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Protein Requirement of Elderly Women: Nitrogen Balance Responses to Three Levels of Protein Intake

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Abstract

Background—For elderly women, insufficient data exist to assess the accuracy of the assumed mean protein requirement of 0.6 g of protein · kg^{-1} · day^{-1}, and the adequacy of the current Recommended Dietary Allowance (RDA) of 0.8 g of protein · kg^{-1} · day^{-1}. The aims of this study were to assess the mean protein requirement and suggested safe and adequate protein intake (protein allowance) of elderly women using a shorter-term nitrogen balance protocol.

Methods—During three separate 18-day trials, 11 elderly women (age range, 70–81 years) were randomly fed eucaloric diets designed to provide either 0.50, 0.75, or 1.00 g of protein · kg^{-1} · day^{-1}. Nitrogen balance was determined at Weeks 2 and 3 (Days 7–10 and 14–17, respectively) of each trial using data from total nitrogen analyses of duplicate food composites, 24-hour urine collections, and stool collections. The mean protein requirement was calculated using linear regression of individual women's data from all three trials and inverse prediction.

Results—At protein intakes of 0.53 ± 0.02, 0.76 ± 0.02, or 1.06 ± 0.05 g of protein · kg^{-1} · day^{-1}, net nitrogen balances during Week 2 were −14.5 ± 3.1, 3.8 ± 2.5 and 23.4 ± 3.3 mg of nitrogen · kg^{-1} · day^{-1}, respectively, for these body weight– and body composition–stable women. At Week 3, the net nitrogen balances were −0.1 ± 2.7, 8.5 ± 3.6 and 42.0 ± 3.0 mg of nitrogen · kg^{-1} · day^{-1}. From Week 2 to Week 3, shifts to more positive nitrogen balances occurred due to decreases in urinary nitrogen excretion. The mean protein requirement at Week 2 was calculated to be 0.70 ± 0.09 g of protein · kg^{-1} · day^{-1} (coefficient of variation [CV] = 13%) and at Week 3 was calculated to be 0.56 ± 0.09 g of protein · kg^{-1} · day^{-1} (CV = 17%). From these data, an adequate protein allowance was estimated to be greater than the RDA at Week 2 (0.90 g of protein · kg^{-1} · day $[d]^{-1}$), and not different than the RDA at Week 3 (0.76 g of protein · kg^{-1} · d^{-1}).

Conclusions—The decrease over time in urinary nitrogen excretion from Week 2 to Week 3 suggests that these elderly women did not achieve a metabolic steady state during this shorter-term nitrogen balance study. Collectively, these data suggest that the total protein needs of elderly women are at or above the current RDA for protein. However, the results of this study indicate that shorter-term nitrogen balance protocols are insufficient to firmly establish the RDA for protein of elderly women, and further research is required using alternative criteria measures.

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The current consensus indicates that the Recommended Dietary Allowance (RDA) for protein of 0.8 g of protein · kg^{-1} · day^{-1} safely and adequately meets the dietary needs of virtually all healthy people at or older than the age of 19 years (1). There is also a general consensus that the protein requirement and suggested safe and adequate protein intake (i.e., protein allowance) of adults should be established primarily from shorter-term (2- to 3-week) nitrogen balance studies (2).

Is the RDA for protein adequate for elderly people? Conclusions from a very limited number of shorter-term nitrogen balance studies are conflicting; some support (3,4) and some question (5–7) the adequacy of 0.8 g of protein · kg^{-1} · day^{-1} for elderly people. Support for the conclusion that the RDA for protein may not adequately meet the dietary needs of many older people is found in a retrospective reanalysis of these shorter-term nitrogen balance data, based on calculations recommended by the 1985 Joint Food and Agriculture Organization, World Health Organization, and the United Nations University Expert Consultation (1). This conclusion (8) has been questioned (9,10), and further research is required to define the protein needs of elderly people.

When conducting shorter-term nitrogen balance experiments, it is important to have the subjects consume a controlled diet providing a set amount of protein for a sufficiently long lead-in period to achieve and maintain a steady state for urinary nitrogen excretion. Rand and colleagues (2) reported that 95% of elderly women achieved steady state for urinary nitrogen excretion within 8 days. All (4,5,8,11) but one (3) of the shorter-term nitrogen balance studies that used multiple protein intakes in older people limited the study period to 11 or fewer days. However, in a 30-day nitrogen balance study where older men and women were provided 0.8 g of protein · kg^{-1} · day^{-1}, Gersovitz and colleagues (6) reported that urinary nitrogen excretion continued to decrease over time from Days 6 to 10, 16 to 20, and 26 to 30. These data suggest that older people may require more than 10 days to achieve steady state when provided a given protein intake. A higher urinary nitrogen excretion by people who have not reached steady state would result in a lower apparent nitrogen balance and greater protein requirement and allowance estimates.

The overall goal of this study was to determine the mean protein requirement and suggested adequate protein allowance of elderly women using a shorter-term nitrogen balance protocol. The nitrogen balance responses of elderly women who were provided with protein intakes deemed to be inadequate, marginal, or adequate were assessed at Week 2 (Days 7–10) and Week 3 (Days 14–17) of three separate 18-day trials. We hypothesized that the mean protein requirement and allowance of elderly women would be higher than the assumed values of 0.6 g of protein · kg^{-1} · day^{-1} and 0.8 g of protein · kg^{-1} ·day^{-1}, respectively (1). We also hypothesized a steady state for protein metabolism would be achieved within 7 days, and maintained through Day 17. The achievement and maintenance of steady state would be shown by no change in urinary nitrogen excretion between Weeks 2 and 3 of the study.

Methods

Subjects

Twelve healthy Caucasian women aged 70 years and older volunteered for this metabolic balance study. Each woman completed a prestudy medical evaluation, which included a medical history, an electrocardiogram, a physical examination, and routine blood and urine chemistries. Women on estrogen replacement therapy ($n = 9$) were eligible to participate in the study; however, women with acute or chronic disease or those using drugs that might interfere with protein or energy metabolism were excluded from participation. At screening, all of the women had clinically normal serum albumin and thyroid function, and were deemed medically stable and capable of successfully completing the study protocol. Each woman received oral

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and written explanations of the purpose and procedures of the study and signed an informed consent acknowledging agreement and understanding of the procedures. Eleven of the women completed all three trials, with the twelfth woman completing only one trial due to a specific health complication unrelated to the study. The study protocol and informed consent were approved by the Human Research Advisory Committee at the University of Arkansas for Medical Sciences.

Experimental Design

Each woman completed three separate 18-day trials and consumed one of three levels of protein intake during a given trial. The order of the trials was randomly assigned for each woman. These protein levels are abbreviated as LPro (low protein, 0.50 g of protein · kg^{-1} · day^{-1}), MPro (medium protein, 0.75 g of protein · kg^{-1} · day^{-1}), and HPro (high protein, 1.00 g of protein · kg^{-1} · day^{-1}). Nitrogen balance was determined from samples taken during two separate collection periods, specifically Week 2 (Days 7–10) and Week 3 (Days 14–17) of each trial. The study was conducted on an outpatient basis, although some of the women resided at a clinical research center to ease the demands of travel and of the urine and stool collections. Each trial was separated by a minimum of 7 days, during which time each woman consumed her customary, self-chosen diet. A description of the experimental design is given in Table 1.

Diet

All of the women consumed meals that followed a 3-day rotating menu. Meats were excluded from the menus because they are higher-protein food sources that are difficult to incorporate into lower-protein menus. However, high-quality animal-based proteins were included in the diet. Total energy intake was provided according to each woman's resting metabolic rate (RMR), predicted from the Harris-Benedict equation for women (12), with a 75% allowance made for habitual daily activity. The nonprotein energy content of the diet consisted of 65% carbohydrate and 35% fat. Meals were provided as follows during each of the three 18-day trials:

1. Day 1: A eucaloric, very low protein diet (mean intake 0.18 ± 0.01 g of protein · kg^{-1} · day^{-1}) used to enhance adaptation to the subsequent protein intakes (13).
2. Days 2–18: Daily menus designed to contain either 0.50, 0.75, or 1.00 g of protein · kg^{-1} · day^{-1} were provided using a basal menu of solid foods (0.40 g of protein · kg^{-1} · day^{-1}) and a protein supplement mixture (Table 2). The protein supplement mixture contributed the remaining amount of protein (either 0.10, 0.35, or 0.60 g of protein · kg^{-1} · day^{-1}), and consisted of cottage cheese with graded quantities of protein powder (ProMod, Ross Laboratories, Columbus, OH) to equal the specified amounts of protein. Water intake was allowed ad libitum.

All of the women agreed to scrape and rinse all dishes, glassware, and utensils with water and to consume the rinsings. For each trial, food homogenates were made of each woman's three daily menus during the second week of the study (Days 8–10) and frozen for total nitrogen analyses. All breakfast meals and the first day of meals beginning the nitrogen balance period (Day 7) were consumed at the research center's dining facility, while other meals (i.e., lunch, dinner, and weekend meals) were packed out.

The energy, protein, carbohydrate, and fat contents of each cycle menu were calculated by Nutritionist V computer software (Nutritionist V, version 1.5, First Databank, Inc, San Bruno, CA). No adjustments to the menus were made during the trials. One multivitamin/multimineral supplement tablet (Advanced Formula Centrum, Lederle Laboratories, Pearl River, NY) was taken daily by each woman. All of the women were asked to refrain from taking any self-

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administered vitamin and/or mineral supplements for at least 1 week before and during the entire period of study, including the times between trials.

Body Composition

Body weight was measured each weekday following an overnight fast and soon after voiding, to the nearest 0.1 kg on an Ohaus scale (model 15S, Ohaus Corporation, Florham Park, NJ). Nude body weight was calculated as the total body weight minus hospital robe weight. Body height was measured to the nearest 0.1 cm, without shoes, using a stadiometer. Body mass index was calculated as weight divided by height^2 (kg/m^2).

Body density was determined by whole body plethysmography during Weeks 2 and 3 of each trial using the Bod Pod body composition system (version 1.68, Life Measurement Instruments, Concord, CA) (14). Each woman was tested while fasting and after voiding, with each result being the average of triplicate measurements. Each woman wore only a tight-fitting bathing suit and a swim cap during the tests. Lung volume was directly measured in 9 of the 11 women. A predicted lung volume was used for two women because they were unable to successfully perform the measured lung volume test. Fat-free mass, percentage of body fat, and fat mass were calculated from body density using the two-compartment model equation developed by Siri (15).

Energy Metabolism

Resting metabolic rate measurements were obtained via indirect calorimetry during Weeks 2 and 3 of each trial. All resting metabolic rate tests were done in the fasting state after each woman had rested in a semirecumbent position for at least 30 minutes. Subsequently, a clear plastic box was placed over the woman's head so that all expired breath could be collected while she rested in a supine position for another 30 minutes. After a 10-minute stabilization period under the hood, 20 consecutive 1-minute measurements were taken. The hood indirect calorimetry system included analyzers for CO_2 (model Uras 4, Hartmann and Braun, Applied Automation, Frankfurt, Germany) and O_2 (model Magnos 4G, Hartman and Braun Applied Automation, Germany). These analyzers measured the reference air against the sample air and gave a reading as the percentage of difference between the two. The rate of air flow was controlled by a precise flow meter (model 5861L, Mass Flowmeter, Brooks Instruments, Hatfield, PA).

The averages of the CO_2 produced and excreted in the breath and the O_2 taken into the lungs and extracted into the body (in L/minute) were then calculated and used to determine the respiratory exchange ratio (CO_2/O_2). Using the respiratory exchange ratio, a conversion factor of kJ per liter of oxygen was determined and used to calculate the RMR (16).

Food, Urine, and Stool Collections

During each of the three trials, the following samples were collected and aliquots frozen and stored for subsequent analyses of total nitrogen content:

- One duplicate composite of each of the three menus (food and protein supplement mixture together);
- One composite of a 3-day stool collection (Days 7–9) (Stool dye markers consisting of encapsulated food coloring [either FD&C Blue No.l Alum Lake 11%–13% or Carmine Red, Warner-Jenkinson Co, Inc, Saint Louis, MO] were given orally at the start and end of each collection period); and
- Eight 24-hour urine collections (Week 2, Days 7–10; Week 3, Days 14–17).

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Nitrogen Analyses

Food, urine, and stool samples were analyzed for total nitrogen content (duplicate analyses of each sample) via the Elementar Macro N Nitrogen analyzer (Elementar Analysensysteme, Hanau, Germany). The National Institute of Standards and Technologies (NIST) Typical (No. 1548a) and Whole Milk Powder (No. 8435) diet reference materials, along with in-house pooled food and urine standards were used as quality controls. According to the NIST reference values, the percentage of nitrogen contents of the Typical and the Whole Milk Powder diets should be 3.03% ± 0.31% and 4.18% ± 0.05%, respectively. In comparison, our Macro N Nitrogen analyzer ran these samples at 2.90% ± 0.29% and 4.13% ± 0.10% for the Typical and Whole Milk Powder diets, respectively. For the in-house pooled food sample, within- and between-assay coefficients of variation were 5.5% and 5.6%, respectively. The within- and between-assay coefficients of variation for the in-house pooled urine standard were 4.6% and 6.5%, respectively. Dietary protein intake (g of protein $\cdot$ kg^{-1} $\cdot$ day^{-1}) was calculated by assuming that each gram of nitrogen was equivalent to 6.25 g of protein.

Apparent Nitrogen Balance and Calculations of Protein Requirement

Nitrogen balance (mg of N $\cdot$ kg^{-1} $\cdot$ day^{-1}) was measured during Week 2 (Days 7–10) and Week 3 (Days 14–17) of each trial and calculated as $I_N - (U_N + F_N + M_N)$, where I_N = dietary nitrogen intake; U_N = daily urinary nitrogen excretion; F_N = daily fecal excretion; and M_N = miscellaneous nitrogen losses, assumed to be 8 mg of nitrogen $\cdot$ kg^{-1} $\cdot$ day^{-1}. This is the formula currently accepted by the World Health Organization (WHO) (17) and the National Research Council (1) to calculate nitrogen balance.

For each woman, the nitrogen-balance data from all three trials (data from Week 2 and Week 3 were treated separately) were used to establish a linear regression equation between protein intake (x-axis) and nitrogen balance (y-axis) (2). The protein intake predicted to result in zero nitrogen balance was used to estimate the protein requirement for each woman (using inverse prediction). The mean protein requirement for this group of elderly women was taken as the average of the protein requirement estimates of the 11 individual subjects. A suggested protein allowance to safely and adequately meet the dietary protein needs of 97.5% of the population of elderly women was estimated to be twice the standard deviation (*SD*) above the mean protein requirement (17).

Clinical Blood Parameters

Fasting blood samples were obtained via venipuncture on the mornings of Days 11 and 18 of each trial. A complete blood count was performed by the Pathology and Laboratory Medicine Service (Central Arkansas Veterans Healthcare System, Little Rock, AR) and included analyses of hemoglobin, hematocrit, red blood cells, and white blood cells. Serum was separated by centrifugation and used for analyses of albumin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and blood urea nitrogen (BUN), using standard chemistry techniques at the same clinical laboratory. Specifically, albumin was determined through an adaptation of the bromocresol purple dye-binding method (18). FSH and LH were analyzed by microparticle enzyme immunoassay. Last, BUN was analyzed using the urease/glutamate dehydrogenase coupled enzymatic technique (19).

Statistical Methods

Group data are reported as mean ± standard error of the mean (*SEM*). Within-trial comparisons (i.e., Week 2 vs Week 3) for all independent variables were made using paired *t* tests (two-sided). If no difference was found within trials, the data from Week 2 and Week 3 were averaged, and the average value was used for among-trial comparisons. Comparisons among the three trials were made using repeated measures analysis of variance. All data processing

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and statistical analyses were done using Microsoft Excel 5.0 (Microsoft Corporation, Redmond, WA) and JMP Statistical Discovery software (SAS Institute, Inc, Cary, NC). Statistical significance was assigned at $p < .05$.

Results

Body Composition, RMR, and Clinical Blood Profile

There were no differences within trial (Week 2 vs Week 3) or among the three trials (LPro, MPro, and HPro) for body weight, percentage of body fat, fat-free mass, or RMR (Table 3). Likewise, with the exception of BUN, there were no differences within trial or between trials for any of the clinical blood parameters measured. The clinical blood parameters for the LPro trial were as follows: hemoglobin (12.6 ± 0.3 g/dl); hematocrit (37.6% ± 0.8%); red blood cell count (4.1 ± 0.11); white blood cell count (5.5 ± 0.41); albumin (3.5 ± 0.1g/dl); FSH (56.8 ± 10.2 IU/l); and LH (28.6 ± 4.7 IU/d). BUN concentrations were 8.2 ± 0.7, 11.6 ±0.8, and 14.5 ± 1.3 mmol/l for the LPro, MPro, and HPro trials, respectively (among-trial difference, $p = .0003$).

Dietary Intake

Each woman consumed the same menus throughout each trial, thus there was no difference within trials in energy, carbohydrate, fat, and protein intakes (Table 3). Mean energy intakes were not different among trials. Carbohydrate ($p < .001$) and fat ($p < .001$) intakes decreased in absolute terms with increasing protein intake. However, the nonprotein ratio of carbohydrate to fat of 65% to 35% was constant for all three trials.

Nitrogen Balance

Dietary nitrogen intake was increased from the LPro to the MPro to the HPro trials. Nitrogen analyses determined that the mean protein intakes for the LPro, MPro, and HPro trials were 0.53 ± 0.01, 0.76 ± 0.02 and 1.06 ± 0.05 g of protein $\cdot kg^{-1} \cdot day^{-1}$, respectively. Twenty-four-hour urinary nitrogen excretion increased with increasing dietary protein intake among trials. Urinary nitrogen excretion was significantly lower at Week 3 compared with Week 2 for Trials 1 ($p < .002$) and 3 ($p < .002$), with a similar trend apparent during Trial 2. Stool nitrogen excretion was not different among trials (data not reported); thus, stool nitrogen excretion was assumed to equal the mean excretion as calculated from the three trials combined (17).

At Week 2 and Week 3, net nitrogen balances increased (i.e., became more positive) with increasing protein intake among trials. A shift toward a more positive net nitrogen balance from Week 2 to Week 3 was apparent within trials and was due to a decrease in urinary nitrogen excretion. This apparent shift was only statistically significant for Trials 1 and 3, with a trend at Trial 2 (Table 4).

The protein requirement for each woman as well as the group mean protein requirement and protein allowance estimates are reported in Table 5. Using data from Week 2, the individual protein requirement estimates ranged from 0.55 to 0.86 g of protein $\cdot kg^{-1} \cdot day^{-1}$, with a mean (±*SD*) of 0.70 ± 0.10 g of protein $\cdot kg^{-1} \cdot day^{-1}$ (coefficient of variation [CV] = 13%). This mean protein requirement for these elderly women was greater than the assumed mean protein requirement of 0.6 g of protein $\cdot kg^{-1} \cdot day^{-1}$ (1). For Week 3, the individual protein requirement estimates ranged from 0.40 to 0.66 g of protein $\cdot kg^{-1} \cdot day^{-1}$ with a mean (±*SD*) of 0.56 ± 0.10 g of protein $\cdot kg^{-1} \cdot day^{-1}$ (CV = 17%). Due to a decrease in urinary nitrogen excretion and a shift toward a more positive nitrogen balance in elderly women, the calculated mean protein requirement was significantly lower at Week 3, compared with Week 2 ($p < .0004$). Based on these data, the protein allowances to safely and adequately meet the dietary protein needs of

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the population of elderly women were estimated to be 0.90 and 0.76 g of protein · kg^{-1} · day^{-1} at Weeks 2 and 3, respectively.

Discussion

This 18-day nitrogen balance study provides the most comprehensive assessment to date of the protein requirement for elderly women. The study was designed and conducted based on the current recommendations for valid nitrogen balance experiments (13,17), and with a full appreciation of the inherent and well-known limitations of the method (17,20,21). The data from Week 2 of the current study are consistent with other published literature (5,6,8) and indicate that the actual protein needs of elderly women are greater than the current RDA. However, the data from Week 3, of which to our knowledge no other published data are available for comparison, indicate that the RDA is adequate for elderly women.

Published studies of the protein requirement for elderly people, based on shorter-term nitrogen balance protocols, are very limited (3–5). Of these previous short-term balance studies, only Uauy and colleagues (5) assessed the protein requirement of elderly women. Uauy and colleagues determined the nitrogen balance responses of seven elderly women to graded levels of protein intake (specifically, 0.52, 0.65, and 0.80 g of protein · kg^{-1} · day^{-1}) from Days 6 to 10 of 10-day trials. From these data, they concluded that the mean protein requirement of these elderly women was 0.83 g of protein · kg^{-1} · day^{-1}. Based on a further reassessment of the data, an estimated protein allowance of 1.04 g of protein · kg^{-1} · day^{-1} was established (8). The data of Uauy and colleagues suggest that the RDA for protein should be higher than the current standard of 0.8 g of protein · kg^{-1} · day^{-1}, a finding supported by the Week 2 nitrogen balance data in the present study.

The study of Uauy and colleagues (5) utilized a 10-day nitrogen balance protocol. Previously, Rand and colleagues (22) determined that, in response to a change in protein intake, a mean time of only 4.5 days was required for the reestablishment of steady state for urinary nitrogen excretion in elderly women. Rand and colleagues also reported that 95% of these elderly women reached equilibrium for urinary nitrogen excretion within 8 days, and 100% within 10 days. However, another study by Rand and colleagues (2) was limited in that it was only 10 days long, and thus did not establish whether urinary nitrogen excretion remained stable for longer periods of time, an attribute consistent with steady state. Indeed, shorter-term nitrogen balance studies (3–5) have been criticized (9) on the premise that they do not provide an adequate period of time for steady state to be reached, contributing to greater variability in daily urinary nitrogen excretion.

In the present study, the finding of a decrease in urinary nitrogen excretion and a shift in net nitrogen balance toward a more positive balance at Week 3, brings into question the notion of whether a steady state indeed occurred by Days 6 to 10 (13,22). A longer-term nitrogen balance study supports that steady state may not be attained within 6 to 10 days (6). Gersovitz and colleagues (6) conducted the longest-term assessment of the RDA in their 30-day nitrogen balance study. Fifteen men and women aged older than 70 years consumed 0.8 g of protein · kg^{-1} · day^{-1} for 30 days. At study Days 1 to 10, 13 of the 15 subjects were in negative nitrogen balance. Although the mean nitrogen balance became more positive over time, 7 of the 15 subjects remained in negative nitrogen balance at Days 21 to 30, indicating that some type of adaptive response occurred. The authors concluded that the RDA of 0.8 g of protein · kg^{-1} · day^{-1} was inadequate for some older people (6).

A metabolic and physiological accommodation can be defined as a survival response in which significant losses occur in bodily functions as a result of an inadequate protein intake (23). In an accommodation, urinary nitrogen excretion would decrease until the achievement of a lower

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steady state, in conjunction with a loss of total body nitrogen (23,24). Previous research with elderly women established that inadequate protein intake resulted in an accommodation, including losses of body cell mass, muscle function, and immune response (25). Castaneda and colleagues (25) observed a similar shift toward a positive nitrogen balance in elderly women fed a low protein diet (0.45 g of protein · kg^{-1} · day^{-1}). These elderly women accommodated to a low-protein diet with a significant shift toward positive nitrogen balance and losses in protein mass and functional capacity by Week 9, specifically when compared with the first measurements taken at Week 3. It is possible that this shift in nitrogen balance observed by Castaneda and colleagues occurred before Week 9, which is consistent with the current study data showing a shift toward a more positive nitrogen balance by Week 3. It is unlikely, however, that the shift observed between trials in the current study data was reflective of an accommodation, because the shift also occurred for the HPro group at a dietary intake of 1.06 ± 0.05 g of protein · kg^{-1} · day^{-1}.

Table 5 illustrates the calculated protein requirement for each individual woman at Week 2 and Week 3. The obvious shift toward a more positive nitrogen balance from these two measurement periods results in a lower apparent protein requirement at Week 3 than at Week 2. The 1985 RDA was set at 2 *SD*s above the group mean protein requirement to safely and adequately meet the protein needs of practically all individuals. Thus, at Week 2, the estimated protein allowance was 0.90 g of protein · kg^{-1} · day^{-1}, a value higher than that of the current RDA. At Week 3, the estimated protein allowance was 0.76 g of protein · kg^{-1} · day^{-1}, a value virtually the same as the WHO estimate of 0.75 g of protein · kg^{-1} · day^{-1} (17), and thus a value not different from the current RDA (1).

In light of the lingering debate over the protein requirement of elderly individuals, it is important to question which data are more appropriate to use to establish the protein requirement for elderly women, Week 2 or Week 3? Some researchers suggest that elderly people should consume 12% to 14% of total energy intake as protein (5,8,13,21,25–27). This recommendation calls for a higher percentage of energy from protein than that of a younger person. To consume 12% of energy from protein, an elderly woman who weighs 65 kg and has an energy requirement of 1900 kcal/day would need to consume 0.88 g of protein · kg^{-1} · day^{-1}. This protein intake would be 10% more than the current RDA and an amount similar to the protein allowance estimated from the Week 2 data.

Munro and colleagues (28) assessed the protein status of 691 free-living elderly men and women divided into groups aged 60 to 75 years old and those aged older than 75 years. The average protein intakes among these groups of elderly people ranged from 1.02 to 1.06 g of protein · kg^{-1} · day^{-1}. Plasma concentrations of albumin, prealbumin, and transferrin were found to decline with age; however, they were not related to low intakes of protein. Munro and colleagues concluded that a low dietary protein intake does not have a detrimental effect in maintaining the health of elderly people. They further stated that their research implies that 1.00 g of protein · kg^{-1} · day^{-1}, a level significantly higher than the current RDA of 0.8 g of protein · kg^{-1} · day^{-1}, is adequate to maintain plasma protein levels and tissue protein content to offset the losses that inevitably occur with aging.

Horwitz and colleagues (29) noted that elderly individuals are more likely to be influenced by various biological, environmental, and social factors that could alter the protein needs of this population. The general statements of the WHO (17) support this view, The WHO statements indicated that, despite insufficient data thus far, the protein needs of adults might be expected to change progressively with aging, in part due to the changes in body composition, functional capacity, physical activity, food intake, and frequency of disease. Thus, protein allowances should ideally enable an elderly person to maintain not only health, but also bodily function and physical and mental capacities. Vellas and colleagues (30) reported that over a 10-year

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period, elderly women who habitually consumed greater than 1.2 g of protein · kg^{-1} · day^{-1} developed fewer health problems than elderly women who consumed less than 0.8 g of protein · kg^{-1} · day^{-1}. Given this, one might conclude that it is better to err on the side of a higher protein intake in elderly individuals.

Conclusion

In summary, data from the current study do not provide a definitive assessment of the mean protein requirement and suggested adequate protein allowance for elderly women. However, the current data do provide the most comprehensive assessment to date, and provide important information for future research. In particular, the decrease in urinary nitrogen excretion from Week 2 to Week 3, and thus the increase in net nitrogen balance, brings into question whether a steady state in nitrogen excretion was reached and maintained by Day 10, as assumed by previous research (22,31). It is apparent that longer-term nitrogen balance studies will be required to establish when steady state is attained and to establish the actual protein requirement of elderly women based on changes in urinary nitrogen excretion and other independent indicators of protein status. Because elderly women make up a large and growing segment of society, it is important to determine not only what is considered an adequate level of protein intake, but also an optimal intake for these individuals.

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Table 1

Approximate Study Schedule

Measure	Study Day																	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Diet	VLP	CD	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
24-h urine collections							x	x	x	x				x	x	x	x	
Stool collections							x	x	x									
Food homogenates								x	x	x								
Body composition							x							x				
Resting metabolic rate							x							x				
Fasting blood draw											x							x

Notes: VLP = very low protein diet; mean intake of 0.18 ± 0.01 g of protein·kg^{-1}·d^{-1} (mean ± *SEM*). CD = controlled diet of either 0.53 ± 0.02, 0.76 ± 0.02, or 1.06 ± 0.05 g of protein · kg^{-1} · day^{-1}.

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Table 2

Protein Supplement Mixture (Example of ProMod® Added to Menus at Each Trial)

	LPro	MPro	HPro
Cottage cheese, g	100	125	125
ProMod®, g	0	2.1	22
Protein, g	5	15	31

Notes: LPro refers to a dietary protein intake of 0.50 ± 0.02 g of protein·kg^{-1}·d^{-1}, MPro refers to 0.76 ± 0.02 g of protein·kg^{-1}·d^{-1}, and HPro refers to a dietary protein intake of 1.06 ± 0.05 g of protein·kg^{-1}·d^{-1}. ProMod® protein powder supplement is composed of D-whey protein concentrate and soy lecithin; 1 oz serving contained 21 g of protein.

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Table 3

Subject Characteristics and Dietary Intake

Characteristic	Trial		
	LPro	MPro	HPro
Age, y	75 ± 4.0		
Height, cm	64.1 ± 0.6		
Weight, kg			
Week 2	72.7 ± 3.9	73.0 ± 4.0	72.8 ± 4.0
Week 3	72.3 ± 3.9	72.8 ± 4.0	72.4 ± 4.0
Body mass index, kg/m^2	27.3 ± 3.8		
Body fat, %†			
Week 2	44.0 ± 1.5	44.4 ± 1.5	44.3 ± 1.6
Week 3	45.0 ± 1.6	45.2 ± 1.5	43.5 ± 1.7
Fat-free mass, kg†			
Week 2	38.8 ± 1.5	40.1 ± 1.6	40.7 ± 2.0
Week 3	39.4 ± 1.6	39.6 ± 1.6	40.1 ± 1.7
Resting metabolic rate, MJ/d	4.99 ± 0.22	4.95 ± 0.18	4.96 ± 0.17
Energy intake, MJ/d	9.31 ± 0.26	9.32 ± 0.27	9.33 ± 0.27
Carbohydrate intake, g/d‡	330 ± 9.5	313 ± 8.8	302 ± 8.6
Fat intake, g/d‡	77 ± 2.0	78 ± 2.0	74 ± 2.0
Protein intake, g/d	36.6 ± 1.8	54.9 ± 2.7	73.2 ± 3.6
(g·kg^{-1}·d^{-1})	0.50 ± 0.01	0.75 ± 2.7	1.01 ± 3.6

Notes: Values are reported as mean ± *SEM*. LPro refers to a measured protein intake of 0.53 ± 0.02 g of protein·kg^{-1}·d^{-1}; MPro refers to 0.76 ± 0.02 g of protein·kg^{-1}·d^{-1}; and HPro refers to 1.06 ± 0.05 g of protein·kg^{-1}·d^{-1}.

† Percentage of body fat and fat-free mass were determined by whole body plethysmography.

‡ Carbohydrate, fat, and protein intakes were different among trials ($p < .0001$).

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Table 4

Total Nitrogen Intake, Excretion, and Balance for Elderly Women

Measure	Trial		
	LPro	MPro	HPro
Dietary nitrogen intake (mg N·kg^{-1}·d^{-1})			
Week 2	84.2 ± 0.8	120.9 ± 1.0	169.9 ± 2.4
Week 3	83.6 ± 0.6	121.4 ± 0.8	171.0 ± 2.8
Nitrogen excretion (mg N·kg^{-1}·d^{-1})			
Urine			
Week 2	71.8 ± 2.9	90.4 ± 2.0	119.6 ± 3.5
Week 3	56.9 ± 2.4†	79.0 ± 0.8	102.2 ± 3.0†
Stool‡	33.1 ± 2.5	same	
Miscellaneous§	8.0	same	
Nitrogen balance‖ (mg N·kg^{-1}·d^{-1})			
Week 2	−14.5 ± 3.1	3.8 ± 2.5	23.4 ± 3.3
Week 3	−0.1 ± 2.7	8.5 ± 3.6	42.0 ± 3.0

Notes: Values are reported as mean ± *SEM*. LPro denotes 0.53 ± 0.02 g of protein·kg^{-1}·d^{-1}; MPro at 0.76 ± 0.02 mg N·kg^{-1}·d^{-1}; and HPro at 1.06 ± 0.05 mg N·kg^{-1}·d^{-1}.

† A comparison of urinary nitrogen excretion in Week 2 vs. Week 3 for LPro ($p < .002$) and HPro ($p < .002$). A comparison between trials at Week 2 and at Week 3 was highly significant.

‡ Stool calculations are the mean nitrogen output from all trials combined and collected during Week 2. Stool nitrogen output was not significantly different among trials.

§ Miscellaneous losses assumed to be 8 mg N·kg^{-1}·d^{-1} (17).

‖ Nitrogen balance, equal to dietary nitrogen intake minus (urine + stool + miscellaneous N excretions).

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Table 5

Individual Mean Protein Requirement

Subject	Week 2 (g of protein·kg^{-1}·d^{-1})	R^2 Value	Week 3 (g of protein·kg^{-1}·d^{-1})	R^2 Value
1	0.86	.992	0.65	.854
2	0.75	.997	0.65	.857
3	0.66	.957	0.42	.950
4	0.72	.994	0.57	.964
5	0.62	.999	0.55	.548
6	0.55	.999	0.47	.999
7	0.81	.927	0.64	.763
8	0.80	.970	0.66	NA[†]
9	0.71	.782	0.40	.570
10	0.60	.886	0.51	.998
11	0.66	.999	0.66	.962
Mean	0.70[‡]		0.56[§]	
SD	0.10		0.10	
SEM	0.03		0.03	
CV	13.5		17.7	
Protein allowance[‖]	0.90		0.76	

Notes: R^2 values indicate the proportion of total variability of the nitrogen balance that can be accounted for by the dietary protein intake. CV = coefficient of variation.

[†] Subject 8 did not collect samples for nitrogen balance during Week 3 of Trial 2; thus, no R^2 value could be calculated.

[‡] Different than the assumed mean protein requirement of 0.6 g of protein·kg^{-1}·d^{-1} (1) at Week 2 ($p < .005$).

[§] Different from the value calculated at Week 2 ($p < .0004$).

[‖] Calculated as 2 *SD*s above the mean.