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Dietary Protein Requirement of Men >65 Years Old Determined by the Indicator Amino Acid Oxidation Technique Is Higher than the Current Estimated Average Requirement[1,2]

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Abstract

Background: The current estimated average requirement (EAR) and RDA for protein of 0.66 and 0.8 g · kg^{-1} · d^{-1}, respectively, for adults, including older men, are based on nitrogen balance data analyzed by monolinear regression. Recent studies in young men and older women that used the indicator amino acid oxidation (IAAO) technique suggest that those values may be too low. This observation is supported by 2-phase linear crossover analysis of the nitrogen balance data.

Objective: The main objective of this study was to determine the protein requirement for older men by using the IAAO technique.

Methods: Six men aged >65 y were studied; each individual was tested 7 times with protein intakes ranging from 0.2 to 2.0 g · kg^{-1} · d^{-1} in random order for a total of 42 studies. The diets provided energy at 1.5 times the resting energy expenditure and were isocaloric. Protein was consumed hourly for 8 h as an amino acid mixture with the composition of egg protein with L-[1-^{13}C]phenylalanine as the indicator amino acid. The group mean protein requirement was determined by applying a mixed-effects change-point regression analysis to F$^{13}CO_2$ (label tracer oxidation in breath $^{13}CO_2$), which identified a breakpoint in F$^{13}CO_2$ in response to graded intakes of protein.

Results: The estimated protein requirement and RDA for older men were 0.94 and 1.24 g · kg^{-1} · d^{-1}, respectively, which are not different from values we published using the same method in young men and older women.

Conclusions: The current intake recommendations for older adults for dietary protein of 0.66 g · kg^{-1} · d^{-1} for the EAR and 0.8 g · kg^{-1} · d^{-1} for the RDA appear to be underestimated by ~30%. Future longer-term studies should be conducted to validate these results. This trial was registered at clinicaltrials.gov as NCT01948492. *J Nutr* doi: 10.3945/jn.115.225631.

Keywords: protein requirement, older men, indicator amino acid oxidation, stable isotope, phenylalanine oxidation

Introduction

Compared with younger men, older men have lower rates of protein flux, synthesis, and breakdown with a reduced contribution of muscle (1–3) to whole-body protein catabolism (2). Although a number of underlying mechanisms contribute to the loss of lean mass, inadequate protein intake is an important risk factor (4, 5). Inadequate protein intake results in altered amino acid metabolism, loss of lean mass, decreased muscle function, and reduced immune response to stress or infection (5–7); and intakes at the current RDA have been shown by some to produce negative nitrogen balance and loss of lean mass in the elderly (8–10). Older adults suffer a higher prevalence of chronic disease than do younger adults, and a recent working group has used this as evidence for recommending protein intakes of 1.2–1.5 g · kg^{-1} · d^{-1} for this age group (11). This recommendation is supported by epidemiologic research, which showed a positive association between higher dietary protein intake and fewer health problems in the

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elderly (12), indicating a beneficial effect of a higher protein intake than current recommendations (13).

The current WHO/FAO (14) and DRI (15) recommendations for protein in healthy older adults are based on nitrogen balance data derived from studies conducted primarily in healthy young adults. The estimates are 0.66 and 0.8 g · kg^{-1} · d^{-1} for the Estimated Average Requirement (EAR)[11] and RDA, respectively (15). The RDA represents the lowest daily intake of protein estimated to meet the need of apparently healthy adults in the population (15). Therefore, comparisons to the RDA should be established in healthy individuals.

Due to limitations of the nitrogen balance method (15–17), the Institute of Medicine has recommended that alternative methods be sought to determine protein requirements. Starting with healthy young men we sought to respond to the Institute of Medicine challenge (18). In addition to applying the indicator amino acid oxidation (IAAO) method, we reanalyzed the world literature by using nitrogen balance to determine protein requirement with the use of 2-phase linear crossover analysis and found that this resulted in an estimate of protein requirement of ~0.91 g · kg^{-1} · d^{-1}, which is similar to that obtained by using the IAAO method. Next, using the minimally invasive IAAO method, we recently assessed the protein requirement of older women (>65 y) (18) and octogenarian women (19) and found it to be higher than current recommendations but not different from estimates derived in young men with the use of the same IAAO method (20). When compared on the basis of fat-free mass (FFM), the estimates derived in older women (18, 19) were higher than those derived in young men (20). However, it is uncertain whether protein requirement estimates derived in women >65 y are applicable to men in the same age category.

The primary goal of the current study was to apply the noninvasive IAAO method to the determination of the protein requirement of healthy men aged >65 y. Furthermore, we compared the protein requirement so derived with the protein requirements of their counterpart older women (18) and young healthy men that were previously determined by our group with the use of the same method (20). We hypothesized that there would be no difference in protein requirement on the basis of body weight among all 3 groups. However, the protein requirement of older men would be higher than that of young men but not different from that of older women when calculated on the basis of FFM (21).

Methods

Subjects. Six free-living older men (>65 y) were recruited and participated in the study beginning in March 2012 at the Clinical Research Center (CRC), The Hospital for Sick Children, Toronto, Canada. Subjects were excluded if they had a recent history of weight loss, chronic disease, or acute illness that could affect protein and amino acid metabolism (e.g., diabetes, cancer, liver or kidney disease, or HIV). Subjects with hypertension were not excluded if their blood pressure was well controlled and their antihypertensive medications were taken as prescribed by their physician. The Research Ethics Board at The Hospital for Sick Children approved all procedures. Informed written consent was obtained from all participating subjects. Subjects received financial compensation for their participation.

[11] Abbreviations used: BIA, bioelectrical impedance analysis; CRC, Clinical Research Center; EAR, Estimated Average Requirement; FFM, fat-free mass; IAAO, indicator amino acid oxidation; REE, resting energy expenditure.

Experimental design. The study design was based on the minimally invasive IAAO protocol (22). Before the studies commenced, each subject visited the CRC, after a 12-h overnight fast, for a prestudy assessment. During that visit, subjects had a blood sample taken for measurements of glucose, creatinine, and urea to assess for diabetes and renal function. Resting energy expenditure (REE) was measured by continuous, open-circuit indirect calorimetry (Vmax Encore metabolic cart; VIASYS), and body composition (FFM and fat mass) was measured by skinfold thickness and bioelectrical impedance analysis (BIA) as previously described (18).

Each amount of protein intake was studied over a 3-d period: 2 adaptation days followed by an IAAO study day (23). During the adaptation days, subjects received a lactose-free milkshake maintenance diet (Scandishake; Scandipharm) that supplied 1.0 g protein · kg^{-1} · d^{-1} and 1.7 × REE (18). Subjects were adapted to a set adequate protein intake, because we previously showed that previous protein intake produced a significant effect on amino acid kinetics on the study day (24). The lactose-free milkshake was chosen because, at the prestudy assessment, 5 of the 6 men reported they were lactose intolerant. In addition, from our previous study in older women (18), we also used a lactose-free milkshake on the adaptation days because many of the women reported that they were lactose intolerant. On the third study day, after a 12-h fast, subjects were randomly assigned to receive test protein intakes ranging from 0.2 to 2.0 g · kg^{-1} · d^{-1} (25). Each subject participated in 7 studies. Each 3-d study period was separated by 1–2 wk.

Study diets. The adaptation diet was weighed in daily portions for each subject and prepared as previously described (18). During the 2 adaptation days, the daily diet was consumed as 4 equal meals. Subjects consumed a daily multivitamin supplement (Centrum Cardio; Wyeth Consumer Health Care) for the duration of all studies. On the adaptation days, subjects were not allowed to consume anything else except for water, plus 1 cup of clear tea or coffee.

On each IAAO testing day, subjects presented to the CRC where they consumed 8 hourly isocaloric meals, with each meal representing 1/12th of the daily energy requirement (22). The experimental diet consisted of a protein-free liquid formula made with protein-free powder (PFD1; Mead Johnson), flavored drink crystals (Tang and Kool-Aid; Kraft Foods), grape seed oil, a crystalline amino acid mixture patterned after egg protein (representing various test protein intake amounts) (**Table 1**), and protein-free cookies. Energy was provided at 1.5 × REE. The carbohydrate content of the diets was adjusted according to the amount of protein intake to keep the diets isocaloric. The study diet provided up to 40% of the energy as fat and variable energy from carbohydrate (37–57%) and protein (3–37%), according to the test protein intake.

Tracer protocol. On each IAAO testing day, the participants consumed 4 hourly meals before the start of the hourly oral tracer protocol. Oral priming doses of 0.176 mg $NaH^{13}CO_3$ · kg^{-1} (99 atom percent excess; Cambridge Isotope Laboratories) and 0.66 mg L-[1-^{13}C]phenylalanine · kg^{-1} were given with the fifth hourly meal. An hourly oral dosing protocol of L-[1-^{13}C]phenylalanine (1.2 mg · kg^{-1} · d^{-1}) was commenced simultaneously with the fifth meal and continued for the remaining 3 h of the study. The quantity of phenylalanine supplied as L-[1-^{13}C]phenylalanine during the last 4 h of the study was subtracted from the diet to provide a total intake of 30 mg phenylalanine · kg^{-1} · d^{-1}. This intake of phenylalanine is in slight excess of the estimated phenylalanine requirement in the presence of an excess of tyrosine (26). Tyrosine was provided at 40 mg · kg^{-1} · d^{-1} to provide an excess of tyrosine, ensuring that the tracer not be delayed in the tyrosine pool (27).

Sample collection and analysis. Breath and urine samples were collected on all oxidation study days (22). Three baseline breath and urine samples were collected 45, 30, and 15 min before the tracer protocol began. Six plateau breath and 4 urine samples were collected at isotopic steady state every 30 min beginning at 2.5 h after the start of the tracer protocol. The collection and storage process has been previously described (18). During each oxidation study day, the rate of carbon dioxide production was measured immediately after the fifth

TABLE 1 Amino acid composition of reference protein and selected test protein intakes[1]

	Reference protein,[2] mg/g	Test protein intake, g · kg^{-1} · d^{-1}							
		0.20	0.50	0.80	1.00	1.20	1.50	1.80	2.00
L-Alanine	61.5	12.3	30.8	49.2	61.5	73.8	92.3	111	123
L-Arginine-HCl[3]	75.1	15.0	37.6	60.1	75.1	90.1	113	135	150
L-Asparagine	33.3	6.66	16.7	26.6	33.3	40.0	50.0	59.9	66.6
L-Aspartic acid	33.3	6.66	16.7	26.6	33.3	40.0	50.0	59.9	66.6
L-Cysteine	22.1	4.42	11.1	17.9	22.1	26.5	33.2	39.8	44.2
L-Glutamine	56.6	11.3	28.3	45.3	56.6	67.9	84.9	102	113
L-Glutamic acid	56.6	11.3	28.3	45.3	56.6	67.9	84.9	102	113
Glycine	33.3	6.66	16.7	26.6	33.3	40.0	50.0	59.9	66.6
L-Histidine	22.7	4.54	11.4	18.2	22.7	27.2	34.1	40.9	45.4
L-Isoleucine	62.8	12.7	31.4	50.2	62.8	75.4	94.2	113	126
L-Leucine	83.3	16.7	41.7	66.6	83.3	100	125	150	167
L-Lysine-HCl[3]	75.7	15.1	37.9	60.6	75.7	90.8	114	136	151
L-Methionine	29.6	5.92	14.8	23.7	29.6	35.5	44.4	53.3	59.2
L-Phenylalanine[4]	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
L-Proline	41.9	8.38	20.9	33.5	41.9	50.3	62.9	75.4	83.8
L-Serine	83.9	16.8	41.9	67.1	83.9	101	126	151	168
L-Threonine	47.1	9.42	23.6	37.7	47.1	56.5	70.7	84.8	94.2
L-Tryptophan	15.6	3.12	7.80	12.5	15.6	18.7	23.4	28.1	31.2
L-Tyrosine[5]	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
L-Valine	70.3	14.1	35.2	56.2	70.3	84.4	105	127	141

[1] Subjects each received an individual protein test amount that ranged from 0.20 to 2.00 g protein · kg^{-1} · d^{-1}.
[2] Represents the egg-protein composition.
[3] Actual amounts of amino acids were as follows: 62.1 mg arginine/g and 60.6 mg lysine/g.
[4] L-Phenylalanine intake was kept constant at 30.0 mg · kg^{-1} · d^{-1}.
[5] L-Tyrosine intake was kept constant at 40.0 mg · kg^{-1} · d^{-1}.

meal for a period of 20 min with the use of an indirect calorimeter (Vmax Encore metabolic cart; VIASYS).

Expired $^{13}CO_2$ enrichment was measured with a continuous-flow isotope ratio mass spectrometer (CF-IRMS 20/20 isotope analyzer; PDZ Europa Ltd.) as previously described (18). Enrichments were expressed as atom percent excess compared with a reference standard of compressed carbon dioxide gas. Urinary L-[1-^{13}C]phenylalanine enrichment was analyzed by an API 4000 triple quadrupole mass spectrometer (Applied Biosystems-MDS Sciex) in positive electrospray ionization mode as previously described (18). Isotopic enrichment was expressed as mole percent excess and calculated from peak area ratios at isotopic steady state at baseline and plateau. CVs between the $^{13}CO_2$ enrichment in the 4 breath samples at plateau were <5% and between the L-[1-^{13}C]phenylalanine in urine at plateau were <10%.

Estimation of isotope kinetics. Phenylalanine flux (μmol · kg^{-1} · h^{-1}) was calculated from the dilution of orally administered L-[1-^{13}C]phenylalanine into the metabolic pool (at steady state) by using enrichment of L-[1-^{13}C]phenylalanine in urine (28). The rate of appearance of $^{13}CO_2$ in breath ($F^{13}CO_2$ μmol · kg^{-1} · h^{-1}) after the oxidation of ingested L-[1-^{13}C]phenylalanine was calculated according to the model of Matthews et al. (29) by using a factor of 0.82 to account for carbon dioxide retained in the body's bicarbonate pool (30). The rate of phenylalanine oxidation (μmol · kg^{-1} · h^{-1}) was calculated from $F^{13}CO_2$ and urinary L-[1-^{13}C]phenylalanine enrichment (29).

Statistical analysis. All of the statistical analyses were performed with SAS for Windows (SAS/STAT version 9.3; SAS Institute). Statistical analyses were performed on primary and derived variables, and data are expressed as means ± SDs. Significance was established at $P \leq 0.05$.

A paired *t* test was used to test for differences in FFM and percentage of body fat to test differences in the 2 different body-composition methods of BIA and skinfold. ANOVA was used to test for differences between the various estimates of body weight and body composition (FFM and percentage of body fat) and differences between mean protein requirements of older men in the current study and older women (18) and younger men (20) from our previous studies.

The order of testing the 7 protein intakes was randomized within subjects, with the amount of protein intake serving as the main treatment effect. The effect of protein intake on phenylalanine flux, oxidation, and $F^{13}CO_2$ was tested by using a mixed linear model with subject as a random variable (PROC MIXED) by using SAS (SAS/STAT version 9.3). Differences in flux between individual subjects were compared by ANOVA, with post hoc analysis by using Bonferroni multiple-comparisons test.

The mean protein requirement was estimated by applying a nonlinear mixed-effects model (PROC NLMIXED) to the $F^{13}CO_2$ data as previously described (18, 31). Observations within subjects were regarded as statistically dependent. CIs were obtained by following the standard asymptotic theory of the maximal likelihood estimation.

Comparison of protein requirements in older adults and young men. The protein requirement estimate from the current study was compared with the protein requirement estimate from our previous studies conducted in healthy young men (20) and older women (18) with the use of ANOVA as detailed above. The requirement was compared on a kilogram body-weight basis and per kilogram of FFM as derived by using BIA.

Results

Subject characteristics. Six free-living older men participated in the study (**Table 2**). Five men were white and 1 was African Canadian. Blood glucose, urea, and creatinine were within clinically normal ranges (3.3–6.1 mmol/L, 2.9–7.1 mmol/L, and <98 μmol/L for glucose, urea, and creatinine, respectively). BMIs (in kg/m^2) ranged from 23 to 33, and body weight was maintained by each subject throughout the entire 4- to 6-mo study period. By ANOVA, there were no differences in either FFM ($P = 0.26$) or percentage of body fat ($P = 0.29$) measured by BIA compared with skinfold techniques.

Phenylalanine flux. Phenylalanine flux was not affected ($P = 0.11$) within each individual by different protein intakes as

TABLE 2 Characteristics of older men who participated in the study[1]

Characteristics	Value
Age, y	71.3 ± 4.50
Weight, kg	87.2 ± 12.7
Height, cm	177 ± 10.3
BMI, kg/m^2	27.7 ± 3.47
FFM-BIA,[2] kg	51.4 ± 5.61[3]
FFM-SF,[4] kg	54.3 ± 8.10[5]
%Fat-BIA[2]	40.6 ± 5.85[3]
%Fat-SF[4]	37.5 ± 6.25[5]
REE,[6] kcal/d	1560 ± 220
Serum glucose, mmol/L	5.13 ± 0.47
Serum urea, mmol/L	7.36 ± 1.52
Serum creatinine, μmol/L	90.2 ± 6.88

[1] All values are means ± SDs, $n = 6$. BIA, bioelectrical impedance analysis; FFM, fat-free mass; REE, resting energy expenditure; SF, skinfold thickness; %Fat, percentage of body fat.
[2] Determined by BIA.
[3] By paired *t* test, there was no difference in FFM measured by BIA and SF, $P = 0.26$.
[4] Determined by skinfold thickness.
[5] By paired *t* test, there was no difference in %Fat measured by BIA and SF, $P = 0.29$.
[6] Determined by open-circuit indirect calorimetry.

required by the IAAO method (**Table 3**). This provides evidence that the precursor pool for the IAAO did not change with changes in protein intake and suggests that changes in phenylalanine oxidation are inversely related to changes in whole-body protein synthesis.

L-[1-^{13}C]Phenylalanine oxidation. The rate of $^{13}CO_2$ released from the oxidation of L-[1-^{13}C]phenylalanine ($F^{13}CO_2$) declined in the older men with increasing protein intakes up to 0.94 g · kg^{-1} · d^{-1} (**Figure 1**). Additional increases in protein intakes did not result in changes in $F^{13}CO_2$ values. This indicates no additional increases in the incorporation of phenylalanine for protein synthesis. Mixed-effects change-point regression analysis of the $F^{13}CO_2$ data resulted in the identification of a breakpoint for the mean protein requirement of 0.94 g · kg^{-1} · d^{-1} (r^2 = 0.64, SE = 0.11). The safe population intake estimated by determining the upper 95% confidence limit of the breakpoint was at 1.24 g · kg^{-1} · d^{-1} with a lower 95% confidence limit of 0.64 g · kg^{-1} · d^{-1}.

Comparison of requirements between older men (current study) and young men and older women. **Table 4** presents the protein requirement estimates and body-composition and metabolic data for the older men in the current study compared with our recently published data in older women (>65 y) (18) and in young men (20) using the IAAO method. The older men in the current study were heavier than the older women and young men in our previous studies (18, 20). However, absolute FFM and REE, although not different between older and younger men, were significantly lower in older women. Although absolute FFM and REE were not different between old and young men, when expressed as a percentage of body weight, FFM was significantly lower in older men than in younger men (59.4% ± 5.42% compared with 81.8% ± 5.84%; $P < 0.0001$) but not different between older men and women (59.4% ± 5.42% compared with 59.9% ± 5.37%). As expected, phenylalanine flux was lower in older men and women than in young men ($P < 0.0001$).

There was no difference in protein requirements between the 3 groups when expressed per kilogram of body weight. However, the protein requirement per kilogram of FFM was higher in older adults (men and women) compared with young men. There was no difference in protein requirement expressed per kilogram of FFM between older men and women.

TABLE 3 Protein intake amounts used in individual men aged >65 y and the effect of protein intake on phenylalanine flux[1]

Subjects	Test protein intakes, g · kg^{-1} · d^{-1}	Phenylalanine flux, μmol · kg^{-1} · h^{-1}
1	0.20, 0.70, 0.80, 0.90, 1.50, 1.80, 1.90	32.7 ± 4.56^{c}
2	0.20, 0.70, 0.80, 0.90, 1.50, 1.80, 1.90	39.1 ± 3.28^{b}
3	0.50, 0.70, 0.90, 0.90, 1.10, 1.80, 2.00	23.3 ± 2.64^{d}
4	0.20, 0.50, 0.60, 0.70, 1.00, 1.50, 2.00	47.4 ± 2.34^{a}
5	0.28, 0.35, 0.50, 0.70, 1.25, 1.40, 2.00	36.1 ± 2.57^{b,c}
6	0.25, 0.95, 1.15, 1.20, 1.30, 1.58, 1.65	36.3 ± 2.51^{b,c}
Mean ± SD	—	35.8 ± 7.85

[1] Values are means ± SDs. No differences ($P > 0.05$) in phenylalanine flux were observed within each subject as a result of the test protein intakes (n = 6 men, each tested 7 times for a total of 42 observations). Means without a common superscript letter within the column differed, $P < 0.05$. Post hoc analysis was performed by using Bonferroni multiple-comparison tests. Subjects participated in a range of protein intakes (0.2–2.0 g · kg^{-1} · d^{-1}); each subject participated in 7 test intakes for a total of 42 studies.

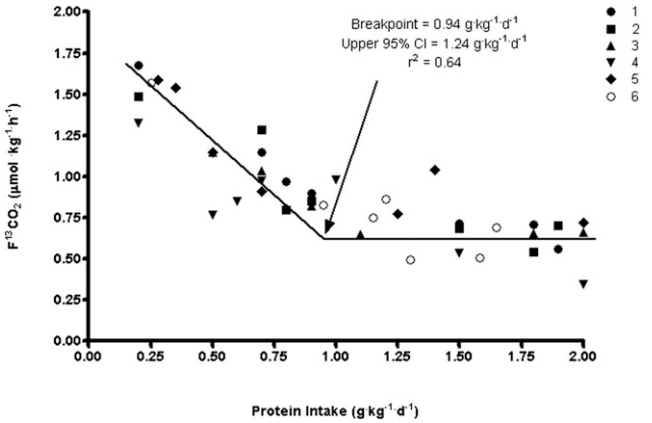

FIGURE 1 Influence of protein intake on production of $^{13}CO_2$ from phenylalanine oxidation ($F^{13}CO_2$) in older men >65 y (n = 6 men, each tested 7 times for 42 observations). The breakpoint represents the estimated mean protein requirement. A mixed-effects change-point regression analysis identified a breakpoint and upper 95% CI comparable to 2 SDs for the relation between protein intake and phenylalanine oxidation to be 0.94 and 1.24 g · kg^{-1} · d^{-1}, respectively. Values are means ± SDs.

Discussion

This is the third in our series of investigations examining protein requirements in older adults. We previously published our estimates of the protein requirement in women who were of similar ages as the men in the current study (>65 y) (18) as well as requirement estimates of octogenarian women (19). The application of the minimally invasive IAAO method to the determination of protein requirement presents a more practical, less invasive approach than nitrogen balance in vulnerable populations such as older adults, in whom exposure to repeated deficient amounts of protein intake for up to 10 d at a time is unfavorable.

When we first applied the IAAO method to determine the protein requirements of young men, we compared our estimate with a reanalysis of the nitrogen balance–based estimates of protein requirements in young men (20) with the use of nonlinear regression. The breakpoints or mean requirements were 0.91 and 0.93 g · kg^{-1} · d^{-1} on the basis of the reanalyzed nitrogen balance data and IAAO data, respectively, which is evidence of the validity of the IAAO method. In the current study, which used the IAAO technique, we estimated the mean and upper 95% confidence limit of the protein requirements of older men (>65 y) to be 0.94 and 1.24 g · kg^{-1} · d^{-1}. This mean estimate does not differ from our estimate in young men (20) or from our recently published data in older women (18) (Table 4). However it is ~30% higher than the current DRI and WHO recommendations of 0.66 and 0.8 g · kg^{-1} · d^{-1} (EAR and RDA, respectively), which are based on monolinear regression analysis of the nitrogen balance data. On the basis of FFM, the current estimated protein requirement is significantly higher in the older adults compared with young adults (20) (Table 4). This can be explained by the significant proportional decrease in FFM in the older men (current experiment) and women (18) compared with younger men (20), which has been described previously by others (2, 3, 32).

TABLE 4 Comparisons between older men (current study), older women, and young men who participated in our previous studies defining protein requirements with the use of the IAAO method[1]

Variable	Older men (current study)	Older women[2]	Young men[3]	*P*
Age, y	71.3 ± 4.50[a]	74.3 ± 7.37[a]	26.8 ± 5.62[b]	<0.0001
n	6	12	8	
Weight, kg	87.2 ± 12.7[a]	63.2 ± 9.82[b]	69.6 ± 10.4[b]	0.0007
BMI, kg/m^2	27.7 ± 3.47[a]	24.8 ± 2.33[a,b]	23.3 ± 2.79[b]	0.023
FFM-BIA, kg	51.4 ± 5.61[a]	37.9 ± 3.70[b]	56.4 ± 5.47[a]	<0.0001
%Fat-BIA	40.6 ± 5.85[a]	39.5 ± 5.09[a]	18.3 ± 5.84[b]	<0.0001
REE				
kcal/d	1560 ± 220[a]	1210 ± 105[b]	1670 ± 105[a]	<0.0001
kcal/kg	15.5 ± 2.51[a]	19.3 ± 2.00[b]	24.2 ± 2.31[c]	<0.0001
Phenylalanine flux				
μmol · kg^{-1} · h^{-1}	35.8 ± 7.85[a]	32.8 ± 11.1[a]	58.5 ± 14.6[b]	<0.0001
μmol · kg FFM^{-1} · h^{-1}	59.1 ± 19.5	55.3 ± 13.1	73.5 ± 17.0	NS
Protein requirement				
g · kg^{-1} BW · d^{-1} (EAR)	0.94	0.96	0.93	NS
g · kg^{-1} FFM · d^{-1}	1.59 ± 0.15[a]	1.62 ± 0.14[a]	1.14 ± 0.09[b]	<0.0001

[1] Values are means ± SDs unless otherwise indicated; $n = 6$ (older men >65 y), $n = 12$ (older women), and $n = 8$ (young men). Comparisons between older men in the current study, older women, and young men were performed by using ANOVA with Bonferroni correction for multiple comparisons. Means in a row without a common superscript letter differed, $P < 0.05$. BIA, bioelectrical impedance analysis; BW, body weight; EAR, Estimated Average Requirement; FFM, fat-free mass; IAAO, indicator amino acid oxidation; REE, resting energy expenditure; %Fat, percentage of body fat.
[2] Data for older women are from reference 16.
[3] Data for young men are from reference 18.

In our recent study in octogenarian women (19) that used the IAAO technique, the mean and upper 95% CI (RDA) protein requirements were determined to be 0.85 and 1.15 g · kg^{-1} · d^{-1}. Calculated on the basis of FFM, the protein requirement was estimated to be 1.41 g · kg^{-1} · d^{-1} in the older women compared with 1.59 g · kg^{-1} · d^{-1} in the current study in older men. As we reasoned earlier, the differences may be due to differences in the equipment used to measure FFM (19). The protein requirement estimates in young men (20) and octogenarian women (19) as well as the current estimate in older men provide consistent results and suggest that current DRI (15) and WHO (12) protein recommendations may underestimate actual needs.

The current RDA for protein is a minimal intake that meets most of the nutritional needs among healthy individuals in the population, but it may not promote optimal health (12) or protect elderly individuals from loss of FFM (9, 33). The current data, and those previously published (18–20), show that for healthy adults (younger and older) the protein requirement is not affected by age or sex on a body-weight basis. Increases in protein requirements on the basis on FFM in older compared with younger adults could be explained by metabolic studies that show differences in protein and amino acid metabolism between older and younger adults. For example, muscle protein synthesis is blunted in older adults compared with younger adults (34), and comparable protein synthesis rates are achieved only in the presence of a higher precursor pool of essential amino acids (35, 36). In addition, daily protein flux is lower in older adults than in younger adults due to a decrease in the contribution of skeletal muscle to whole-body protein catabolism (2). Because skeletal muscle supplies a significant percentage of the amino acids required to promote protein synthesis, this could also be a key contributor to the need for a higher protein requirement in older compared with younger individuals. Of interest is the similarity in protein requirement on the basis of FFM between older men and women (Table 4) despite a higher FFM in the older men.

Several reports provide evidence for a higher protein requirement in the elderly than suggested by current recommendations (12, 33, 37–39). Epidemiologic evidence points to an association between higher protein intake and slower bone loss (37), higher bone density (38, 39), muscle strength (33), and fewer health problems (12) in older adults. Recent cross-sectional observational analysis of the impact of dietary protein on body composition and physical performance showed that women who had a mean protein intake of 1.2 g · kg^{-1} · d^{-1}, which is higher than the current RDA (0.8 g · kg^{-1} · d^{-1}) had lower rates of osteoarthritis and fractures and better physical performance than did women in the low-protein group who had a mean protein intake of 0.7 g · kg^{-1} · d^{-1}, which is closer to the current RDA (40). More recent data from the Framingham Offspring Study showed a positive association between protein intake and higher lean muscle mass and muscle strength of quadriceps muscles in both men and women. The mean protein intake reported in that study was 0.91 and 1.1 g · kg^{-1} · d^{-1} for men and women in the entire cohort, but when analyzed by quartiles the intake ranged from 0.7 to 1.2 and 0.8 to 1.32 g · kg^{-1} · d^{-1} for men and women, respectively. The authors concluded that maintaining adequate protein intake may help preserve muscle mass and strength in men and women (41).

A criticism of the IAAO method with regard to its validity for determining protein requirement is that the rate of oxidation of the tracer does not indicate the oxidation of the rest of the dietary protein, only its own excess in relation to the overall pattern of demand (42). Although we acknowledge that the pattern of oxidation of the tracer reflects its excess relative to the overall pattern of demand of all of the other amino acids, the tracer itself is never limiting but is provided at a constant intake above its requirement. Therefore, the response of the tracer to the changes in protein intake in the current study is reflective of the increasing uptake of all amino acids for protein synthesis as the intake of protein is increased, followed by an unchanging pattern of oxidation after the protein requirement is met.

The IAAO method is widely accepted as a valid method for the determination of amino acid requirements and applies a fundamental physiologic principle of whole-body protein synthesis; if 1 amino acid is missing or limiting in the diet, protein synthesis is limited by that essential amino acid. The level of synthesis is measured by a surrogate; usually phenylalanine provided in the diet at the level of its requirement (43). Whole-body protein synthesis is limited not only by the intake of ≥1 essential amino acid but also by a suboptimal intake of total protein in the diet. The physiologic response to alteration in protein intake has been evaluated by studying whole-body nitrogen metabolism, which is presented in the many nitrogen balance studies published on protein requirement (2, 3, 14, 15). The current study as well as our previously published studies on protein requirement represent an approach in which protein metabolism is evaluated via a different endpoint, that of carbon oxidation. We propose that it is an appropriate endpoint for studying protein requirements because the study of protein metabolism fundamentally represents the combined evaluation of the sum total of amino acid metabolism, which has shown rapid response to changes in amino acid intake and whose metabolism has become more widely understood by the study of carbon oxidation and the use of ^{13}C-labeled essential amino acids (44, 45). Admittedly, the relevance of the higher requirement estimates can only be truly understood in longer-term

trials. Such studies are difficult to conduct. Therefore, more practical and feasible studies evaluating functional markers of adequacy of protein intake might serve to bridge the gap and provide relatively valid answers to the current dilemma. It is reasonable that such studies must and should be conducted with urgency.

In conclusion, it is well established that dietary protein is essential for the health and functional well-being of aging adults, yet controversy continues with regard to how much protein older adults need to meet their requirements and support physiologic vitality. The current findings in men aged >65 y suggest that the current EAR recommendation is underestimated by ~30%. Future longer-term studies based on functional indexes are needed to validate these results.

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