# The Recommended Dietary Allowance for Protein May Not Be Adequate for Older People to Maintain Skeletal Muscle

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**Background.** Inadequate dietary protein intake results in loss of skeletal muscle mass. Some shorter-term nitrogen balance studies suggest that the Recommended Dietary Allowance (RDA) of protein may not be adequate for older people. The aim of this study was to assess the adequacy of the RDA of protein for older people by examining longer-term responses in urinary nitrogen excretion, whole-body protein metabolism, whole-body composition, and mid-thigh muscle area.

*Methods.* This was a 14-week precisely controlled diet study. Ten healthy, ambulatory men and women, aged 55 to 77 years, were provided eucaloric diets that contained 0.8 g protein· $kg^{-1}$ · $day^{-1}$ . The study was conducted at a General Clinical Research Center using an outpatient setting for 11 weeks and an inpatient setting for 3 weeks. The main outcome measures included urinary nitrogen excretion, postabsorptive and postprandial whole-body leucine kinetics via infusion of L-[1-<sup>13</sup>C]-leucine, whole-body density via hydrostatic weighing, total body water via deuterium oxide dilution, and mid-thigh muscle area via computed tomography scans.

**Results.** Mean urinary nitrogen excretion decreased over time from Weeks 2 to 8 to 14 (p = .025). At Week 14, compared with Week 2, there were no changes in postabsorptive or postprandial leucine kinetics (turnover, oxidation, incorporation into protein via synthesis, release via breakdown, or balance). Whole-body composition (% body fat, fat-free mass, and protein + mineral mass) did not change over time in these weight-stable subjects. Mid-thigh muscle area was decreased by  $-1.7 \pm 0.6 \text{ cm}^2$  (p = .019) at Week 14 compared with Week 2. The loss of mid-thigh muscle area was associated with the decrease in urinary nitrogen excretion (Spearman r = .83, p = .010).

**Conclusions.** The maintenance of whole-body leucine metabolism and whole-body composition is generally consistent with a successful adaptation to the RDA for protein. However, the decrease in mid-thigh muscle area and the association with decreased urinary nitrogen excretion are consistent with a metabolic accommodation. These results suggest that the RDA for protein may not be adequate to completely meet the metabolic and physiological needs of virtually all older people.

THE current Recommended Dietary Allowance (RDA) for protein, 0.8 g protein  $kg^{-1}$  day<sup>-1</sup>, is set as the safe and adequate intake for virtually all healthy men and women aged 19 years and older (1). Specific to the protein RDA for older adults (age >50 years), several important considerations were recognized. First, very little data were available to help set an RDA for protein in older adults, and the data that were available (2–5) were contradictory. Thus, the protein RDA for older men and women was basically an extrapolation from nitrogen balance studies in young men. Second, whereas significant changes in body composition, food intake, physical activity, and the frequency of disease occur with aging, how such changes affect protein metabolism and dietary protein needs was largely unknown. The consensus was that, because of age-associated changes in body composition, primarily the loss of muscle mass (sarcopenia), an RDA of 0.8 g protein·kg<sup>-1</sup>·day<sup>-1</sup> would be higher per unit of fat-free mass in older people than in younger people and should allow for any decrease in the efficiency of protein utilization (1).

The current consensus (1,6) is that adult protein allowances should be established from shorter-term (2-3-week) nitrogen balance studies. However, longer-term (several weeks to several months) nitrogen balance studies should be highly valuable in assessing dietary protein adequacy by additional criteria, such as changes in protein metabolism, body composition, physical status, and functional status. Results of the limited number of shorter-term nitrogen balance studies in older adults are conflicting, with some supporting (4,5) and others questioning (2,3,7) the adequacy of 0.8 g protein  $kg^{-1}$  day<sup>-1</sup>. Retrospective re-analyses (8) of these shorter-term nitrogen balance data, on the basis of calculations recommended by the 1985 Joint FAO/WHO/UNU Expert Consultation (6), support the conclusion that the protein RDA may not be adequate for many older and elderly adults. These conclusions (8) have been questioned (9,10).

To date, the longest-term nitrogen balance study in older men and women fed the RDA of 0.8 g protein  $kg^{-1} day^{-1}$ was 4 weeks (2). Three out of seven men and four out of eight women were in negative nitrogen balance during Days 26 to 30 of the study, and the authors concluded that 0.8 gprotein·kg<sup>-1</sup>·day<sup>-1</sup> was not adequate for a majority of men and women aged 70 years and older (2). More recently, Castaneda and colleagues (11,12) assessed the longer-term metabolic and physiological changes of 66- to 79-yearold women who consumed either 0.45 or 0.92 g protein·kg<sup>-1</sup>·day<sup>-1</sup> (56% or 115% of the RDA) for 9 weeks. The marked negative nitrogen balance, loss of body cell mass and muscle mass, reduced muscle strength, and impaired immune responses all indicated that 0.45 g protein·kg<sup>-1</sup>·day<sup>-1</sup> was inadequate and compromised the physical and functional capacities of these elderly women. In contrast, the apparent adequacy of 115% of the protein RDA for elderly women was supported by the maintenance of body composition, functional capacity, and immune responses.

The present study assesses the adequacy of the RDA for protein in older people in a longer-term nitrogen balance study. Older men and women were fed diets providing 0.8 g protein·kg<sup>-1</sup>·day<sup>-1</sup> and sufficient energy to maintain body weight continuously for 14 weeks. The criteria of adequacy included measurements of urinary nitrogen excretion, wholebody protein metabolism, body composition, energy metabolism, and muscle strength and function. It is hypothesized that the protein RDA is not adequate to maintain these indicators of metabolic, physical, and functional status.

#### Methods

#### Subjects

Four men and six women, aged 55 to 77 years, participated in this metabolic study. The study was completed at the General Clinical Research Center (GCRC) located at the University Park campus of The Pennsylvania State University. Prestudy medical evaluations, which included a medical history questionnaire, a physician-administered physical examination, routine blood and urine chemistries, and a resting and resistance exercise electrocardiogram, were used to exclude people with clinically abnormal thyroid, liver, kidney, or heart function. All six of the women studied were postmenopausal and were not taking any estrogen replacement medications. Each subject signed an informed consent agreement. The study protocol and informed consent agreement were reviewed and approved by the Institutional Review Board, The Pennsylvania State University, University Park, PA and by the Clinical Investigation Committee, The Milton S. Hershey Medical Center, Hershey, PA.

#### Experimental Design

The study design was a 14-week precisely controlled dietary intake trial. Testing and evaluations were completed at study Weeks 2, 8, and 14, as indicated. During these weeks, each subject was in residency at the GCRC. The remainder of the study was conducted on an outpatient basis.

#### Diet

Each subject's diet was designed to provide 0.8 g protein·kg<sup>-1</sup>·day<sup>-1</sup> and sufficient dietary energy to maintain body weight. The diet was provided as a rotating cycle of three daily menus of lacto-ovo-vegetarian foods, as described by Campbell and colleagues (13). Whereas animal striated tissues (i.e., beef, pork, poultry, fish) were not used, animal-based proteins were provided in the forms of milk-based proteins ( $28.5\% \pm 1.2\%$  of total protein) and egg-based proteins ( $10.2\% \pm 0.8\%$  of total protein). The non-protein portion of each of the three menus contained 60% of energy from carbohydrate and 40% of energy from fat. Water, decaffeinated coffee, and decaffeinated tea were allowed ad libitum.

The total energy intake of each subject was initially set on the basis of the sex-specific Harris-Benedict equation (14) of resting energy expenditure plus an allowance of 0.7 times this predicted resting energy expenditure to account for the energy expenditure of physical activity. Total energy intake was adjusted during the nonresidency periods as necessary to maintain body weight within  $\pm 0.5$  kg of each subject's mean body weight during Days 4 to 15. The adjustments to total energy intake were done either by adding or subtracting protein-free or very-low-protein foods and beverages from each subject's daily menus while maintaining the nonprotein energy ratio at 60% carbohydrate to 40% fat. The energy and macronutrient contents of the daily menus were calculated using Nutritionist IV software (version 4.0; N-squared Computing, First Data Band, San Bruno, CA) assuming that the metabolizable energy content of protein, carbohydrate, and fat were 16.7, 16.7, and 37.7 kJ/g, respectively.

### Resting Energy Expenditure

Postabsorptive resting energy expenditure was measured at Weeks 2 and 14 by indirect calorimetry, as described by Campbell and colleagues (13).

## **Body Composition**

Postabsorptive, nude body weight was measured (model 2181; Toledo Scale, Toledo, OH) to the nearest 0.1 kg each weekday during the study, as described previously (13). Body height was measured to the nearest 0.1 cm with a wall-mounted stadiometer one morning during Week 1.

Whole-body density was measured by hydrostatic weighing (15), with lung residual volume measured by nitrogen dilution (16) during the underwater procedure. Total body water was measured by the deuterium oxide dilution technique (17) using a 20.0 g dose of deuterium oxide and a fixed-filter single-beam infrared spectrophotometer (Miran 1FF; Foxboro Analytical, South Norwalk, CT) for analyses of prepared urine samples, as described by Campbell and colleagues (18). Percent body fat was calculated from body density and total body water using the 3-compartment equation of Siri (19), as described previously (18). Fat-free mass (FFM) was calculated as body mass minus fat mass, and protein plus mineral mass was calculated as FFM minus body water mass.

Mid-thigh muscle and fat areas were measured by image analyses (IMAGE, version 1.60; National Institutes of Health, Bethesda, MD) of computed tomography (CT) scans (Picker 2000 operating at 130 kVp; Picker International Inc., Cleveland, OH). The CT scans were taken of the dominant leg at Weeks 2 and 14; a 10-mm CT slice was taken midway between the inguinal crease and the lower pole of the patella. At Week 2, the exact location of the mid-thigh CT slices were determined from bony landmarks on the femur and were used to identify the identical slice location for the Week-14 scans. Digital Imaging and Communication in Medicine (DIACOM) software (version 3.0) was used to maintain spatial and density calibration during the transfer of the images from the CT scanner to a SUN (SPARK) station for imaging analysis. Digitized images, analyzed in a blinded fashion, were used to calculate the total muscle and subcutaneous fat areas. The Hounsfield units used to detect muscle and fat were -30 to +150 and -250 to -40, respectively. Computed tomography data from two subjects were inadvertently lost during processing. Therefore, results are presented for eight subjects (3 men and 5 women).

#### Muscle Strength and Power

Dynamic concentric muscle strength was measured as the maximum force that each subject could move through a full range of motion one time only for the seated chest press, seated arm pull, seated unilateral knee extension, and seated bilateral leg curl exercises (Keiser Sports Health Equipment, Fresno, CA). Upper-body strength was calculated as the sum of the linear forces (newtons) of the chest press and arm pull exercises. Lower-body strength was calculated as the sum of the angular forces (newton  $\times$  meter) of the knee extension and leg curl exercises. Peak leg power was measured using a NUMAZ (University of Nottingham, UK) power rig (20). Subjects were asked to exert a maximal push of both legs to a large foot pedal while in a seated position with folded arms. The highest value (watts) from 10 trials was recorded, with each trail separated by a minimum of 60 seconds of rest.

#### Nitrogen Intake and Excretion

At Weeks 2, 8, and 14, samples from duplicate menu composites, four consecutive 24-hour urine collections, and four-day fecal collections (made between two dye markers) were collected, processed, and aliquots stored frozen at  $-20^{\circ}$ C. All food, urine, and feces samples were analyzed for total nitrogen using an Elementar Macro N (Elementar Analysensytene GmBh, Hanau, Germany) nitrogen analyzer. The National Institute of Standards and Technologies Total Diet standard reference material and in-house pooled food and urine standards were used as quality controls. Dietary protein intake was calculated from the food nitrogen data assuming a factor of 6.25 g protein-g nitrogen<sup>-1</sup>.

#### Infusion Procedure

At the end of Weeks 2 and 14, a primed constant infusion of L- $[1^{-13}C]$ leucine was performed, as described previously (21), to determine postabsorptive and postprandial leucine kinetics. After baseline blood and expired breath samples were collected, the infusion of the isotope was begun with the intravenous administration of priming doses of NaH<sup>13</sup>CO<sub>3</sub> (2.35  $\mu$ mol·kg<sup>-1</sup>) and L- $[1^{-13}C]$ leucine (7.6  $\mu$ mol·kg<sup>-1</sup>). This was followed immediately by a continuous infusion of L- $[1^{-13}C]$ leucine (7.6  $\mu$ mol·kg<sup>-1</sup>) using a calibrated syringe pump (model 55-2222; Harvard Apparatus, Natick, MA).

The constant infusion was continued for 8 hours, with all subjects in the postabsorptive state during the first 4 hours and in the postprandial state during the last 4 hours. During the postprandial period, each subject consumed a formula beverage hourly (minutes 240, 300, 360, and 420 of the infusion procedure) providing one-twelfth of their daily protein and energy intakes at baseline. The dietary leucine concentration of each formula beverage was estimated assuming factors of 6.38 g protein/g nitrogen and 95 mg leucine/g protein (6). All blood and breath samples were obtained and processed using standard methods (21,22). The <sup>13</sup>C enrichment of plasma  $\alpha$ -ketoisocaproic acid (KIC) was determined by gas chromatography mass spectrometry (model HP 5989; Hewlett-Packard, Palo Alto, CA) using the silvlquinoxalinol derivative (22). The expired air samples were analyzed for <sup>13</sup>CO<sub>2</sub> enrichment by isotope ratio mass spectrometry (model cira 2; VG Isogas, VG Instruments, Middlewich, UK). Prior to and during the fourth and eighth hours of the infusion, the rate of CO<sub>2</sub> production was measured by indirect calorimetry using a ventilated hood system.

#### Calculations

All kinetics parameters are expressed as µmol leucine kg<sup>-1</sup> hour<sup>-1</sup>. The parameters included leucine turnover, leucine oxidation, leucine incorporation into protein (synthesis), leucine release from protein (breakdown), and leucine intake from the diet. Leucine turnover was calculated as described by Matthews and colleagues (23), with the intracellular leucine pool estimated by the plasma  $[^{13}C]$ KIC enrichment at plateau (24). The rate of leucine oxidation was calculated as described previously (23), assuming fractional bicarbonate retention factors in the postabsorptive and postprandial states of 0.70 and 0.82, respectively (25). Synthesis was calculated as turnover minus oxidation. Breakdown was calculated as turnover minus intake. In the postabsorptive state intake from diet was zero, and breakdown equaled turnover. In the postprandial state intake was corrected for the amount of leucine estimated to be removed during the first pass through the splanchnic tissues (50%) (26). Predicted 24-hour leucine balance (input – output,  $\mu$ mol·kg<sup>-1</sup>) was calculated from leucine intake (dietary) and leucine oxidation, as described by El-Khoury and colleagues (27).

#### Serum Albumin

A postabsorptive-state serum sample collected at baseline of the infusion procedure was analyzed for albumin concentration using an automated analyzer technique at a clinical medical laboratory (American Medical Laboratory, Chantilly, VA).

#### Statistical Methods

Values are reported as means  $\pm$  *SEM*. The effect of time was assessed either by paired *t* test (Week 2 vs Week 14) or one-factor repeated measures analysis of variance (ANOVA) (Weeks 2, 8, and 14). For these paired *t* tests, statistical significance was assigned if p < .05 (two-sided). For the one-factor repeated measures ANOVA, when a statistically significant time effect was established, separate

comparisons (paired *t* tests) were done among time points (i.e., Weeks 2 vs 8, Weeks 2 vs 14, and Weeks 8 vs 14). For these comparisons, the Bonferroni correction was applied, and statistical significance was assigned if p < .017 (two-sided). For the leucine kinetics data, a two-factor repeated measures ANOVA was used to assess the main effects of metabolic state (postabsorptive vs postprandial) and time (Weeks 2 vs 14) and the metabolic state-by-time interaction. For these tests, statistical significance was assigned if p < .05. The degree of linear association between variables was established by using the Spearman Rho nonparametric ranked correlation (Prob > IRhol, p < .05). All data were processed using Microsoft Excel 5.0 (Microsoft, Redmond, WA). Statistical evaluations were done using JMP software (version 3.2.2; SAS Institute, Inc., Cary, NC).

#### RESULTS

Table 1 presents the group mean values for the descriptive characteristics, body composition, mid-thigh muscle and fat areas, resting metabolic rate, and serum albumin concentration, at Week 2 and Week 14. Body weight, BMI, body density, total body water, % body fat, FFM, proteinmineral mass, mid-thigh circumference and fat area, resting metabolic rate, and serum albumin were not different at Week 14 compared with Week 2. Mid-thigh muscle area was decreased by  $-1.7 \pm 0.6$  cm<sup>2</sup> (p = .019; n = 8) at Week 14 compared with Week 2.

Upper body strength ( $672 \pm 98$  N vs  $657 \pm 89$  N; n = 8), lower body strength ( $297 \pm 42$  Nm vs  $288 \pm 33$  Nm; n = 8), and leg peak power ( $222 \pm 47$  W vs  $216 \pm 44$  W; n = 7) were not different at Week 14 compared with Week 2.

From computer-based diet analyses, the group mean dietary intakes at Week 2 were  $9.99 \pm 0.75$  MJ energy/d,  $52.9 \pm$ 3.0 g protein/d,  $347 \pm 43.3$  g carbohydrate/d, and  $95.5 \pm$ 7.4 g fat/d and were not significantly different at Week 8 and Week 14 (Table 2). On the basis of nitrogen analysis, the group mean protein intakes at Weeks 2, 8, and 14 were  $0.821 \pm 0.008$ ,  $0.819 \pm 0.009$ , and  $0.813 \pm 0.009$  g pro-

Table 1. Subject Characteristics

Parameter	Week 2	Week 14	
Age, y <sup>†</sup>	66 ± 3 (range 55–77)		
Height, cm	$167 \pm 3$		
Weight, kg	$67.7 \pm 4.1$	$67.7 \pm 4.0$	
Body mass index, kg/m <sup>2</sup>	$24.2 \pm 1.0$	$24.2 \pm 0.9$	
Body density, kg/l	$1.020 \pm 0.006$	$1.019 \pm 0.006$	
Total body water, 1	$28.9 \pm 2.3$	$27.8 \pm 3.0$	
Body fat, %	$38.7 \pm 2.8$	$40.1 \pm 3.0$	
Fat-free mass, kg	$42.1 \pm 3.1$	$41.0 \pm 3.0$	
Protein-mineral mass, kg	$12.5 \pm 0.9$	$12.6\pm0.9$	
Thigh circumference, cm	$50.9 \pm 1.6$	$50.9 \pm 1.6$	
Thigh muscle area, cm <sup>2</sup>	$100.4 \pm 8.0$	98.7 ± 7.5*	
Thigh fat area, cm <sup>2</sup>	$76.8 \pm 8.0$	$78.3 \pm 14.1$	
Resting metabolic rate, MJ/d	$5.67 \pm 0.33$	$5.65 \pm 0.38$	
Albumin (serum), g/l	39 ± 3	$40 \pm 3$	

*Note*: Data are presented as mean  $\pm$  *SEM*, n = 10 subjects, except for thigh circumference, muscle area, and fat area (n = 8 subjects).

\*Significantly different from Week 2; p = .019.

<sup>†</sup>The ages of the subjects were 55, 56, 58, 62, 63, 68, 71, 72, 76, and 77.

Table 2. Dietary Energy, Macron	utrient	Intakes,	and
Nitrogen Excretion	ons		

Parameter	Week 2	Week 8	Week 14
Dietary Intakes			
Energy			
MJ/d	$9.99 \pm 0.75$	$10.99 \pm 1.08$	$10.95 \pm 0.99$
kcal/d	$2388 \pm 180$	$2626\pm259$	$2617\pm236$
Protein, g/d	$52.9 \pm 3.0$	$52.9 \pm 2.9$	$53.0 \pm 2.9$
Carbohydrate, g/d	$347.2 \pm 43.3$	$364.6 \pm 39.7$	$362.2\pm35.4$
Fat, g/d	$95.5 \pm 7.4$	$105.3\pm10.2$	$106.3 \pm 9.5$
Nitrogen Intakes and Excretions	(mg N·kg <sup>-1</sup> ·d <sup>-1</sup>	<sup>1</sup> )	
Nitrogen intake	$131.3 \pm 1.3$	$131.0\pm1.5$	$130.1 \pm 1.5$
Urinary nitrogen excretion*	$102.9\pm4.4$	$87.7 \pm 4.1$	80.2 ± 4.3**
Fecal nitrogen excretion	$23.7\pm2.0$	$25.2\pm2.3$	$22.1\pm2.4$

Note: Data are presented as mean  $\pm$  SEM.

\*Time effect; p = .025.

\*\*Different from Week 2; p = .005.

tein·kg<sup>-1</sup>·day<sup>-1</sup>. These levels of protein intake represent about 102% of the current RDA of protein (1).

Dietary nitrogen intake was  $131.3 \pm 1.3$  g N·kg<sup>-1</sup>·day<sup>-1</sup> at Week 2 and was not different over time (i.e., at Week 8 and Week 14; Table 2). At Week 2, urinary nitrogen excretion of each subject did not change over the four days of collection (i.e., no significant slope was observed when regression analysis was performed), consistent with each subject being in steady state. Over time, urinary nitrogen excretion decreased (p = .025), and fecal nitrogen excretion was not different. Subsequent paired t test analyses (with Bonferroni correction) showed that urinary nitrogen excretion was different between Week 2 and Week 14 (p = .005). From Week 2 to Week 14, the change in urinary nitrogen excretion was associated with the change in mid-thigh muscle area (r = .83, p = .010; Table 3 and Figure 1).

Table 4 presents the whole-body leucine kinetics data. Leucine turnover and oxidation were higher, and synthesis and breakdown were not different in the postprandial state versus the postabsorptive state. Postabsorptive leucine balance was negative, and postprandial leucine balance was positive. Net leucine balance was negative. There were no significant changes over time or metabolic state- (postabsorptive vs postprandial) by-time interactions for any of the leucine kinetics parameters.

#### DISCUSSION

When longer-term metabolic balance studies are used to assess the adequacy of protein intake, it is important to distinguish between adaptation and accommodation (28–31). Adaptation, an appropriate and desired response, refers to metabolic changes that occur in response to changes in protein intake and result in the establishment of a new steady state without a compromise or loss in physiological function. Accommodation, a survival response, refers to further metabolic changes in response to the decreased protein intake that the body undergoes to establish steady state, but only with a compromise or loss in physiological function.

Waterlow (30,31) and Young and colleagues (28,29) have proposed that it might be possible to distinguish between adaptation and accommodation by measuring longer-term changes in urinary nitrogen excretion in response to mar-

		Urinary Nitrogen Excretion		Leg Muscle Area		
Subject	Week 2 (g N/d)	Change at Week 14 (g N/d)	%	Week 2 (cm <sup>2</sup> )	Change at Week 14 (cm <sup>2</sup> )	%
1	$7.36 \pm 0.28$	-3.07	-42	104.5	-2.82	-2.7
2	$5.01 \pm 0.49$	-0.48	-10	89.9	-1.42	-1.6
3	$4.78 \pm 0.22$	+0.77	+16	69.5	+0.22	+0.3
4	$5.13 \pm 0.12$	-0.13	-3	82.1	-1.13	-1.4
5	$7.04 \pm 0.73$	-1.37	-19	82.0	0.79	+1.0
6	$6.91 \pm 1.19$	-2.25	-33	130.4	-3.31	-2.4
7	$7.07 \pm 0.81$	-1.67	-24	120.8	-3.02	-2.5
8	$9.98 \pm 0.80$	-4.93	-49	123.9	-3.41	-2.8
Group	$6.66\pm0.60$	$-1.84 \pm 0.53$	$-21 \pm 8$	$100.4 \pm 8.1$	$-1.74\pm0.57$	$-1.5 \pm 0.5$

Table 3. Changes in Steady-State Urinary Nitrogen Excretion and Leg Muscle Area

*Note*: Data are presented as mean  $\pm$  *SEM*.

ginal or inadequate protein intakes. Upon changing from a higher (completely adequate) to a lower protein intake, urinary nitrogen excretion decreases within approximately 1 week to a new steady state. Rand and colleagues (32) showed that a new steady state was achieved in both young men and elderly women in an average of 4.5 days, with 95% of all subjects achieving steady state within 8 days. For subjects who metabolically adapt, this new steady state would be maintained, and urinary nitrogen excretion would remain fairly constant over longer periods of time. In contrast, for subjects who metabolically accommodate, urinary nitrogen excretion would gradually decrease over time, presumably due to decreased total body nitrogen, until a new lower steady state was achieved. This accommodation is subtle and may require several years to achieve a new steady state (31).

To distinguish between adaptation and accommodation, three criteria must be met. First, was steady state achieved and was urinary nitrogen excretion stable after the subjects consumed the lower-protein diet? Second, was urinary nitrogen excretion maintained (consistent with adaptation) or decreased (consistent with accommodation) in the subjects over longer periods of time? Third, was it possible to show that the change in urinary nitrogen excretion over time was associated with an adverse change in physiological function consistent with accommodation? In the present study, all subjects presumably achieved steady state at Week 2, as ev-



Change in Urinary Nitrogen Nxcretion, %

Figure 1. Correlation between changes in urinary nitrogen excretion and leg muscle area in older people who consumed the Recommended Dietary Allowance for protein  $(0.8 \text{ g protein} \cdot \text{kg}^{-1} \cdot \text{day}^{-1})$  for 14 weeks. idenced by the absence of significant changes in urinary nitrogen excretion over time from Day 8 to Day 11. Second, from Week 2 to Week 14, the group mean urinary nitrogen excretion decreased by  $-1.84 \pm 0.53$  g N/d ( $-21\% \pm 8\%$ ), but with a wide within-group variability in response (+16% to -49%) (Table 3). Third, the changes over time in urinary nitrogen excretion were associated with changes in leg muscle area (Figure 1). The subjects who showed greater reductions in urinary nitrogen excretion over time also experienced greater losses of mid-thigh skeletal muscle area. Thus, these data may be interpreted as accommodation.

This interpretation is made with all due caution. For example, one would expect that subjects experiencing accommodation due to an inadequate protein intake, reflected by a decrease over time in urinary nitrogen excretion (28,29,31), might also show adverse changes in whole-body composition, serum albumin concentration, or muscle strength and power. The apparent maintenance of these parameters is generally consistent with the interpretation that the subjects

Table 4. Whole-Body Leucine Kinetics

Parameter	Week 2	Week 14	
Leucine Kinetics, µmol L	eucine·kg <sup>-1</sup> ·h <sup>-1</sup>		
Turnover*			
Fasting	$98.9 \pm 3.1$	$94.3 \pm 3.4$	
Fed	$116.0 \pm 4.3$	$114.7 \pm 4.1$	
Oxidation**			
Fasting	$19.6 \pm 1.4$	$17.9 \pm 1.4$	
Fed	$33.4 \pm 1.2$	$31.1 \pm 1.4$	
Synthesis			
Fasting	$79.3 \pm 2.7$	$76.4 \pm 2.6$	
Fed	$82.6 \pm 4.5$	$83.6 \pm 3.6$	
Breakdown			
Fasting	$98.9 \pm 3.1$	$94.3 \pm 3.4$	
Fed	$94.9 \pm 4.0$	93.7 ± 4.0	
Balance <sup>†</sup> **			
Fasting	$-19.6 \pm 1.4$	$-17.9 \pm 1.4$	
Fed	$8.9 \pm 1.8$	$10.8 \pm 1.4$	
Total	$-10.7 \pm 2.2$	$-7.1 \pm 2.0$	

*Note*: Data are presented as mean  $\pm$  *SEM*.

 $^{\dagger}$ Balance = leucine intake (dietary) - leucine oxidation; total balance = fasting balance + fed-state balance.

\*p = .002; \*\*p < .001; Fed state different from fasting state (i.e., main effect of metabolic state).

successfully adapted to this protein intake by increasing the efficiency of nitrogen retention and nitrogen availability without resorting to the breakdown of body proteins. It is generally recognized that nitrogen balance data are inherently biased toward showing a more positive balance (33), associated with either incomplete dietary protein intake or incomplete collection of nitrogen excretions. However, it seems unlikely that the decrease over time in urinary nitrogen excretion was due to a systematic shift in food consumption or urine collection during the study. It is noted, also, that computed tomography scanning is a more sensitive and accurate method of detecting subtle body composition changes than measures of whole-body density or total body water (34).

It is important to point out the limitations of the current study. Future studies that aim to assess protein intake adequacy of older people might include a larger number of subjects, higher and lower protein control groups, and a younger age control group. Another consideration might be to extend the baseline period to achieve an initial steady state for urinary nitrogen excretion beyond 8 to 10 days. Whereas Rand and colleagues (32) reported that 95% of young men and elderly women achieved steady state within 8 days, their study was conducted for only 10 days. There is some suggestion that it might take longer than 10 days to adjust to a given protein intake and to achieve steady state (2). In the present study, we did not determine the pattern of change in urinary nitrogen excretion between Week 2 and Week 8.

Prior to the current study, a nitrogen balance study in 15 men and women aged 70 years or older who consumed 0.8 g protein kg<sup>-1</sup> day<sup>-1</sup> for 30 days provided the longest-term assessment of the RDA for protein in older people (2). Gersovitz and colleagues (2) showed that nitrogen balance, negative for 13 of the 15 subjects at Days 1 through 10, increased over time due to a reduction in urinary nitrogen excretion but remained negative in 7 subjects at Days 21 through 30. Body weight trended down over time, but no significant changes in muscle mass (assessed via urinary creatinine excretion) or body cell mass (assessed via <sup>40</sup>Kpotassium scans) were detected. From these data, the authors concluded that the RDA of 0.8 g protein  $kg^{-1} day^{-1}$ was inadequate for some older people but that the shift in nitrogen balance over time and no measurable changes in body composition indicated adaptation to the diet. The authors cautioned, however, that the relatively short-term study period limited the ability to quantify subtle changes in muscle or body cell mass.

Castaneda and colleagues (11) reported that postmenopausal women who consumed 0.45 g protein·kg<sup>-1</sup>·day<sup>-1</sup> (56% of the RDA) for 9 weeks experienced 8% declines in body cell mass and skeletal muscle mass along with declines in muscle strength and function. These results clearly showed 0.45 g protein·kg<sup>-1</sup>·day<sup>-1</sup> to be an inadequate protein intake and to cause physiological accommodation in older women. In contrast, postmenopausal women who consumed 0.92 g protein·kg<sup>-1</sup>·day<sup>-1</sup> (115% of the RDA) maintained body cell mass and skeletal muscle mass over the 9-week period. These results indicated that a protein intake greater than the RDA was adequate for these older women to adapt and maintain physiological status. The lack of a substantial change over time in whole-body leucine kinetics in response to the diet is consistent with previous research (12). The accommodation to marginal protein intake is subtle and takes place over many months (30,31). The increase in nitrogen loss that led to a measurable decrement in leg muscle over 14 weeks resulted from an imbalance between muscle protein synthesis and breakdown. However, the imbalance between synthesis and breakdown over any period of a few hours that would eventually lead to a loss of 1 kg of muscle over 14 weeks (1.8 g nitrogen·d<sup>-1</sup> × 98 days × 6.25 g protein·g nitrogen<sup>-1</sup>) would not be expected to be detectable with the isotopic technique. Thus, a loss of 1.84 g nitrogen·d<sup>-1</sup> would correspond to approximately 5.8 µmol leucine·kg<sup>-1</sup>·hour<sup>-1</sup>.

Sarcopenia, the age-associated loss of skeletal muscle mass, is indeed a multi-factorial syndrome (35–37). We do not presume that inadequate dietary protein intake is the only cause of sarcopenia. However, inadequate protein intake causes considerable losses of muscle mass in elderly people (11), and the present data suggest that more subtle, but significant, losses of muscle occur with longer-term consumption of the protein RDA in older people. Thus, habitual consumption of marginal amounts of protein by older people may contribute to sarcopenia. Research showing no association between protein intake and skeletal muscle size in elderly people were conducted using healthy, free-living, predominantly Caucasian subjects, the majority of whom consumed diets providing protein in excess of the RDA (38,39).

We (8,40) and other researchers (9,10,41) agree that there are insufficient data to establish a mean protein requirement and a suggested safe and adequate protein allowance for older persons, and the present study was not designed to accomplish these tasks. Tentatively, however, retrospective reassessments of published shorter-term nitrogen balance studies (8), using the currently standard nitrogen balance formula (6), estimate a mean protein requirement of 0.9 gprotein  $kg^{-1} day^{-1}$  and suggest a protein allowance of at least 1.0 g protein kg<sup>-1</sup> day<sup>-1</sup> for older people. An intake of 1.0 g protein  $kg^{-1} day^{-1}$  was shown to be adequate to maintain protein status in healthy, free-living older people in the United States (39) and in western European countries (42,43). A 10-year longitudinal study in initially healthy elderly women showed that women who habitually consumed greater than 1.2 g protein kg<sup>-1</sup> day<sup>-1</sup> developed fewer health problems than those who consumed less than 0.8 g protein·kg<sup>-1</sup>·day<sup>-1</sup> (44).

#### **Conclusions**

Many of the results of this 14-week assessment of 0.8 g protein·kg<sup>-1</sup>·day<sup>-1</sup> suggest that this protein intake is adequate for older people. However, a gradual decline in urinary nitrogen excretion and loss of body cell mass with longer-term consumption of inadequate protein (0.45 g protein·kg<sup>-1</sup>·day<sup>-1</sup>), reported by Castaneda and colleagues (11), is consistent with the concept of metabolic and physiological accommodation (28,29,31). The gradual decline in urinary nitrogen excretion over time in older people who consumed 0.80 g protein·kg<sup>-1</sup>·day<sup>-1</sup> reported by Gersovitz and colleagues (2) suggests that the RDA might be marginally inadequate and result in longer-term accommodation in skeletal muscle. The loss of skeletal muscle reported in the present study supports this suggestion. The RDAs are defined as "the levels of intake of essential nutrients that, on the basis of scientific knowledge, are judged ... to be adequate to meet the known nutrient needs of practically all healthy persons" (1). Research should continue to question whether the RDA for protein is indeed adequate to meet the dietary needs of older people.

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