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Dietary Protein Requirement of Female Adults >65 Years Determined by the Indicator Amino Acid Oxidation Technique Is Higher Than Current Recommendations[1–3]

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Abstract

Background: Studies on protein requirements in vulnerable groups such as older adults are few, and results are conflicting.

Objective: The main objective of this study was to determine the protein requirements of free-living women >65 y by measuring the oxidation of L-[1-^{13}C]phenylalanine to $^{13}CO_2$ in response to graded intakes of protein.

Methods: Twelve subjects participated in the study, with protein intakes ranging from 0.2 to 2.0 g · kg^{-1} · d^{-1} for a total of 82 studies. The diets provided energy at 1.5 times each subject's resting energy expenditure and were isocaloric. Protein was given as an amino acid mixture on the basis of the egg protein pattern, except for phenylalanine and tyrosine, which were maintained constant across the protein intake amounts. All subjects were adapted for 2 d before the study day to a protein intake of 1.0 g · kg^{-1} · d^{-1}. The mean protein requirement was determined by applying a mixed-effects change-point regression analysis to F$^{13}CO_2$ (label tracer oxidation in $^{13}CO_2$ breath), which identified a breakpoint in the F$^{13}CO_2$ in response to graded amounts of protein.

Results: The mean estimated average requirement (EAR) and upper 95% CI (approximating the RDA) protein requirement of women >65 y were 0.96 and 1.29 g · kg^{-1} · d^{-1}, respectively.

Conclusion: These estimates of protein requirements for older women are higher than the current EAR and RDA based on nitrogen balance data, which are 0.66 and 0.80 g · kg^{-1} · d^{-1}, respectively. This trial was registered at clinicaltrials.gov as NCT01604980. *J Nutr* 2015;145:18–24.

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

Introduction

Aging is associated with metabolic and physiologic changes, as well as changes in body composition, with an increase in fat mass (FM)[11] and a decrease in lean mass (1–3). Age-related loss of lean mass is of particular concern because of its link to increased physical frailty and decreased ability to perform routine activities of daily living (4, 5). Although a number of underlying mechanisms contribute to the loss of lean mass, inadequate protein intake is an important risk factor (6, 7). Inadequate protein intake results in altered amino acid (AA) metabolism, loss of lean mass, decreased muscle function, as well as reduced immune response (7–9). Older adults, particularly women, tend to consume less protein than the rest of the population (10, 11). Recent evidence showed that older adults in the highest quintile of protein intake lost 40% less lean body mass over a 3-y period than those in the lowest quintile (7). Still more recent evidence showed better physical performance among postmenopausal women with higher protein intakes than those with intakes <0.8 g · kg^{-1} · d^{-1} (12).

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[3] Supplemental Table 1 is available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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[11] Abbreviations used: AA, amino acid; BIA, bioelectrical impedance analysis; CIU, Clinical Investigation Unit; EAR, Estimated Average Requirement; FM, fat mass; FFM, fat-free mass; IAAO, indicator amino acid oxidation; REE, resting energy expenditure.

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One in every 8 (13%) North Americans is >65 y old, which is an increase of >15% since 2000 (13). Persons reaching 65 y have an average life expectancy of an additional 19 y. Therefore, optimal health is of importance in this age group and is significantly affected by nutritional status. The current recommendation for protein for older adults according to the recent DRI (14) is set at the same level as for healthy young adults on the basis of nitrogen balance data. In 2002, the Estimated Average Requirement (EAR) and RDA of 0.66 and 0.8 $g \cdot kg^{-1} \cdot d^{-1}$ of good-quality protein, respectively, were recommended by the Food and Nutrition Board (14). These recommendations were based on a meta-analysis of 19 nitrogen balance studies (15) conducted in mostly healthy young adults, in which a linear regression analysis was fitted to the data. The limitations of the nitrogen balance method have been well described (16,17). In addition, Rand et al. (15) agreed that the physiologic relation between nitrogen intake and balance is not linear over a range of intakes from low to high and suggested alternative statistical approaches that can be applied to the analysis of nitrogen balance data—among them, the biphasic linear model. By using that statistical approach and the inclusion of nitrogen balance studies previously omitted from Rand et al.'s analysis in subjects who had higher intakes of nitrogen, our group performed a reanalysis of the nitrogen balance data and found a mean requirement estimate of 0.91 $g \cdot kg^{-1} \cdot d^{-1}$ (18). This estimate is very similar to 0.93 $g \cdot kg^{-1} \cdot d^{-1}$ derived by using our indicator amino acid oxidation (IAAO) protocol (18).

In general, studies determining protein requirements in older adults showed conflicting results. Although some studies suggested a higher requirement than current recommendations, others suggested a lower or similar requirement (19–22). A recent consensus report concluded that current protein recommendations for older adults are inadequate and that healthy older people should consume an average daily intake of 1.0 to 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ of protein (23). Data using the IAAO method and published by us (24) are in agreement with this consensus.

The purpose of this study, therefore, was to apply the noninvasive IAAO method to the determination of the protein requirement of older women (>65 y). Furthermore, we aimed to compare the protein requirement derived with the protein requirement of young healthy adults previously determined by our group by using the same method (18). We hypothesized that there would be no difference in protein requirement on a kilogram of body weight basis, but that older adults would have an increased protein requirement when calculated on the basis of lean body mass.

Methods

Subjects. Twelve free-living older women (>65 y) were recruited and started participation in the study beginning in October 2011 at the Clinical Investigation Unit (CIU), The Hospital for Sick Children, Toronto, Canada. There were no subjects with recent history of weight loss, chronic disease, or acute illness that could affect protein and AA metabolism (e.g., diabetes, cancer, liver or kidney disease, HIV, acute cold or flu, hypo- or hyperthyroidism, or rheumatoid arthritis treated with anti-inflammatory medications). Subjects with hypertension were not excluded if their blood pressure was well controlled and if they took their medication as prescribed by their physician. The Research Ethics Board at The Hospital for Sick Children approved all procedures. Informed written consent was obtained from the participating subjects. Subjects received financial compensation for their participation.

Experimental design. The study design was based on the minimally invasive IAAO protocol (25). Before the studies commenced, each subject visited the CIU at The Hospital for Sick Children, after a 12-h overnight fast, for a prestudy assessment. During that visit, subjects had a blood sample taken for measurement 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 [FM and fat-free mass (FFM)] was measured by skinfold-thickness analysis, bioelectrical impedance analysis (BIA), and by using the BodPod (Cosmed USA). In our previous protein requirement study in young men, we used BIA and skinfold analysis to measure body composition (18). The availability of the BodPod at our institution provided a quick, convenient method to measure body composition in older adults (no electrodes or calipers, which might damage delicate skin). Previous evidence showed less marked systematic differences among BIA, skinfold, and body density measures in elderly subjects (26). However, body density was measured by underwater weighing in that study (26). We therefore performed all 3 measures of body composition so that we could compare the results in the elderly. In addition, we needed to use BIA in this study to compare the protein requirements of young men from our previous study (18) on an FFM basis.

Four skinfold thicknesses (triceps, biceps, subscapular, and suprailiac) were measured to the nearest 1 mm with a Harpenden caliper to obtain estimates of FM (27). BIA (28) was performed by using a fixed-frequency analyzer (50 kHz; BIA model 101A: RJL Systems). Resistance (R) and reactance (X_c) measurements were made by using a 4-terminal bioelectrical impedance analyzer. Three readings of R and Xc (in Ω) were taken for each subject, and equations described previously were used to calculate FFM (29).

Each amount of protein intake was studied over a 3-d period: 2 adaptation days followed by an IAAO study day (30). During the adaptation days, subjects received a lactose-free milkshake maintenance diet (Scandishake; Scandipharm) supplying 1.0 g protein $\cdot kg^{-1} \cdot d^{-1}$ and 1.7 × REE. The lactose-free milk was made by treating homogenized milk with lactase enzyme (10 drops/L) and allowed to sit for 24 h in the refrigerator at 4°C. On the third oxidation study day, after a 12-h fast, subjects were randomly assigned to receive test protein intakes ranging from 0.2 to 2.0 $g \cdot kg^{-1} \cdot d^{-1}$) (31). Most subjects participated in 7 studies: 2 subjects in 2 studies, 2 subjects in 6 studies, 5 subjects in 7 studies, 1 subject in 10 studies, and 2 subjects in 11 studies, for a total of 83 IAAO studies. Each 3-d study period was separated by 1 to 2 wk.

Study diets. The adaptation diet was weighed in daily portions for each subject and supplemented with additional protein (Beneprotein; Nestle Clinical Nutrition) and carbohydrate (Polycose; Abbott Nutrition), depending on each subject's requirement (1.7 × the individual's REE). The milkshakes were prepared with the lactase-treated homogenized milk, which contained 3.25% fat. 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. The diet provided all of the subject's micronutrient needs on the basis of the current DRI. On the adaptation days, subjects were not allowed to consume anything except water, plus 1 cup of clear tea or coffee.

On oxidation study day 3, subjects presented at the CIU at The Hospital for Sick Children where they consumed 8 hourly isocaloric meals, with each meal representing one-twelfth of the daily energy requirement (25). The experimental diet consisted of the following: 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 AA mixture, patterned after egg protein (representing various 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 35% of energy as fat and variable energy from carbohydrate (39–58%) and protein (2–32%) according to the test protein intake.

Tracer protocol. On each oxidation study day, the participants consumed hourly meals for 4 h before the start of the oral tracer infusion protocol. Oral priming doses of 0.176 mg $NaH^{13}CO_3$/kg (99 atom percent excess; Cambridge Isotope Laboratories) and 0.66 mg

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

		Test protein intake, g/kg							
	Reference protein,[2] mg/g	0.2	0.5	0.8	1.0	1.2	1.5	1.8	2.0
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	125.9	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 0.2 to 2.0 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}.

L-[1-^{13}C]phenylalanine/kg were given with the fifth hourly meal. An hourly oral dosing protocol of L-[1-^{13}C]phenylalanine (1.2 mg · kg^{-1} · h^{-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} · h^{-1} (18, 24, 32). Tyrosine was provided at 40 mg · kg^{-1} · d^{-1} to ensure an excess of tyrosine (33).

Sample collection and analysis. Breath and urine samples were collected on all oxidation study days (25). 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. Breath samples were collected in disposable Exetainer tubes (Labco) with a collection mechanism (Easy-Sampler; Quintron) that permitted the removal of dead-space air. Breath samples were stored at room temperature, and urine samples were stored at −20°C until analysis. During each study day, the rate of carbon dioxide production (VCO_2) was measured immediately after the fifth meal for a period of 20 min with 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). 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 (31). Isotopic enrichment was expressed as mole percent excess and calculated from peak area ratios at isotopic steady state at baseline and plateau. The CV between the $^{13}CO_2$ enrichment in the 4 breath samples at plateau was <5% and the CV between the L-[1-^{13}C] phenylalanine in urine at plateau was <8%.

Estimation of isotope kinetics. The whole-body phenylalanine flux was calculated as previously described (31, 34), according to the stochastic model of Matthews et al. (35). Isotopic steady state in the tracer enrichment at baseline and plateau was represented as unchanging values of L-[1-^{13}C]phenylalanine in urine and $^{13}CO_2$ in breath.

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 (31, 35). 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. (35) by using a factor of 0.82 to account for carbon dioxide retained in the body's bicarbonate pool (36). 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 (31, 35).

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

ANOVA was used to test for differences among the various estimates of body composition (fat and FFM), and correlation analysis was performed to test for associations. Student's *t* test was used to determine differences between mean protein requirements of older adults in the current study and younger men from our previous study (18).

Protein intakes were completely 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 between individual fluxes were compared by ANOVA, with post hoc analysis using the Bonferroni multiple-comparisons test.

In our previous studies in which we determined indispensable AA (34, 37–42) and protein (18, 31) requirements, statistical analysis of the data was performed by applying a biphasic linear regression crossover analysis to determine the breakpoint (EAR). We then derived the 95% CI with the application of Filler's theorem (43). Recently, an independent group applied a new statistical approach to data from one of our previously published studies (44) in which they narrowed the width of the CI of the breakpoint (45, 46). They suggested that the application of

Filler's theorem to the derivation of the CI is invalid, because it relies on the assumption that all observations are statistically independent (46). We therefore considered their approach and applied their statistical method to our current data (45, 46) because this statistical approach is believed to provide a more precise estimate of the breakpoint and the CI.

The mean protein requirement was estimated by applying a nonlinear mixed-effects model (PROC NLMIXED; SAS Institute) to the oxidation and $F^{13}CO_2$ data. Observations within subjects were regarded as statistically dependent. CIs were obtained by following the standard asymptotic theory of the maximal likelihood estimation. The model minimizing the Akaike information criterion was regarded as the model with the best fit (46). The following statistical model was used, accounting for correlations within observations from the same subject:

$$Y_{id} = \beta 0 + b_i + \beta_1 l(x_{id} > x_{cp})(x_{id} - x_{cp}) + \varepsilon_{id} \quad (1)$$

Y_{id} = $F^{13}CO_2$ or phenylalanine oxidation at the dose of the protein of i, x_{id} is the dose amount of the test protein intake of the *i*-th subject, ϵ_{id} are random errors that are independently normally distributed with a mean of 0 and variance of σ^2. $\beta 0$ is the left line intercept, b_i is the random intercept that incorporates within-subject correlation, $\beta_1 1$ is the left line slope, X_{cp} is the breakpoint, and the slope for x_{id} is 0 for x_{id} more than the breakpoint.

Comparison protein requirement in older vs. young adults. The protein requirement estimate from the current study was compared with the protein requirement estimated from our previous study conducted in healthy young men (18). The requirement was compared on a kilogram body weight basis and per kilogram of FFM as derived by using BIA.

Results

Subject characteristics. Twelve free-living older women participated in the study. Eleven women were Caucasian and one was of Asian descent. All subjects engaged in some level of physical activity. Subject characteristics are presented in **Table 2**. Blood glucose, urea, and creatinine were within the normal range (3.3–6.1 mmol/L, 2.9–7.1 mmol/L, and <98 μmol/L for glucose, urea, and creatinine, respectively). Their ages ranged from 65 to 85 y, and BMIs (in kg/m^2) ranged from 21.4 to 29. By ANOVA, there were no differences in FFM measured by BIA, skinfold thickness, or BodPod (P = 0.63). There were also no differences in percentage fat measured by the 3 methods (P = 0.51). The correlation analysis showed an 85–87% correlation among the estimates. The women maintained their body weight over the entire period in which they participated in the study.

Phenylalanine flux. Phenylalanine flux was not affected (P = 0.3) within each individual by different protein intakes as required by the IAAO method (**Table 3**). The phenylalanine flux was 32.8 ± 11.1 μmol · kg^{-1} · h^{-1} (mean ± SD).

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 women with increasing protein intakes up to 0.96 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 that there were 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 0.96 g · kg^{-1} · d^{-1} (r^2 = 0.58). The safe population intake estimated by determining the upper 95% confidence limits of the breakpoint was at 1.29 g · kg^{-1} · d^{-1} with a lower CI of 0.65 g · kg^{-1} · d^{-1}.

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

Characteristics	Value
Age, y	74.3 ± 7.4
Weight, kg	63.2 ± 9.8
Height, cm	159 ± 7.8
BMI, kg/m^2	24.8 ± 2.3
Waist-to-hip ratio	0.87 ± 0.06
FFM, kg	
BIA	37.9 ± 3.7
SF	37.7 ± 5.7
BP	36.4 ± 5.5
%Fat	
BIA	39.5 ± 5.1
SF	39.6 ± 4.4
BP	41.5 ± 3.8
REE,[2] kcal/d	1210 ± 105
Blood glucose, mmol/L	4.9 ± 0.7
Blood urea, mmol/L	7.0 ± 1.3
Blood creatinine, μmol/L	67.0 ± 11.5

[1] All values are means ± SDs, n = 12. By ANOVA, there was no difference in FFM measured by each of the 3 methods (BIA, SF, BP), P = 0.63. By ANOVA, there was no difference in %Fat measured by each of the 3 methods (BIA, SF, BP), P = 0.51. BIA, bioelectrical impedance analysis; BP, BodPod (Cosmed USA); FFM, fat-free mass; REE, resting energy expenditure; SF, skinfold analysis.
[2] Determined by open-circuit indirect calorimetry.

Comparison of requirements in older vs. young adults. **Table 4** compares the protein requirement estimate, body composition (BIA), and metabolic data of the older women in the current study with that of 8 men from our previous IAAO study in young men (18). There was no significant difference in mean body weight, BMI, or protein requirements per kilogram of body weight. However, older women had a lower FFM than did younger men: 37.9 vs. 56.4 kg (P < 0. 01). The protein requirement per kilogram of FFM was significantly higher in older women than in younger

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

Subject	Test protein intakes, g · kg^{-1} · d^{-1}	Phenylalanine flux, μmol · kg^{-1} · h^{-1}
1	0.2, 0.8, 1.1, 1.35, 1.7,1.8	42.3 ± 10.5^{a}
2	0.4, 0.5, 0.8, 1.0, 1.2, 1.45, 1.7	26.7 ± 10.4^{b,d,f}
3	0.2, 0.3, 0.4, 0.5, 0.9, 1.0, 1.2, 1.5, 1.6, 1.8	28.0 ± 8.39^{b,f}
4	0.3, 0.5, 0.6, 0.8, 1.0, 1.15, 1.3, 1.4, 1.7, 2.0	32.5 ± 12.8^{a,b,c,d}
5	0.2, 0.4, 0.6, 0.75, 0.8, 1.6	40.8 ± 11.6^{a,b,c,d}
6	0.2, 0.3, 0.6, 0.8, 1.0, 1.1, 1.3, 1.7, 2.0	28.1 ± 3.88^{b,c,f}
7	0.3, 0.9, 1.2, 1.3, 1.4, 1.8, 2.0	32.4 ± 0.90^{a,b,c,d,e}
8	1.2, 2.0	38.0 ± 0.25^{a,b,c,d,e}
9	1.1, 1.6	26.9 ± 4.96^{a,b,f}
10	0.8, 1.45, 1.5, 1.55, 1.6,1.8	21.0 ± 2.46^{e,f}
11	0.4, 0.8, 1.1, 1.2, 1.3, 1.8, 2.0	41.8 ± 9.61^{a}
12	0.2, 0.9, 1.09, 1.1, 1.5, 1.8	34.2 ± 12.2^{a,b,c,d}
All	—	32.8 ± 11.1

[1] Values are means ± SDs. No significant differences (P > 0.05) in phenylalanine flux were observed within each subject because of various test protein intakes. Values without a common superscript letter within the column were significantly different, 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 a minimum of 2 test intakes for a total of 82 studies.

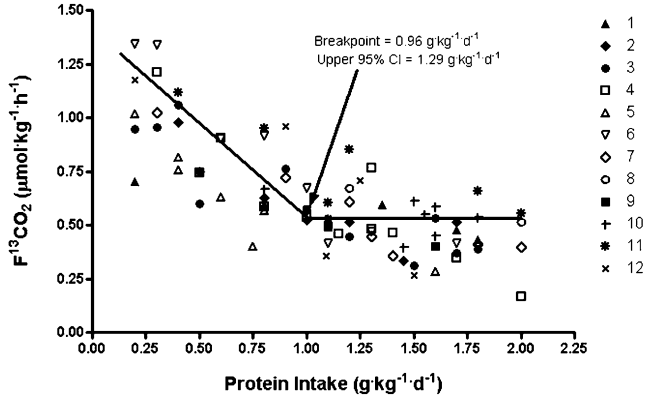

FIGURE 1 Influence of protein intake on production of $^{13}CO_2$ from phenylalanine oxidation ($F^{13}CO_2$) in older women (>65 y). Individual values are for 12 women (n = 82 observations). Individual values for each subject are represented by different symbols. The breakpoint represents the estimated mean protein requirement. A mixed-effects change-point regression analysis identified a breakpoint and upper 95% CI for the relation between protein intake and phenylalanine oxidation to be 0.96 and 1.29 g · kg^{-1} · d^{-1}, respectively.

men (P = 0.04). Conversely, REE and phenylalanine flux were significantly lower (P < 0.01) in the older adults. **Supplemental Table 1** provides the individual FFM values from BIA for the younger adults (18) and the older adults in the current study.

Discussion

This is the second study, to our knowledge, to determine protein requirements in older adults directly by using an alternative method to nitrogen balance. The first, to our knowledge, was recently published by 4 of the current authors (24) in which it was determined that the protein requirement in independent-living octogenarian women with the use of the minimally invasive IAAO method was 0.85 and 1.15 g · kg^{-1} · d^{-1} mean and RDA, respectively. The mean and RDA for protein in women aged 65–85 in the current study were estimated to be 0.96 and 1.29 g · kg^{-1} · d^{-1}, respectively (Figure 1). These results are higher (47% and 60%) than the current EAR and RDA, which are 0.66 and 0.80 g · kg^{-1} · d^{-1}, respectively (14).

TABLE 4 Comparisons between older women (current study) and young men who participated in a protein requirement study conducted by our group using the IAAO method[1]

Variable	Older women	Young men[2]	P
Age, y	74.3 ± 7.4	26.8 ± 5.7	<0.01
n	12	8	
Weight, kg	63.2 ± 9.8	69.6 ± 10.5	NS
BMI, kg · m^{-2}	24.8 ± 2.3	23.3 ± 2.8	NS
FFM–BIA, kg	37.9 ± 3.7	56.4 ± 5.4	<0.01
REE, kcal · d^{-1}	1210 ± 105	1670 ± 105	<0.01
Phenylalanine flux, μmol · kg^{-1} · h^{-1}	32.8 ± 11.1	58.5 ± 14.6	<0.01
Protein requirement (EAR), g · kg^{-1} BW · d^{-1}	0.96	0.93	NS
Protein requirement, g · kg^{-1} LBM · d^{-1}	1.62 ± 0.14	1.14 ± 0.09	<0.01
%Fat (BIA)	39.5 ± 5.1	18.7 ± 6.2	<0.01

[1] Values are means ± SDs, n = 12 (older women >65 y) and n = 8 (young men). Comparisons between older women in the current study and young men were performed by t test. BIA, bioelectrical impedance analysis; BW, body weight; EAR, Estimated Average Requirement; FFM, fat-free mass; IAAO, indicator amino acid oxidation; LBM, lean body mass; REE, resting energy expenditure.
[2] Data for young men are from reference 18.

Although the requirement estimates in the current study are not different from those in young adults from our previous study (18) when compared per kilogram of body weight, the current estimates are higher per kilogram of FFM (Table 3). Expressed as a fraction of FFM, the EAR for protein in older adults in the current study was 1.6 compared with 1.14 g · kg^{-1} FFM · d^{-1} in young adults (18). Expressed as a percentage of calories, the current results and those from our previous study in young adults (18) provide protein at 13% and 10% of calories for older and young adults, respectively. The current DRI estimate of 0.66 g · kg^{-1} · d^{-1} only represents 9% of calories for older adults and 7% for younger adults. This is lower than the Acceptable Macronutrient Distribution Range of 10–35% of calories from protein (14).

In our recent study in octogenarian women (24), the mean and population-safe (RDA) protein requirements were 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}. This estimate is 88% of the current estimate based on FFM. However, the body composition measurements in the previous study (24) were performed with a different instrument (Tanita 2202 UM-016, Tanita, Tokyo, Japan) than that used in the current study. However, taken together, these previous estimates of protein requirements (18, 24), along with the current estimate, suggest that current protein recommendations are inadequate.

We appreciate that opposing views exist regarding the current protein recommendations in general. Although some maintain that there is little justification for revision of the current recommendations for elderly subjects, citing a decrease in requirements with age rather than an increase (47), others disagree (23, 48), citing differences in protein metabolism with aging, including impairment in anabolic response in older compared with younger individuals. The basis for the current DRI protein requirement estimate lies in the results from studies that used nitrogen balance, which is well recognized to be an imprecise method (16, 49). In addition, nitrogen balance measures minimal rather than optimal protein requirement (50), and the RDA is "an estimate of the minimum daily average dietary intake level that meets the nutrient requirements of nearly all (97–98%) healthy individuals in a particular life stage and gender group" (14). This was demonstrated in a study of glutathione synthesis rates in response to habitual vs. recommended protein intake (51). The significantly lower glutathione synthesis in the presence of apparent nitrogen balance observed with the recommended vs. habitual protein intake is evidence that the requirement derived from nitrogen balance studies is inadequate. In addition, controlled consumption of an energy-balanced diet at the RDA for protein in older adults aged 54–78 y resulted in decreased FFM, midthigh muscle area, and total body water (52), suggestive of inadequacy of the current RDA. In a more recent cross-sectional observational analysis of the relation of dietary protein on body composition and physical performance, women who consumed a protein intake at the RDA or higher had lower rates of osteoporosis and better physical performance than did women in the low-protein group (12). Although these are only associations, they provide further evidence to suggest that older adults >65 y might benefit from a higher protein intake than the current RDA. Clearly, in an age in which an ever-increasing percentage of the population is >65 y (13), optimal health promoted by optimal protein intakes should be the more desired option, if only for the purpose of reduction in health care costs associated with sarcopenia.

Varying differences in metabolism in older individuals could indeed account for higher protein requirements per kilogram of

FFM. By using stable isotope kinetics to measure muscle protein synthesis in young and older individuals in response to an AA/glucose supplement, Volpi et al. (53) found a blunted response of protein synthesis in older compared with younger individuals. In a more recent study, stable isotopes were again used to measure muscle protein synthesis in young compared with older adults (54). The results showed similar muscle protein synthesis rates in both young and old individuals, despite a substantially higher plasma AA concentration in the elderly subjects. These studies suggest that a larger precursor pool of AAs is required in older subjects to drive a similar anabolic response in muscle tissue. Still others showed that the rate of protein digestion affects protein synthesis differently in younger vs. older individuals (54). In older individuals, protein gains are greater with more rapidly digested protein. Also, a pulse diet in which 80% of the protein was provided in one meal was associated with a more positive nitrogen balance than in a spread diet in which the protein intake was divided into 4 meals (55). The pulse diet was associated with increased protein turnover and maintenance of FFM, whereas the spread-diet recipients lost muscle mass despite a 1.6 $g \cdot kg^{-1}$ FFM protein intake equivalent to that received by the pulse-diet participants.

The IAAO method has been criticized on methodologic grounds (56, 57); the key concern being that with a constant intake of phenylalanine and tyrosine, the rate of oxidation of the tracer phenylalanine is only reflective of its own excess or limitation rather than the oxidation of the dietary protein intake. The criticisms were thoroughly addressed in a reply to the authors (56) and again in our recent publication on protein requirement in octogenarian women (24). Briefly, we argued that the oxidation of the indicator phenylalanine is reflective of its excess in relation to the overall pattern of demand. The overall demand is dependent on the availability of the test amount of the total AAs that are available to optimize protein synthesis—because IAAO is the reciprocal of whole-body protein synthesis. Because the indicator AA is fed above its requirement, it is never limiting for whole-body protein synthesis. Therefore, the pattern of its oxidation, rather than the absolute rate, is the important factor.

We agree that the short-term nature of the IAAO method, although a superior method for determining protein requirements in a vulnerable population such as the elderly, does not provide evidence that the increased protein requirement estimate so derived makes any difference to long-term health (57). However, results generated using this approach provide validation to the increasing school of thought that the current protein recommendation for older adults is too low. It also provides an empirical basis for the design of future research to test the higher estimated protein requirement in long-term functional studies.

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