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[1–4]

Robin M Daly, Stella L O'Connell, Niamh L Mundell, Carley A Grimes, David W Dunstan, and Caryl A Nowson

ABSTRACT

Background: Physical inactivity, inadequate dietary protein, and low-grade systemic inflammation contribute to age-related muscle loss, impaired function, and disability.

Objective: We assessed the effects of progressive resistance training (PRT) combined with a protein-enriched diet facilitated through lean red meat on lean tissue mass (LTM), muscle size, strength and function, circulating inflammatory markers, blood pressure, and lipids in elderly women.

Design: In a 4-mo cluster randomized controlled trial, 100 women aged 60–90 y who were residing in 15 retirement villages were allocated to receive PRT with lean red meat (~160 g cooked) to be consumed 6 d/wk [resistance training plus lean red meat (RT+Meat) group; $n = 53$] or control PRT [1 serving pasta or rice/d; control resistance training (CRT) group; $n = 47$)]. All women undertook PRT 2 times/wk and received 1000 IU vitamin D_3/d.

Results: The mean (± SD) protein intake was greater in the RT+Meat group than in the CRT group throughout the study (1.3 ± 0.3 compared with 1.1 ± 0.3 g · kg^{-1} · d^{-1}, respectively; $P < 0.05$). The RT+Meat group experienced greater gains in total body LTM (0.45 kg; 95% CI: 0.07, 0.84 kg), leg LTM (0.22 kg; 95% CI: 0.02, 0.42 kg), and muscle strength (18%; 95% CI: 0.03, 0.34) than did the CRT group (all $P < 0.05$). The RT+Meat group also experienced a 10% greater increase in serum insulin-like growth factor I ($P < 0.05$) and a 16% greater reduction in the proinflammatory marker interleukin-6 (IL-6) ($P < 0.05$) after 4 mo. There were no between-group differences for the change in blood lipids or blood pressure.

Conclusion: A protein-enriched diet equivalent to ~1.3 g · kg^{-1} · d^{-1} achieved through lean red meat is safe and effective for enhancing the effects of PRT on LTM and muscle strength and reducing circulating IL-6 concentrations in elderly women. This trial was registered at the Australian Clinical Trials Registry as ACTRN12609000223235. *Am J Clin Nutr* 2014;99:899–910.

INTRODUCTION

Sarcopenia or the age-related loss in muscle mass, strength, and function has been shown to contribute to many chronic diseases (1, 2). Although there is no single cause of sarcopenia, physical inactivity, protein malnutrition, and an increase in systemic low-grade inflammation have all been implicated as causes (1, 2). To prevent muscle loss and promote hypertrophy, it is essential that a net positive muscle protein balance is achieved [ie, muscle protein synthesis (MPS)[5] must exceed muscle protein breakdown (MPB)]. Although progressive resistance training (PRT) is one strategy that can enhance MPS and promote an increase in muscle mass and strength, there has been considerable evidence in young and middle-aged adults that dietary protein can augment the skeletal muscle responses to PRT (3, 4). However, compared with young adults, older adults appear to experience a blunted response, termed anabolic resistance, to the stimulatory effects of small doses (~5–10 g) of protein (amino acids) (3, 5, 6). Thus, it has been suggested that older adults require a higher dose of protein to stimulate an increase in MPS and promote muscle hypertrophy in response to PRT (3, 5, 7). Indeed, results from a meta-analysis of randomized controlled trials revealed that protein ingestion (through either supplementation or diet) at a dose >1.2 g · kg^{-1} · d^{-1} enhanced the effects of PRT on muscle mass and strength in older adults (8).

The source or quality of protein can also have differing effects on MPS and the anabolic response to PRT. Although most previous research has focused on effects of milk proteins, particularly whey and casein, there has been evidence that red meat, which is a source

[1] From the Centre for Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences, Deakin University, Melbourne, Australia (RMD, SLO, NLM, CAG, DWD, and CAN), and the Baker IDI Heart and Diabetes Institute, Melbourne, Australia (DWD).

[2] The sponsor had no role in the design and conduct of the study; collection, management, and interpretation of the data; or preparation of the manuscript.

[3] Supported by a competitive, peer-reviewed grant from Meat and Livestock Australia Ltd, a National Health and Medical Research Council Career Development Award (ID 425849; to RMD), and an Australian Research Council Future Fellowship (FT 100100918; to DWD).

[4] Address reprint requests and correspondence to RM Daly, Centre for Physical Activity and Nutrition Research, Deakin University, 221 Burwood Highway, Burwood, Victoria 3125, Australia. E-mail: robin.daly@deakin.edu.au.

[5] Abbreviations used: BMD, bone mineral density; CRT, control resistance training; CSA, cross-sectional area; DXA, dual-energy X-ray absorptiometry; eGFR, estimated glomerular filtration rate; FM, fat mass; FSST, 4-square step test; IGF-I, insulin-like growth factor I; LTM, lean tissue mass; MPB, muscle protein breakdown; MPS, muscle protein synthesis; PRT, progressive resistance training; RM, repetition maximum; RT+Meat, resistance training plus lean red meat; STS, sit-to-stand test; TUG, timed-up-and-go test.

Received April 11, 2013. Accepted for publication January 9, 2014.

First published online January 29, 2014; doi: 10.3945/ajcn.113.064154.

of protein that contains complete and balanced proportions of all 8 essential amino acids, can also stimulate a rise in MPS at rest and after PRT provided that an adequate dose is consumed (5, 7, 9). In Australia, red meat is a major source of dietary protein, but adults aged >60 y have been reported to consume 34% less meat than that consumed by adults aged 25–44 y (10). This finding may be related to concerns about the potential adverse health effects of increased saturated fat and a subsequent increased risk of heart disease or type 2 diabetes (10). It has also been suggested that a diet that is high in red meat may increase systemic inflammation because of the associated increase in iron stores and/or saturated fat intake (11), but an 8-wk trial in older adults showed no adverse effects of an increased intake of red meat on inflammatory markers (11). Therefore, the primary aim of this 4-mo cluster randomized controlled trial was to investigate whether a modest increase in dietary protein intake achieved through the consumption of lean red meat 2 times/d, combined with PRT in a vitamin D-replete state results in a greater increase in total body and regional lean tissue mass (LTM), muscle size and strength, and functional performance and a reduction in markers of inflammation than with PRT alone in elderly women. Secondary aims were to investigate effects of the intervention on bone mineral density (BMD), muscle density, a surrogate marker of intermuscular adiposity, blood pressure, and blood lipid concentrations.

SUBJECTS AND METHODS

Participants

Women aged ≥60 y who were residing independently in self-care retirement villages within metropolitan Melbourne were recruited into this 4-mo study. In these self-care retirement villages, people live independently in apartments or units but share common-room facilities. Participants were excluded from the study on the basis of the following criteria: participation in resistance exercise (>1 wk) and/or moderate-intensity exercise ≥150 min/wk over the past 3 mo, acute or terminal illness, unstable metabolic or cardiovascular disease, a history of low-trauma fracture in the past 12 mo, type 1 diabetes, renal impairment [estimated glomerular filtration rate (eGFR) <45 mL/min]; BMI (in kg/m^2) >40, the use of medication that might have affected muscle metabolism (corticosteroids or thyroxine), substantial weight loss (>5 kg) in the past 6 mo, any condition that might have limited participation in the trial, or an inability to commit to the program. A total of 186 women from 15 retirement villages were prescreened via the telephone; 100 women were deemed eligible and willing to participate in the study (**Figure 1**). The average number of women per village was 7 (range: 2–11 women). All eligible women obtained clearance from their local physicians before participation in the program to ensure that they were free of any contraindicating medical conditions to exercise on the basis of the American College of Sports Medicine guidelines (12). The study recruitment and intervention were conducted in 2 cohorts over 2 y (from January 2009 to December 2010), with the intervention commencing at the same time each year (May to July). Thirty-eight women were recruited into the study in year 1 (2009), and 62 women were recruited into the study in year 2 (2010). The study was approved by the Deakin University Human Ethics Committee and registered with the Australian Clinical Trials Registry (ACTRN12609000223235). All participants provided written informed consent before participating in the study.

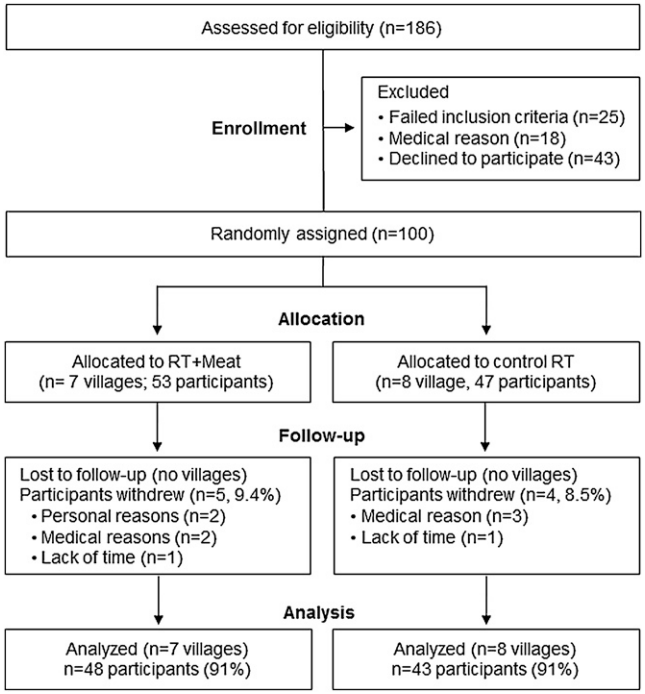

FIGURE 1. Flowchart of participants through the study. RT, resistance training; RT+Meat, resistance training plus lean red meat.

Study design

The study was a 4-mo cluster randomized controlled trial in which 100 elderly women were randomly allocated to one of the following 2 groups: *1*) PRT with two 80-g servings of cooked lean red meat/d (RT+Meat group; n = 53)] or *2*) control PRT in which participants were provided with and advised to consume ≥1 serving (~75 g cooked) rice and/or pasta/d that provided ~25–35 g carbohydrates [control resistance training (CRT) group; n = 47]. Because the intervention was conducted within retirement villages, all participants were randomly assigned by cluster (eg, village) to minimize the potential contamination across the 2 diet groups and enhance feasibility. The random assignment (by clusters) was conducted by an independent statistician with the use of a computer-generated randomization of study numbers. Outcome assessments for all participants were performed by the same research staff, but not all staff were blinded to the group allocation.

Exercise intervention

All participants enrolled in the study were prescribed an individually tailored and supervised progressive resistance and balance-agility training program. All training was conducted at retirement villages in small groups (≤8 women/group) under the supervision of a qualified exercise specialist. Each session lasted ~45–60 min and consisted of the following activities: *1*) a 5–10-min warm-up period that consisted of rhythmic exercises (eg, marching, side stepping, and line dancing), *2*) 30–40 min moderate-intensity PRT and low-impact balance-agility exercises, and *3*) a 5–10-min cool-down period that consisted of a series of stretching exercises. For the first 2 wk, participants completed 3 sets of 12 repetitions by using relatively light resistance to familiarize themselves with the equipment [free weight (dumbbells and ankle weights) and a Swiss ball], training protocol, and

correct execution of exercises. Thereafter, participants completed 3 sets of 8–12 repetitions for 8 different upper and lower body exercises at an intensity that corresponded to 14–16 (somewhat hard to hard) on the Borg Rating of Perceived Exertion scale. Some examples of exercises used throughout the program included squats, lunges, box step-ups, leg extensions, standing leg curls, hip abductions, calf raises, shoulder press, upright row, bicep curls, wall pushups, and triceps kickbacks.

To deliver the exercise program, qualified exercise trainers drove a custom-built Weights on Wheels mobile van that contained the resistance-training equipment to each retirement village 2 times/wk for 4 mo (32 sessions/person in total). When possible, the sessions were conducted before lunch or dinner so that participants in the RT+Meat group could consume their red meat meal as soon as possible after the training. Exercise compliance was computed from daily exercise cards completed by the women at each retirement village and checked by trainers after each session.

Dietary intervention

Lean red meat group

Women allocated to the protein-enriched diet (RT+Meat) were supplied with ~220 g (raw weight) lean red meat to be consumed 6 d/wk for 4 mo. This amount equated to ~160 g cooked meat/d (~45 g protein) or ~1.3 g · kg^{-1} · d^{-1} for an average woman who weighs 78 kg. Women prepared the meals themselves and consumed the meat across 2 meals (ie, lunch and dinner; ~80 g cooked serving/meal). The meat was supplied in labeled 110-g portion packs and trimmed of visible fat, and participants could select from a variety of veal, lamb, or beef cuts that were delivered frozen every 2–4 wk. Participants who were living with a partner also received sufficient food supplies for their partner. All participants received individual counseling sessions with a dietitian, recipes, and written dietary instructions for the consumption of the red meat. Participants recorded all meat consumed per day on a compliance calendar, which was collected every month.

Control group

Women randomly assigned to the control group were instructed to consume their usual diets and consume ≥1 serving carbohydrates (rice and pasta)/d. This control diet was not designed as an isoenergetic diet, and the rice and pasta were provided to assist in keeping their dietary protein intakes toward the lower range of a usual intake and to ensure that both groups received the same level of attention. Participants in this group were supplied with packs of pasta and rice every 2–4 wk and advised to consume ≥1 serving (~0.5 cups or ~250 mL) cooked rice, pasta, or potato/d (~25–35 g carbohydrates) and focus on having larger servings of bread, cereals, rice and vegetables and small servings of protein foods (eg, meat and fish). All women were also provided with individual counseling sessions, recipes, and written dietary instructions for the consumption of a carbohydrate-rich diet and supplies for their partner when appropriate. As with the red meat group, a checklist of carbohydrate-rich meals (pasta and rice) was completed daily to measure compliance.

Vitamin D_3 supplementation

To ensure vitamin D sufficiency (serum 25-hydroxyvitamin D concentration >75 nmol/L), all participants were required to consume one 1000-IU vitamin D_3 capsule (Blackmores)/d. These capsules were supplied at the start of the study, and compliance was checked by counting returned capsules at the end of the 4 mo.

Anthropometric measures, body composition, and BMD

Height was assessed by using a stadiometer (Holtain Ltd), and weight was measured on a digital scale (BWB 800; Wedderburn Australia,). Total body and regional (arm and leg) body composition [LTM, fat mass (FM), and percentage of body fat] and lumbar spine (L1–L4) and proximal femur (femoral neck and total hip) areal BMD were assessed by using dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy; GE Lunar Corp) with software version 12.30.008. Muscle and fat cross-sectional areas (CSAs) and muscle density, as a surrogate measure of intermuscular adiposity, of the nondominant distal femur (25% site) were assessed by using peripheral quantitative computed tomography (Stratec XCT 3000 scanner; Stratec Medical) with the methods previously reported (13). Briefly, after a scout view of the distal end of the femur was performed, scans were placed at 4% and 25% positions of the femur. The slice thickness was 2.3 mm, and the voxel size was set at 0.3 mm with a scanning speed of 10 mm/s. The subcutaneous fat CSA was determined by selecting the area with thresholds −40 to +40 mg/cm^3 hydroxyapatite density (contour mode 3, peel mode 1), and the muscle CSA was determined by subtracting the total bone CSA (threshold: 280 mg/cm^3; contour mode 1, peel mode 2) and subcutaneous fat CSA from the total area of the distal femur (threshold: −40 mg/cm^3; contour mode 3, peel mode 1) (13). For consistency, all DXA and peripheral quantitative computed tomography scans were analyzed by a single investigator. The short-term CV for repeated measurements of total body lean mass and FM in our laboratory ranged from 1.0% to 1.7%. The CV for BMD measures ranged from 0.6% to 1.0%, and femur muscle CSA was 1.3%.

Muscle balance and function

The 4-square step test (FSST), timed-up-and-go test (TUG), and 30-s sit-to-stand test (STS) were used to assess muscle function. The FSST provides a measure of dynamic standing balance and stepping speed in 4 different directions (14). To complete this test, participants were required to step forward, sideways, and backward over 4 canes that rest flat on the floor in a cross formation by moving *1*) in a clockwise direction and *2*) in a counterclockwise direction to return to the starting position (14). Participants were instructed to complete the task as quickly as possible without touching or stepping on the canes and, if possible, to face forward during the entire sequence. Subjects were also instructed to ensure that both feet made contact with the floor in each square. After one practice trial, participants completed the test, and the time (in s) taken to complete the sequence was measured with a stopwatch and recorded as the final score. The TUG is a measure of dynamic balance during the following 3 commonly performed functional activities: standing up from and sitting down in a chair, walking, and turning (15, 16). For this test, participants were seated in a chair (height: 45 cm) that was placed at the end of a marked 3-m walkway. On the command "go," participants were instructed to stand up, walk at a comfortable speed for 3 m, turn back to the chair, and sit down. To minimize any ceiling effects and make the

test more challenging, participants were also instructed to start counting backward from 100 by 3s on the command "go." A stopwatch was used to record the time taken to complete the test. All participants were given a practice trial before they completed the timed test. Finally, the 30-s STS provides a measure of lower-extremity muscle strength and was administered by using a chair without arms. With participants starting from a sitting position in the chair, with arms folded across the chest, they were instructed to stand fully upright and return to the seated position at their own pace as many times as possible in 30 s. The final score was the number of complete stands recorded in 30 s. All testing was conducting in research laboratories at Deakin University.

Muscle strength

One-repetition maximum (RM) leg-extension strength was estimated from a 3-RM strength test on a leg-extension machine (Free Motion Fitness) at Deakin University. Before the determination of 3-RM muscle strength, participants completed a 5-min warm-up session on an exercise bike. To determine the 3-RM, each participant initially performed a warm-up set of 8–10 repetitions with a light load. After the successful completion of an additional 6–8 repetitions at a heavier weight selected by the instructor and after a brief rest (~2 min), the workload was increased incrementally until only 3 repetitions with correct technique could be completed. The following formula was used to predict 1-RM strength (17):

$$1-\mathrm{RM} = 100 \times (3-\mathrm{RM\,load}) \div [48.8 + 53.8 \times e^{(-0.075\times 3)}] \quad (1)$$

Medical history and medication use

Information on the medical history, smoking status, and alcohol consumption of each subject was determined by using a questionnaire at baseline and every 4 wk. Participants were categorized as never smokers, ex-smokers, and current smokers. The current weekly intake of beer, wine, or hard liquor was used to tabulate grams of alcohol consumed per week. Information on the use of medication was determined by using a questionnaire and confirmed by a personal interview (at baseline and 4 mo) or telephone (at 1, 2, and 3 mo).

Diet and physical activity

Nutrient intakes were assessed at baseline and every 4 wk by using telephone-facilitated 24-h dietary recalls performed by trained research dietitians. Participants were asked to estimate food quantities in household measures through the use of standardized actual size food-model booklets that were provided to participants before the commencement of the study. Dietary data were analyzed with Australia-specific dietary analysis software (Foodworks version 6.0.2562; Xyris Software). The total leisure and recreational physical activity time (h/wk) was assessed at baseline and 4 mo by using the Community Healthy Activities Model Program for Seniors physical activity questionnaire developed and validated for use with older adults (18).

Serum and urinary measurements

Fasted, resting morning (0800–1000) venous blood samples were collected at baseline and 2 and 4 mo from each participant's antecubital vein at a National Association of Testing Authorities/Royal College of Pathologists Australasia accredited pathology laboratory. Participants also completed a timed 24-h urine collection at each of these time points. Serum IL-6, IL-10, and TNF-α were measured by using the Milliplex high-sensitivity human cytokine panel (Millipore Billerica) per the manufacturers' recommendations with an intraassay CV <7% and interassay CV <10%. Serum adiponectin was assayed by using a Procarta kit (Affymetrix) with an intraassay CV <7% and interassay CV <10%. Serum insulin-like growth factor I (IGF-I) was determined by using an Immulite 2000 IGF-I chemiluminescent immunometric assay (Siemens Health care Diagnostics) with an intraassay CV of 3.1% and interassay CV of 6.2%. Serum 25-hydroxyvitamin D was assessed by using validated liquid chromatography–mass spectrometry–mass spectrometry method on an Applied Biosystems 4000 Q Trap liquid chromatography–mass spectrometry–mass spectrometry system (RDDT Laboratories). The intraassay CV was 3.9%, and the interassay CV was 2.8%. HDL-cholesterol concentrations were determined with an Olympus analyzer by using an OSR6287 HDL-cholesterol reagent (Olympus Diagnostica). The intraassay CV was 0.96%, and the interassay CV was 1.6%. Total serum cholesterol, triglycerides, and serum and urinary urea and creatinine were analyzed by using standardized techniques at a National Association of Testing Authorities/Royal College of Pathologists Australasia–accredited pathology laboratory. LDL cholesterol was calculated by using Friedewald's formula. The eGFR was calculated by using the participants' serum creatinine, age, and sex according to the following abbreviated Modification of Diet in Renal Disease formula, which is now used by most laboratories in Australia (19):

$$\mathrm{eGFR}\ (\mathrm{mL\cdot min^{-1}\cdot 1.73\,m^{-2}}) = 175 \times [\mathrm{serum\ creatinine}\ (\mu\mathrm{mol/L}) \times 0.0113]^{-1.154} \times (\mathrm{age})^{-0.203} \times 0.742\ (\mathrm{if\ female}) \quad (2)$$

Blood pressure measurement

After a 5-min rest period seated in a quiet room, systolic and diastolic blood pressures were measured by using an automated blood pressure monitor (A&D Instruments). Three measurements were taken on the left arm with a 2-min interval between readings; the mean of the final 2 readings was used in the analysis.

Statistical analysis

Sample-size calculations were based on the expected difference between groups for the change in the primary outcome measure of total body LTM measured by using DXA. With the assumption of an average cluster size (participants per village) of 8 and an intracluster correlation coefficient of 0.04 (20), we estimated that to have 80% power of detecting a difference (±SD) in total body LTM of 0.8 ± 1.7 kg at the 2-tailed test with an α level of 0.05, we would require 50 women in each group (100 participants from ~13 villages), with allowance for a possible 15% dropout rate. All statistical analyses were conducted with Stata Statistical Software (release 11.1; StataCorp LP). Baseline characteristics between the groups were compared by using independent *t* tests for continuous variables, the Mann-Whitney nonparametric test for continuous data

that was not normally distributed (ie, duration of hormone-replacement therapy and alcohol intake), and chi-square tests for categorical variables. Changes in body composition, bone density, muscle function and strength, and various hormonal, inflammatory, lipid and biochemical markers were expressed as either the absolute or percentage of change from baseline. Between-group differences were calculated by subtracting within-group changes from baseline for the RT+Meat group from within-group changes for the CRT group after 2 and 4 mo. Because of nonnormality, the following variables were log transformed before analysis: 1-RM strength, TUG, 30-s STS, IGF-I, serum urea, serum creatinine, 25-hydroxyvitamin D, 24-h urinary urea, 24-h urinary creatinine, serum IL-6, IL-10, TNF-α, adiponectin, high-sensitivity C-reactive protein, LDL cholesterol, and triglycerides. The percentage of change in these traits represented the absolute difference from baseline in log-transformed data multiplied by 100. Generalized linear mixed models with random effects were used to analyze differences between RT+Meat and CRT groups after 2 and 4 mo when appropriate. This model appropriately adjusted for the variability both between clusters (villages) and within a cluster (participants within the same village). The group (RT+Meat or CRT) was the fixed effect, and clusters (retirement village) and the unit of analysis (participants) were included as random effects to account for the clustered design. Because of differences between groups, the change in physical activity was included as a covariate for all outcome measures, and baseline muscle strength and baseline serum IL-6 were included as covariates for muscle strength and IL-6, respectively. Similarly, the baseline carbohydrate percentage of energy, fat intake (g/d), and fat percentage of energy were included as covariates for each of these measures in the analysis. For blood pressure and all lipid measures, the antihypertensive medication use and lipid-lowering medication use were also included as covariates, respectively. All data were analyzed by using an intention-to-treat approach. $P < 0.05$ was used to determine statistical significance. All data are presented as means ± SD or 95% CIs unless otherwise stated.

RESULTS

Baseline characteristics

Baseline characteristics of the 2 groups are shown in **Table 1.** On average, women were aged 73 y (range: 60–90 y) with a BMI of 28. Eight (8%) of the women (RT+Meat: $n = 2$; CRT: $n = 6$) had an eGFR between 45–59 mL/min which could have represented mildly reduced kidney function in this age group. Forty-four women (44%) reported a history of the use of hormone-replacement therapy (mean duration: 9.9 ± 8.1 y), and 31% of women were ex-smokers or current smokers (only one current smoker). Approximately one-third of the women were taking a lipid-lowering medication (38%), and 58% of women were taking an antihypertensive medication.

Study attrition

Nine (9%) of 100 women withdrew from the study over the 4-mo period (RT+Meat: $n = 5$; CRT: $n = 4$) (Figure 1). Reasons for withdrawal included medical (viral pneumonia: $n = 1$; osteoarthritis: $n = 2$; unexpected surgery: $n = 1$; and back pain: $n = 1$), work or personal time commitments ($n = 2$), and personal reasons unrelated to the study ($n = 2$). Subjects who withdrew from the trial did not differ significantly in age or any baseline anthropometric or body-composition variable from women who completed the intervention (data not shown).

TABLE 1
Baseline characteristics of participants in RT+Meat and CRT groups[1]

Characteristic	RT+Meat	CRT
n	53	47
Age (y)	72.1 ± 6.4[2]	73.6 ± 7.7
Height (cm)	160.1 ± 5.1	158.7 ± 5.4
Weight (kg)	70.0 ± 11.3	68.4 ± 11.5
BMI (kg/m^2)	27.7 ± 3.9	27.6 ± 4.8
Age at menopause (y)	49.3 ± 6.5	47.7 ± 5.3
Use of HRT [*n* (%)]	24 (45%)	20 (43%)
Duration of HRT use (y)	9.2 ± 8.1	10.7 ± 8.2
Ex-smoker or current smoker [*n* (%)]	17 (32)	14 (30)
Physical activity (h/wk)	9.3 ± 5.6	8.1 ± 4.0
Alcohol (g/d)[3]	7.7 ± 6.5	10.8 ± 8.5
Antihypertensive therapy [*n* (%)]	30 (57)	28 (60)
Lipid-lowering therapy [*n* (%)]	20 (43)	18 (34)
IGF-1 (ng/mL)	15.4 ± 5.6	15.3 ± 6.2
25-Hydroxyvitamin D (nmol/L)	68.4 ± 26.8	70.0 ± 31.6
IL-6 (pg/mL)	23.3 ± 88.1	20.6 ± 89.0
TNF-α (pg/mL)	6.91 ± 3.38	7.81 ± 3.32
hs-CRP, mg/L	3.63 ± 7.49	2.69 ± 5.23
IL-10 (pg/mL)	37.3 ± 94.0	51.3 ± 261.8
Adiponectin (μg/mL)	6.6 ± 13.8	12.7 ± 17.5

[1] Independent *t* tests or Mann-Whitney nonparametric tests (continuous variables) and chi-square tests (categorical variables) were used to examine between-group differences at baseline. CRT, control resistance training; HRT, hormone-replacement therapy; hs-CRP, high-sensitivity C-reactive protein; IGF-I, insulin-like growth factor I; RT+Meat, resistance training plus lean red meat.

[2] Mean ± SD (all such value).

[3] Alcohol represents the mean intake for women who reported consuming alcohol (RT+Meat: $n = 26$; CRT: $n = 28$).

Program adherence and compliance

The mean attendance at the exercise classes over the 4-mo of training for all women was 74% and did not differ between RT +Meat [75% (95% CI: 68%, 82%)] and CRT [72% (95% CI: 64%, 80%)] groups. The mean compliance for consumption of the daily portions of lean red meat and carbohydrates as assessed by a self-report calendar was 81% (95% CI: 76%, 86%) and 100%, respectively. Participants took, on average, 92% of the vitamin D supplements allocated [RT+Meat: 92% (95% CI: 89%, 96%); CRT: 93% (95% CI: 90%, 95%)].

Adverse events and kidney function

There were no serious injuries or adverse events associated with the program. Four women reported aggravated knee or back pain or soreness as a result of the exercise training, but all were able to continue with a modified program. The consumption of lean red meat was not associated with a deterioration in kidney function. After 2-mo, 7 women in the study (RT+Meat: $n = 5$; CRT: $n = 2$) had changed from a normal baseline to mildly decreased eGFR (range: 45–59 mL · min^{-1} · 1.73 m^{-2}), but by 4-mo, only one woman (in the RT+Meat group) exhibited this alteration. The

remaining women either maintained their baseline status of normal or mildly decreased eGFR across the study or, in the case of 2 women (RT+Meat: $n = 1$; CRT: $n = 1$), improved their eGFR status from mildly decreased to normal at both 2 and 4 mo.

Dietary intake

Throughout the study, the dietary protein intake (g/d, $g \cdot kg^{-1} \cdot d^{-1}$, or the percentage of energy from protein) was greater in the RT+Meat group than in the CRT group (all $P < 0.01$ to <0.001). The average ($\pm$ SD) dietary protein intake in the RT+Meat group throughout the study (weeks 4, 8, 12, and 16) was 1.29 ± 0.30 compared with 1.15 ± 0.35 $g \cdot kg^{-1} \cdot d^{-1}$ ($P < 0.05$) in the CRT group. The intake of carbohydrates was ~20–40 g greater throughout the study in the CRT group than in the RT+Meat group (**Table 2**). The dietary zinc intake increased in the RT+Meat group throughout the study and was greater than in the CRT group at each time point (P-group-by-time interaction < 0.001). In contrast, there were no consistent differences in total energy, fat, saturated fat, or iron intakes between groups at any time throughout the study, with the exception of fat intake, which was greater in the CRT group at weeks 12 and 16.

Physical activity

The total leisure time physical activity increased significantly in the CRT group compared wtih the RT+Meat group throughout the study [mean ($\pm$SD) change at 4 mo: 2.8 ± 5.1 compared with -0.3 ± 4.7 h/wk, respectively; $P < 0.01$].

TABLE 2
Estimated dietary intakes in RT+Meat ($n = 53$) and CRT ($n = 47$) groups at baseline and 4, 8, 12, and 16 wk[1]

	Baseline	Week 4	Week 8	Week 12	Week 16
Total energy (kJ/d)					
RT+Meat	6160 ± 1513	6060 ± 1569	6300 ± 1490	6125 ± 1887	6340 ± 1570
CRT	6612 ± 1593	6674 ± 1869	6404 ± 1919	6727 ± 2224	7111 ± 1813
Interaction	—	NS	NS	NS	NS
Protein (g/d)					
RT+Meat	73.4 ± 23.2	88.3 ± 17.5***	88.3 ± 20.0***	86.4 ± 23.6**	89.7 ± 22.8***
CRT	76.1 ± 26.6	75.0 ± 26.2	73.1 ± 23.4	73.5 ± 26.3	76.9 ± 27.3
Interaction	—	<0.05	<0.01	<0.01	=0.05
Protein (% of energy)					
RT+Meat	19.7 ± 4.4	25.0 ± 5.8***	24.1 ± 6.5†	23.9 ± 5.0†	23.9 ± 5.6***
CRT	18.9 ± 5.0	18.6 ± 4.5	19.3 ± 4.7	18.9 ± 5.7	17.9 ± 4.0
Interaction	—	<0.001	<0.05	<0.01	<0.001
Carbohydrates (g/d)					
RT+Meat	170.6 ± 48.0	151.7 ± 64.5	157.0 ± 41.1	168.4 ± 73.3	166.1 ± 52.4
CRT	172.0 ± 57.3	191.5 ± 67.9	183.8 ± 63.6	186.3 ± 72.3	190.6 ± 59.5
Interaction	—	<0.05	NS	NS	NS
Carbohydrates (% of energy)					
RT+Meat	44.3 ± 7.6#	38.9 ± 9.8***	39.6 ± 6.8**	42.9 ± 7.1	41.3 ± 7.1*
CRT	40.7 ± 7.9	45.3 ± 10.0**	45.9 ± 9.4**	44.0 ± 9.1	42.1 ± 8.0
Interaction	—	<0.001	<0.001	<0.05	NS
Fat (g/d)					
RT+Meat	52.8 ± 22.2#	51.7 ± 20.3	54.6 ± 20.7	47.6 ± 19.7	52.7 ± 19.2
CRT	61.2 ± 19.2	56.1 ± 24.7	52.3 ± 26.5	59.8 ± 32.5	66.4 ± 23.6
Interaction	—	NS	NS	<0.05	<0.05
Fat (% of energy)					
RT+Meat	30.0 ± 8.4#	30.3 ± 8.8	30.5 ± 7.2	27.6 ± 6.5	29.6 ± 6.7
CRT	33.4 ± 8.1	29.8 ± 9.5*	28.3 ± 9.7**	30.9 ± 9.3	33.2 ± 7.3
Interaction	—	NS	<0.05	NS	NS
Saturated fat (g/d)					
RT+Meat	20.5 ± 10.6	19.2 ± 9.6	21.2 ± 9.7	18.2 ± 8.3	20.0 ± 9.7
CRT	24.0 ± 11.4	21.3 ± 11.1*	19.3 ± 9.7	22.1 ± 13.2	22.8 ± 8.9
Interaction	—	NS	NS	NS	NS
Iron (mg/d)					
RT+Meat	10.7 ± 3.6	12.1 ± 4.4	11.7 ± 2.8	12.1 ± 4.6	11.8 ± 2.7
CRT	10.5 ± 3.8	10.8 ± 4.4*	9.9 ± 3.5	10.2 ± 4.0*	11.0 ± 3.3
Interaction	—	NS	NS	NS	NS
Zinc (mg/d)					
RT+Meat	10.0 ± 3.9	14.9 ± 4.6***	14.0 ± 3.9***	14.9 ± 5.1***	14.2 ± 4.6***
CRT	9.9 ± 3.8	9.4 ± 3.9	9.6 ± 3.6	9.2 ± 3.8	9.8 ± 3.8
Interaction	—	<0.001	<0.001	<0.001	<0.001

[1] All values are means ± SDs. P values for interaction terms and within-group changes were derived from the generalized linear mixed models with random effects after adjustment for the change in physical activity and baseline values for carbohydrates (percentage of energy) and fat (g/d and percentage of energy). Within-group change relative to baseline, ***$P < 0.001$, **$P < 0.01$ *$P < 0.05$. #Compared with CRT at baseline, $P < 0.05$. CRT, control resistance training; RT+Meat, resistance training plus lean red meat.

Body composition

After 4 mo, there was a significant 0.5-kg (95% CI: 0.1, 0.8-kg) greater gain in total body LTM in the RT+Meat group than in the CRT group (P-group-by-time interaction = 0.007) (**Table 3**). This result was largely attributed to a greater gain in leg LTM [mean change (95% CI): RT+Meat: 0.33 kg (0.19, 0.47 kg); CRT: 0.11 kg (−0.04, 0.26 kg); P-group-by-time interaction < 0.05] because there was no difference between groups for the change in arm LTM [mean change (95% CI): RT+Meat: 0.06 kg (0.00, 0.12 kg); CRT: 0.04 kg (−0.2, 0.11 kg); P-group-by-time interaction = 0.61]. For muscle CSA at the 25% femur, the 1.4% greater gain in the RT+Meat group was not significantly different from that in the CRT group ($P = 0.397$) (Table 3). For both total body FM and the percentage of body fat, there were no between-group differences for the change over time, but there were significant time effects (Table 3). Additional post hoc analyses revealed that there was a significant 0.5-kg (95% CI: −0.9, −0.03-kg) decrease in FM ($P < 0.05$) and a 0.8% (95% CI: −1.3%, −0.3%) ($P < 0.01$) absolute reduction in the percentage of body fat in the RT+Meat group. For femur muscle density and subcutaneous fat, there were no significant within- or between-group differences for the change over 4 mo.

Muscle strength and function

The RT+Meat group had an average 18% (95% CI: 3%, 34%) greater increase in leg-extension muscle strength compared with that in the CRT group (P-group-by-time-interaction = 0.01) (Table 3). Both groups experienced similar significant exercise-induced improvements in all measures of muscle function (TUG, FSST, and 30-s STS) (Table 3).

Biochemical, hormonal, and inflammatory measures

There were no differences at baseline in any biochemical, hormonal, or inflammatory markers (Table 1, **Table 4**). In comparison with the CRT group, the proinflammatory cytokine IL-6 decreased significantly in the RT+Meat group after 4 mo (P-group-by-time interaction < 0.05) (**Figure 2**). There were no other between-group differences for the other cytokines or adiponectin, but there was a significant time effect for TNF-α. An additional post hoc analysis revealed that there was a 7.8% (95% CI: −15.7%, 0.0%) reduction in TNF-α in the RT+Meat group after 4 mo ($P < 0.05$). For serum IGF-I, there was a greater increase in the RT+Meat group than CRT group after 2 mo [net difference: 6.9% (95% CI: 0.0%, 14.2%); $P = 0.05$] and 4 mo [10.2% (95% CI: 3.1%, 17.3%); $P < 0.05$] (Figure 2). Serum 25-hydroxyvitamin D concentrations increased similarly by 10.4 nmol/L (95% CI: 4.1, 16.7 nmol/L) and 13.1 nmol/L (95% CI: 7.3, 19.0 nmol/L) in RT+Meat and CRT groups, respectively, after 4 mo. For both serum and urinary urea, there was a greater increase in the RT+Meat group than in the CRT group after 2 mo (both $P < 0.05$) but not after 4 mo (Table 4). Although there was a trend for a greater increase in urinary creatinine in the RT+Meat group than in the CRT group after only 2 mo (P-group-by-time interaction = 0.052), this difference did not persist after 4 mo ($P = 0.116$).

BMD

For femoral neck, total hip and lumbar spine BMDs, there were no significant time or group-by-time interaction effects for the change after 4 mo (Table 3).

Blood pressure and lipids

For systolic and diastolic blood pressures and all lipid variables, there were no significant between group differences for changes after 2 or 4 mo, but there was a main effect for time for systolic blood pressure, total cholesterol, and triglycerides (**Table 5**).

DISCUSSION

The findings from this 4-mo cluster randomized controlled trial indicated that the combination of PRT with a protein-enriched diet achieved through an increased intake of lean red meat led to greater gains in total body and leg LTM and leg muscle strength as well as an increase in serum IGF-I and a reduction in the proinflammatory marker IL-6 compared with PRT alone in community-dwelling elderly women. The consumption of two ~80-g servings of cooked lean red meat (beef, veal, and lamb) on most days of the week was well tolerated by the elderly women as indicated by the high compliance (81%) and had no adverse effects on kidney function, blood pressure, or blood lipids.

To achieve an increase in skeletal muscle mass requires a positive net-protein balance (ie, the rate of MPS must exceed MPB). As highlighted in a number of reviews, the postexercise ingestion of protein (amino acids) can enhance MPS above rates observed with exercise alone and inhibit MPB, which, if repeated over time, can promote muscle hypertrophy (21–23). However, there have been mixed findings from available intervention trials with regard to the optimal amount (dose) of protein needed to achieve a positive net-protein balance and augment anabolic muscle responses to PRT, particularly in older adults (20, 24–29). Although these mixed findings may have been related in part to the short-term follow-up (<3 mo), small sample sizes, and/or varying doses of protein used in many of these trials, the findings from our study indicated that a daily intake of ~1.3 g protein/kg can enhance the effects of PRT on LTM and leg muscle strength in older women after 4 mo. This result was consistent with results from a meta-analysis of randomized controlled trials which reported that a protein dose >1.2 g/kg was effective for augmenting the effects of PRT on fat-free mass and muscle strength in adults aged >50 y (8). In the meta-analysis, the weighted mean difference in fat-free mass between PRT-plus-protein-supplementation and placebo groups was 0.48 kg, which was equivalent to the 0.5-kg net gain observed in the RT+Meat group in our study.

The quality or type of protein consumed can also play an important role in the anabolic response to PRT (3, 21, 23). Red meat is a high-quality source of protein that contains complete and balanced proportions of all 8 essential amino acids and has been shown to acutely stimulate MPS after PRT in older adults in a dose-response manner (5, 7, 30). In line with these acute findings, we showed that the consumption of ~160 g cooked lean red meat (~45 g protein) on most days of the week combined with PRT was associated with greater gains in total body and leg LTM and muscle strength compared with PRT alone. However, in our study, the dose of protein (red meat) was divided into 2 servings, with participants advised to consume red meat at lunch and dinner.

TABLE 3
Baseline values and the within-group changes in RT+Meat and CRT groups for body composition, muscle strength, muscle function, and bone mineral density and net between-group differences for the change relative to baseline[1]

	RT+Meat		CRT			*P*		
	Baseline (n = 53)	Change (n = 48)	Baseline (n = 47)	Change (n = 43)	Net difference	Group	Time	Group × time
Body composition								
Total body FM (kg)	30.1 ± 8.2	−0.5 (−0.9, −0.03)	28.9 ± 9.6	−0.3 (−0.7, 0.2)	−0.2 (−0.4, 0.8)	0.651	0.042	0.179
Percentage of body fat	42.6 ± 5.9	−0.8 (−1.3, −0.3)	41.2 ± 7.7	−0.4 (−0.8, 0.1)	−0.4 (0.3, −1.1)	0.476	0.005	0.073
Total body LTM (kg)	37.3 ± 4.3	0.6 (0.3, 0.8)	37.3 ± 3.5	0.1 (−0.4, 1.1)	0.5 (0.1, 0.8)	0.758	0.003	0.007
Femur muscle CSA (mm^2)	4047 ± 835	5.4 (3.7, 7.1)	4075 ± 623	4.0 (2.2, 5.8)	1.4 (−1.0, 3.9)	0.565	0.001	0.397
Femur sFat CSA (mm^2)	8148 2163	0.2 (−1.2, 1.5)	8112 ± 2945	0.7 (−1.0, 2.3)	−0.5 (−2.6, 1.6)	0.971	0.441	0.684
Femur MD (mg/cm^3)	74.0 ± 2.4	0.8 (0.0, 1.6)	74.4 ± 3.1	0.4 (−0.6, 1.5)	0.4 (−0.9, 1.7)	0.973	0.074	0.588
Muscle strength								
Leg extension (kg)	32.0 ± 11.2	28 (18, 39)	28.1 ± 10.1[#]	10 (−1, 21)	18 (3, 34)	0.014	0.005	0.010
Muscle function								
4-square step test (s)	7.7 ± 1.5	5.4 (1.6, 9.2)	7.7 ± 1.6	6.5 (1.4, 11.7)	−1.1 (−7.4, 5.1)	0.915	0.001	0.274
Timed-up-and-go test (s)	9.1 ± 3.3	−14.4 (−19.5, −0.1)	9.1 ± 2.4	–16.4 (−23.7, −0.1)	−2.0 (−6.6, 10.6)	0.667	0.001	0.798
30-s sit-to-stand test	13.1 ± 3.1	18.5 (11.1, 25.8)	12.5 ± 3.0	27.0 (19.4, 34.5)	−8.4 (−18.9, 1.9)	0.472	0.001	0.190
Bone mineral density								
Femoral neck (g/cm^2)	0.815 ± 0.107	0.2 (−0.5, 0.9)	0.852 ± 0.147	–0.5 (−1.1, 0.04)	0.7 (−0.1, 1.6)	0.205	0.573	0.092
Total hip (g/cm^2)	0.866 ± 0.134	0.2 (−0.4, 0.9)	0.897 ± 0.152	–0.2 (−0.9, 0.4)	0.4 (−0.5, 1.4))	0.593	0.937	0.164
Lumbar spine (g/cm^2)	1.073 ± 0.177	–0.3 (−1.1, 0.4)	1.105 ± 0.184	–0.3 (−1.0, 0.4)	0.0 (−1.0, 1.0)	0.980	0.091	0.880

[1] All baseline values are means ± SDs. All change values are within-group mean absolutes (total body FM, LTM, and percentage of body fat) or percentages (femur subcutaneous fat CSA, muscle strength, muscle function, and bone mineral density) of changes from baseline; 95% CIs in parentheses. Net differences (95% CIs) were calculated by subtracting within-group changes from baseline for the RT+Meat group from within-group changes for the CRT group after 4 mo. Independent t tests were used to examine between-group differences at baseline. P values for group, time, and group-by-time interaction terms were derived from generalized linear mixed models with random effects after adjustment for the change in physical activity (and baseline muscle strength for leg-extension strength). [#]Compared with RT + Meat, $P = 0.06$. CRT, control resistance training; CSA, cross-sectional area; FM, fat mass; LTM, lean tissue mass; MD, muscle density; RT+Meat, resistance training plus lean red meat; sFat, subcutaneous fat.

TABLE 4
Baseline values and within-group changes in RT+Meat and CRT groups for serum and urinary urea and creatinine and net between-group differences for the change relative to baseline[1]

	Study group			*P*		
	RT+Meat	CRT	Net difference	Group	Time	Group × time
Serum urea						
Baseline (mmol/L)	5.94 ± 1.31	6.16 ± 1.56	—	—	—	—
△ 2 mo	13.3 (6.2, 20.4)	4.4 (−2.3, 11.1)	8.9 (−0.8, 18.7)	0.563	0.001	0.035
△ 4 mo	8.8 (2.5, 15.2)	3.5 (−2.8, 9.7)	5.3 (−3.5, 14.2)		0.007	0.072
Serum creatinine						
Baseline (μmol/L)	68.8 ± 10.8	70.2 ± 10.0	—	—	—	—
△ 2 mo	2.3 (−1.1, 5.6)	1.8 (−1.1, 4.6)	0.5 (−3.9, 4.8)	0.335	0.101	0.936
△ 4 mo	−2.0 (−7.3, 3.4)	−0.7 (−3.0, 1.6)	1.2 (−7.2, 4.7)		0.303	0.893
Urinary urea						
Baseline (mmol/24 h)	378 ± 118	376 ± 134	—	—	—	—
△ 2 mo	13.4 (6.4, 20.3)	−0.8 (−10.1, 8.4)	14.2 (5.6, 30.2)	0.073	0.008	0.026
△ 4 mo	2.4 (−6.3, 11.1)	−1.1 (−9.8, 7.5)	3.5 (6.1, −8.6)		0.566	0.609
Urinary creatinine						
Baseline (mmol/24 h)	7.81 ± 1.78	7.74 ± 1.71	—	—	—	—
△ 2 mo	10.9 (4.5, 17.3)	2.1 (−3.6, 7.7)	8.8 (4.3, 0.2)	0.081	0.001	0.052
△ 4 mo	4.5 (−2.6, 11.5)	−2.2 (−9.5, 5.0)	6.7 (5.0, −3.3)		0.369	0.116

[1] All baseline values are unadjusted means ± SDs. All change values (95% CIs) were calculated from the absolute difference from baseline in log-transformed data multiplied by 100. Net differences (95% CIs) were calculated by subtracting within-group changes from baseline for the RT+Meat group from within-group changes for the CRT group after 2 and 4 mo. Independent *t* tests were used to examine between-group differences at baseline. *P* values for group, time, and group-by-time interaction terms were derived from generalized linear mixed models with random effects after adjustment for the change in physical activity. *P* values for all measures were based on log-transformed data. CRT, control resistance training; RT+Meat, resistance training plus lean red meat.

Although we did not monitor the timing or size of each serving, it has been proposed that the ingestion of ~25–30 g protein with each meal may be more effective for stimulating MPS and provide a greater 24-h protein anabolic response than would an unequal protein distribution (31). Although additional studies are needed to test this hypothesis, this possibility may explain the mixed findings from previous trials that investigated interactive effects of PRT with a diet high in red meat on body composition and muscle strength in older adults (24, 28, 32, 33).

In a small 12-wk trial of 19 men aged 51–69 y, Campbell et al (24) reported that PRT combined with a meat-containing diet (~1.0 g protein/kg) was more effective for improving lean mass than for individuals who consumed a lactoovegetarian diet (~0.8 g protein/kg). Although these findings were consistent with our results, this study suggested that a daily protein intake of ~1.0 g/kg can enhance the effects of PRT on muscle mass in older adults. However, these results must be interpreted with caution because of the small sample size and nonrandomized nature of the dietary intervention. Indeed, in a follow-up study by the same group that involved 21 men (mean age: 65 y), there were no significant differences in fat-free mass and muscle size or strength after 12 wk of PRT with a meat or lactoovegetarian diet; the mean daily protein intake in both groups ranged from 1.03 to 1.17 g/kg during the intervention (28). Thus, we suggest that our study, with its larger sample size and longer duration, provided the strongest evidence thus far that a high–red meat diet combined with PRT represents a safe and practical strategy to augment the effects of PRT on LTM and muscle strength in elderly women.

It has been suggested that both age-related muscle loss and the blunted response of ageing muscle to the provision of protein may be related to an increase in systemic inflammation. Indeed, there has been evidence that normal ageing is associated with a persistent increase in proinflammatory cytokines, which have been associated with muscle weakness, atrophy, and disability (34–37). It has also been shown that the inflammatory markers IL-6 and TNF-α can inhibit MPS and attenuate exercise-induced gains in muscle mass (38, 39). Although exercise training, particularly aerobic training, can reduce systemic and local inflammation (40), the effects of increased dietary protein on inflammation remain equivocal (11, 41, 42). There has been some evidence that an increased protein intake may indirectly affect muscle via a reduction in inflammation mediated by an increase in circulating IGF-I, whereby high concentrations of IL-6 decrease serum IGF-I, and low IGF-I concentrations stimulate IL-6, which suggest that IL-6 may oppose the effects of IGF-I on muscle (34, 43). In our study, we showed that PRT alone had no effect on any marker of inflammation, but the proinflammatory cytokine IL-6 was reduced and serum IGF-I concentrations were increased in the RT+Meat group compared with the CRT group. Consistent with these findings, the results from an 18-mo trial in 41 elderly outpatients with sarcopenia showed that nutritional supplementation with 8 g essential amino acids led to a greater gain in lean mass with a parallel increase in IGF-I and a reduction in TNF-α compared with the placebo control group (44). Collectively, these findings indicate that the mechanism(s) by which increased dietary protein can enhance the effects of PRT on muscle may be via an increase in serum IGF-I and a concomitant reduction in circulating inflammatory markers.

Although the findings from this study support several previous reports that older adults are likely to require a protein intake of ~1.2–1.5 $g \cdot kg^{-1} \cdot d^{-1}$ to enhance the effects of PRT on

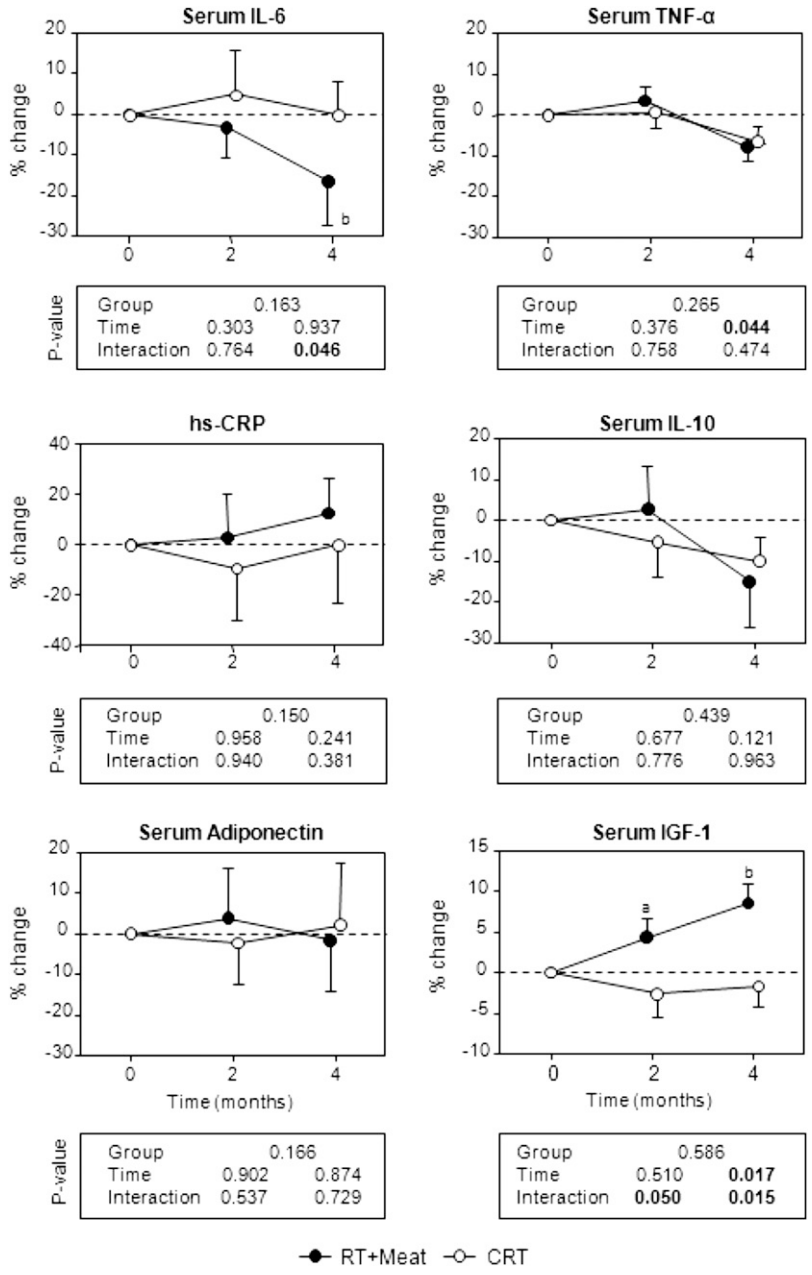

FIGURE 2. Mean (±SE) percentages of changes in serum IL-6, TNF-α, hs-CRP, IL-10, adiponectin, and IGF-I in the RT+Meat (n = 48) and CRT (n = 43) groups. [a]$P = 0.054$; [b]$P < 0.05$ significant between group difference from baseline (group-by-time interaction) after adjusting for change in physical activity (and baseline values for IL-6). P values for the group, time, and interaction terms were derived from the generalized linear mixed models with random effects. CRT, control resistance training; hs-CRP, high-sensitivity C-reactive protein; IGF-I, insulin-like growth factor I; RT+Meat, resistance training plus lean red meat.

muscle (8, 45), we acknowledge that our assessment of dietary protein intake was an approximate estimate derived only from the self-reported intake. However, the finding that 24-h urinary urea concentrations increased after 2 mo in the RT+Meat group supported the dietary data that the protein intake increased in this group. The result that there were no between-group differences after 4 mo may have been attributed to a reduction in compliance with the consumption of the red meat. On average, the mean intake of red meat (servings/d) decreased from 86% during the first 2 mo to 75% thereafter. Although this result suggested that adherence to 2 daily servings of lean red meat on most days of the week may not be sustainable for many older adults, additional research is needed to determine whether similar muscle benefits could be achieved with a less frequent consumption of red meat (eg, after each training session only). In addition, the finding that mean dietary protein intakes only differed by ~15 g between the groups throughout the study, despite the red meat providing an additional 45 g protein, could have been attributed in part to the fact that this was a meal-replacement trial and not a protein-supplementation study. That is, participants were asked to incorporate 2 servings of red meat into their daily diet and not take additional protein over and above their habitual

TABLE 5
Baseline values and within-group changes in RT+Meat and CRT groups for blood pressure and blood lipid concentrations and net between-group differences for the change relative to baseline[1]

	Study group			*P*		
	RT+Meat	CRT	Net difference	Group	Time	Group × time
Systolic blood pressure						
Baseline (mm Hg)	137.0 ± 15.8	134.9 ± 24.7	—	—	—	—
△ 2 mo	—	—	—			
△ 4 mo	−6.6 (−10.9, −2.4)	−3.5 (−8.5, 1.4)	−3.1 (−9.5, 3.3)	0.663	0.008	0.359
Diastolic blood pressure						
Baseline (mm Hg)	75.2 ± 9.3	74.3 ± 8.4	—	—	—	—
△ 2 mo	—	—	—			
△ 4 mo	−3.5 (−5.9, −1.1)	−0.3 (−3.9, 3.3)	−3.2 (−7.4, 0.9)	0.773	0.180	0.219
Total cholesterol						
Baseline (mmol/L)	5.46 ± 1.22	5.56 ± 1.02	—	—	—	—
△ 2 mo	−0.18 (−0.38, 0.03)	−0.20 (−0.32, −0.07)	0.02 (−0.23, 0.27)	0.748	0.061	0.361
△ 4 mo	−0.25 (−0.46, −0.04)	−0.22 (−0.37, −0.07)	−0.03 (−0.29, 0.23)		0.015	0.813
HDL cholesterol						
Baseline (mmol/L)	1.49 ± 0.32	1.60 ± 0.34	—	—	—	—
△ 2 mo	−0.04 (−0.08, 0.00)	−0.02 (−0.07, 0.02)	−0.02 (−0.07, 0.04)	0.071	0.308	0.813
△ 4 mo	−0.01 (−0.05, 0.03)	−0.02 (−0.07, 0.03)	0.01 (−0.05, 0.08)		0.556	0.541
LDL cholesterol						
Baseline (mmol/L)	3.39 ± 1.01	3.36 ± 0.96	—	—	—	—
△ 2 mo	−0.09 (−0.27, 0.08)	−0.15 (−0.27, −0.03)	0.06 (−0.16, 0.27)	0.958	0.583	0.386
△ 4 mo	−0.19 (−0.38, 0.00)	−0.20 (−0.35, −0.05)	0.01 (−0.23, 0.25)		0.201	0.646
Triglycerides				—	—	—
Baseline (mmol/L)	1.41 ± 1.00	1.32 ± 0.57	—			
△ 2 mo	−0.17 (−0.32, −0.02)	−0.05 (−0.15, 0.05)	−0.12 (−0.31, 0.07)	0.875	0.016	0.793
△ 4 mo	−0.11 (−0.30, 0.08)	0.01 (−0.10, 0.13)	−0.12 (−0.35, 0.10)		0.018	0.465

[1] All baseline values are unadjusted means ± SDs. Change values (95% CIs) represent within-group mean absolute changes from baseline. Net differences (95% CIs) were calculated by subtracting within-group changes from baseline for the RT+Meat group from within-group changes for the CRT group after 2 and 4 mo. Independent *t* tests were used to examine between-group differences at baseline. *P* values for group, time, and group-by-time interaction terms were derived from generalized linear mixed models with random effects after adjustment for the change in physical activity, use of an antihypertensive medication (for blood pressure), and use of a lipid-lowering medication (for all lipid measures). *P* values for LDL cholesterol and triglycerides were based on log-transformed data. CRT, control resistance training; RT+Meat, resistance training plus lean red meat.

intake. Another limitation of our study was that not all research staff involved in the testing were blinded to the group allocation, which may have introduced some observational bias.

In conclusion, this study indicates that the consumption of a diet enriched with lean red meat (beef, lamb, and veal) equivalent to a dietary protein intake of $\sim 1.3\ g \cdot kg^{-1} \cdot d^{-1}$ was safe and effective for enhancing the effects of PRT on total body and leg LTM, leg muscle strength, and serum IGF-I in community-dwelling elderly women. Although the quantity and frequency of red meat consumed by the women in our study was more than that currently recommended by dietary guidelines for older Australians (46), there was no indication that this dose increased inflammation or the saturated fat intake or negatively influenced kidney function, blood pressure, or blood lipid concentrations. There was evidence of a reduction in concentrations of serum IL-6, which is a proinflammatory marker, in the RT+Meat group. From a public health perspective, these findings indicate that the elderly require a higher dietary protein intake to maximize the anabolic response to resistance training, and lean red meat can be safely used to modestly increase dietary protein in older people.

We are very grateful to the managers and volunteers of the following retirement-lifestyle villages who took part in the Women's Health Ageing and Muscle study: Burwood Terrace, Salford Park, Bedford Heights Estate, Knox Village, Waterford Park Retirement Village, Yarbatt Place, Wantirna Village, Prospect Hill, Cherry Tree Grove, Balmoral Gardens, Cumberland View, Walmsley, Sackville Grange, Tudor Village, and Lexington Gardens. We thank the Melbourne Health Shared Pathology Service, Royal Melbourne Hospital, for assistance with the measurement of IGF-I.

The authors' responsibilities were as follows—RMD, CAN, and DWD: designed the research; NLM, SLO, and CAG: conducted the research; CAG: created the database; RMD and SLO: wrote the manuscript; RMD, SLO, and CAN: interpreted results; RMD: analyzed data and had primary responsibility for the final content of the manuscript; and all authors: critically reviewed the manuscript and read and approved the final version of the manuscript. None of the authors had a conflict of interest.

REFERENCES

1. Fielding RA, Vellas B, Evans WJ, Bhasin S, Morley JE, Newman AB, Abellan van Kan G, Andrieu S, Bauer J, Breuille D, et al. Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. J Am Med Dir Assoc 2011;12:249–56.
2. Forbes SC, Little JP, Candow DG. Exercise and nutritional interventions for improving aging muscle health. Endocrine 2012;42:29–38.
3. Breen L, Phillips SM. Skeletal muscle protein metabolism in the elderly: interventions to counteract the 'anabolic resistance' of ageing. Nutr Metab (Lond) 2011;8:68.
4. Hulmi JJ, Lockwood CM, Stout JR. Effect of protein/essential amino acids and resistance training on skeletal muscle hypertrophy: a case for whey protein. Nutr Metab (Lond) 2010;7:51.
5. Robinson MJ, Burd NA, Breen L, Rerecich T, Yang Y, Hector AJ, Baker SK, Phillips SM. Dose-dependent responses of myofibrillar

protein synthesis with beef ingestion are enhanced with resistance exercise in middle-aged men. Appl Physiol Nutr Metab 2013;38:120–5.
6. Yang Y, Breen L, Burd NA, Hector AJ, Churchward-Venne TA, Josse AR, Tarnopolsky MA, Phillips SM. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. Br J Nutr 2012;108:1780–8.
7. Symons TB, Sheffield-Moore M, Mamerow MM, Wolfe RR, Paddon-Jones D. The anabolic response to resistance exercise and a protein-rich meal is not diminished by age. J Nutr Health Aging 2011;15: 376–81.
8. Cermak NM, Res PT, de Groot LC, Saris WH, van Loon LJ. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. Am J Clin Nutr 2012;96:1454–64.
9. Symons TB, Schutzler SE, Cocke TL, Chinkes DL, Wolfe RR, Paddon-Jones D. Aging does not impair the anabolic response to a protein-rich meal. Am J Clin Nutr 2007;86:451–6.
10. McLennan W, Podger A. National nutrition survey. Food eaten. Australia 1995. Canberra, Australia: Australian Bureau of Statistics, 1999.
11. Hodgson JM, Ward NC, Burke V, Beilin LJ, Puddey IB. Increased lean red meat intake does not elevate markers of oxidative stress and inflammation in humans. J Nutr 2007;137:363–7.
12. American College of Sports Medicine. ACSM guidelines for exercise testing and prescription. 6th ed. Philadelphia, PA: Lippincott, Williams & Wilkins, 2000.
13. Eser P, Hill B, Ducher G, Bass S. Skeletal benefits after long-term retirement in former elite female gymnasts. J Bone Miner Res 2009;24: 1981–8.
14. Dite W, Temple VA. A clinical test of stepping and change of direction to identify multiple falling older adults. Arch Phys Med Rehabil 2002; 83:1566–71.
15. Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. J Am Geriatr Soc 1991;39: 142–8.
16. Shumway-Cook A, Brauer S, Woollacott M. Predicting the probability for falls in community-dwelling older adults using the Timed Up & Go Test. Phys Ther 2000;80:896–903.
17. Wathen D. Load assignment. In: Beachle TR, ed. Essentials of strength training and conditioning. Champaign, IL: Human Kinetics, 1994: 416–30.
18. Stewart AL, Mills KM, King AC, Haskell WL, Gillis D, Ritter PL. CHAMPS physical activity questionnaire for older adults: outcomes for interventions. Med Sci Sports Exerc 2001;33:1126–41.
19. Mathew TH, Johnson DW, Jones GRD. Chronic kidney disease and automatic reporting of estimated glomerular filtration rate: revised recommendations. Med J Aust 2007;187:459–63.
20. Kukuljan S, Nowson CA, Sanders K, Daly RM. Effects of resistance exercise and fortified milk on skeletal muscle mass, muscle size, and functional performance in middle-aged and older men: an 18-mo randomized controlled trial. J Appl Physiol 2009;107:1864–73.
21. Burd NA, Tang JE, Moore DR, Phillips SM. Exercise training and protein metabolism: influences of contraction, protein intake, and sex-based differences. J Appl Physiol 2009;106:1692–701.
22. Kumar V, Atherton P, Smith K, Rennie MJ. Human muscle protein synthesis and breakdown during and after exercise. J Appl Physiol 2009;106:2026–39.
23. Tang JE, Phillips SM. Maximizing muscle protein anabolism: the role of protein quality. Curr Opin Clin Nutr Metab Care 2009;12:66–71.
24. Campbell WW, Barton ML Jr, Cyr-Campbell D, Davey SL, Beard JL, Parise G, Evans WJ. Effects of an omnivorous diet compared with a lactoovovegetarian diet on resistance-training-induced changes in body composition and skeletal muscle in older men. Am J Clin Nutr 1999;70:1032–9.
25. Campbell WW, Leidy HJ. Dietary protein and resistance training effects on muscle and body composition in older persons. J Am Coll Nutr 2007;26:696S–703S.
26. Candow DG, Chilibeck PD, Facci M, Abeysekara S, Zello GA. Protein supplementation before and after resistance training in older men. Eur J Appl Physiol 2006;97:548–56.
27. Esmarck B, Andersen JL, Olsen S, Richter EA, Mizuno M, Kjaer M. Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. J Physiol 2001;535: 301–11.
28. Haub MD, Wells AM, Tarnopolsky MA, Campbell WW. Effect of protein source on resistive-training-induced changes in body composition and muscle size in older men. Am J Clin Nutr 2002;76:511–7.
29. Verdijk LB, Jonkers RA, Gleeson BG, Beelen M, Meijer K, Savelberg HH, Wodzig WK, Dendale P, van Loon LJ. Protein supplementation before and after exercise does not further augment skeletal muscle hypertrophy after resistance training in elderly men. Am J Clin Nutr 2009;89:608–16.
30. Williams PR. Nutritional composition of red meat. Nutr Diet 2007;64 (suppl 4):S113–9.
31. Paddon-Jones D, Rasmussen BB. Dietary protein recommendations and the prevention of sarcopenia. Curr Opin Clin Nutr Metab Care 2009;12:86–90.
32. Iglay HB, Thyfault JP, Apolzan JW, Campbell WW. Resistance training and dietary protein: effects on glucose tolerance and contents of skeletal muscle insulin signaling proteins in older persons. Am J Clin Nutr 2007;85:1005–13.
33. McLennan PL, Tapsell LC, Owen AJ, Gutteridge IF. The influence of red meat intake upon the response to a resistance exercise-training program in older Australians. Asia Pac J Clin Nutr 2003;12(suppl):S17.
34. Barbieri M, Ferrucci L, Ragno E, Corsi A, Bandinelli S, Bonafe M, Olivieri F, Giovagnetti S, Franceschi C, Guralnik JM, et al. Chronic inflammation and the effect of IGF-I on muscle strength and power in older persons. Am J Physiol Endocrinol Metab 2003;284:E481–7.
35. Cesari M, Penninx BW, Pahor M, Lauretani F, Corsi AM, Rhys Williams G, Guralnik JM, Ferrucci L. Inflammatory markers and physical performance in older persons: the InCHIANTI study. J Gerontol A Biol Sci Med Sci 2004;59:242–8.
36. Ferrucci L, Penninx BW, Volpato S, Harris TB, Bandeen-Roche K, Balfour J, Leveille SG, Fried LP, Md JM. Change in muscle strength explains accelerated decline of physical function in older women with high interleukin-6 serum levels. J Am Geriatr Soc 2002;50:1947–54.
37. Visser M, Pahor M, Taaffe DR, Goodpaster BH, Simonsick EM, Newman AB, Nevitt M, Harris TB. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. J Gerontol A Biol Sci Med Sci 2002;57:M326–32.
38. Toth MJ, Matthews DE, Tracy RP, Previs MJ. Age-related differences in skeletal muscle protein synthesis: relation to markers of immune activation. Am J Physiol Endocrinol Metab 2005;288:E883–91.
39. Peake JM, Kukuljan S, Nowson CA, Sanders K, Daly RM. Inflammatory cytokine responses to progressive resistance training and supplementation with fortified milk in men aged 50+ years: an 18-month randomized controlled trial. Eur J Appl Physiol 2011;111: 3079–88.
40. Nicklas BJ, Brinkley TE. Exercise training as a treatment for chronic inflammation in the elderly. Exerc Sport Sci Rev 2009;37:165–70.
41. Santesso N, Akl EA, Bianchi M, Mente A, Mustafa R, Heels-Ansdell D, Schunemann HJ. Effects of higher- versus lower-protein diets on health outcomes: a systematic review and meta-analysis. Eur J Clin Nutr 2012;66:780–8.
42. Azadbakht L, Esmaillzadeh A. Red meat intake is associated with metabolic syndrome and the plasma C-reactive protein concentration in women. J Nutr 2009;139:335–9.
43. De Benedetti F, Alonzi T, Moretta A, Lazzaro D, Costa P, Poli V, Martini A, Ciliberto G, Fattori E. Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. J Clin Invest 1997;99:643–50.
44. Solerte SB, Gazzaruso C, Bonacasa R, Rondanelli M, Zamboni M, Basso C, Locatelli E, Schifino N, Giustina A, Fioravanti M. Nutritional supplements with oral amino acid mixtures increases whole-body lean mass and insulin sensitivity in elderly subjects with sarcopenia. Am J Cardiol 2008;101:69E–77E.
45. Wolfe RR, Miller SL, Miller KB. Optimal protein intake in the elderly. Clin Nutr 2008;27:675–84.
46. National Health and Medical Research Council (NHMRC). Australian dietary guidelines. Canberra, Australia: National Health and Medical Research Council, 2013.