg/m^2). Whole-body volume and mass were measured by using a plethysmography system (Bod Pod; Life Measurement Instruments, Concord, CA) on days 7 and 14 of each trial, and fat-free mass, fat mass, and percentage body fat were calculated from body density by using Siri's 2-compartment model equation (19). Within groups, there were no differences between days 7 and 14 within trial or between trials; thus, individual values were averaged for presentation.

Food, urine, and stool collections

During all 3 trials, the following samples were collected and processed, and aliquots were stored at −20 °C. Duplicate portions of all foods and beverages (except ad libitum water intake) that each subject consumed from the daily menus (on days 7–10 of each trial, 12 d of food and beverages total for the study) were homogenized in a stainless steel blender dedicated to the task, and aliquots were stored frozen. On days 7–9 of each trial, complete stool collections were made for 3 d. All collections were pooled into a stainless steel blender dedicated to the task and homogenized (2 parts stool to 1 part water), and aliquots were stored frozen. The accuracy of the collections was enhanced by having each subject orally consume a stool dye marker made of encapsulated food coloring (either FD&C Blue No. 1 Alum Lake 11–13% or Carmine Red; Warner-Jenkinson Co, Saint Louis

MO) at the start and end of the 3-d collection periods. Samples were continuously collected until the end-of-collection marker was visually identified by a research technician. Fecal collections were not made during study days 14–17 to avoid logistical challenges and burdens to participants and staff if an end-of-collection fecal marker was not passed until after day 18. We do not feel this seriously compromised the calculation of nitrogen balance because previous research documented that weekly fecal nitrogen excretion remains constant when older persons consume the RDA for protein for 4 wk (12). Eight 24-h urine collections were made during days 7–10 and 14–17 of each trial. Data from the day 14–17 urine collection periods were used for the nitrogen balance calculations because the subjects had longer to establish a steady state (20).

Nitrogen analyses

Food, stool, and urine aliquots were analyzed for total nitrogen concentration with a Leco model FP-528 analyzer (Saint Joseph, MI). The FP-528 Determinator is a microprocessor-based software-controlled instrument that determines the nitrogen content of a sample during a 3-phase analyze cycle: *1*) purge—removal of any atmospheric gases from the encapsulated sample, *2*) burn—oxygen-based rapid combustion of the sample in an 850 °C furnace, and *3*) analyze—the combustion product, nitrogen in a helium carrier, is measured by a thermal conductivity cell. The instrument was calibrated daily with the use of an EDTA standard, and a soy flour standard was used to confirm accuracy. On purchase from Leco, the nitrogen content of these standards was validated by comparison with National Institute of Standards and Technologies (NIST) Typical (no. 1548a) and Whole Milk Powder (no. 8435) diet reference materials. The stability of the instrument was assessed by reanalyzing EDTA every 6–10 samples. On a representative week, the within- and between-assay CVs for the soy standard were 4.1% and 4.4%, respectively. The within- and between-assay CVs for the reanalyzed EDTA samples were 5.8% and 0.7%, respectively. The protein content of each daily menu was calculated by using the conversion factor of 6.25 g protein/g nitrogen.

Nitrogen balance and calculations of protein requirement

Each subject's apparent nitrogen balance (mg nitrogen · kg body wt^{-1} · d^{-1}) during the LPro, MPro, and HPro trials was calculated as $I_N - (U_N + F_N + M_N)$, where I_N is daily dietary nitrogen intake, U_N is daily urinary nitrogen excretion, F_N is daily fecal nitrogen excretion, and M_N is daily miscellaneous nitrogen excretions, assumed to be 5 mg N · kg body wt^{-1} · d^{-1}. The estimate of 5 mg N · kg body wt^{-1} · d^{-1} for M_N was deemed the most appropriate to use when conducting nitrogen balance experiments in temperate climates (3) and was measured in older men (6). This formula was used to calculate nitrogen balance for the data set (3) that provided the foundation for the 2002/2005 Institute of Medicine report of the Dietary Reference Intakes for protein in the United States and Canada (2).

Each subject's nitrogen balance data from all 3 trials were linearly regressed with dietary protein intake, and inverse prediction (21) was used to estimate the protein intake that corresponded with nitrogen equilibrium. This value was considered to equal the subject's protein requirement. The mean requirements of dietary protein for the YM, OM, YW, and OW groups were calculated as the mean ± SD (with 5% trimming, 2.5% trim from each tail). The adequate protein allowance, which is comparable with the RDA, was calculated as the estimated average requirement plus twice the pooled SD, estimated by the bootstrap procedure (22).

Clinical blood variables

Fasting blood samples collected on day 12 of each trial were analyzed for leukocyte content, and aliquots of serum were used to determine the concentrations of urea nitrogen (blood urea nitrogen; BUN), albumin, and aspartate aminotransferase. These analyses were performed using standard clinical procedures at Pathology and Laboratory Medicine Service, Central Arkansas Veterans Healthcare System, Little Rock, AR, or at the Laboratory Corporation of America (LabCorp, Burlington, NC).

Statistical methods

Values are reported as means ± SDs. Two-factor analysis of variance was used to compare subject characteristics and protein requirements. When a significant age-by-sex interaction was established, between-group comparisons were made by using Student's *t* test. For diet, urine, and blood variables, a three-factor, repeated-measures analysis of variance was used to assess the main effects of dietary protein intake (LPro, MPro, and HPro; within-subject effect), age (younger and older; between-subject effect), sex (male and female; between-subject effect), and their interactions. A one-sample *t* test was used to compare the estimated adequate protein allowance to the RDA. Significance was determined at $P < 0.05$. SAS 9.1.3 for Windows (SAS Institute Inc, Cary, NC) statistical software was used to perform the statistical analyses. Effect size was calculated based on the method of Cohen (23).

RESULTS

Subject characteristics, dietary intakes, and clinical blood profiles

For both sexes, height and weight did not differ significantly between the younger and older subjects, whereas percentage body fat was lower and fat-free mass was higher in the younger subjects (**Table 1**), as previously reported (17, 24). Body weights at week 2 compared with week 3 differed by 0.31 ± 0.41% (73.7 ± 13.4 and 73.4 ± 13.5 kg, respectively), based on a comparison of mean fasting body weights from week 2 (days 8–11) compared with week 3 (days 15–18 for all subjects and all 3 trials). The effect size for the difference between the mean weight at week 3 and the mean weight at week 2 was 0.021. This indicates that the mean body weight at week 3 is at the 0.508 percentile of the distribution of body weight at week 2. We concluded that this difference was very small. Individually, each subject's mean body weight at week 3 compared with week 2 differed by <1%.

Protein intake (g protein · kg body wt^{-1} · d^{-1}) was purposefully not different between the 4 groups and increased from LPro to MPro to HPro. The dietary intakes of energy, carbohydrate, and fat were higher in the younger than in the older subjects, higher for the men than for the women, and not appreciably different between the 3 trials. Fiber intakes were higher for the younger than for the older subjects and for the men than for the women and increased from LPro to MPro to HPro. Serum albumin concentration, blood leukocyte content, and serum aspartate

TABLE 1
Subject characteristics and dietary intake[1]

Variable and trial	Group			
	YM ($n = 11$)	YW ($n = 12$)	OM ($n = 8$)	OW ($n = 11$)
Age (y)	29 ± 7^a	30 ± 8^a	72 ± 6^b	75 ± 4^b
Height (cm)	179.4 ± 5.8^j	169.6 ± 5.8^k	173.1 ± 4.0^j	162.8 ± 5.3^k
Weight (kg)	79.9 ± 15.7^j	65.5 ± 8.7^k	78.6 ± 11.6^j	72.8 ± 13.1^k
BMI (kg/m^2)	$24.8 \pm 4.4^{a,b}$	22.8 ± 2.5^a	$26.2 \pm 3.5^{a,b}$	27.8 ± 4.1^b
Body fat (%)	$20.8 \pm 7.4^{a,j}$	$30.3 \pm 7.0^{a,j}$	$28.4 \pm 7.1^{b,k}$	$44.4 \pm 5.6^{b,k}$
Fat-free mass (kg)	$62.0 \pm 8.5^{a,j}$	$44.8 \pm 2.8^{a,k}$	$55.5 \pm 5.4^{b,j}$	$39.2 \pm 5.1^{b,k}$
Energy intake (MJ/d)				
LPro	$13.7 \pm 2.7^{a,j}$	$10.4 \pm 0.6^{a,k}$	$11.3 \pm 1.8^{b,j}$	$9.3 \pm 0.9^{b,k}$
MPro	$13.5 \pm 2.3^{a,j}$	$10.4 \pm 0.7^{a,k}$	$11.4 \pm 1.5^{b,j}$	$9.3 \pm 0.9^{b,k}$
HPro	$13.5 \pm 2.5^{a,j}$	$10.4 \pm 0.6^{a,k}$	$11.8 \pm 1.9^{b,j}$	$9.3 \pm 0.9^{b,k}$
Protein intake (g · kg body wt^{-1} · d^{-1})				
LPro	0.51 ± 0.01^x	0.50 ± 0.04^x	0.51 ± 0.01^x	0.50 ± 0.02^x
MPro	0.77 ± 0.02^y	0.74 ± 0.02^y	0.77 ± 0.02^y	0.76 ± 0.03^y
HPro	1.02 ± 0.02^z	0.98 ± 0.02^z	1.01 ± 0.02^z	1.01 ± 0.03^z
Carbohydrate intake (g · kg body wt^{-1} · d^{-1})				
LPro	$6.43 \pm 0.84^{a,j}$	$5.96 \pm 0.54^{a,k}$	$5.40 \pm 0.94^{b,j}$	$4.61 \pm 0.47^{b,k}$
MPro	$6.20 \pm 0.91^{a,j}$	$5.76 \pm 0.52^{a,k}$	$5.22 \pm 0.60^{b,j}$	$4.36 \pm 0.46^{b,k}$
HPro	$6.02 \pm 0.86^{a,j}$	$5.57 \pm 0.51^{a,k}$	$5.27 \pm 1.28^{b,j}$	$4.22 \pm 0.47^{b,k}$
Fat intake (g · kg body wt^{-1} · d^{-1})				
LPro	1.54 ± 0.22^a	1.42 ± 0.13^a	1.28 ± 0.22^b	1.08 ± 0.12^b
MPro	1.46 ± 0.21^a	1.38 ± 0.13^a	1.24 ± 0.15^b	1.08 ± 0.13^b
HPro	1.41 ± 0.19^a	1.34 ± 0.12^a	1.24 ± 0.26^b	1.03 ± 0.12^b
Fiber intake (g/d)				
LPro	27 ± 5^x	21 ± 2^x	26 ± 4^x	19 ± 2^x
MPro	30 ± 5^y	22 ± 2^y	28 ± 2^y	18 ± 2^y
HPro	35 ± 5^z	24 ± 2^z	35 ± 5^z	18 ± 2^z

[1] All values are $\bar{x} \pm$ SD (untrimmed data). YM, younger men; OM, older men; YW, younger women; OW, older women; LPro, lower protein (0.50 g · kg body wt^{-1} · d^{-1}); MPro, medium protein (0.75 g · kg body wt^{-1} · d^{-1}); HPro, higher protein (1.00 g · kg body wt^{-1} · d^{-1}). Means within a row with different superscript letters are significantly different by age (a and b) and by sex (j and k), $P < 0.05$. Means within a column with different superscript letters (x, y, and z) are significantly different, $P < 0.05$. Two-factor ANOVA, 3-factor ANOVA, and Student's t tests were used.

aminotransferase concentration—markers of protein status, infection, and liver function, respectively—were within the ranges of clinical normalcy for all groups at all trials; the clinical blood profile data are not shown, but were reported previously (24). The BUN concentration was lower in the younger than in the older subjects, higher in the men than in the women, and increased from LPro to MPro to HPro (**Figure 1**).

Nitrogen balance

Dietary nitrogen intakes, urinary nitrogen excretion, and apparent nitrogen balance were not different between the 4 groups and increased from LPro to MPro to HPro (**Table 2**). For all 4 groups, stool nitrogen excretion was not different between the 3 trials (data not shown), and the mean excretion was calculated and used to determine nitrogen balance.

Average requirement and adequate allowance for protein

The estimated protein requirements are presented in **Table 3**, and individual subject data are presented in Supplementary Table 1 under "Supplemental data" in the online issue. The protein requirement (g protein · kg body wt^{-1} · d^{-1}) was not related to total energy intake (MJ/d; $r^2 = 0.014$, $P = 0.45$) or to the change in body weight from week 2 to week 3 (delta kg; $r^2 = 0.048$, $P = 0.16$) in any of the subjects. When expressed on a per kilogram body weight basis, there were no significant differences between the younger and older subjects (men and women combined; $P =$

0.565) or between the men and women (younger and older subjects combined; $P = 0.849$). The 95% CIs for the mean difference in the protein requirements of the younger and older subjects
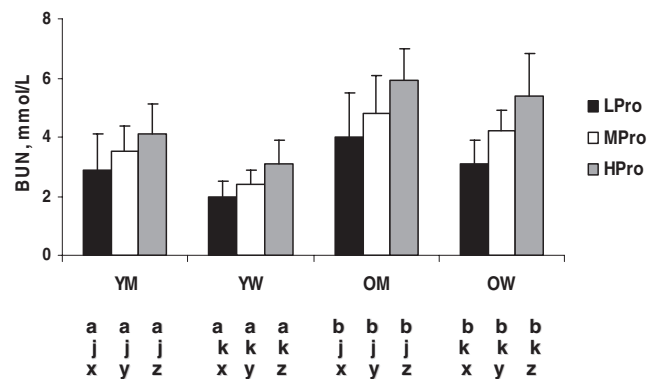

FIGURE 1. Blood urea nitrogen (BUN) concentrations when younger men (YM), younger women (YW), older men (OM), and older women (OW) consumed diets that contained 0.50 (LPro), 0.75 (MPro), and 1.00 (HPro) g protein · kg body wt^{-1} · d^{-1}. BUN was higher in the older than in the younger subjects and in the men than in the women and increased progressively from lower protein (LPro) to medium protein (MPro) to higher protein (HPro) ($P < 0.05$). The statistical significance of these effects are depicted by lowercase letters below the x axis. Means within a variable without a common lowercase letter differ by age (a and b) and by sex (j and k), $P < 0.05$. Trials within a variable without a common lowercase letter (x, y, z) differ, $P < 0.05$. Statistical analyses were conducted by using 3-factor ANOVA and Student's t tests.

TABLE 2
Total nitrogen intake, excretion, and balance for younger and older men and women[1]

| | Group | | | |
Variable and trial	YM (n = 11)	YW (n = 12)	OM (n = 8)	OW (n = 11)
	$mg\ N \cdot kg\ body\ wt^{-1} \cdot d^{-1}$			
Dietary nitrogen intake				
LPro	87 ± 6^x	85 ± 6^x	85 ± 4^x	84 ± 2^x
MPro	120 ± 7^y	121 ± 5^y	127 ± 5^y	121 ± 3^y
HPro	153 ± 8^z	160 ± 10^z	161 ± 8^z	171 ± 9^z
Urinary nitrogen excretion				
LPro	63 ± 12^x	73 ± 9^x	68 ± 9^x	57 ± 8^x
MPro	80 ± 21^y	89 ± 20^y	83 ± 12^y	90 ± 9^y
HPro	97 ± 21^z	109 ± 18^z	109 ± 12^z	102 ± 10^z
Stool nitrogen excretion[2]				
All trials	22 ± 3	16 ± 4	23 ± 3	19 ± 6
Miscellaneous nitrogen excretion[3]				
All trials	5	5	5	5
Nitrogen balance				
LPro	-4 ± 13^x	-8 ± 11^x	-11 ± 9^x	3 ± 9^x
MPro	12 ± 18^y	11 ± 18^y	16 ± 10^y	9 ± 12^y
HPro	29 ± 19^z	30 ± 19^z	24 ± 18^z	45 ± 10^z

[1] All values are $\bar{x} \pm$ SD (untrimmed data). YM, younger men; OM, older men; YW, younger women; OW, older women; LPro, lower protein (0.50 g · kg body wt$^{-1} \cdot$ d^{-1}); MPro, medium protein (0.75 g · kg body wt$^{-1} \cdot$ d^{-1}); HPro, higher protein (1.00 g · kg body wt$^{-1} \cdot$ d^{-1}). Means within a column with different superscript letters (x, y, and z) are significantly different, $P < 0.05$ (3-factor ANOVA and Student's t tests).

[2] Values are the mean nitrogen outputs from the LPro, MPro, and HPro trials combined, which were not significantly different between the 3 trials. The decision to pool the stool nitrogen data from the 3 trials was made after observing larger-than-expected within-subject variability between the trials. In hindsight, this variability should have been expected from 3-d stool collection periods. We concluded that it was appropriate to pool data to obtain a better estimate of the subject's stool nitrogen loss and that this decision did not compromise the study because published data show that stool nitrogen was not significantly influenced by protein intake (6, 10, 13) or changed over time in subjects who consumed a known and constant amount of protein for several weeks (12).

[3] Miscellaneous nitrogen excretion was assumed to be 5 mg N · kg body wt$^{-1} \cdot$ d^{-1} (3).

were $(-0.11, 0.06)$ and $(-0.08, 0.09)$ for the difference in the men compared with the women. A statistically significant age-by-sex interaction ($P = 0.002$) was observed, and subsequent analyses showed that the mean protein requirement was lower for the older women than for the older men, whereas there was no difference between the younger women and younger men. When the protein requirement data were expressed on a per kilogram fat-free mass basis, there were no differences between the young and older subjects ($P = 0.070$) or between the men and women ($P = 0.053$), and there was no age-by-sex interaction ($P = 0.253$). For all subjects combined, the adequate protein allowance was estimated to be 0.85 ± 0.21 g protein · kg body wt$^{-1} \cdot$ d^{-1}, a value that was not statistically different from the RDA of 0.80 g protein · kg body wt$^{-1} \cdot$ d^{-1} (2).

DISCUSSION

Although the nitrogen balance method is the foundation for the current RDA of protein (2, 3), and the present study was conducted using an established experimental design and procedures (15), caution is warranted when interpreting these findings because of the inherent and well-known limitations of the method (2, 8, 25). Dietary protein requirement research is at an interesting and important junction. A recommendation was made that the nitrogen balance method no longer be considered the "gold standard" for assessing dietary adequacy; however, this remains the only method with sufficient data to determine protein needs and no validated or accepted alternative method has been established (2). We share this view and conducted this study because the protein needs of older persons are not known with confidence, and a paucity of nitrogen balance data in older persons exists.

Contrary to our hypothesis, the results of this study suggest that there are no differences in the need for dietary protein between younger and older adults. Cheng et al (4) are the only other researchers to directly assess the effect of age on nitrogen balance responses to different protein intakes. They reported that the nitrogen balance responses were comparable in eight 23–29-y-old male prisoners and in seven 60–73-y-old male nursing home residents who consumed wheat-soy-milk–based liquid formula beverages that contained 0.4, 0.8, or 1.6 g protein · kg body wt^{-1} · d^{-1} during three 11-d trials. Both age groups were equally responsive to changes in protein intake, with increased nitrogen excretions and apparent nitrogen retention with increasing protein intake. The estimated need for protein was not different between the younger and older men, and the authors concluded that the RDA of 0.8 g protein · kg body wt^{-1} · d^{-1} should be adequate. Retrospective reanalyses of the Cheng et al data confirmed that age did not influence the need for protein (8, 10). However, interpretations of these data differ; some have concluded that the protein needs of both age groups are not different from the RDA (7, 8) and others that the needs are higher than the RDA (10, 14, 26).

The meta-analysis of nitrogen balance studies by Rand et al (3) that provided the foundation for the current RDA of protein (2) included individual subject data from 19 studies with a combined total of 235 subjects, but only 1 study with 14 subjects who were older (13). The median nitrogen requirements of the younger and

TABLE 3
Estimated protein requirements of younger and older men and women[1]

Subjects	$\bar{x} \pm SD$	Median	97.5th percentile	CI for the mean
Protein estimate (g protein · kg body wt^{-1} · d^{-1})				
Younger men ($n = 10$)	0.54 ± 0.15	0.51	0.83[2]	(0.43, 0.64)
Younger women ($n = 11$)	0.67 ± 0.12	0.67	0.89[2]	(0.59, 0.74)
Older men ($n = 8$)	0.65 ± 0.09	0.66	0.83[2,3]	(0.57, 0.73)
Older women ($n = 11$)	0.53 ± 0.11	0.57	0.75[2]	(0.45, 0.60)
All younger subjects ($n = 21$)	0.61 ± 0.14	0.62	0.89	—
All older subjects ($n = 19$)	0.58 ± 0.12	0.62	0.81	—
All men ($n = 18$)	0.59 ± 0.14	0.61	0.85	—
All women ($n = 22$)	0.60 ± 0.13	0.62	0.85	—
All subjects ($n = 40$)	0.59 ± 0.13	0.62	0.85	—
Protein estimate (g protein · kg FFM^{-1} · d^{-1})				
Younger men ($n = 11$)	0.73 ± 0.24	0.70	1.21	(0.57, 0.90)
Younger women ($n = 10$)	0.92 ± 0.15	0.91	1.22	(0.81, 1.03)
Older men ($n = 8$)	0.92 ± 0.14	0.91	1.20	(0.80, 1.04)
Older women ($n = 11$)	0.96 ± 0.20	1.00	1.36	(0.82, 1.09)
All younger subjects ($n = 21$)	0.82 ± 0.22	0.82	1.25	—
All older subjects ($n = 19$)	0.94 ± 0.18	0.96	1.29	—
All men ($n = 19$)	0.81 ± 0.22	0.81	1.24	—
All women ($n = 21$)	0.94 ± 0.18	0.96	1.29	—
All subjects ($n = 40$)	0.88 ± 0.21	0.89	1.28	—

[1] FFM, fat-free mass.
[2] Group-by-sex interaction, $P = 0.002$.
[3] Significantly different from the value of 0.75 in older women in a post hoc analysis (2-factor ANOVA and Student's t tests).

older subjects (data from men and women combined) were estimated to be 104 and 131 mg N · kg body wt^{-1} · d^{-1}, respectively (0.65 and 0.82 g protein · kg body wt^{-1} · d^{-1}); however, this apparent 26% difference was not statistically confirmed. Analyses of group data from studies that measured obligatory nitrogen losses or nitrogen balance responses of groups of subjects who consumed different amounts of protein also did not show an age effect on protein needs. Rand et al very cautiously stated that "whereas there is a suggestion that... the healthy elderly may have a somewhat higher requirement [for protein], there is not enough evidence to make different recommendations" (3). Earlier evaluations of the published nitrogen balance data from older persons by several researchers echoed this tentativeness: some critical reviews supported the adequacy of the current RDA (7, 8) and others questioned it (10, 14, 26). Collectively, the findings from the current study, from Cheng et al (4), and from the meta-analysis by Rand et al (3) indicate that, at the whole body level, age does not appreciably affect the need for dietary protein.

The potential limitations of these previous studies with regard to subject characteristics and metabolic states, experimental designs and methods, and the assumptions and formulas used to calculate nitrogen balance and estimate protein requirements have been discussed (2, 3, 7, 8, 10, 14, 26). For example, incomplete consumption of the foods provided and incomplete collection of urine and feces would inherently bias the nitrogen balance determination toward more positive values, which translate to a lower protein requirement. Imbalances between energy intake and energy need for weight maintenance also influence nitrogen balance (27). Inadequate energy intake would increase nitrogen excretions, lower nitrogen balance, and increase protein requirement. We share the view that the length of time of controlled feeding before measuring nitrogen balance is an especially important factor that influences the findings among studies (7, 8).

Most short-term nitrogen balance studies used 10- or 11-d protocols, with measurements made during the final 3–5 d of each trial. Whereas some data support the adequacy of this length of time to reestablish steady state at a given protein intake in older people (28), other data indicate that changes in urinary nitrogen excretion occur when 15- to 30-d protocols are used (12, 20, 29). It is interesting to note that most of the nitrogen balance data that support higher protein needs for older people were obtained from 10- or 11-d protocols, whereas data that support the adequacy of the RDA (6), including the current results, are from studies that measured nitrogen balance during the third week of dietary control. The decrease in urinary nitrogen excretion from week 2 to week 3 of each trial in the older women from the present study (20) highlights this issue and is consistent with the opinion that a longer period of adaptation to a given protein intake is an experimental design strength for nitrogen balance–based assessments of the protein needs of older adults (8).

Linear regression was used to interpolate zero nitrogen balance and to predict individual protein requirements based on the recommendation and use of this mathematical technique for the US Food and Nutrition Board (Institute of Medicine) determination of protein needs (2, 3). Alternative methods of statistical evaluation of nitrogen balance data exist (eg, log model, asymptotical exponential model, and biphasic linear model), but the linear model is deemed appropriate to use when nitrogen balance data are obtained from relatively few data points (3 for the current study) at protein intakes known to produce nitrogen balance responses close to zero balance (ie, the range of linearity for nitrogen balance responses) (3). The use of the alternative methods of statistical evaluation listed above resulted in higher estimates of protein requirements than did linear regression (3), a finding that was recently confirmed for the biphasic model (30). The biphasic model increases the protein requirement estimate

by 20% (3) to 40% (30). This model appears to address the weakness of the nitrogen balance technique that positive nitrogen balances measured when higher protein intakes are consumed are not plausible (30). A positive nitrogen balance value is likely an artifact of the inherent bias for a subject's nitrogen intake to be overestimated and nitrogen excretions to be underestimated and does not reflect an accrual of protein mass. The validity of the biphasic model-based protein requirement estimate is severely limited by the lack of nitrogen balance data from studies in which protein intakes were above the estimated breakpoint of 0.91 g protein $\cdot$ kg body wt$^{-1}\cdot$ d^{-1} reported by Humayun et al (30).

It is appropriate to consider results from longer-term experiments when evaluating the protein needs of older people. It is well established that inadequate protein intake results in detrimental accommodative responses. For example, weight-stable older women who consumed 56% of the RDA for protein for 9 wk were in a profound negative nitrogen balance (-1.1 g nitrogen/d) and experienced significant decreases in lean body mass (-4.6%), muscle mass (-13.5%), muscle fiber area (-32.7%), muscle strength (-12.0% for chest press exercise), and immune responses (-50% for the skin test response to the number of antigens 24 h after implantation) (11, 31, 32). Specific to the adequacy of the RDA, 29 older men and women who consumed the RDA for protein for 14 wk (with or without resistance exercise training) experienced decreased urinary nitrogen excretion and a positive shift in nitrogen balance from near equilibrium at baseline (study week 2) to positive at week 14 (33, 34). Whole-body leucine oxidation decreased and net leucine balance increased among all subjects. These nitrogen and leucine balance responses are consistent with adaptive responses to improve the efficiency of protein retention and utilization. The maintenance of resting energy expenditure and protein status (serum albumin concentration) among all subjects, and the maintenance and increase in skeletal muscle strength in subjects who remained sedentary or performed resistance training, respectively, also generally support the adequacy of this protein intake. Potentially adverse accommodation responses were indicated by decreases in whole-body fat-free mass (all subjects) and midthigh muscle area (sedentary group), findings that suggest that the RDA for protein might be marginally inadequate. More research is needed to assess whether the RDA is indeed an acceptable protein intake to meet the minimum needs of older persons and to evaluate whether modestly higher "optimum" protein intakes would more effectively counter sarcopenia, especially in conjunction with exercise training (35), and promote long-term health (36).

The comparable protein intake–related changes in BUN confirm previous observations in younger and older men (4, 37). They are also consistent with the equal adaptability of the younger and older subjects in the present study for albumin synthesis (24). The BUN concentrations among all of the subjects were well below the clinical threshold for renal disease or severe dehydration. The finding that BUN was higher in the older than in the younger subjects conflicts with the data of Cheng et al (4), who reported lower BUN in older than in younger men.

Conclusion

This study provides the most comprehensive nitrogen balance–based assessment of the protein needs of older men and women ever published. The results indicate that the requirement for dietary protein is not different between apparently healthy younger and older adults, and that the recommended dietary allowance of 0.8 g protein $\cdot$ kg body wt$^{-1}\cdot$ d^{-1} is adequate to meet the minimum dietary needs of virtually all older persons.

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