

Effect of protein source on resistive-training-induced changes in body composition and muscle size in older men¹⁻³

Mark D Haub, Amanda M Wells, Mark A Tarnopolsky, and Wayne W Campbell

ABSTRACT

Background: Aging is associated with reductions in muscle mass and strength, but nutrition and exercise interventions can delay this progression and enhance the quality of life.

Objective: We examined whether the predominant source of protein consumed by older men influenced measures of muscle size and strength, body composition, resting energy expenditure, and skeletal muscle creatine concentrations in response to 12 wk of resistive training.

Design: After consuming a lactoovovegetarian (LOV) diet for 2 wk, 21 men aged 65 ± 5 y were randomly assigned to either consume a beef-containing (BC) diet ($n = 10$) or to continue the LOV diet ($n = 11$) throughout resistive training. The BC diet included $0.6 \text{ g protein} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ from beef and the LOV diet included $0.6 \text{ g protein} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ from textured vegetable protein (soy) sources. The remaining protein in the diets came from self-selected LOV sources.

Results: The mean total protein intake for both groups ranged from 1.03 to $1.17 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ during the intervention. Men in both groups had improvements (14–38%) in maximal dynamic strength of all the muscle groups trained with no significant difference between groups. With resistive training, cross-sectional muscle area of the vastus lateralis increased in both groups ($4.2 \pm 3.0\%$ and $6.0 \pm 2.6\%$ for the LOV and BC groups, respectively) with no significant difference between groups. Body composition, resting energy expenditure, and concentrations of muscle creatine, phosphocreatine, and total creatine did not differ significantly between groups or change over time.

Conclusions: These data suggest that increases in muscle strength and size were not influenced by the predominant source of protein consumed by older men with adequate total protein intake. *Am J Clin Nutr* 2002;76:511–7.

KEY WORDS Vegetarian, omnivore, meat, beef, soy, textured vegetable protein, protein source, aging, elderly men, muscle strength, strength training, resistive training, exercise, muscle hypertrophy, creatine

INTRODUCTION

As preventive therapy, resistive training is effective at increasing both strength and muscle mass in older people (1–3). Some limited data suggest that the nutrients an older person consumes during a resistive-training program can influence muscle hypertrophy. Meredith et al (4) found that older men who completed a 12-wk resistive-training program and consumed a nutritional

supplement drink (2.34 MJ) daily gained more muscle mass than did age-matched men who did not consume the nutritional drink. Campbell et al (5) showed that different protein intakes influenced the relative uptake and efficiency of nitrogen utilization in older people during a 12-wk resistive-training program. However, contrary to previous research in the same laboratory (1, 4), these older subjects did not experience resistive-training-induced muscle hypertrophy (5). It was proposed that the differences in the muscle hypertrophy response among these studies might be related to differences in nutrient intakes and dietary control. More specifically, it was questioned whether consumption of a controlled lactoovovegetarian (LOV) diet (5), compared with self-selected habitual diets (which presumably contained meat) (1, 4), diminished the muscle hypertrophy response.

Meat consumption may enhance protein synthesis and muscle hypertrophy by providing creatine in the diet. One study found that exogenous creatine may enhance protein synthesis (6). These increases in creatine and phosphocreatine availability may allow the individual to perform more exercise over time, thereby increasing muscle hypertrophy. Limited data exist concerning creatine loading via controlled ingestion of food sources such as red meat. Furthermore, it was suggested that vegetarians may not be able to perform high-intensity exercises optimally because they do not consume meat and therefore they have lower creatine concentrations (7).

Regarding the dietary protein source, Campbell et al (8) subsequently showed that consumption of an omnivorous diet, compared with an LOV diet, promoted resistive-training-induced

¹ From the Department of Human Nutrition, Kansas State University, Manhattan, KS (MDH); the Nutrition, Metabolism, and Exercise Laboratory, Donald W Reynolds Center on Aging, University of Arkansas for Medical Sciences and Central Arkansas Veterans Healthcare System, North Little Rock, AR (MDH and AMW); the Department of Medicine, Division of Neurology, McMaster University Medical Centre, Hamilton, Canada (MAT); and the Department of Foods and Nutrition, Purdue University, West Lafayette, IN (WWC).

² Supported by the National Cattlemen's Beef Association, the National Institutes of Health (R29 AG13409, R01 AG15750, and M01 RR14288), the US Department of Agriculture (98-35200-6151), and a grant from the University of Arkansas for Medical Sciences Student Research Fund.

³ Address reprint requests to MD Haub, Department of Human Nutrition, 143B Justin Hall, Kansas State University, Manhattan, KS 66506. E-mail: haub@humeck.ksu.edu.

Received February 20, 2001.

Accepted for publication September 24, 2001.

TABLE 1
Energy and macronutrient contents of high-protein foods (beef and textured vegetable protein products) provided to subjects in a 3-d menu rotation¹

Food item	Weight	Energy	Protein	Carbohydrate	Fat
	<i>g</i>	<i>MJ</i>	<i>g</i>	<i>g</i>	<i>g</i>
BC diet					
Day 1: cube steak	319	3.34	53.4	0	61.3
Day 2: ground beef	249	2.94	53.4	0	57.7
Day 3: beef tips	176	1.36	53.3	0	19.9
Daily average	248	2.55	53.4	0	46.3
LOV diet					
Day 1					
Vegetable sausage ²	100	0.77	21.1	5.3	7.9
Vegetable hamburger ³	148	1.36	32.3	11.6	13.9
Total	248	2.13	53.4	16.9	21.8
Day 2					
Vegetable sausage ²	100	0.77	21.1	5.3	7.9
Vegetable chicken ⁴	254	2.25	32.3	53.6	21.5
Total	354	3.02	53.4	58.9	29.4
Day 3					
Vegetable sausage ²	100	0.77	21.1	5.3	7.9
Vegetable hot dog ⁵	167	0.98	32.2	17.6	1.5
Total	267	1.75	53.3	22.9	9.4
Daily average	290	2.30	53.4	32.9	20.2

¹BC, beef-containing; LOV, lactoovovegetarian. All products were provided in amounts that yielded 0.6 g protein · kg body wt⁻¹ · d⁻¹, calculated for an 89-kg man.

²Breakfast Patties (Morningstar Farms, Worthington Foods Inc, Worthington, OH).

³Grillers (Morningstar Farms).

⁴Chik Patties (Morningstar Farms).

⁵Veggie Dog (Morningstar Farms).

increases in muscle mass in older men. These data suggest that meat consumption enhanced muscle mass accretion in older men who underwent resistive training. The design of this study (8), however, did not involve random assignment of subjects or control of meat intake to provide an opportunity to assess whether a specific type of meat was responsible for the overall effect.

Therefore, the objective of the current study was to test whether consuming a meat-containing diet, compared with an LOV diet, influenced whole body composition and muscle size in older men in response to a 12-wk period of resistive training. Measurements of muscle strength, resting energy expenditure, and muscle creatine stores were also made.

SUBJECTS AND METHODS

Subjects

Twenty-six men were recruited and enrolled. Each subject had a physical examination before acceptance into the study. The examination included a written medical history, resting electrocardiogram, and clinical blood and urine chemistries. The results were used to exclude men with medical conditions that might place them at risk if they participated in the study. Before the study began, all of the subjects provided written, informed consent in accordance with the Institutional Review Board at the University of Arkansas for Medical Sciences.

Three subjects decided to withdraw from the study during the first 2 wk because they were unwilling to commit to the data collection schedule. One other subject's data were removed from the statistical analyses because he was unable to finish the exercise program as a result of a viral infection that he acquired during the last 4 wk of the training program. Another subject's data were

removed after the analyses of iron status showed that he was iron-deficient throughout the study. Therefore, a total of 21 men (aged 65 ± 5 y) completed the study protocol as designed.

The sample size was determined from the data of Campbell et al (8). On the basis of the means and variances of those data, to achieve a power (1 - β) = 0.80 at *P* < 0.05, 9 subjects were needed to detect changes in body composition. The power of the present study was ≈0.50 on the basis of the differences between groups in midhigh cross-sectional area.

Experimental design and dietary control

The duration of the entire protocol was 15 wk. During the initial week of testing (week 1), the men consumed their habitual diets with no dietary control or constraints. During the next 2 wk (weeks 2 and 3), all subjects were counseled and asked to self-select a LOV diet. In addition, each subject was given selected food items (Morningstar Farms, Worthington Foods Inc, Worthington, OH; **Table 1**) that were individually weighed to the nearest 0.1 g to yield 0.6 g protein · kg⁻¹ · d⁻¹ from textured vegetable protein (soy) products. For the remaining 12 wk of the study (weeks 4–15), the men were randomly assigned to either continue the LOV diet (*n* = 11) or to begin the beef-containing (BC) diet, a self-selected LOV diet supplemented with beef (*n* = 10). The men who were randomly assigned to the LOV group continued to consume the same portions that were provided during the first 2 wk. The men assigned to the BC group were provided with 0.6 g protein · kg⁻¹ · d⁻¹ from beef (Table 1). The remainder of their energy intake was from self-selected LOV food sources.

The subjects recorded all the foods and beverages that they consumed, including the supplemental protein, for 24 h on 3 consecutive days (1 weekend day and 2 weekdays) during 3 different

weeks of the study (weeks 1, 3, and 15). Each subject was counseled by a registered dietitian on how to report portion sizes and record food entries. The food records were analyzed with NUTRITIONIST V software (N-Squared Computing, First Data Bank Inc, San Bruno, CA) to determine the energy and macronutrient intakes. One subject failed to record his diet during week 15.

The daily portions of high-protein foods were weighed and distributed to the subjects in frozen form. The protein contents of these food items were determined from the labels on the product packages. Each subject prepared and cooked the products as desired on the basis of personal preference. Throughout the 14 wk of dietary intervention, the subjects were routinely asked about their adherence to the dietary guidelines. They were told to adjust, if necessary, their self-selected energy intakes on the basis of changes in their body weight (measured before each exercise session, 3 d/wk) so that they would remain weight stable.

Resistive training

After the 2-wk LOV diet, the subjects began the resistive-training program. The training sessions were performed 3 d/wk on non-consecutive days. However, on one occasion each, 11 subjects had to train on 2 consecutive days or on weekend days because they missed a regular session to fulfill other commitments (eg, a work-related trip or a funeral). An exercise specialist supervised each training session individually. The exercises performed were the unilateral seated leg extension, unilateral seated leg flexion, bilateral leg press, arm pull, and seated chest press. Initially, we determined the maximum weight each subject could lift or push (the one-repetition maximum, or 1-RM) for each exercise.

The sessions began with a 10-min warm-up period on a cycle ergometer set at 20–50 W at a pedaling rate of 40–70 rev/min, followed by 5–10 min of passive stretching of the muscle groups to be exercised. For each exercise, subjects performed a warm-up set of 4 repetitions with the resistance at 40% of the 1-RM. This was followed by 2 sets of 8 repetitions and a third set performed to volitional fatigue; for these 3 sets, resistance was at 80% of the 1-RM. The resistance for subsequent sessions was increased by 5% when ≥ 12 repetitions were performed during the third set of a given exercise.

Subjects rested for 60 s between sets and for 3–5 min between the different exercises. During each workout session, subjects alternated between upper-body exercises (arm pull and seated chest press) and lower body exercises (unilateral seated leg extension, unilateral seated leg flexion, and bilateral leg press). The exercises were performed on pneumatically adjusted resistance exercise equipment (K400; Keiser Inc, Fresno, CA) with digital displays of force output. This equipment provides an eccentric and concentric phase throughout the range of motion. The 1-RM procedure was repeated on the final day of the resistive-training program to assess changes in strength. All subjects completed 31–33 out of a possible 33 workout sessions.

Body composition

Each subject underwent body-composition assessments during weeks 1, 3, and 15 of the study. Body density was measured by plethysmography (Bod Pod; Life Measurements, Concord, CA) after an overnight fast (9). The subjects wore minimal clothing (a swimsuit) and a tight-fitting swim cap to minimize volume attributable to hair, if necessary. The predicted value for lung volume was used to account for thoracic volume (10). Fat-free mass and percentage body fat were calculated from body density by using

the 2-compartment model equation of Siri (11). We used the average of 3 measurements in the statistical analyses.

Resting energy expenditure

Indirect calorimetry was used to determine resting energy expenditure (REE; in MJ/d) during weeks 1, 3, and 15. Testing was done between 0600 and 0830 after a 10-h overnight fast. REE testing during weeks 3 and 15 was done within 30 min of the time when the week 1 REE testing was performed. Two of the men in the BC group and one man in the LOV group could not complete the procedure because they were unable to minimize movements while the hood was in place.

Subjects were asked to lay supine and rest for 30 min before data collection. While the subjects were resting, the oxygen sensor (Magnus 16; Hartmann and Braun, Applied Automation, Frankfurt, Germany) and carbon dioxide sensor (Uras 14; Hartmann and Braun) were calibrated. We used separate sensors for inspired (ambient) and expired gases. This allowed for continuous monitoring of changes in ambient oxygen and carbon dioxide concentrations; the number of people in the procedure room during testing varied, ranging from 2 to 7 people. A free-flowing hood with a drape was positioned over each subject's head. The hood was connected to tubing that directed the expired gas to the analyzers. A turbine blower (EG & G Rotron, Saugerties, NY) was used to pull ambient air through the hood and subsequently to pull the mixed gases (ambient and expired) through the tubing to the sampling port. A factory-calibrated flowmeter (Mass Flowmeter, model 5861L; Brooks Instruments, Hatfield, PA) was placed in-line, according to factory specifications, to constantly measure the flow rate (L/min) to account for dilution. The initial 5 min of data were not used when calculating REE. For 2 subjects during their week-15 data collection period, there was a technical problem with the oxygen analyzers; their data were removed from the statistical analyses of REE.

Muscle metabolites

During weeks 1, 3, and 15, muscle samples were obtained from the dominant side vastus lateralis with a 6-mm Bergstrom biopsy needle (Microsurgical Instruments, Lake Forest, IL) and the aid of suction (12). Briefly, a site on the subject's leg was chosen for sampling and was anaesthetized with 1% lidocaine (≈ 4 mL total) given intradermally. Once the muscle tissue had been extracted, any blood, fat, or connective tissue was blotted from the sample before freezing. The samples were frozen between 60 and 90 s after extraction (13) and were stored in liquid nitrogen until analyzed. A week-15 sample was not obtained from one subject in the BC group because the initial biopsy attempt failed to produce a muscle sample and the subject refused to allow a second attempt.

Analyses of muscle phosphocreatine and creatine contents were performed with methods described by Tarnopolsky and Parise (14). Briefly, samples were lyophilized overnight and stored at -80°C until subsequent analyses. Samples were powdered and any blood or connective tissue remnants were removed; then 5–10 mg muscle powder were weighed out and placed in a 1.5-mL polyethylene tube. The metabolites were extracted into 0.5 mol/L perchloric acid combined with 1 mmol/L EDTA and were neutralized with 2 mol/L KHCO_3 . The subsequent assays for phosphocreatine and creatine were performed by using a modification of methods described previously (15). We modified these methods further according to Tarnopolsky and Parise (14), who



TABLE 2
Descriptive characteristics of the subjects¹

	BC diet (n = 10)	LOV diet (n = 11)
Age (y)	63 ± 3	67 ± 6
Weight (kg)	89.5 ± 8.7	89.1 ± 6.3
BMI (in kg/m ²)	28.1 ± 3.2	28.3 ± 2.1
Body fat (%)	31.3 ± 6.9	29.3 ± 6.0

¹ $\bar{x} \pm SD$. BC, beef-containing; LOV, lactoovovegetarian. There were no significant differences between groups.

used a fluorometer (Mini 150; Shimadzu RF, Kyoto, Japan) with an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Creatine and phosphocreatine values are reported as mmol/kg dry mass. The intraassay CVs for creatine and phosphocreatine were 4.4% and 1.7%, respectively. Total creatine content was calculated as the sum of creatine plus phosphocreatine contents.

Cross-sectional muscle area

During weeks 3 and 15, computed tomography scans of the dominant side mid thigh were performed on a GE CTI scanner (General Electric, Milwaukee) with the following parameters: 120 kVp, 200 mA, 512 × 512 matrix, slice thickness of 10 mm, and a field of view of 26 cm. A phantom was scanned weekly to insure validity and reliability. The location for each scan was determined by placing a lead marker at one-half the distance between the distal edge of the patella and the midline of the inguinal crease while the subject was lying on the scanning bed. After the scout scan, this location was measured digitally with the image software by using the crest of the head of the femur as the initial point and the lead marker as the endpoint. The distance and angle were recorded and used to determine the section to be scanned during the postintervention scan.

The images were saved on an optical disk and downloaded to the radiology workstation, where the image was transferred (via file transfer protocol) to a remote location for image analysis. With a method described previously (16), the images were analyzed for muscle area by a single investigator, in a blinded fashion, by using SCION IMAGE software, version Beta-3b (Scion Corporation, Frederick, MD). Muscle area (cm²) was calculated automatically by summing the tissue's pixels in the selected regions and multiplying by the pixel surface area. Duplicate measurements to determine within-subject variability were not done to avoid repeated exposure to radiation and the financial costs of repeating the test. However, the femur area of these subjects changed minimally (0.1 ± 0.4%) and these data had a CV of 3.4%.

Statistical analyses

Unless otherwise noted, values are expressed as means ± SDs. Independent *t* tests were used to compare week-1 values between groups for each variable. Time, group, and group-by-time interaction effects were assessed by using two-way repeated-measures analysis of variance to compare weeks 1 to 3 and weeks 3 to 15. All statistical analyses were performed with STATISTICA computer software, version 5.5 (Statsoft, Tulsa, OK). Statistical significance was defined as *P* ≤ 0.05.

RESULTS

The descriptive characteristics of the 21 men who completed the protocol are shown in **Table 2**. There were no significant differences between groups for any of these variables.

TABLE 3
Energy and protein intakes before and during the resistive-training intervention in 10 men consuming a beef-containing (BC) diet and 10 men consuming a lactoovovegetarian (LOV) diet¹

Diet ²	Week 1	Week 3	Week 15
Energy intake (MJ/d)			
LOV	9.26 ± 1.9	9.37 ± 1.8	9.33 ± 1.4
BC	8.91 ± 2.5	9.09 ± 2.1	9.07 ± 2.3
Total protein intake (g/d) ³			
LOV	92.9 ± 18.6	103.3 ± 11.7	100.8 ± 13.2
BC	88.9 ± 17.5	98.3 ± 16.5	92.0 ± 26.5
Relative protein intake (g · kg ⁻¹ · d ⁻¹) ³			
LOV	1.06 ± 0.2	1.17 ± 0.1	1.15 ± 0.1
BC	1.00 ± 0.2	1.10 ± 0.2	1.03 ± 0.3
Self-selected protein intake (g · kg ⁻¹ · d ⁻¹) ⁴			
LOV	—	0.57 ± 0.1	0.55 ± 0.1
BC	—	0.50 ± 0.2	0.43 ± 0.3
Protein sources (% of total protein intake)			
LOV			
Beef	21 ± 14	NR	NR
Other meats	24 ± 11	NR	NR
Soy	NR	52 ± 5	53 ± 7
Dairy and eggs	18 ± 7	15 ± 6	17 ± 9
Other	36 ± 9	34 ± 10	30 ± 6
BC			
Beef	18 ± 13	NR	57 ± 10
Other meats	32 ± 14	NR	NR
Soy	1 ± 4	56 ± 7	NR
Dairy and eggs	17 ± 10	15 ± 9	17 ± 9
Other	31 ± 11	28 ± 5	25 ± 8

¹ $\bar{x} \pm SD$. NR, none recorded.

²BC, LOV diet supplemented with 0.6 g protein · kg⁻¹ · d⁻¹ from beef; LOV, LOV diet supplemented with 0.6 g protein · kg⁻¹ · d⁻¹ from textured vegetable protein (soy) products.

³There were no significant main effects of group or group-by-time interactions between weeks 1 and 3 and no significant main effects of group or time or group-by-time interactions between weeks 3 and 15.

⁴Self-selected protein intake = relative protein intake – provided protein (0.6 g · kg⁻¹ · d⁻¹); all protein during week 1 was self-selected.

Diet

For energy intake, there were no significant differences between or within groups throughout the intervention according to the 3-d food records (**Table 3**). For protein intake, there was a main effect of time, with increases in reported intakes for both groups between weeks 1 and 3; there was no group or time effect at week 15. Subjects in the BC group consumed 57 ± 10% of their total protein from the supplemental beef and subjects in the LOV group consumed 53 ± 7% of their total protein from the supplemental texturized vegetable protein during the 12-wk period of resistive training. These analyses were performed with data from the week-15 food records.

Strength

At week 3 (preintervention), there were no significant differences between the LOV and BC groups in maximal strength for any of the exercises (**Table 4**). All subjects then underwent the 12-wk resistive-training intervention, and at week 15 (postintervention), both groups showed increased maximal dynamic strength of all

TABLE 4

Muscle strength before (week 3) and after (week 15) the resistive-training intervention in 10 men consuming a beef-containing (BC) diet and 11 men consuming a lactoovovegetarian (LOV) diet¹

	Week 3	Week 15
Unilateral seated leg extension (Nm) ²		
LOV	121 ± 29	164 ± 28
BC	111 ± 22	138 ± 28
Unilateral seated leg flexion (Nm) ²		
LOV	111 ± 24	140 ± 20
BC	109 ± 25	139 ± 23
Bilateral leg press (N) ²		
LOV	1339 ± 212	1589 ± 161
BC	1280 ± 358	1544 ± 481
Seated chest press (N) ²		
LOV	449 ± 58	511 ± 61
BC	450 ± 103	554 ± 76
Arm pull (N) ²		
LOV	603 ± 21	741 ± 26
BC	606 ± 39	744 ± 27

¹ $\bar{x} \pm SD$. Nm, Newton meter; N, Newton.

²Significant main effect of time ($P < 0.05$), but no significant main effect of group or group-by-time interaction.

the muscle groups trained (improvements of 14–38%). There were no significant differences in strength gains between groups.

Body composition

Body weight, fat-free mass, and fat mass were not significantly different between the BC and LOV groups before the intervention (week 1) and remained unchanged at weeks 3 and 15 (Table 5). There were no significant differences in response to the resistive-training intervention between the 2 groups.

The cross-sectional area of the midhigh (vastus lateralis) muscle did not differ significantly between the LOV and BC groups at week 3 (Figure 1). At week 15, after the resistive-training intervention, subjects in both groups had significant increases (4.2 ± 3.0% and 6.0 ± 2.6% for the LOV and BC groups, respectively) in midhigh cross-sectional area with no significant difference between groups.

Resting energy expenditure

There were no significant between-group differences in REE before the intervention (Table 5). REE did not change significantly over time, either within or between groups, or when analyzed in terms of relative values (MJ · kg fat-free mass⁻¹ · d⁻¹; data not shown).

Muscle metabolites

Muscle contents of creatine, phosphocreatine, and total creatine did not differ significantly between groups at weeks 1 or 3 (Table 5). At week 15, after the 12-wk resistive-training intervention, there were no significant changes in muscle contents of creatine, phosphocreatine, or total creatine in either group.

DISCUSSION

The main finding of this study was that resistive-training-induced muscle hypertrophy did not differ significantly between older men who consumed a diet with beef as the predominant source of protein and similar subjects who consumed an LOV diet

TABLE 5

Body composition, resting energy expenditure, and muscle metabolites before and during the resistive-training intervention in 10 men consuming a beef-containing (BC) diet and 11 men consuming a lactoovovegetarian (LOV) diet¹

	Week 1	Week 3	Week 15
Body weight (kg)			
LOV	89.5 ± 8.8	89.4 ± 8.9	89.3 ± 9.7
BC	89.1 ± 7.2	89.4 ± 7.1	89.5 ± 7.5
Fat mass (kg)			
LOV	27.6 ± 7.5	27.3 ± 7.7	28.4 ± 8.5
BC	27.1 ± 7.8	27.8 ± 7.8	27.7 ± 8.0
Fat-free mass (kg)			
LOV	61.5 ± 5.8	61.7 ± 5.0	60.8 ± 4.9
BC	62.5 ± 5.1	62.1 ± 4.0	61.9 ± 5.2
Resting energy expenditure (MJ/d)			
LOV ²	5.86 ± 0.8	5.91 ± 0.7	6.08 ± 0.9
BC ³	5.84 ± 0.9	5.69 ± 0.6	6.01 ± 0.5
Muscle creatine content (mmol/kg dry wt)			
LOV	48.8 ± 8.4	51.3 ± 10.8	44.1 ± 9.2
BC ⁴	47.4 ± 11.9	45.4 ± 8.9	47.9 ± 3.6
Muscle phosphocreatine content (mmol/kg dry wt)			
LOV	69.1 ± 14.6	65.8 ± 9.0	75.2 ± 16.6
BC ⁴	66.0 ± 15.8	72.0 ± 11.9	72.7 ± 16.0
Muscle total creatine content (mmol/kg dry wt)			
LOV	117.9 ± 13.7	117.1 ± 13.2	119.3 ± 17.9
BC ⁴	113.5 ± 15.6	117.4 ± 17.5	120.6 ± 15.2

¹ $\bar{x} \pm SD$. There were no significant main effects or interactions.

² $n = 10$.

³ $n = 8$.

⁴ $n = 9$.

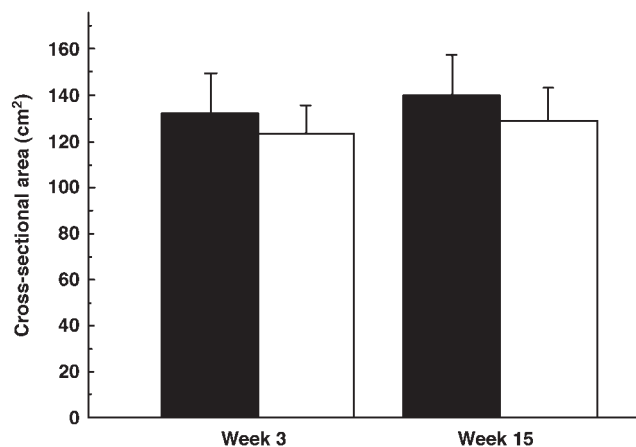


FIGURE 1. Mean (± SD) midhigh muscle cross-sectional area, as measured with computed tomography, at week 3 (preintervention) and week 15 (postintervention) in the beef-containing (BC) diet group (■, $n = 10$) and the lactoovovegetarian (LOV) diet group (□, $n = 11$). At week 15, after the resistive-training intervention, subjects in both groups had significant increases (4.2 ± 3.0% and 6.0 ± 2.6% for the LOV and BC groups, respectively) in midhigh cross-sectional area with no significant difference between groups. Thus, there was no significant main effect of group and no significant group-by-time interaction, but the main effect of time was significant ($P < 0.05$).

with soy as the predominant source of protein. This finding was contrary to our original hypothesis that muscle hypertrophy would be greater in the BC group than in the LOV group, and is different from previous research in our laboratory (8).

Our previous research (8) showed that older men who consumed an omnivorous diet (ie, including protein from beef, poultry, pork, and fish) experienced different whole-body and skeletal-muscle responses to a 12-wk resistive-training program compared with men who self-selected an LOV diet (ie, containing no striated tissue but including protein from dairy products and eggs). Differences between the previous and present studies included 1) the degree of dietary control (completely self-selected versus self-selected with portioned quantities of protein, respectively); 2) the types of striated-tissue protein tested (omnivorous versus beef only, respectively); 3) the quantities of protein provided (≤ 1.0 g protein \cdot kg⁻¹ \cdot d⁻¹ versus ≥ 1.0 g protein \cdot kg⁻¹ \cdot d⁻¹, respectively); 4) the frequency of resistive exercise (2 times/wk versus 3 times/wk, respectively); 5) the methods of whole-body-composition assessment (hydrostatic weighing versus plethysmography, respectively); and 6) the methods of muscle size assessment (muscle fiber histochemistry versus computed tomography scanning, respectively).

This protocol can be generalized in that all the foods (supplemented and self-selected) are readily available to consumers and can be prepared in a self-selected manner. It appears that the amount of protein consumed (in g \cdot kg⁻¹ \cdot d⁻¹) may be crucial with regard to the potential differential effects of the protein source (omnivorous or LOV) during periods of resistive training in older men. Further studies are needed; these studies should include women, use a longer training duration, and vary the protein content to better evaluate the influence of the protein source on factors involved in muscle hypertrophy.

The average increases in midthigh cross-sectional muscle area from weeks 3 to 15 ($4.2 \pm 3.0\%$ and $6.0 \pm 2.6\%$ for the LOV and BC diets, respectively) are comparable to the muscle hypertrophy results reported by others (17, 18) and support the effectiveness of the 12-wk resistive-training protocol for inducing muscle hypertrophy.

Regarding total protein intake, previous research (19) suggests that older adults need a higher protein intake than the current recommended dietary allowance (RDA) of 0.8 g protein \cdot kg⁻¹ \cdot d⁻¹ (20). Research also suggests that long-term consumption of the RDA for protein might result in skeletal muscle atrophy (21) consistent with metabolic and physiologic accommodations to inadequate protein intake. If this is true, marginal protein intake might also explain the decrease in fat-free mass and the trend toward less hypertrophy of type II muscle fiber in a group consuming an LOV diet, as reported by Campbell et al (8). The lack of a detectable difference in midthigh cross-sectional muscle area between the BC and LOV groups in the present study may indicate that total protein intakes were adequate to meet the demands of protein synthesis.

The resistive-training protocol used in the present study effectively increased muscle strength in both groups. The increases in strength are consistent with the results of other investigations of similar training regimens and subject populations (8, 22, 23). This finding was expected given that in general, initial strength gains induced by resistive training result from enhanced neuromuscular recruitment of the myofibrils needed to perform the required exercise (24) and do not necessarily result from muscle hypertrophy.

The lack of change in REE may be explained by the absence of change in fat-free mass. In accord with our results, Taaffe et al


(25) observed that strength increased by 30–40% as a result of resistive training in older women, without significant changes in fat-free mass or REE. Studies that showed an increase in REE after participation in a resistive-training program (8, 19, 23) also showed an increase in fat-free mass.

As with many studies on resistive training, the relatively short duration of training (12 wk) and the methods used to assess body composition and muscle size are important factors, and potential limitations, of the present investigation. We chose to use 12 wk of resistive training on the basis of previous research showing that significant muscle hypertrophy can occur within this time period (8, 26) and that significant diet-related differences in muscle hypertrophy responses may also occur within this time frame (4, 8). Regarding body-composition assessment, not all methods are equally sensitive and accurate for detecting subtle changes in soft tissue. For the present study, we decided to use computed tomography scanning as the primary method for detecting changes in skeletal muscle on the basis of research by Nelson et al (27). They reported that this technique was one of the most sensitive ways to detect changes in soft tissue after resistive training in older women. The significant increases in midthigh muscle area measured in the present study support the use of computed tomography scanning for this purpose. Given these factors, however, one might question whether muscle hypertrophy would have been greater in the BC group than in the LOV group if the resistive-training period had extended beyond 12 wk. Future studies should use a longer training duration and a larger sample size to insure adequate statistical power, as the statistical power for the present investigation was 0.50 on the basis of the cross-sectional-area data.

The resting muscle creatine values (creatine, phosphocreatine, and total creatine) of the present study are in agreement with those of Tarnopolsky and Parise (14) but are higher than the phosphocreatine and total creatine values reported by Campbell et al (8). In the current study, the observed muscle creatine values did not support our hypothesis that skeletal muscle creatine content would increase at week 15 in the BC group. Raw beef contains ≈ 30 mmol creatine/kg dry wt (28), but exposing meat to high temperatures for an extended period of time catalyzes the conversion of creatine to creatinine, as observed when meat is rendered for processing as animal food. This can greatly diminish the amount of creatine available for consumption (≤ 3 mmol/kg dry wt) (28). Therefore, depending on the cooking methods chosen, the subjects may have ingested more creatinine than creatine. The finding that there was no significant difference between groups for muscle creatine content appears to indicate that there is a feedback mechanism of exogenous creatine absorption on endogenous synthesis that maintains a constant myocellular content (29).

Another interesting finding in the current study was the lower muscle total creatine content in this group of older men compared with values reported previously for middle-aged (mean: 49 y) sedentary men and women studied in the same laboratory with the same analytic methods (14). The total creatine values in the current study were 11.5% lower, and the values reported by us previously in a similar group of men were 21% lower (8) than those of the younger group studied by Tarnopolsky and Parise (14). These findings, in combination with the observation that creatine supplementation did not have a differential effect on strength gains in the older men and women who performed resistive exercise (30), could indicate a relative deficiency in the creatine transporter (31) in older people.



In summary, the primary aim of this study was to determine whether consumption of striated tissue in the form of beef influenced muscle hypertrophy in older men during 12 wk of resistive training. These results indicate that the predominant source of dietary protein (beef or soy), when consumed by older men in amounts that averaged $1.1 \text{ g protein} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, did not influence the extent of muscle hypertrophy during 12 wk of resistive training. 

We thank Stephanie Davey and Hannah Morse for assisting in the data collection; James D Fluckey and Lyndon JO Joseph for their technical assistance; and the staff of the GCRC Metabolic Kitchen, led by Nadine Carnell, for preparing the portioned foods and counseling the subjects.

REFERENCES

1. Fiatarone MA, Marks EC, Ryan ND, Meredith CN, Lipsitz LA, Evans WJ. High-intensity strength training in nonagenarians. Effects on skeletal muscle. *JAMA* 1990;263:3029–34.
2. Fiatarone MA, O'Neill EF, Ryan ND, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med* 1994;330:1769–75.
3. Frontera WR, Meredith CN, O'Reilly KP, Knuttgen HG, Evans WJ. Strength conditioning in older men: skeletal muscle hypertrophy and improved function. *J Appl Physiol* 1988;64:1038–44.
4. Meredith CN, Frontera WR, O'Reilly KP, Evans WJ. Body composition in elderly men: effect of dietary modification during strength training. *J Am Geriatr Soc* 1992;40:155–62.
5. Campbell WW, Crim MC, Young VR, Joseph LJ, Evans WJ. Effects of resistance training and dietary protein intake on protein metabolism in older adults. *Am J Physiol* 1995;268:E1143–53.
6. Ingwall JS. Creatine and the control of muscle-specific protein synthesis in cardiac and skeletal muscle. *Circ Res* 1976;38:1115–23.
7. Shomrat A, Weinstein Y, Katz A. Effect of creatine feeding on maximal exercise performance in vegetarians. *Eur J Appl Physiol* 2000;82:321–5.
8. Campbell WW, Barton ML Jr, Cyr-Campbell D, et al. Effects of an omnivorous diet compared with a lactoovo vegetarian diet on resistance-training-induced changes in body composition and skeletal muscle in older men. *Am J Clin Nutr* 1999;70:1032–9.
9. McCrory MA, Gomez TD, Bernauer EM, Mole PA. Evaluation of a new air displacement plethysmograph for measuring human body composition. *Med Sci Sports Exerc* 1995;27:1686–91.
10. McCrory MA, Mole PA, Gomez TD, Dewey KG, Bernauer EM. Body composition by air-displacement plethysmography by using predicted and measured thoracic gas volumes. *J Appl Physiol* 1998;84:1475–9.
11. Siri WE, ed. *Body composition from fluid spaces and density: analysis of methods*. Washington, DC: National Academy of Sciences, 1961.
12. Evans WJ, Phinney SD, Young VR. Suction applied to a muscle biopsy maximizes sample size. *Med Sci Sports Exerc* 1982;14:101–2.
13. Soderland K, Hultman E. Effects of delayed freezing on content of phosphagens in human skeletal muscle biopsy samples. *J Appl Physiol* 1986;61:832–5.
14. Tarnopolsky MA, Parise G. Direct measurement of high-energy phosphate compounds in patients with neuromuscular disease. *Muscle Nerve* 1999;22:1228–33.
15. Harris R, Hultman E, Nordesjo L. Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. *Methods and variance of values*. *Scand J Clin Lab Invest* 1974;33:109–20.
16. Mitsiopoulos N, Baumgartner RN, Heymsfield SB, Lyons W, Gallagher D, Ross R. Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. *J Appl Physiol* 1998;85:115–22.
17. Hurley BF, Redmond RA, Pratley RE, Treuth MS, Rogers MA, Goldberg AP. Effects of strength training on muscle hypertrophy and muscle cell disruption in older men. *Int J Sports Med* 1995;16:378–84.
18. Sipilä S, Suominen H. Effects of strength and endurance training on thigh and leg muscle mass and composition in elderly women. *J Appl Physiol* 1995;78:334–40.
19. Campbell WW, Crim MC, Young VR, Evans WJ. Increased energy requirements and changes in body composition with resistance training in older adults. *Am J Clin Nutr* 1994;60:167–75.
20. National Research Council. *Recommended dietary allowances*. 10th ed. Washington, DC: National Academy Press, 1989.
21. Campbell WW, Trappe TA, Wolfe RR, Evans WJ. The recommended daily allowance for protein may not be adequate for some older people to maintain skeletal muscle. *J Gerontol A Biol Sci Med Sci* 2001;56:M373–80.
22. Jozsi AC, Campbell WW, Joseph L, Davey SL, Evans WJ. Changes in power with resistance training in older and younger men and women. *J Gerontol A Biol Sci Med Sci*, 1999;54:M591–6.
23. Pratley R, Nicklas B, Rubin M, et al. Strength training increases resting metabolic rate and norepinephrine levels in healthy 50- to 65-yr-old men. *J Appl Physiol* 1994;76:133–7.
24. Sale DG. Neural adaptations to resistance training. *Med Sci Sports Exerc* 1988;21(suppl):S135–45.
25. Taaffe DR, Pruitt L, Reim J, Butterfield G, Marcus R. Effect of sustained resistance training on basal metabolic rate in older women. *J Am Geriatr Soc* 1995;43:465–71.
26. Charette SL, McEvoy L, Pyka G, et al. Muscle hypertrophy response to resistance training in older women. *J Appl Physiol* 1991;70:1912–6.
27. Nelson ME, Fiatarone MA, Layne JE, et al. Analysis of body-composition techniques and models for detecting change in soft tissue with strength training. *Am J Clin Nutr* 1996;63:678–86.
28. Harris RC, Lowe JA, Warnes K, Orme CE. The concentration of creatine in meat, offal and commercial dog food. *Res Vet Sci* 1997;62:58–62.
29. Walker JB. Metabolic control of creatine biosynthesis: effect of dietary creatine. *J Biol Chem* 1960;235:2357–61.
30. Bermon S, Venembre P, Sachet C, Valour S, Dolisi C. Effects of creatine monohydrate ingestion in sedentary and weight-trained older adults. *Acta Physiol Scand* 1998;164:147–55.
31. Guimbal C, Kilimann M. A Na^+ -dependent creatine transporter in rabbit brain, muscle, heart, and kidney. cDNA cloning and functional expression. *J Biol Chem* 1993;268:8418–21.

