Two-Year Whey Protein Supplementation Did Not Enhance Muscle Mass and Physical Function in Well-Nourished Healthy Older Postmenopausal Women1–3

Kun Zhu,4,5* Deborah A Kerr,6 Xingqiong Meng,7 Amanda Devine,8 Vicky Solah,6 Colin W Binns,6 and Richard L Prince 4,5

4Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth, Australia; 5School of Medicine and Pharmacology, University of Western Australia, Perth, Australia; 6School of Public Health, Curtin University, Perth, Australia; 7Flinders Centre for Innovation in Cancer, School of Medicine, Flinders University, Adelaide, Australia; and 8School of Exercise and Health Sciences, Edith Cowan University, Perth, Australia

Abstract

Background: Protein may play a role in preventing muscle loss with aging. To our knowledge, there have been no long-term randomized controlled trials to examine the effects of increased dietary protein intake on muscle health in community-dwelling older women.

Objective: In this study, we evaluated the effects of whey protein supplementation on muscle mass and physical function in community-dwelling older Australian women.

Methods: In this 2 y randomized, double-blind, placebo-controlled trial, women aged 70–80 y (mean 74.3 ± 2.7 y) were randomly assigned to either a high protein drink containing 30 g of whey protein (n = 109) or a placebo drink containing 2.1 g protein (n = 110) daily. Dual-energy X-ray absorptiometry appendicular skeletal muscle mass, upper arm and calf (38% tibia) muscle cross-sectional area, physical function including hand grip strength, lower limb muscle strength and Timed Up and Go test, and 24 h urinary nitrogen were measured at baseline, 1 y, and 2 y.

Results: A total of 196 women with at least one follow-up measurement were included in this analysis. Baseline mean BMI was 26.7 ± 3.9 kg/m² and protein intake was 76 ± 17 g/d (1.1 ± 0.3 g · kg body weight−1 · d−1). A mean increase in protein intake of ~20 g/d in the protein group was confirmed by the estimates from 24 h urinary nitrogen. Over the 2 y in both groups there was a significant decrease in the upper arm (mean ± SE: −5.59 ± 0.75 cm²) and calf (−0.77 ± 0.11 cm²) muscle area, as well as hand grip strength (−1.30 ± 0.3 kg) (all P < 0.05), but appendicular skeletal muscle mass did not change significantly. There were no significant effects of the protein intervention on any of the muscle mass or physical function measures (all P > 0.05) at 1 and 2 y.

Conclusion: This study showed that in protein-replete, healthy, ambulant, postmenopausal older women, 30 g/d of extra protein did not improve the maintenance of muscle mass or physical function despite evidence of deterioration in muscle measurements in the upper limb. This trial was registered at the Australian New Zealand Clinical Trials Registry as ACTRN012607000163404. J Nutr 2015;145:2520–6.

Keywords: whey protein, muscle mass, muscle strength, physical function, older women

Introduction

Aging is associated with a progressive loss of muscle mass (sarcopenia), which can lead to reduced muscle strength and an increased risk of falls. As much as one-third of muscle mass can be lost during the 3 decades after the age of 50 y (1). The etiology of skeletal muscle loss with aging is unclear, but factors such as declining physical activity, altered protein synthesis and turnover, and reductions in serum insulin-like growth factor I (IGF-I)9 have been suggested (2). Furthermore, altered protein synthesis in older people may result from a reduced ability of aging skeletal muscle to respond to anabolic stimuli, such as insulin

1 This study was supported by the Australian National Health and Medical Research Council (Project grant: 458629) and the University of Western Australia Research Grants Scheme.
2 Author disclosures: K Zhu, DA Kerr, X Meng, A Devine, V Solah, CW Binns, and RL Prince, no conflicts of interest. None of the funding agencies had any role in the conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript.
3 Supplemental Tables 1 and 2 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
5 To whom correspondence should be addressed. E-mail: kun.zhu@uwa.edu.au.
9 Abbreviations used: Health ABC, Health, Aging, and Body Composition; IGF-I, insulin-like growth factor I; RCT, randomized controlled trial; TUG, Timed Up and Go.
and amino acid availability, rather than from reduced basal muscle protein synthesis (3).

Increased availability of amino acids has been shown to have positive effects on muscle anabolism (4), and oral amino acid supplementation could improve lean body mass in older adults (5, 6). Besides increasing amino acid availability, protein intake may also affect muscle anabolism through its positive effects on the production and action of IGF-I (7). A study with elderly women showed that marginal protein intake was associated with loss of muscle mass and reduced plasma IGF-I concentration (8). Cross-sectional (9, 10) and longitudinal cohort (11, 12) studies have shown positive associations between dietary protein intake and maintenance of lean body mass or muscle mass with aging. However, observational studies cannot determine the causal relation, and a few short-term protein intervention studies (6–12 wk) with a relatively small sample size (n = 40–66) have yielded conflicting results (13–15). To our knowledge, there have been no well-designed long-term randomized controlled trials (RCTs) of sufficient power to examine the effects of increased dietary protein intake on muscle mass and strength in community-dwelling older women.

The aim of this study was to evaluate the effect of 2 y whey protein supplementation on muscle mass, muscle area, and physical function in community-dwelling Australian women aged 70–80 y in a randomized placebo-controlled setting. We hypothesized that increased protein intake has beneficial effects on the maintenance of muscle mass and physical function. The effects of the intervention on bone structure and blood pressure have been reported previously (16, 17).

Methods

Participants

Study participants were recruited from April–September 2007 with the use of a population-based approach in which a random selection of women (n = 6065) aged 70–80 y on the electoral roll in the metropolitan area of Perth, Western Australia, received a letter inviting them to join the study. Over 98% of women of this age are on the Western Australian electoral roll. Of the 829 women who responded to the letter, 256 attended clinic screening and 219 women who met the inclusion criteria joined the study. The inclusion and exclusion criteria have been published previously for the bone-related outcomes of this study (16). In brief, women who had previous osteoporotic fracture; had metabolic bone disease apart from osteoporosis; currently or within the last year were taking medication for osteoporosis (including hormone replacement therapy) apart from calcium or vitamin D; were taking steroid tablets in the previous 3 mo or had taken >7 g in total in their lifetime; had a high protein intake as assessed by FFQ (>1.5 g · kg body weight$^{-1}$ · d$^{-1}$); and had any other condition that may affect the participation of the study were excluded. All procedures followed were in accordance with institutional guidelines and were conducted at the Sir Charles Gairdner Hospital in Perth. Study participants were followed up for 2 y, and the trial ended in September 2009, when the last patient was seen. The study was approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee, and all participants provided written informed consent. The study was conducted in compliance with the ethical principles of the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practices Guidelines and registered with the Australian New Zealand Clinical Trials Registry (ACTRN01260700163404).

Study design

A 2 y randomized, double-blind, placebo-controlled, prospective, parallel study was undertaken. Eligible participants were randomly assigned to 1 of 2 treatment groups (protein and placebo groups). The study used a computer-generated randomization sequence with a block size of 10 to assign participants to either a protein drink or a placebo drink in a ratio of 1:1. The randomization code was generated by one of the investigators (CWB), who did not have direct contact with participants, and the code was kept at the School of Public Health, Curtin University. In addition to assigning participants in the intervention, Curtin University staff labeled the drinks and organized the delivery of the study drinks to participants’ homes. The study participants and researchers at the Sir Charles Gairdner Hospital responsible for recruitment and assessment of outcome measures remained blinded to group assignment. Assessments were made at baseline, year 1, and year 2. This paper followed the CONSORT (Consolidated Standards of Reporting Trials) reporting guidelines (18).

Drink supplements

The supplemental drinks were developed by an experienced food technologist (VS). The test drinks were provided in 3 flavors, coffee, chocolate, and strawberry. The nutrient content of the test drinks is shown in Supplemental Table 1. Test drinks were delivered to participants’ homes every 3 mo, with participants being instructed to take a daily test drink before breakfast for the duration of the study.

Protein supplement. Participants in the protein group received a 250 mL skim milk–based high-protein supplement drink reconstituted with cold water from a powder, which provided 30 g of protein, 600 mg of calcium, and 3.2 kJ/mL. The high-protein product used skim milk plus whey protein isolate (Alacen 894, Fonterra Brands) to increase protein content.

Placebo supplement. Participants in the placebo group received a 250 mL skim milk–based supplement drink reconstituted with cold water from a powder, which provided identical calories and calcium (600 mg of calcium and 3.2 kJ/mL), but only contained 2.1 g protein. The extra energy content in the placebo drink was supplied by carbohydrate. Adherence to the study drinks was established by counting empty containers returned at the clinic visit at 1 and 2 y.

Appendicular skeletal muscle mass

Total body composition was measured by DXA with the use of a Hologic Discovery A fan-beam densitometer. The scans were analyzed with the use of Hologic QDR (version 12.6) software and estimates of appendicular skeletal muscle mass (defined as lean mass of arms and legs) were obtained. The CV at this site was <2% in our laboratory. Adjusted appendicular skeletal muscle mass (kilograms per meter squared) was calculated as appendicular skeletal muscle mass (kilograms) divided by height (meters squared).

Anthropometry

Anthropometry measurements were performed with subjects in a hospital gown and without shoes. Standing height was measured with the use of a wall-mounted stadiometer to the nearest 0.1 cm, and body weight with the use of an electronic scale to the nearest 0.1 kg. Mid–upper arm girth was measured at the level of the mid–acromiale–radial site on the right arm by a tape to the nearest 0.1 cm. Triceps skinfold was measured by a Harpenden skinfold caliper (John Bull British Indicator) to the nearest 0.1 cm according to a standard protocol (19).

Cross-sectional muscle area

Upper arm muscle area was derived from the measurements of mid–upper arm girth and triceps skin fold with the use of the following formulas (20): 1) upper arm muscle area (centimeters squared) = [mid–upper arm girth (centimeters) − π × triceps skinfold (centimeters)]$^2$/4π, and 2) Corrected upper arm muscle area (centimeters squared) = upper arm muscle area − 6.5. Calf muscle cross-sectional area was measured by peripheral quantitative computed tomography with the use of a Stratec XCT (Stratec Medizintechnik). The position was chosen at 38% of the tibia length proximal to the ankle joint. The peripheral quantitative computed tomography scans were analyzed with the use of Stratec 2000 software, version 6.0. All analysis was done by one researcher (XM). The CV at this site was 1.5% as assessed by repositioning and reanalysis of the scans of 30 patients.
Physical function
Hand-grip strength was assessed by a hand dynamometer on the dominant hand. Ankle dorsiflexion, knee flexor, knee extensor, hip abductor, hip flexor, hip extensor, and hip adductor strength were assessed with the use of a strain gauge. The subjects were requested to exert a maximal muscle contraction against the strain gauge after one practice. The best of 3 attempts was recorded for each muscle group. The CV error was 7% for hand-grip strength and between 14% and 20% for the different lower limb muscle groups. Mobility functioning was measured by the Timed Up and Go (TUG) test, which required the subjects to be timed while getting up, walking 3 meters, turning, returning to the chair, and sitting down again (21). The CV error was 6% for the TUG test.

Dietary intake
Dietary intake was assessed by a 3 d weighed food record (2 weekdays and 1 weekend day) as previously reported (16). The food record was analyzed with the use of the AUSNUT99 database (Foodworks Professional edition version 3.02) (22) by nutritionists trained in dietary assessment.

24 h urinary nitrogen
24 h urine samples were collected at baseline, 1 y, and 2 y for the assessment of urinary nitrogen for monitoring compliance. The participants collected a 24 h urine sample on day 3 of the food recording period in a 5 L plastic collection bottle that contained 20 mL of 1 M HCl. Urinary nitrogen concentration was determined with the use of the Kjeldahl method, which involves 3 steps: digestion, neutralization, and titration (23). The estimated dietary protein intake in gram equalled 6.25×(N + 2), where N is the number of grams of total urinary nitrogen in 24 h urine samples and 2 g is the estimated nitrogen excretion by routes other than urine (24, 25).

Other assessments
Physical activity level was assessed by the International Physical Activity Questionnaire short form (26). Demographic information for participants, including health history, education, past occupation, and smoking history, were collected with the use of a demographic questionnaire. A mini-mental state test was administered at baseline with the aim of excluding those participants who demonstrated significant cognitive impairment.

Adverse events
With the use of a previously validated method (27), participants were asked to fill out an adverse event diary in which each contact with a physician was recorded. At 6 mo intervals, the diary was returned to the study center at clinic visit or by mail. The adverse events were coded with the use of the International Classification of Primary Care system database of disease coding, a validated method of event recording developed for use in general practice (28).

Sample size calculation
Power calculations were performed before the commencement of the study. A sample size of 85 in each group was required to detect a difference of 3% on change in appendicular skeletal muscle mass, the primary outcome variable of the study, assuming a standard deviation of 6% based on our previous study, at 90% power and 5% level of significance. A 3% difference was considered to be reasonable based on our previous epidemiologic study (12). The subject number was increased to 110 per group (total of 220) to allow for a 30% predicted dropout rate, which we reported in previous studies of a similar age group.

Statistical analysis
Descriptive statistics are reported in the text and tables as means ± SDs and differences as means ± SEMs for all variables unless otherwise stated. The normality of continuous variables was checked through the construction of histograms. Baseline characteristics and compliance between the groups were compared by Student’s t test as appropriate. Treatment, time, and interaction effects during the 24 mo study period were examined with the use of linear mixed-effects model analysis with time since baseline as the timeline, group effects as fixed, and subject effects as random, and a first-order autoregressive covariance structure. Significant treatment and time interaction in the linear mixed model analysis indicates significant treatment effects. In addition, the treatment effects at years 1 and 2 were analyzed with the use of ANCOVA, with baseline values and age as covariates, and the interactions with baseline protein intake or physical activity were tested. The time effects at 1 and 2 y were evaluated by 1-factor repeated-measures ANOVA in each group. The primary outcome variable was appendicular skeletal muscle mass as assessed by DXA, and the secondary outcome variables were muscle area and physical function, including muscle strength measurements and TUG. The normality and independence of the residuals and the homogeneity of variance of each model were checked by residual plots (normal probability plot and plot of residuals vs. treatment and predicted values). P values < 0.05 (2-tailed) were regarded as statistically significant. All data were analyzed by IBM SPSS, version 20.

Results
Participant characteristics. Participant flow throughout the study is shown in Figure 1. The 196 subjects who had at least one follow-up assessment were included in the analysis. There were no significant differences between the protein and placebo groups in any baseline characteristics (Table 1). At study entry, the mean age of participants was 74.3 ± 2.7 y, with a mean BMI of 26.7 ± 3.9 kg/m² and mean protein intake of 76 ± 17 g/d (1.1 ± 0.3 g·kg body weight⁻¹·d⁻¹). Seventeen (8.7%) participants had a protein intake below the Australian recommendation of the Estimated Average Requirement (0.75 g·kg body weight⁻¹·d⁻¹) and 55 (28.1%) had a protein intake below the Recommended Dietary Intake (0.94 g·kg body weight⁻¹·d⁻¹) for women aged >70 y (29). Only 5 subjects (2.6%) had a protein intake below the WHO recommended population average requirement of 0.66 g·kg body weight⁻¹·d⁻¹ for adults (30). During the 2 y, 16 subjects in the protein group and 22 subjects in the placebo group withdrew from the study, and 17 subjects in each group discontinued the test drink (Figure 1). There was no significant difference between the protein group and the placebo group in the number of subjects who discontinued the test drink or were lost to follow up. The compliance rate with the test drink, as determined from empty test drink containers returned, was higher in the protein group than in the placebo group (87.1% compared with 80.8%, P = 0.03).

Protein intake. In the 174 subjects with complete urinary nitrogen and dietary intake data collected at baseline, 1 y, and 2 y, we evaluated the change in protein intake with supplementation. Protein intake was 17–23 g/d higher in the protein group than in the placebo group at 1 and 2 y, as reflected by both protein intake estimated from 24 h urinary nitrogen and 3 d food record (Supplemental Table 2). Although calculated protein intake was lower when estimated from 24 h urinary nitrogen compared with that estimated from food records, differences in intake of the protein and placebo groups were similar when using the two methods, both confirming an increased protein intake of ~20 g/d with the intervention.

Effects on muscle mass and muscle area. At year 1, in both groups, body weight and appendicular skeletal muscle mass measured by DXA increased from baseline, but these changes were no longer significant at year 2 (Table 2). At year 1, upper arm muscle area decreased from baseline in the protein group, whereas at year 2 there was a significant decrease in upper arm muscle area and calf muscle area at 38% tibia in both groups.
There were no significant effects from the protein intervention on changes in body weight, DXA appendicular skeletal muscle mass, and muscle area of upper arm and at 38% tibia over 2 y (Table 2).

### Effects on muscle strength and mobility

At years 1 and 2, hand-grip strength decreased significantly in both groups, whereas the majority of the lower limb muscle strength measures improved significantly in both groups (ranging from 1.10 to 3.67 kg), except for hip extensor strength, which only increased in the placebo group, and hip adductor strength, which decreased in both groups (Table 3). TUG performance improved significantly in both groups at 2 y. There were no significant effects from the protein intervention on changes in hand-grip strength, lower limb strength and TUG performance over the 2 y (Table 3).

In further per-protocol analysis in those with compliance $\geq 80\%$ ($n = 113$), the results were similar to that of the intention-to-treat analysis in the whole cohort for muscle mass and strength variables (data not shown).

### Interactions with baseline protein intake and physical activity

We tested the intervention by baseline protein intake interaction for muscle mass and strength measures at years 1 and 2. The interaction terms were not significant for any variables listed in Tables 2 and 3 except for hip abductor strength at year 1 ($P = 0.003$), which is likely to have resulted from a type I error with the multiple testing performed.

The physical activity levels at year 2 did not change significantly from those at baseline in either the protein or the placebo group, and there were no significant differences between the 2 groups ($424 \pm 406$ compared with $385 \pm 356$ metabolic...
equivalent task–min/wk; \( P = 0.56 \). We tested the intervention by baseline physical activity level interaction for muscle mass and strength measures at years 1 and 2. The interaction terms were not significant for any variables listed in Tables 2 and 3 except for adjusted appendicular skeletal muscle mass at year 1 \( (P = 0.047) \), which again is likely to have resulted from a type I error.

**Adverse events.** During the study period, there were no significant differences between the protein group and the placebo group in the rate of incident cancer \( (protein, 5.0\% \text{ and control, } 5.3\%) \), type 2 diabetes \( (protein, 3.0\% \text{ and control, } 1.1\%) \), diarrhea \( (protein, 4.0\% \text{ and control, } 1.1\%) \), esophageal reflux \( (protein 2.0\% \text{, control 3.3\%}) \) or fracture \( (protein 3.0\%, \text{control } 3.2\%) \). Two participants in the protein group reported constipation.

**Discussion**

In this 2 y RCT, we found that in community-dwelling women aged 70–80 y at baseline with a mean protein intake of 76 ± 17 g/d \( (1.1 ± 0.3 \text{ g · kg body weight}^{-1} \cdot \text{d}^{-1}) \), there were no significant differences between the group that received the high-protein drink (containing 30.1 g protein per 250 mL) and the group that received the low-protein, high-carbohydrate drink (containing 2.1 g protein per 250 mL) daily in changes in appendicular skeletal muscle mass, muscle area of upper arm and calf, muscle strength, and TUG performance. There was, however, a significant decline in upper arm and calf muscle area and hand grip strength in both groups, indicating a possible age-related decline.

Aging is associated with progressive loss of muscle mass and physical function. Over the 2 y, we observed a reduction in the upper arm and calf muscle areas and a decrease in hand-grip strength in women in both the protein and the placebo groups, indicating deterioration in muscle health with aging. However, appendicular skeletal muscle mass remained stable over the study period and TUG performance and some lower limb strength measures improved. Because both groups improved in the TUG and lower limb strength measurements and there were no significant changes in physical activity levels over the study period, a “learning effect” cannot be ruled out. However, the double-blind, randomized, placebo-controlled design of the present study ensured that any “learning effect” had happened in both groups and did not introduce bias to the intervention effects.

In the present study, we used whey protein, a rapidly digested protein, because it has been shown to be beneficial in preventing body protein loss in the elderly compared with slowly digested protein \( (31) \). Previous studies showed that a moderate-to-large serving of amino acids or protein could increase muscle protein synthesis \( (4, 32–34) \), and oral amino acid supplementation has been shown to improve lean body mass in older people \( (5, 6) \). However, in the present study, although the intervention led to a 20 g/d increase in protein intake as confirmed by 24 h urinary nitrogen excretion and an 8% increase in serum IGF-I, as reported previously \( (16) \), there were no significant effects on muscle mass, muscle area, or physical function measurements. These findings are in contrast with previous longitudinal cohort studies that showed a positive association between protein

**TABLE 2**  Body weight, muscle mass, and muscle area at baseline and changes after 1 and 2 y in older postmenopausal women who received protein or placebo drinks

<table>
<thead>
<tr>
<th></th>
<th>Baseline values</th>
<th>Change (1 y–baseline)</th>
<th>Change (2 y–baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein ( (n = 101) )</td>
<td>Placebo ( (n = 95) )</td>
<td>Protein ( (n = 101) )</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>66.8 ± 11.0</td>
<td>66.6 ± 11.3</td>
<td>0.60 ± 0.21 *</td>
</tr>
<tr>
<td>Lean mass of arms, kg</td>
<td>3.8 ± 0.6</td>
<td>3.9 ± 0.6</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Lean mass of legs, kg</td>
<td>12.4 ± 1.9</td>
<td>12.7 ± 1.9</td>
<td>0.18 ± 0.05 *</td>
</tr>
<tr>
<td>Appendicular skeletal muscle mass, kg</td>
<td>16.2 ± 2.4</td>
<td>16.6 ± 2.4</td>
<td>0.20 ± 0.06 *</td>
</tr>
<tr>
<td>Adjusted appendicular skeletal muscle mass, kg/m²</td>
<td>6.3 ± 0.7</td>
<td>6.5 ± 0.8 engage</td>
<td>0.09 ± 0.02 *</td>
</tr>
<tr>
<td>Corrected upper arm muscle area, cm²</td>
<td>33.5 ± 10.3</td>
<td>34.9 ± 10.2</td>
<td>-2.93 ± 0.96 *</td>
</tr>
<tr>
<td>Calf muscle area at 38% tibia, cm²</td>
<td>30.5 ± 5.3</td>
<td>31.9 ± 6.2</td>
<td>-0.01 ± 0.13</td>
</tr>
</tbody>
</table>

\( ^* \) Values are means ± SDs or means ± SEMs for changes. *Change from baseline, \( P < 0.05 \). Groups did not differ at baseline and there were no group × time interactions in the linear mixed-effects model analysis, indicating that there were no significant treatment effects.

**TABLE 3**  Physical function at baseline and changes after 1 and 2 y in older postmenopausal women who received protein or placebo drinks

<table>
<thead>
<tr>
<th></th>
<th>Baseline values</th>
<th>Change (1 y–baseline)</th>
<th>Change (2 y–baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein ( (n = 99) )</td>
<td>Placebo ( (n = 94) )</td>
<td>Protein ( (n = 99) )</td>
</tr>
<tr>
<td>Hand-grip strength, kg</td>
<td>21.7 ± 5.2</td>
<td>21.7 ± 5.5</td>
<td>-0.87 ± 0.40 *</td>
</tr>
<tr>
<td>Ankle dorsiflexion strength, kg</td>
<td>10.5 ± 4.4</td>
<td>10.2 ± 4.2</td>
<td>1.10 ± 0.38 *</td>
</tr>
<tr>
<td>Knee flexor strength, kg</td>
<td>9.1 ± 3.6</td>
<td>9.7 ± 3.7</td>
<td>1.81 ± 0.38 *</td>
</tr>
<tr>
<td>Knee extensor strength, kg</td>
<td>15.4 ± 5.3</td>
<td>16.1 ± 7.2</td>
<td>2.08 ± 0.54 *</td>
</tr>
<tr>
<td>Hip extensor strength, kg</td>
<td>16.9 ± 6.6</td>
<td>16.2 ± 6.6</td>
<td>-0.62 ± 0.57</td>
</tr>
<tr>
<td>Hip abductor strength, kg</td>
<td>11.1 ± 4.3</td>
<td>11.0 ± 5.0</td>
<td>0.67 ± 0.41</td>
</tr>
<tr>
<td>Hip flexor strength, kg</td>
<td>12.0 ± 4.6</td>
<td>11.8 ± 4.6</td>
<td>1.51 ± 0.44 *</td>
</tr>
<tr>
<td>Hip adductor strength, kg</td>
<td>12.0 ± 5.6</td>
<td>12.1 ± 5.9</td>
<td>-2.06 ± 0.53 *</td>
</tr>
<tr>
<td>Timed Up and Go, s</td>
<td>7.9 ± 1.3</td>
<td>8.0 ± 1.5</td>
<td>-0.14 ± 0.13</td>
</tr>
</tbody>
</table>

\( ^* \) Values are means ± SDs or means ± SEMs for changes. *Change from baseline, \( P < 0.05 \). Groups did not differ at baseline and there were no group × time interactions in the linear mixed-effects model analysis, indicating that there were no significant treatment effects.
intake and the maintenance of lean body mass with aging (11, 12), but consistent with a 12 wk RCT of protein supplementation (210 g/d of ricotta cheese) in sarcopenic elderly men and women over 60 y of age, which did not observe any significant effect on appendicular skeletal muscle mass and hand-grip strength (13). However, the same type of supplementation (210 g/d of ricotta cheese) for 12 wk was shown to improve appendicular skeletal muscle mass and balance test scores in nonsarcopenic elderly men and women (15), and, in malnourished hospitalized elderly patients, protein pulse (i.e., sudden burst of protein intake) feeding (72% of dietary protein, 1.3 g · kg body weight \(^{-1} \cdot d^{-1}\) on average, consumed in one meal at 1200) was effective in improving lean body mass and skeletal muscle mass (14).

One explanation for the lack of effects on muscle measurements in the present study could be the high habitual protein intake of the study population. In the Health, Aging, and Body Composition (Health ABC) study, which examined 2066 American men and women aged 70–79 y, energy-adjusted protein intake was significantly associated with change in lean body mass over 3 y; losses in lean body mass and appendicular skeletal muscle mass were ~40% lower in subjects in the highest quintile than they were in those in the lowest quintile of energy-adjusted protein intake (11). The mean protein intake in female subjects in the Health ABC study was 60.9 g/d (0.9 g · kg body weight \(^{-1} \cdot d^{-1}\)), whereas the mean protein intake in the present study was 76 g/d (1.1 g · kg body weight \(^{-1} \cdot d^{-1}\)), which is approaching that of the 5th quintile (1.2 g · kg body weight \(^{-1} \cdot d^{-1}\)) in the Health ABC study. In a study by Aleman-Mateo et al. (15) in nonsarcopenic older adults in which a positive protein intervention effect was observed, the baseline protein intake was estimated to be 0.9 g · kg body weight \(^{-1} \cdot d^{-1}\), which increased to 1.2 g · kg body weight \(^{-1} \cdot d^{-1}\) with supplementation. In fact, in our study, appendicular skeletal muscle mass did not change significantly over 2 y in either the protein or the placebo groups (Table 2), although a decrease in muscle area was observed at both upper arm and lower leg. In addition, in our study, we did not find significant interactions between the intervention and baseline protein intake for muscle mass and strength measures at year 1 and year 2, but with the low number of subjects (n = 55; 28.1%) who had a protein intake below the Recommended Dietary Intake of 0.94 g · kg body weight \(^{-1} \cdot d^{-1}\) for women of this age, our study is not powered to test this hypothesis. It is possible that protein intervention is more effective in older adults with relatively low protein intake and who have more pronounced loss of muscle mass with aging.

Another argument for the lack of effect could be that in our study the intervention was not carried out in combination with resistance training. A meta-analysis published in 2012 summarized the findings of 22 RCTs (680 subjects), and concluded that during prolonged resistance-type training (>6 wk), protein supplementation could increase fat-free mass and 1-repetition maximum leg press strength compared with the placebo group in both younger and older subjects (35). However, it is worth noting that most of the trials included in the meta-analysis (15 of 22) were conducted in men only, and although in younger subjects protein supplementation also led to greater gains in type I and II muscle fiber cross-section area, in older subjects (>50 y), such effects were not observed (35). Since the publication of the meta-analysis, an intervention study in 100 elderly women aged 60–90 y living in retirement villages showed that providing a protein-enriched diet with the use of red meat (160 g cooked) for 4 mo could enhance the effects of resistance training on lean body mass and muscle strength (36). The baseline protein intake of the abovementioned study was 1.1 g · kg body weight \(^{-1} \cdot d^{-1}\) (36), similar to that of our study. However, the characteristics of women living in retirement villages might be different from those the women from our study. These women were community-living, their age range was wider (60–90 compared with 70–80 y), and 44% had a history of using hormone-replacement therapy, which has been shown to play a role in preserving muscle in postmenopausal women (37).

The strengths of our study included the randomized controlled design, study participants who were representative of large numbers of individuals living in Western countries, the high retention and compliance rates, and the use of a range of validated assessment techniques for muscle mass, muscle area, and physical function. A limitation of our study is that the study population included community-dwelling older Caucasian women; therefore, the findings may not be applicable to other populations. In addition, although women with a high protein intake (>1.5 g · kg body weight \(^{-1} \cdot d^{-1}\)) were excluded at study entry, the participants still had a relatively high habitual dietary protein intake, which may explain the lack of a protein intervention effect on muscle mass and strength. Furthermore, our protein intervention was not combined with resistance training. However, the purpose of the present study was to investigate the effects of protein intervention alone on muscle health in older women.

In conclusion, in healthy ambulant women with a baseline protein intake well above the current Australian recommended Estimated Average Requirement of 0.75 g · kg body weight \(^{-1} \cdot d^{-1}\), we found that extra protein of 30 g/d did not enhance muscle mass and physical function, despite some evidence of deterioration in upper arm and calf muscle area and hand-grip strength. Taking together the findings of our study and previous trials, it seems the effectiveness of protein intervention on muscle health in older adults would depend on participants’ nutrition status and habitual protein intake, whether carried out in combination with resistance training, and the type and amount of protein intervention. Therefore, whereas protein intervention has the potential to improve muscle health in older people, the most effective way of intervention and the population most sensitive to the intervention deserve further study.

Acknowledgments
We thank Linda Schollum of Fonterra Brands Limited for her assistance with providing the whey protein isolate. KZ, DAK, AD, VS, CWB, and RLP designed the research; KZ, DAK, XM, AD, VS, CWB, and RLP conducted the research; KZ analyzed the data; XM performed the peripheral quantitative computed tomography measurements of the tibia muscle area; VS developed the test drink and provided oversight of the test drink powder production; and KZ, DAK, and RLP wrote the paper. KZ had primary responsibility for the final content. All authors read and approved the final manuscript.

References
36. Daly RM, O’Connell SL, Mundell NL, Grimes CA, Dunstan DW, Nowson CA. Protein-enriched diet, with the use of lean red meat, combined with progressive resistance training enhances lean tissue mass and muscle strength and reduces circulating IL-6 concentrations in elderly women: a cluster randomized controlled trial. Am J Clin Nutr 2014;99:899–910.