

Research and Professional Briefs

Effects of Whey and Fortified Collagen Hydrolysate Protein Supplements on Nitrogen Balance and Body Composition in Older Women

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ABSTRACT

Many elderly people have a low intake of dietary protein, yet their protein requirement may be higher than the current Recommended Dietary Allowance. High-quality protein supplements may be useful to enhance nitrogen retention and increase the availability of essential amino acids in elderly people. We compared the nitrogen balance of two protein supplements (Resource Beneprotein Instant Protein Powder, Nestlé HealthCare Nutrition, Minnetonka, MN, a whey protein concentrate; or Pro-Stat 101, Medical Nutrition USA, Englewood, NJ, a concentrated, fortified, collagen protein hydrolysate) varying in type but not amount of protein content using a crossover study design. The study consisted of two 15-day diet trials separated by a ≥ 1 -week washout period. Nine healthy elderly women (age 71 ± 1 years) were provided a euca-

loric diet containing approximately the protein Recommended Dietary Allowance of 0.8 g/kg body weight/day. The supplements constituted about half of the total protein provided to each subject. Nitrogen balance responses were assessed over days 6 to 10 and days 11 to 14 of each trial. Measured nitrogen content of the foods indicated that subjects consumed 0.81 ± 0.02 g protein/kg/day and 0.85 ± 0.05 g/kg/day for the whey and fortified collagen protein trials, respectively. Body weight decreased ($P = 0.02$) after consumption of the whey supplement, with no significant changes in body weight or composition resulting from the consumption of the collagen supplement. Nitrogen excretion was higher during the whey supplement trial than during the collagen trial ($P = 0.047$). Therefore, a concentrated, fortified, hydrolyzed collagen protein supplement maintained nitrogen balance and preserved lean body mass during 15 days of consumption of a relatively low-protein diet.

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Adequate dietary protein is essential to maintain lean body mass and provide adequate amounts of amino acids for protein synthesis in all tissues. Sarcopenia is the age-associated loss of muscle mass (1) and is associated with an increase in body fat, decreased basal metabolic rate and daily energy needs, loss of bone mass, and reduced strength and functional status (2). A growing body of evidence indicates that aging may be associated with increased need for dietary protein (3-5) and that consumption of a eucaloric diet providing the Recommended Dietary Allowance (RDA) for protein (0.8 g/kg body weight/day) results in a significant loss of muscle mass in healthy older men and women (6). Several nutrition surveys demonstrate that a significant percentage of free-living, community-dwelling elderly people as well as those living in long-term care facilities consume less than the current RDA for protein (7,8), which may result in the loss of skeletal muscle mass (9,10) and subsequent morbidity, functional decline, and mortality.

Given that many elderly people consume a relatively low-protein diet at the same time that dietary protein requirements are likely increased, a high quality, low-fat protein supplement has been shown to reduce complications and decrease mortality for those in a hospital setting (10). Both whey and fortified collagen are marketed and used in long-term care settings to increase dietary protein intake in elderly people with low food intake (11). The purpose of this study was to compare two commonly used protein supplements on nitrogen balance and

changes in body composition in older women. Our hypothesis was that a supplement of whey protein would result in a similar nitrogen balance compared to a collagen-based protein hydrolysate supplement.

SUBJECTS AND METHODS

Design and General Overview

This was a double-blind, crossover study; each subject completed two separate 15-day trials, with the order of the trials assigned in a randomized, balanced fashion. Each trial provided a total dietary protein intake of approximately 0.8 g/kg body weight/day. Approximately half of the protein was administered as a supplement and consisted of either Protein A (Resource Beneprotein Instant Protein Powder, Nestlé HealthCare Nutrition, Minnetonka, MN) or Protein B (Pro-Stat 101, Medical Nutrition USA, Englewood, NJ). The supplements were prepared as described below to match them for portion size, energy content, and palatability. Resource Beneprotein is a concentrated source of 100% whey protein isolate, and has a protein digestibility-corrected amino acid score (PDCAAS) of 100, and Pro-Stat 101 is a concentrated, enzyme-hydrolyzed collagen protein fortified with L-tryptophan with a PDCAAS of ~36. The remainder of the diet is described below. Nitrogen balance was determined from food and urine samples obtained during days 6 to 10 and 11 to 14 of each trial. The study was conducted on an outpatient basis, with subjects eating breakfast under supervision each weekday in the Clinical Research Center; food for remaining meals was packaged for each subject to consume at home. The trials were separated by a minimum 7-day wash-out period, during which time each subject consumed her habitual, self-chosen diet. The study protocol and informed consent form were approved by the Institutional Review Board at the University of Arkansas for Medical Sciences.

Participants

Eleven women between the ages of 65 and 85 years were recruited. 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. Two women withdrew from the study for reasons unrelated to the protocol, leaving a final sample size of nine women (all white) who completed both dietary periods.

MEASURES AND PROCEDURES

Study Diet

All of the women consumed meals that followed a 3-day rotating menu consisting of commonly consumed foods along with the different protein supplements. High-quality animal-based proteins were included in the food (ie, nonsupplement-based) portion of the diet. Total energy content of the diet was provided according to the 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 two 15-day trials:

day 1: A eucaloric very-low-protein diet (mean intake 0.18 ± 0.01 g protein/kg body weight/day), used to enhance adaptation to the subsequent protein intakes (13); and

days 2 through 15: Daily menus containing ~0.8 g protein/kg body weight/day were provided using a standardized menu of nonsupplement-based foods (intended to provide 0.4 g protein/kg/day) and a protein supplement mixture given with each meal. The protein supplement mixture consisted of protein A or protein B (amounts matched for protein content), plus added light cream, sugar, and flavoring to match the supplements for energy content and palatability. The supplements contributed the remaining amount of dietary protein to provide a total protein intake of approximately 0.8 g/kg/day. Water and no-energy, noncaffeinated beverages were allowed ad libitum.

All of the women were asked to scrape and rinse all dishes, glassware, and utensils with water and to consume the rinsing to ensure complete intake of provided foods. The estimated energy, protein, carbohydrate, and fat contents of each cycle menu were calculated by Pro-Nutra computer software (version 3.2, 2007, Viocare Technologies, Princeton, NJ).

Body Composition Assessments

Body weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 cm. Body mass index was calculated as kg/m^2 .

Body density was determined by whole body plethysmography initially and on day 15 of each trial of nitrogen balance data collection using the BOD POD body composition system (version 1.91, 2002, Life Measurement Instruments, Concord, CA) (14). Fat-free mass, percent body fat, and fat mass were calculated from body density using the two-compartment model equation of Siri (15).

Energy Metabolism Assessment

RMR measurements were obtained over a 30-minute period via indirect calorimetry (VMax 29N, SensorMedics Corp, Yorba Linda, CA) initially and on day 15 of each trial. All RMR tests were performed in the fasting state after each woman had rested in a semirecumbent position for at least 30 minutes. No steady state criteria for terminating the RMR test or for processing the calorimetry results was used, but data from the first 10 minutes of the measurement period were generally excluded from subsequent analyses.

Food and Urine Sample Analyses

The following samples were collected during each trial, and aliquots stored frozen for subsequent analyses of total nitrogen content:

- One duplicate composite of each of the three menus (food and protein supplement mixture together) during the second week of the study (days 8 through 10).
- 24-hour urine samples.

Subjects collected their urine over each 24-hour period into individual chilled containers, which were returned

for processing. Fecal collections were not made in this study. Differences in nitrogen balance subsequent to changes in dietary protein intake have been shown to be a result of differences in urinary nitrogen excretion with no differences observed in fecal nitrogen losses (16,17). Assumed values for fecal nitrogen loss were used in our calculations based on previous data collected by our group (17).

Nitrogen Analysis and Balance Calculations

Food composite and urine samples were analyzed for total nitrogen content (triplicate analyses of each sample) via the TruSpec N Nitrogen Determinator (version 1.62, 2006, LECO Corporation, St Joseph, MI). A 0.5% glycine solution standard was used as quality control for both types of sample analyses and measured before, during, and after each assay. Within- and between-assay coefficients of variation for the food standards were 1.9% and 2.3%, respectively, and those for the urine standards were 2.1% and 2.2%, respectively. Dietary protein intake was calculated from the food nitrogen data assuming a factor of 6.25 g protein/g nitrogen and was compared to estimated protein intake calculated via ProNutra.

Nitrogen balance (mg nitrogen/kg/day) was measured during days 6 to 10 and days 11 to 14 of each trial. Balance was 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 5 mg/kg/day according to World Health Organization recommendations (16). Daily fecal excretion was assumed to be 33.1 mg/kg/day based on the mean excretion calculated by Morse and colleagues (17) who observed no differences in fecal nitrogen between low and high protein intakes (0.5 to 1.0 g/kg/day).

In total, four nitrogen-balance periods were examined: Protein A supplement trial days 6 to 10 and 11 to 14 and protein B supplement trial days 6 to 10 and 11 to 14. The total duration of each intervention and measurement period was based on published recommendations (18). Habitual dietary intake was not assessed; however, potential baseline differences in protein status should not substantially influence the outcomes because the order of supplementation was roughly balanced, and thus any baseline effects would be expected to be distributed equally between the two dietary supplement interventions.

Statistical Analysis

Comparisons were made within and between trials for body composition and protein intake using paired *t* tests (two-tailed). Nitrogen balance comparisons within each trial (days 6 to 10 vs days 11 to 14) and between periods (days 6 to 14) were made using paired *t* tests (two-tailed). Potential differences between Protein A and Protein B supplements were examined using an analysis of variance with repeated measures (5 days' pooled urinary nitrogen excretion during the last 10 days of each dietary period). Group data were reported as mean \pm standard error of the mean. Data processing and statistical analyses were done using Microsoft Excel 2003 (Microsoft Corp, Redmond, WA) and SPSS (version 12.0, 2003, SPSS

Inc, Chicago, IL). Sample size and dietary protein intake to achieve nitrogen balance was determined from our previous research (18-21).

RESULTS

Dietary Intake

No differences were found in energy, carbohydrate, fat, or protein intake between the dietary periods (see the Table). The protein content of food calculated from nitrogen analysis of food homogenates was 0.82 ± 0.04 g protein/kg/day for protein A and protein B trials combined.

Body Composition and RMR

There were no differences between trials for changes in body fat content or RMR. However, body weight decreased ($P=0.02$) within the whey protein trial (see the Table). Percent body fat was not found to be significantly different. Note that the observed differences in percentage body fat were smaller than the expected reliability of between-day BOD POD measurements (22). For the Protein B trial, no changes in body weight or composition were observed.

Nitrogen Balance

There were no differences in urinary nitrogen excretion from days 6 to 10 to days 11 to 14 for either trial (see the Table). Nitrogen excretion was higher during the Protein A trial than during the protein B trial ($P=0.047$). However, there were no statistically significant differences in nitrogen balance between the two trials (see the Figure). The observed power in our study (in regards to the nitrogen balance data) was 0.204, given the sample size (nine subjects), the observed difference between mean nitrogen balance values during each diet period (12.47 mg/kg/day), and the standard deviation of this difference (29.3). We did observe a significant difference in nitrogen excretion between the two diet periods, suggesting our study was powered appropriately to detect significant differences between dietary periods.

It may be informative to examine the 95% confidence interval of the difference in nitrogen balance between the two diet periods (23,24). The width of the interval can help determine whether a study has excluded or failed to exclude a potentially important value (23). If the interval is narrow (ie, excluding all values that would be considered clinically or practically important) then the nonsignificant results could be considered conclusive—no difference between treatments exists. If the upper and/or lower boundaries of the interval encompass values deemed clinically or practically important, then the data would be considered inconclusive about a potential treatment effect. In our study, the 95% confidence interval of the difference in nitrogen balance between the two diet periods was (-10.05, 35.00 mg/kg/day), and thus values that could be considered clinically important are included within the interval. Thus, our study fails to exclude a potentially important treatment difference regardless of the observed power.

Table. Characteristics, body composition, dietary intake, and total nitrogen intake and excretion of healthy elderly women during each dietary intervention period of a trial to determine whether high-quality protein supplements may be useful to enhance nitrogen retention and increase the availability of essential amino acids in elderly people

Characteristic	Protein A supplementation period	Protein B supplementation period
	← mean ± standard error of the mean →	
Age (y)	71.3 ± 1.0	
Body mass index	29.2 ± 1.3	
Height (cm)	161.5 ± 1.6	
Weight (kg)		
Day 1	75.3 ± 3.5	75.0 ± 3.4
Day 15	74.5 ± 3.5 ^a	74.5 ± 3.6
Change	-0.81 ± 0.28	-0.50 ± 0.27
Body fat (%)		
Day 1	43.7 ± 2.0	43.2 ± 2.0
Day 15	43.6 ± 1.9	43.3 ± 2.6
Change	-0.10 ± 0.20	-0.24 ± 0.76
Fat-free mass (kg)		
Day 1	41.9 ± 1.1	42.1 ± 1.0
Day 15	41.5 ± 1.0	41.8 ± 0.8
Change	-0.38 ± 0.21	-0.31 ± 0.34
Resting metabolic rate		
Day 1	6.25 ± 0.28	6.18 ± 0.20
Day 15	6.02 ± 0.21	6.11 ± 0.25
Change	-0.23 ± 0.23	-0.07 ± 0.19
Energy intake	9.87 ± 0.24	9.85 ± 0.24
Carbohydrate intake (g/d)	337.5 ± 6.8	336.9 ± 6.7
Fat intake (g/d)	81.6 ± 1.9	81.6 ± 1.9
Protein intake (g/d)	76.7 ± 3.2	76.6 ± 3.2
Measured protein intake (g/kg/d)	0.81 ± 0.02	0.85 ± 0.05
Nitrogen intake (mg/kg/d)		
Days 6-10	130.5 ± 3.4	136.3 ± 8.2
Days 11-14	128.0 ± 3.7	135.4 ± 7.4
Days 6-14	129.2 ± 3.5	135.9 ± 7.8
Nitrogen excretion (mg/kg/d)		
Urine		
Days 6-10	102.0 ± 4.8	96.6 ± 6.2
Days 11-14	108.9 ± 5.5	102.8 ± 7.1
Days 6-14 ^b	105.5 ± 4.7	99.7 ± 6.4
Stool ^c	33.1	33.1
Miscellaneous ^d	5.0	5.0

^aSignificant difference between day 1 and day 15 body weight during the Protein A supplementation period ($P=0.02$).

^bSignificant difference in urinary nitrogen excretion over days 6-14 between Protein A and Protein B supplementation periods ($P=0.047$).

^cCalculations from Morse and colleagues (17).

^dMiscellaneous losses assumed to be 5 mg nitrogen/kg/d (13).

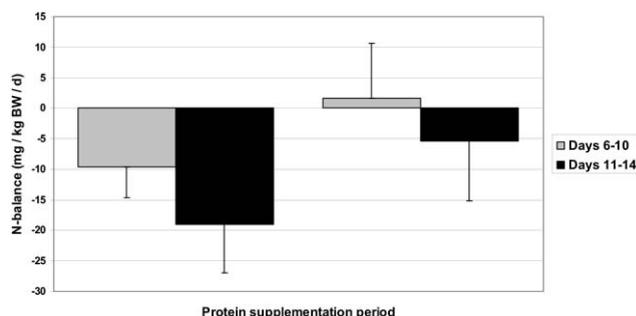


Figure. Mean nitrogen balance (N-balance) for days 6 to 10 (shaded bars) and days 11 to 14 (solid bars) of the protein A (whey protein; left) and protein B (fortified collagen protein; right) supplementation trials. Differences between time periods and supplements were not significant.

etary protein intake close to the current RDA, both whey and fortified collagen hydrolysate protein supplements support nitrogen balance. During the Protein A trial, subjects experienced a significant reduction in body weight with no change in body fat, suggesting a potential decrease in lean body mass. In both trials, energy intake was adequate as reflected by preservation of total body fat content. These data also support previous studies that suggest that the current RDA for protein may be inadequate to maintain nitrogen balance in older people.

In our study, the duration of each diet trial was 15 days based on the assumption that steady state would be achieved within 6 to 10 days (25). Although there was a trend for increasingly negative nitrogen balance comparing the last days of the dietary period with those of days 6 to 10, the two balance periods were not statistically different, suggesting the achievement of a steady state.

Inadequate protein intake has been shown to result in accommodation in other long-term nitrogen balance studies. Campbell and colleagues (6) administered a eucaloric diet providing 0.8 g protein/kg/day for 14 weeks to men and women aged 55 to 75 years. Mean urinary nitrogen excretion decreased over time and those who showed greater reduction in urinary nitrogen excretion over time also experienced greater losses of mid-thigh skeletal muscle area, indicating an accommodation. These data strongly suggested that the protein RDA of 0.8 g protein/kg/day is inadequate to meet the protein needs of older people.

The conclusions of a limited number of short-term nitrogen balance studies in elderly people have been mixed regarding the adequacy of the RDA (17,26-28). Of these, two studies (17,27) that assessed the protein needs of elderly women demonstrated increased protein requirements compared to previous estimates for younger people. Campbell and colleagues (4) recommend protein intakes of 1.00 to 1.25 g protein/kg/day to meet the needs of most elderly people. The data from our study, combined with these previous estimates of dietary protein requirements of older people, strongly indicate that the current RDA is inadequate or marginal even when a eucaloric diet is consumed.

Average energy intakes in each of the studies described above were about 30 to 32 (25), 35 to 38 (6), 28 (27,29), and 30 kcal/kg (17). Body weight during each of the stud-

DISCUSSION

This study was designed to compare the effects of two different dietary protein supplements on nitrogen balance in older women. The results suggest that at a di-

ies remained relatively stable. Urinary nitrogen excretion decreased over time, resulting in adaptation or accommodation and the conclusion that the RDA of 0.8 g protein/kg/day may be inadequate for older people. In our study, average energy intake calculated by the nutrient analysis software during both dietary periods was about 31 to 32 kcal/kg/day and the women in this study did not lose body fat, demonstrating adequate energy intake. Urinary nitrogen excretion tended to increase from days 6 to 10 to days 11 to 14 during the protein A period ($P=0.139$) and during the protein B period ($P=0.118$); thus, a longer trial may have allowed the observation of a potentially greater decline in nitrogen balance.

Collagen hydrolysate supplementation enhances wound healing in elderly people in long-term care (30). A previous study (31) examining whey versus a collagen hydrolysate as the only sources of protein during a very-low-energy diet demonstrated that subjects losing weight during whey feeding were in more negative nitrogen balance than when consuming the collagen hydrolysate. Effective recycling of both essential and nonessential amino acids occurs in human beings to maintain protein synthetic rate (32). However, at inadequate or marginal dietary protein levels, both essential and nonessential amino acid intake may limit protein synthetic rate. Katsanos and colleagues (33) showed that an amino acid supplement has a smaller effect on muscle protein accretion in older compared to younger adults when small amounts (7 g) are consumed as a supplement. That study suggested that dietary protein has a smaller effect on stimulating muscle protein synthesis with advancing age. In our study, nitrogen balance declined during both dietary conditions, suggesting that the protein RDA may not be adequate to maintain even short-term nitrogen balance in older women.

Although collagen has a low PDCAAS, it contains a high proportion of dispensable amino acids that either have a low molecular weight or contain more than 1 nitrogen atom, which means that the nitrogen content of collagen may be higher than whey on a per-gram basis (11). Because subjects in our study were provided about half of their dietary protein requirement using high-quality foods, it is possible that this combination (ie, a diet comprised of foods containing sufficient amounts of indispensable amino acids necessary to meet specific protein synthesis needs and a nitrogen-rich collagen supplement necessary to meet nonspecific nitrogen needs) was sufficient to maintain nitrogen balance despite the low PDCAAS of the supplement.

CONCLUSIONS

Older women consumed food supplemented with equal amounts of protein from different sources. Total dietary protein intake was not different between the two trials and energy intake was adequate, as evidenced by no change in body fat mass in either diet group. Nitrogen balance was not different between the two groups, indicating equivalence of the protein A and protein B supplements. Although there was a small but significant decrease in body weight (with no change in body fat) as a result of consuming protein A, women who consumed a diet containing protein B demonstrated no change in body composition and maintained nitrogen balance. Although

differences between the supplement intervention periods were small, indicating that additional studies in larger samples of nutritionally at-risk populations are needed, these data suggest that the collagen-based protein is equivalent to whey protein as a supplement. Fortified collagen-based protein and whey may provide important therapeutic nutrition strategies to prevent negative nitrogen balance and to minimize subsequent morbidity and mortality in vulnerable elderly populations.

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