The effects of dietary protein intake on appendicular lean mass and muscle function in elderly men: a 10-wk randomized controlled trial

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ABSTRACT

Background: The Recommended Daily Allowance (RDA) for protein intake in the adult population is widely promoted as 0.8 g · kg^{-1} · d^{-1}. Aging may increase protein requirements, particularly to maintain muscle mass.

Objective: We investigated whether controlled protein consumption at the current RDA or twice the RDA (2RDA) affects skeletal muscle mass and physical function in elderly men.

Design: In this parallel-group randomized trial, 29 men aged >70 y [mean ± SD body mass index (in kg/m^2): 28.3 ± 4.2] were provided with a complete diet containing either 0.8 (RDA) or 1.6 (2RDA) g protein · kg^{-1} · d^{-1}, aimed to balance energy needs. Before treatment and after 10 wk of intervention, whole-body and appendicular lean mass were measured by using dual-energy X-ray absorptiometry. Knee-extension peak power was measured with dynamometry.

Results: Both groups were found to have been in a moderate negative energy balance (mean ± SD RDA: 209 ± 213 kcal/d; 2RDA 145 ± 214 kcal/d; $P = 0.427$ for difference between the groups). In comparison with RDA, whole-body lean mass increased in 2RDA ($P = 0.001$; 1.49 ± 1.30 kg, $P < 0.001$ compared with −0.55 ± 1.49 kg, $P = 0.149$). This difference was mostly accounted for by an increase in trunk lean mass found in 2RDA (+1.39 ± 1.09 kg, $P < 0.001$). Appendicular lean mass also decreased in RDA compared with 2RDA ($P = 0.022$), driven by a reduction in RDA (−0.64 ± 0.91 kg, $P = 0.005$ compared with 0.11 ± 0.57 kg, $P = 0.592$). Adjusting for energy imbalances did not alter these findings. Knee-extension peak power was also differently affected ($P = 0.012$; 26.6 ± 47.7 W, $P = 0.015$ in 2RDA compared with −11.7 ± 31.0 W, $P = 0.180$ in RDA).

Conclusions: Consumption of a diet providing 2RDA for protein compared with the current guidelines was found to have beneficial effects on lean body mass and leg power in elderly men. These effects were not explained by differences in energy balance. This trial was registered at the Australia New Zealand Clinical Trial Registry (www.anzctr.org.au) as ACTRN12616000310460. *Am J Clin Nutr* 2017;106:1375–83.

Keywords: nutrient requirements, dietary protein, skeletal muscle, older adults, whole foods

INTRODUCTION

Skeletal muscle is required for locomotion and the performance of activities of daily living (1). Declines in skeletal muscle mass can increase the risk of metabolic disease (2) and mortality associated with serious illness (3) as well as the risk of frailty (4). Muscle mass and physical function begin to decline around the fifth decade of life, and the losses accelerate with advancing age (5). Severe muscle loss and impaired functionality lead to the onset of sarcopenia, a condition associated with frailty, loss of independence, and a greater mortality risk (6).

Central to the maintenance of adequate muscle mass is habitual dietary protein intake (7). Consuming the highest quintile (1.2 g · kg^{-1} · d^{-1}) of dietary protein has been shown to be associated with a smaller muscle loss in older adults (8). This is incongruous with health agencies, including the WHO (9) and the USDA (10), which have established the Recommended Daily Allowance (RDA) of 0.8 g protein · kg^{-1} · d^{-1}. This value was set to achieve a positive or neutral nitrogen balance for 97.5% of the adult population but does not take into account any other metric of health status (11).

These recommendations have been generalized to older adults despite some nitrogen balance studies suggesting a higher protein requirement in older adults (11, 12). Recent reevaluations of the protein requirements with the use of the indicator amino acid oxidation technique suggest that nitrogen balance methodology may underestimate protein needs by as much as 30% (13, 14). There is also evidence of anabolic resistance in the elderly, whereby skeletal muscle synthesis in response to ingested protein is suppressed at any given ingested dose, relative to younger individuals (15–17). With this uncertainty, protein intakes of up

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Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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Abbreviations used: CSA, cross-sectional area; DXA, dual-energy X-ray absorptiometry; RDA, Recommended Daily Allowance; SPPB, short physical performance battery; TUG, timed up-and-go test; 2RDA, twice the Recommended Daily Allowance.

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to twice the current RDA (2RDA) have been recommended as optimal for older adults at risk of sarcopenia (18).

There are limited data from randomized controlled trials on the impact of different protein intakes on skeletal muscle mass and function in the elderly. To date, clinical interventions have analyzed the actions of protein supplements rather than changes in habitual diet to modify protein intakes (19). Although supplements may be beneficial in certain situations, dietary guidelines and common practice are based on the consumption of whole foods rather than isolated macronutrients (20). Therefore, the aim of the present study was to determine whether the consumption of a well-controlled diet containing either RDA (0.8 g · kg^{-1} · d^{-1}) or 2RDA (1.6 g · kg^{-1} · d^{-1}) of protein for 10 wk would affect lean mass and muscle strength in healthy men aged >70 y differently. Although muscle mass is a major determinant of physical function in the elderly (21), it has been demonstrated that muscle strength declines at a rate greater than the loss of muscle mass (22). Hence, measures of physical function including muscle power (23) are important for the assessment of the efficacy of dietary intervention. Therefore, the secondary aim of this study was to determine whether the amount of protein intake altered measures of physical function.

METHODS

Subjects

Thirty-one healthy men aged >70 y were recruited to take part in the study (**Table 1**) by using advertisements placed in local newspapers. Participants were nonsmokers who had BMIs (in kg/m^2) ranging from 18 to 35 and did not take any dietary supplements for ≥1 mo preceding the trial. All participants were able to perform activities of daily living independently without mobility aids. The exclusion criteria included a prior history of cancers, diabetes, thyroid diseases, or conditions affecting neuromuscular function, and participants who completed >4 h/wk of structured physical activity (organized sport, resistance training, or vigorous intensity aerobic exercise). Similarly, those with restricted eating habits, including vegetarians and those with allergies (e.g., nuts, fish, dairy), were not included in the study.

The study was approved by the Southern Health and Disability Ethics Committee (New Zealand; 15/STH/236) and was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from all participants before they were enrolled in the trial. The study was prospectively registered with the Australian and New Zealand Clinical Trial Registry (www.anzctr.org.au) as ACTRN12616000310460.

Experimental design

The trial used a parallel-group design with individual participant random assignment conducted by using sequences generated by www.random.org. Allocation (1:1 ratio) was conducted by using a locked spreadsheet that assigned participants to treatment groups. Investigators were involved in both diet preparation and participant testing, so they were not blinded to group allocation. Group allocation was not discussed with participants; however, it was likely clear to them, based on the food they were provided, the group to which they were assigned. A priori primary outcomes were defined as changes in lean body mass and isometric knee-extension strength. Before commencement of the intervention participants were given a tool to estimate portion size using their hand size as a guide (25) and recorded their dietary intake for 3 consecutive days and collected their full urine output over a 24-h period. Participants also

TABLE 1
Subject characteristics[1]

	RDA (n = 15)	2RDA (n = 14)
Age, y	74.7 ± 3.9 (70–81)	73.7 ± 3.3 (70–79)
Height, cm	172.8 ± 8.2 (157–187)	171.7 ± 5.5 (163–182)
Weight, kg	85.3 ± 20.1 (49.3–111.8)	82.5 ± 7.9 (70.5–98.1)
BMI, kg/m^2	28.4 ± 5.1 (18.9–35.3)	28.2 ± 3.3 (24.1–33.9)
BMR,[2] kcal/d	1595 ± 330 (948–2019)	1561 ± 129 (1341–1796)
Physical activity level,[3] TEE/BMR	1.59 ± 0.28 (1.02–1.96)	1.68 ± 0.28 (1.30–2.40)
Estimated TEE,[4] kcal/d	2498 ± 537 (1707–3358)	2591 ± 230 (2295–3357)
Habitual energy intake,[5] kcal/d	3132 ± 1056 (1836–5731)	2224 ± 647 (1183–3515)
Medication usage, n		
Statin	4	6
ACE inhibitor	4	3
Aspirin	4	2
Calcium channel blocker	1	2
Proton pump inhibitors	0	2
α-Blocker	1	2
β-Blocker	1	2
Xanthine oxidase inhibitor	1	1

[1] Values are means ± SDs (range). The RDA is 0.8 g protein · kg^{-1} · d^{-1}; 2RDA is 1.6 g protein · kg^{-1} · d^{-1}. ACE, angiotensin-converting enzyme; BMR, basal metabolic rate; RDA, Recommended Daily Allowance; TEE, total energy expenditure; 2RDA, twice the Recommended Daily Allowance.

[2] Estimated with Harris–Benedict equation (24).

[3] TEE divided by BMR estimated by using a Fitbit accelerometer.

[4] Harris–Benedict equation–derived BMR multiple of Fitbit-derived physical activity level.

[5] Estimated from 3-d records.

wore an accelerometer on their wrist for 5 d to estimate their level of physical activity. Physical function, body composition, thigh muscle cross-sectional area (CSA), and muscle strength were measured as described below. Analyses and tests were then repeated after a 10-wk period of dietary control. All testing was conducted at the University of Auckland Nutrition and Mobility Clinic between April and October 2016. After pre-intervention testing participants were provided with a controlled diet that contained either 0.8 (RDA) or 1.6 g protein · kg^{-1} · d^{-1} (2RDA) for 10 wk. During the intervention participants were instructed to maintain their normal lifestyle, and prepared meals were delivered to their homes.

Dietary control

All food consumed by the participants during the 10-wk trial was provided by investigators, with the exception of black coffee and tea. Lunch and dinner were provided in prepared form requiring only reheating by participants. Some breakfast meals required minimal preparation by the participants. Portioned snacks were also provided and included fresh fruit, dried fruit, and nuts.

All participants consumed 28–31% of energy from fat; those randomly assigned to the RDA group consumed 0.8 g protein · kg^{-1} · d^{-1}, and those randomly assigned to 2RDA group consumed 1.6 g protein · kg^{-1} · d^{-1}. The difference was made up of carbohydrates. Both diets met local recommendations for the intake of fruits and vegetables (26). All diets were omnivorous and adhered to Eating and Activity Guidelines for New Zealand Adults (27). Protein and energy were distributed between breakfast, lunch, and dinner as 30%, 30%, and 40%, respectively. Compliance records were completed to ensure that all provided food was consumed, and food selection was adjusted based on participants' preferences to maintain high compliance. Records completed by participants during weeks 5, 6, 9, and 10 of the study were compared with the diets provided to calculate the proportion of total energy and protein provided that was consumed by participants. The compliance records consisted of a menu checklist where participants indicated the percentage of each menu item they consumed. Participants were asked to record any nonstudy food they consumed.

Habitual diets (based on 3-d diet records) and diets consumed during the trial were analyzed by using Foodworks software (Version 8; Xyris) by a dietetics graduate student. The energy content of the intervention diets was individually calculated to match subjects' estimated energy needs based on the Harris-Benedict equation and adjusted for physical activity level (28, 29) assessed by wrist-worn accelerometers (Fitbit Charge HR); these devices have been shown to perform similarly to validated devices for the energy expenditure range of the study participants (30). Estimated energy needs were calculated before the commencement of the invention diet and were adjusted fortnightly based on participant satiety to ensure participants consumed adequate energy relative to protein intake. Accelerometers were worn for 5-d periods before the start of the intervention and at weeks 5 and 10. The net energy balance was calculated by using the chemical energy equivalents for changes in fat mass (9434 kcal/kg) and fat-free mass (1815 kcal/kg); it was assumed that changes in body energy stores reflect energy balance over the 10-wk intervention (31).

Imaging and physical function

Body weight was measured without shoes in light clothing on a digital scale (Tanita DH-351) after participants voided their bladder, and height was measured on a stadiometer without shoes. Full-body dual-energy X-ray absorptiometry (DXA) scans (Lunar Prodigy; GE) were segmented into trunk and limbs automatically by the software; this segmentation was verified and adjusted as necessary by a single investigator (SMM). Participants wore light clothing without metal fasteners and were positioned with their arms by their sides and separated from their trunk. All participants were fully contained within the DXA field of view. Thigh muscle CSA at 50% femur length of the dominate leg was assessed by using a Stratec XCT 3000 peripheral quantitative computed tomography with software version 6.20C (Stratec Medizintechnik). Participants were positioned supine with the test leg centered within the machine's gantry and anchored by a foot rest with straps to limit movement during each scan. Images were then exported to ImageJ (NIH), and the muscle area was manually determined by a single investigator (SMM). At least 3 d before the experimental strength, power, and physical function measurements, participants completed the identical measurements to familiarize them with the testing apparatus and procedures. Isometric muscle strength of the knee extensors was tested by using a Biodex System 4 dynamometer with the knee angle set to 90° of flexion. Three maximal contractions were performed for each movement with 30 s of rest in between and the highest values were used for analysis. Participants then performed a test to determine the maximal power output of their knee extensors using the same Biodex positioning. Three isotonic repetitions were performed each with 30%, 40%, and 50% of the participant's maximal isometric strength. Participants were instructed to perform each repetition at maximal speed, power was calculated as the product of torque and velocity, and the maximal power recorded across all trials was taken as the participant's peak power (32). Isometric grip strength was measured by using a Jamar dynamometer (Patterson Medical) with the grip set to position 2; 3 trials were preformed with each hand, and the highest values recorded for each hand were then averaged. Physical function was measured by using the short physical performance battery (SPPB) (33) and timed up-and-go test (TUG) (34).

Urine analysis

On the day of urine collection participants did not collect their first morning urination but collected all subsequent urine produced that day including the first morning urination the following day. Samples were weighed before a 10-mL aliquot was stored at −20°C for further analysis. Urine urea, creatinine, and uric acid were measured with a Cobas c311 analyzer (Roche Diagnostics). Total protein intake was estimated based on urinary nitrogen excretion and corrected for nonurinary losses with a standard factor of 4 g (35).

Statistical analysis

The sample size of 15 participants/group was calculated based on detecting a between-group difference of 800 g whole body lean mass using the test-retest variability of 765 g

previously observed in our laboratory as the SD, a power of 80%, and an alpha of 5%. Changes in dietary intake and body mass were assessed by 2-factor ANOVA with time (before compared with after) as a repeated factor and diet (RDA compared with 2RDA) as a between subject factor. Two-factor ANCOVA with energy deficit as a covariate, time (before compared with after) as a repeated factor, and diet (RDA compared with 2RDA) as a between-subject factor was used to assess changes in body composition and physical function. Normality was assessed with the Shapiro-Wilk test, and non-normally distributed data were log transformed before further analysis. Student's *t* test was used to compare the degree of energy deficit or surplus between groups. Post hoc comparisons were conducted by using Sidak corrections. All analysis was conducted by using SPSS (IBM) version 23. Alpha was set at $P \leq 0.05$. Unadjusted means ± SDs are shown in the tables and text.

RESULTS

Thirty-one subjects were randomly assigned to the 2 dietary interventions (**Figure 1**); of these, 1 subject (assigned to the RDA group) withdrew his consent before the start of the dietary intervention, and 1 participant was removed from the study at week 5 of the intervention because of lack of compliance with the diet (2RDA). Subsequent analysis was performed only on those participants who completed the study per protocol. Compliance was 98.9% for both protein and energy intake in the RDA group. Compliance was 97.5% and 98.4% for protein and energy intakes, respectively, in the 2RDA group. Compliance was not different between groups. No adverse events related to the diets were reported during the study.

Dietary intake

Dietary intake assessed before (habitual) and during the intervention (controlled) is shown in **Table 2**. According to this the RDA group decreased their energy intake by 14% from their self-reported habitual intake, and the 2RDA group increased their energy intake by 25% from their self-reported habitual intake. However, based on changes in body composition, both groups were in slight energy deficit of 209 ± 213 and 145 ± 214 kcal/d in the RDA and 2RDA groups, respectively. There was no difference in energy deficit between groups ($P = 0.427$). Total protein and animal-source protein intake was decreased in the RDA group and increased in the 2RDA group relative to habitual intake. When protein intake was estimated by using urinary nitrogen excretion, the same pattern was observed. As a consequence of dietary control, the RDA group decreased the percentage of energy intake from fat and increased the percentage of energy intake from carbohydrates, whereas the percentage of energy from carbohydrates and fat was statistically unchanged in the 2RDA group. The number of steps taken per day before the start of the intervention was 7879 ± 3106 and 8740 ± 2699 in RDA and 2RDA groups, respectively ($P = 0.641$) and did not change during the intervention ($P = 0.593$).

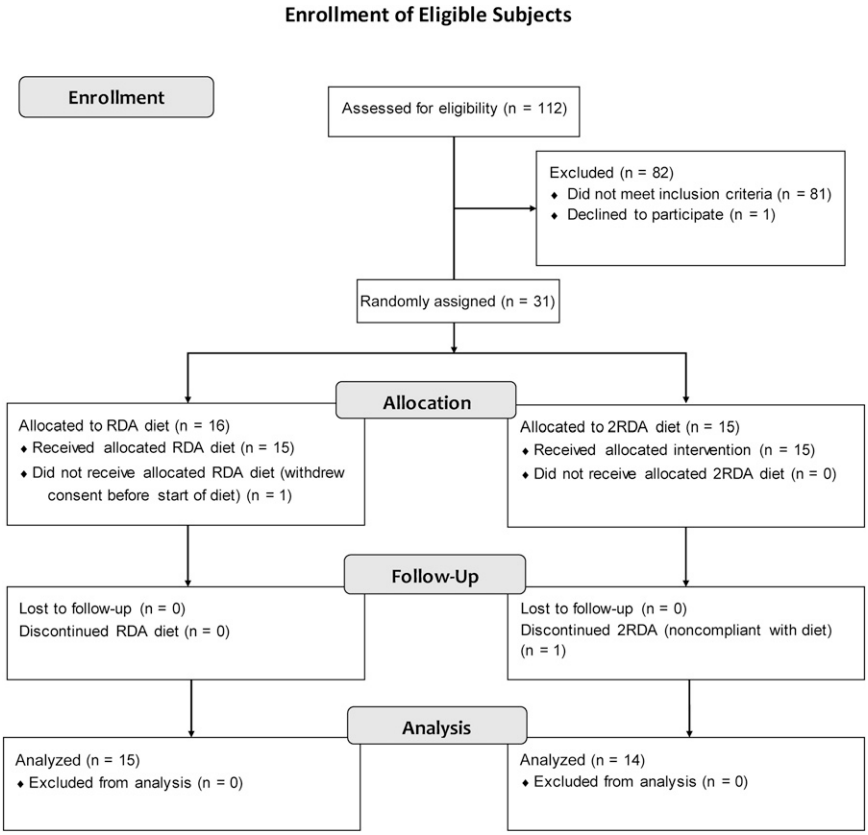

FIGURE 1 Consolidated Standards of Reporting Trials flow diagram of subject enrollment and random assignment to and analysis of study intervention groups. RDA, Recommended Daily Allowance; 2RDA, twice the Recommended Daily Allowance.

TABLE 2
Dietary intake[1]

	RDA		2RDA		Effect[2]		
	Pre	Post	Pre	Post	Time	Diet	Time × diet
Energy intake, kcal	3132 ± 1056	2695 ± 598[#]	2224 ± 647[†]	2779 ± 171[#]	0.705	0.060	0.003*
Protein intake							
Total, g/d	101 ± 30	74 ± 15[#]	88 ± 25	136 ± 10[†,#]	0.041*	0.001*	<0.001*
Relative, g · kg^{-1} · d^{-1}	1.2 ± 0.4	0.9 ± 0.1[#]	1.1 ± 0.3	1.7 ± 0.1[†,#]	0.056	<0.001*	<0.001*
Animal source, g/d	58 ± 20	35 ± 11[#]	53 ± 23	97 ± 6[†,#]	0.016	<0.001*	<0.001*
Plant source, g/d	43 ± 16	48 ± 9	35 ± 9	47 ± 4	0.002*	0.177	0.185
Animal-to-plant ratio	1.5 ± 0.6	0.7 ± 0.1[#]	1.6 ± 0.8	2.1 ± 0.1[†,#]	0.281	<0.001*	<0.001*
Estimated by nitrogen balance, g · kg^{-1} · d^{-1}	1.3 ± 0.2	0.9 ± 0.2[#]	1.3 ± 0.2	1.5 ± 0.1[†,#]	0.098	<0.001*	0.001*
Energy from protein, %	14.1 ± 4.6	11.7 ± 1.6[#]	17.0 ± 4.5	20.6 ± 1.6[†,#]	0.476	<0.001*	0.001*
Carbohydrate intake							
Total, g/d	288 ± 107	368 ± 94	264 ± 102	340 ± 30	0.001*	0.309	0.946
Relative, g · kg^{-1} · d^{-1}	3.5 ± 1.2	4.4 ± 1.0	3.3 ± 1.5	4.2 ± 0.6	0.001*	0.493	0.915
Energy from carbohydrates, %	38.6 ± 10.2	56.6 ± 2.6[#]	48.8 ± 11.1[†]	51.1 ± 2.3[†]	<0.001*	0.217	<0.001*
Fat intake							
Total, g/d	161 ± 86	91 ± 19[#]	75 ± 31[†]	84 ± 6	0.015*	0.002*	0.002*
Energy from fat, %	44.5 ± 10.8	31.7 ± 1.3[#]	31.4 ± 7.4[†]	28.3 ± 1.3[†]	<0.001*	<0.001*	0.007*
Saturated fat, g/d	57 ± 27	33 ± 8[#]	29 ± 13[†]	28 ± 4[†]	0.005*	<0.001*	0.011*
Ethanol, g/d	8.8 ± 15.7	—	9.6 ± 8.6	—	—		—
Fiber intake, g/d	34 ± 14	57 ± 9	33 ± 15	50 ± 5	<0.001*	0.236	0.306

[1] Values are means ± SDs. The RDA is 0.8 g protein · kg^{-1} · d^{-1}; 2RDA is 1.6 g protein · kg^{-1} · d^{-1}. *Significant main effect or interaction, $P < 0.05$. [#]Different from pre-intervention within the same group, $P < 0.05$. [†]Different between diets at indicated time point. P values were determined by using the Sidak post hoc procedure. RDA, Recommended Daily Allowance; 2RDA, twice the Recommended Daily Allowance.
[2] Calculated by 2-factor repeated-measures ANCOVA with energy deficit as a covariate.

Body composition

After correction for energy deficit there was no difference in the magnitude of total-body mass change between groups ($P = 0.174$; **Table 3**). Total fat mass was decreased similarly in both groups by 1.5 ± 1.5 and 1.4 ± 1.6 kg in the RDA and 2RDA groups, respectively, but after correction for energy deficit the decrease was greater in the 2RDA group ($P = 0.001$). The percentage of body fat decreased by 1.0% ± 1.0% and 1.6% ± 1.5% in the RDA and 2RDA groups, respectively, but after correction for energy deficit the decrease was greater in the 2RDA group ($P = 0.004$).

Whole-body lean mass increased in the 2RDA group compared with the RDA group ($P = 0.001$). Whole-body lean mass was unchanged in the RDA group (−0.55 ± 1.49 kg, $P = 0.149$) but increased (1.49 ± 1.30 kg, $P < 0.001$) in the 2RDA group (**Figure 2**A). The increase in lean mass in the 2RDA group was mainly accounted for by an increase in lean mass of the trunk (Figure 2B). Appendicular lean mass was decreased in the RDA group ($P = 0.003$) but unchanged in the 2RDA group ($P = 0.403$; Figure 2C). Composition of each individual limb is shown in **Supplemental Table 1**. Thigh muscle CSA corrected for energy deficit did not change over the course of the study (−539 ± 786 and −120 ± 292 mm^2) in the RDA and 2RDA groups, respectively ($P = 0.112$; Table 3).

Physical function and strength

Isometric knee-extension maximal strength did not change after correction for energy deficit (−8.6 ± 24.2 and 7.5 ± 22.9 Nm)

TABLE 3
Body composition[1]

	RDA		2RDA		Effect[2]		
	Pre	Post	Pre	Post	Time	Diet	Time × diet
Body mass,[3] kg	85.3 ± 20.1	83.2 ± 19.1	82.5 ± 7.9	82.0 ± 8.0	<0.001*	0.899	0.174
Fat mass, kg	25.4 ± 11.5	23.9 ± 11.0[#]	23.5 ± 6.8	21.8 ± 6.8[#]	<0.001*	0.647	0.001*
Lean body mass, kg	56.6 ± 9.8	56.0 ± 9.7	55.7 ± 5.4	57.2 ± 5.0[#]	0.033	0.958	0.001*
Body fat, %	28.3 ± 8.6	27.3 ± 8.8[#]	27.9 ± 6.7	26.3 ± 6.8[#]	0.026*	0.968	0.004*
Appendicular lean mass, kg	24.4 ± 7.7	23.8 ± 4.4[#]	25.0 ± 2.5	25.2 ± 2.5	0.643	0.493	0.022*
Thigh muscle CSA, mm^2	14,678 ± 3148	14,139 ± 2743	14,814 ± 1650	14,639 ± 1511	0.545	0.575	0.112

[1] Values are means ± SDs. The RDA is 0.8 g protein · kg^{-1} · d^{-1}; 2RDA is 1.6 g protein · kg^{-1} · d^{-1}. *Significant main effect or interaction, $P < 0.05$. [#]Different from pre-intervention within the same group, $P < 0.05$. [†]Different between diets at indicated time point. P values were determined by using the Sidak post hoc procedure. CSA, cross-sectional area; Post, postintervention; Pre, pre-intervention; RDA, Recommended Daily Allowance; 2RDA, twice the Recommended Daily Allowance.
[2] Calculated by 2-factor repeated-measures ANCOVA with energy deficit as a covariate.
[3] Measured by using a digital scale whiles participants were wearing light clothing.

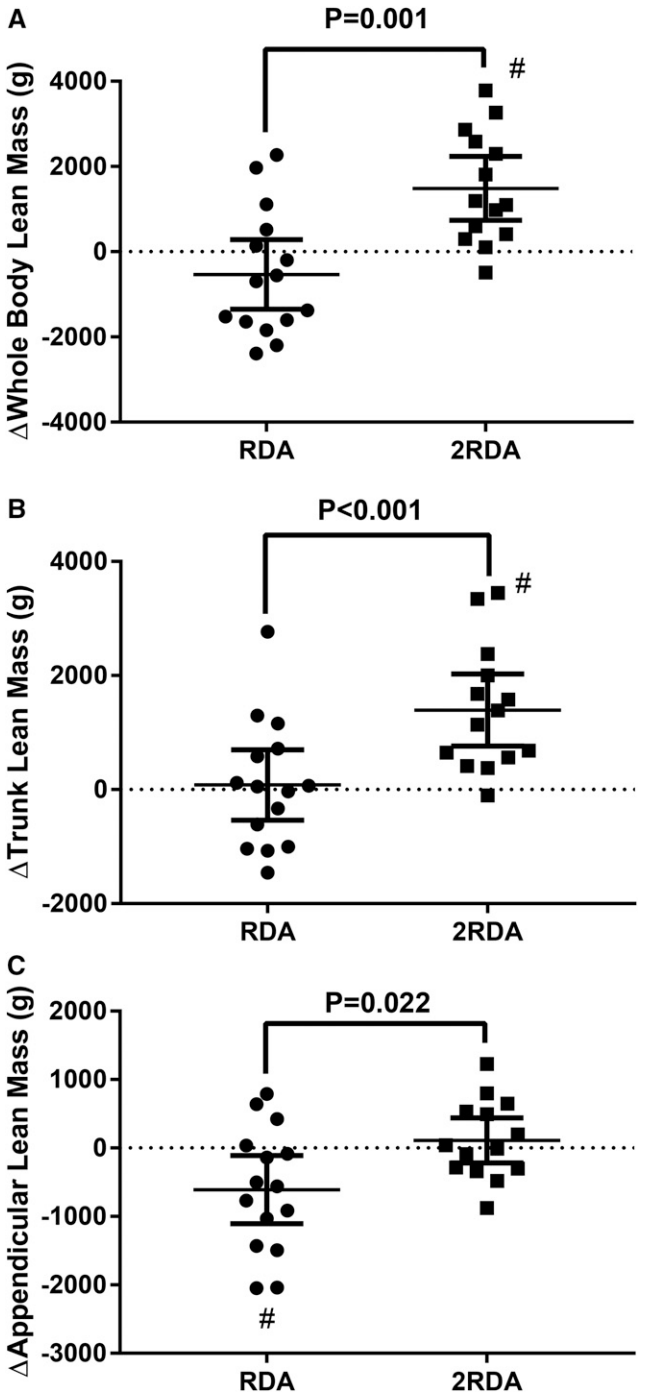

FIGURE 2 Absolute change in lean mass of the whole body (A), trunk (B), and appendicular region (C). Circles represent individual subjects who consumed the RDA diet (0.8 g protein · kg^{-1} · d^{-1}, n = 15), and squares represent individual subjects who consumed the 2RDA diet (1.6 g protein · kg^{-1} · d^{-1}, n = 14). Central lines represent group means, and error bars represent 95% CIs. #Different from baseline $P < 0.05$ by 2-factor ANCOVA. RDA, Recommended Daily Allowance; 2RDA, twice the Recommended Daily Allowance; Δ, absolute change.

in the RDA and 2RDA groups, respectively (**Table 4**). The SPPB score and TUG time were not normally distributed, so they were log normalized before statistical analysis and were not altered by either dietary intervention. Grip strength was unaltered by either intervention (Table 4). There was one apparent outlier in the grip-strength measurements of the 2RDA group who decreased his grip strength by 25 kg, which is >3 SDs from the group mean; if this participant is removed a between-group difference in grip strength is observed (**Figure 3**, P = 0.003). Peak knee-extension power was not normally distributed and was therefore log transformed before statistical analysis. It was increased in the 2RDA group compared with the RDA group (P = 0.012). Peak knee-extension power was unaltered in the RDA group −11.7 ± 31.0 W (P = 0.180) but increased in the 2RDA group 26.6 ± 47.7 W (P = 0.015).

DISCUSSION

Dietary protein intake is an important regulator of skeletal muscle mass in the elderly (8, 36, 37). The current study examined the effects of a controlled diet in free-living elderly men designed to provide either the RDA for protein (0.8 g · kg^{-1} · d^{-1}) or 2RDA (1.6 g protein · kg^{-1} · d^{-1}) on body composition and strength. In free-living men aged >70 y, 10 wk of a controlled diet meeting the current RDA for protein but imposing an ~200-kcal/d energy deficit and reduction in protein intake from their habitual intake caused a loss of appendicular lean mass of ~600 g. Protein consumption of 2RDA did not alter appendicular lean mass but increased both knee-extension peak power output and whole-body lean mass. Thus, in this randomized controlled trial the current dietary recommendation for protein was insufficient to maintain muscle mass and physical function in older men.

Participants in this study habitually consumed on average more protein (~1.1–1.2 g · kg^{-1} · d^{-1}) than the population representative a national dietary survey (~1.0 g · kg^{-1} · d^{-1}) of elderly New Zealand males (aged >70 y) (38). The men were omnivorous, with an animal-to-plant protein ratio of ~1.5. However, in the present study 26% of men reported habitually consuming less protein than the RDA, whereas 14% of the men reported consuming a habitual diet that was equal to or greater than 2RDA with similar distribution between groups. It cannot be ruled out that, because 12 of 15 men in the RDA group decreased their protein intake, the observed reduction in lean mass could at least partly have occurred in the early phase of the intervention before habituation to a reduction in protein intake (39). The men were able to adhere to the controlled diet with >97% of energy and protein provided being consumed. Intake records and urinary nitrogen excretion analysis both suggested that participants in the RDA group actually consumed 0.9 g protein · kg^{-1} · d^{-1}, a consequence of our intention that all diets achieve ≥0.8 g protein · kg^{-1} · d^{-1}. It should be noted that the RDA calculated based on nitrogen balance studies is 0.84 g protein · kg^{-1} · d^{-1} (9). Therefore, the discrepancy between the expected and actual intake in the RDA group is likely minimal.

Both study diets were omnivorous and contained dairy, meat, and fish. To accommodate the prescribed protein and energy specifications the RDA and 2RDA diets provided fewer and more, respectively, animal products than participants' habitual diets. The inevitable differences in protein source between the diets also meant concomitant differences in protein quality (40). Thus, the type or quality of protein may have been responsible for some observed effects. Providing higher-quality protein (i.e., more animal products) without increasing total energy

TABLE 4
Physical function[1]

	RDA		2RDA		Effect[2]		
	Pre	Post	Pre	Post	Time	Diet	Time × diet
SPPB score	11.2 ± 0.9	11.1 ± 0.8	10.7 ± 1.3	11.1 ± 1.1	0.586	0.249	0.185
TUG, s	8.8 ± 1.8	8.9 ± 1.7	9.0 ± 1.5	9.3 ± 1.6	0.515	0.777	0.313
Grip strength, kg	75.3 ± 22.0	70.9 ± 21.8	68.4 ± 19.1	67.7 ± 17.1	0.200	0.547	0.167
Knee extension MVC, Nm	159 ± 64	150 ± 59	147 ± 44	155 ± 38	0.261	0.993	0.120
Knee extension peak power, W	321 ± 114	309 ± 140	314 ± 109	341 ± 110[#]	0.773	0.440	0.012*

[1] Values are means ± SDs. The RDA is 0.8 g protein · kg^{-1} · d^{-1}; 2RDA is 1.6 g protein · kg^{-1} · d^{-1}. *Significant main effect or interaction, $P < 0.05$. [#]Different from pre-intervention within the same group, $P < 0.05$. [†]Different between diets at indicated time point. P values were determined by using the Sidak post hoc procedure. MVC, maximal voluntary contraction; Post, postintervention; Pre, pre-intervention; RDA, Recommended Daily Allowance; SPPB, short physical performance battery; TUG, timed up-and-go test; 2RDA, twice the Recommended Daily Allowance.

[2] Calculated by 2-factor repeated-measures ANCOVA with energy deficit as a covariate.

would be difficult to achieve in practice while consuming standard whole foods and meeting targets for fat intake. Exact protein-quality matching would only have been possible with a contrived diet consisting of foods made primarily of individual macronutrients, which would have greatly reduced the ecological validity of the study.

The present study aimed to balance protein consumption across 3 daily meals (30%, 30%, and 40%); yet participants' habitual protein consumption was distributed as 20%, 30%, and 50%. It is possible that the change in protein distribution may have partially mediated the observed changes in lean mass. It has been suggested that it may be more productive to define protein requirements per meal rather than per day (41). In support, studies have shown a balanced rather than skewed protein distribution is beneficial for maintenance of muscle mass (42, 43).

This study provided participants with diets designed to meet their current energy needs (24). Many participants reported consuming habitual diets at baseline, which contained either too much or too little energy to meet their estimated requirements. This finding could be a reflection of true imbalances between energy intake and requirements or could reflect known inaccuracies of self-reported intake (44). Based on changes in body composition, both groups were in an energy deficit of ~200 kcal/d. This observed energy deficit is likely due to the provision of less-energy-dense foods in both intervention groups compared with habitual diets, which achieved satiety at a lower energy intake (45).

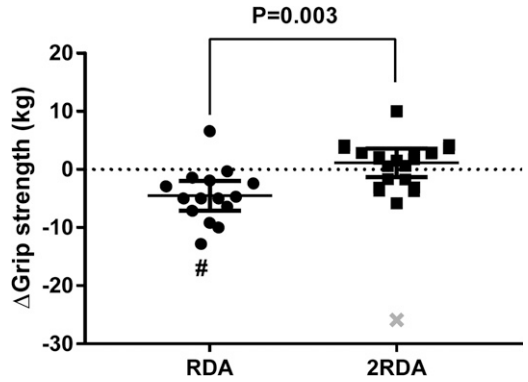

FIGURE 3 Absolute change in isometric grip strength. Circles represent individual subjects who consumed the RDA diet (0.8 g protein · kg^{-1} · d^{-1}, $n = 15$), and squares represent individual subjects who consumed the 2RDA diet (1.6 g protein · kg^{-1} · d^{-1}, $n = 13$). Central lines represent group means, and error bars represent 95% CIs. [#]Different from baseline $P < 0.05$ by 2-factor ANCOVA. A single outlier was not included in the statistical analysis but is shown with a gray ×. RDA, Recommended Daily Allowance; 2RDA, twice the Recommended Daily Allowance; Δ, absolute change.

The confounding effects of an energy deficit on the loss of appendicular muscle mass cannot be discounted; however, this degree of energy deficit is less than what is recommended for weight loss (500–750 kcal/d in older adults) (46) and did not differ between groups. It has also been demonstrated previously that high-protein diets may preserve lean mass during periods of energy deficit. Both groups displayed comparable absolute fat loss, whereas only the RDA group lost appendicular muscle mass. Correcting for the degree of energy deficit did not alter the findings, suggesting that differences in energy intake per se were not responsible for group differences in body composition. Accompanying the ~90% difference in protein intake in the 2RDA diet compared with the RDA diet, there was also an ~10% lower fat intake in the 2RDA group, which cannot be discounted as a contributor to the observed body composition differences. The study was also limited by a lack of a control group, with the maintenance of habitual diet; thus, it is unknown what alterations in weight or body composition would have been evident after 10 additional wk of habitual diet.

Our results show consistent increases in lean mass among the group consuming the 2RDA diet compared with the RDA diet, primarily driven by increases in lean mass in the trunk region. Although this change could be explained by a gain in musculature, it is more likely that nonmuscle lean tissue in this region made a substantial contribution. Animal studies have reported hypertrophy of organs, such as the liver and kidney, in response to high-protein diets (47, 48). MRI would have been required to definitively identify the contribution of skeletal muscle, viscera, and gut to the observed changes in lean mass indistinguishable with the use of DXA as in the current study. In contrast, the appendicular lean mass decline in the RDA group is likely more reflective of skeletal muscle mass than whole-body lean mass (49) because there is minimal tissue that is not muscle in the limbs that would be segmented as lean mass by the DXA (50). Our results suggest that appendicular lean mass loss can be caused by insufficient protein intake, yet higher protein intake does not necessarily induce muscle hypertrophy in the absence of increased physical activity. Resistance training (51) or other high-intensity exercise (52) is normally required to induce muscle

hypertrophy, which may be further increased with additional protein intake; this response has, however, been shown to be blunted in older adults (53).

In the present study, the common SPPB and TUG were used to assess whole-body physical function. The recruited elderly men exhibited a high level of physical functioning (54), and neither of these tests was altered by dietary intervention. Because these tests lack sensitivity and display a celling effect in highly functional adults (55), the assessment of muscle power may provide a more sensitive assessment of physical function (23, 56). Our results show an increase in muscle power only in the 2RDA group, indicating a possible increase in physical function. This was independent of detectible changes in muscle size, making the mechanism unclear; however, greater changes in muscle power than in size have also been reported previously with aging (57).

There is increasing epidemiologic evidence for the beneficial impact of higher-protein diets for the preservation of skeletal muscle mass and function in the elderly (8). Most previous intervention studies have manipulated protein intake with supplements rather than whole foods (19). In this study, the careful control of protein intake to achieve the RDA (0.8 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1}) for 10 wk led to a reduction in total protein intake from the habitual 1.2 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1} accompanied by an $\sim$200-kcal/d energy deficit and resulted in a loss of appendicular lean mass and grip strength. In contrast, raising the intake to 1.6 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1} (2RDA for protein), with a comparable energy deficit, increased whole-body lean mass and knee-extension peak power output, without changing appendicular lean mass or thigh muscle cross-sectional area.

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