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MODERATELY INCREASED PROTEIN INTAKE PREDOMINATELY FROM EGG SOURCES DOES NOT INFLUENCE WHOLE BODY, REGIONAL, OR MUSCLE COMPOSITION RESPONSES TO RESISTANCE TRAINING IN OLDER PEOPLE

H.B. IGLAY, J.W. APOLZAN, D.E. GERRARD, J.K. EASH, J.C. ANDERSON , W.W. CAMPBELL

Correspondence and requests for reprints should be addressed to: Wayne W. Campbell, PhD, Department of Foods and Nutrition, Purdue University, 700 West State Street, West Lafayette, IN 47907-2059; Phone 765-494-8236; Fax 765-494-0674; Email: campbellw@purdue.edu. Authors' affiliations: Department of Foods & Nutrition, Purdue University, 700 West State St, West Lafayette, IN 47907-2059 (HBI, JWA, JCA, WWC); Department of Animal Science, Purdue University, 915 West State St, West Lafayette, IN 47907-2054 (DEG, JKE)

Abstract: The effects of increased dietary protein on resistance training (RT)-induced changes in body composition and skeletal muscle fiber size are uncertain in older people. Objectives: We hypothesized that the ingestion of more animal-based foods, especially eggs, to achieve a higher protein intake would enhance RT-induced changes in body composition. Setting: West Lafayette, IN. Participants: 36 older people (age 61±1 y; mean ± SEM). Intervention: Subjects completed RT three d/wk for 12 weeks, and consumed omnivorous diets that contained either 0.9±0.1 (lower protein) or 1.2±0.0 (higher protein) g protein $\cdot$ kg^{-1} $\cdot$ d^{-1} (12±3 and 17±5 % of energy intakes, respectively), with the higher protein intake achieved by consuming more eggs, meats, and dairy foods. The lower and higher protein diets contained 213±21 and 610±105 mg cholesterol/d, respectively. Measurements: Strength, body composition, serum lipid-lipoprotein profile, urinary creatinine, skeletal muscle fiber type and size. Results: Among all subjects, over time (i.e. with RT) body weight was unchanged, lean mass (1.1±0.2 kg) increased, and fat mass (-1.4±0.2 kg) decreased (all changes $P<0.05$). Regional (i.e. trunk, legs, arms) lean mass increased and fat mass decreased. Whole body muscle mass (24-h urinary creatinine excretion) increased, but skeletal muscle (vastus lateralis) type 1, type 2a, and type 2x fiber cross-sectional areas did not change from baseline. Serum total and LDL cholesterol decreased ($P<0.05$) and HDL cholesterol and triacylglycerol were unchanged. Dietary protein and cholesterol intakes did not influence these responses to RT. Conclusion: Consumption of diets that contained moderately higher protein and variable amounts of cholesterol did not differentially affect body composition, skeletal muscle fiber size, or serum lipid-lipoprotein profile responses to resistance training in older people.

Key words: Diet, elderly, fat-free mass, lipid-lipoprotein profile, eggs.

Introduction

Sarcopenia, the age related decline in skeletal muscle mass, can have devastating effects on older people (1). This decline in skeletal muscle mass is associated with a decrease in strength, and causes a decline in functional ability that affects many aspects of life (1). While the specific contributing factors to sarcopenia are uncertain and likely multifacited, preventing or delaying the loss of skeletal muscle mass is considered critical for older people to maintain a higher quality life (2). Resistance training improves strength (3, 4) whole body composition (2, 5-12), and skeletal muscle fiber size (cross-sectional area) (13, 14) in older people. A higher dietary protein intake, achieved by consuming an omnivorous diet, enhanced the resistance training- induced improvements in body composition, including gains in whole body fat-free mass and muscle mass, in older men (8). However, other research (5, 7, 8, 15-17) does not support an ergogenic effect of higher dietary protein intake on resistance training-induced changes in whole body composition in older people who otherwise consume adequate protein. These studies included only minimal regional body composition assessments and very limited data on skeletal muscle fiber size. In one study, type II muscle fiber size tended to increase to a greater extent with consumption of a higher protein, omnivourous vs. lower protein, lacto-ovovegetarian diet in older men (8).

We recently reported that older men and women who consumed adequate and moderately higher protein (achieved by consuming more animal-based foods, especially eggs) during a 12-week period of resistance training improved whole body composition and indexes of the lipid-lipoprotein profile, glucose tolerance, and skeletal muscle insulin signaling proteins (18). The primary objective of this report is to expand these findings to include regional body composition and skeletal muscle fiber cross-sectional areas. Also, subsequent to the completion of the clinical phase of the present study Riechman et al. (19) published data suggesting that higher dietary and serum cholesterol enhances fat-free mass gains with resistance training by improving cellular signaling or enhancing skeletal muscle fiber repair after resistance training (19). Though uncommon, the negative effect of statins on skeletal muscle (20) suggests that cholesterol may influence muscle response to resistance training. Therefore, a secondary and retrospective objective of this study was to assess possible relationships between dietary and serum cholesterol and changes in body composition and skeletal muscle size.

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Animal-based proteins from egg, meat, and dairy food sources were included in all of the subjects' diets (lower and higher protein groups) and were used to increase the protein intake of the higher protein group because they are considered high-quality proteins that are efficiently digested (true digestibility scores ≥97%) (21, 22). Use of animal-based foods, especially egg products, to increase protein intake also resulted in the subjects in the higher protein group consuming higher dietary cholesterol.

Methods and Materials

Subjects

Fifty men and women were recruited for this study from the greater Lafayette, IN, area. Recruitment criteria included the following: 1) age range 50-80 years; 2) self-reported body mass index of normal weight, overweight or class I obesity (23); 3) non-diabetic and not on insulin replacement therapy; 4) clinically normal kidney, liver and cardiac functions; 5) not currently taking anti-inflammatory steroid medications; 6) no hip replacement; 7) no habitual resistance training in the past six months; and 8) women were at least two years post-menopausal. Participants signed an informed consent agreement and received a monetary reimbursement. The study protocol and informed consent agreement were reviewed and approved by the Purdue University Institutional Review Board. Thirty-six of the fifty participants successfully completed the study protocol. Additional details of the pre-study recruitment and medical screening procedures and the reasons for the 28% dropout incidence have been published (18).

Experimental Design

The 14-week study was arranged as follows: Weeks 1-2, pre-intervention measurements, habitual diet and activity maintained; Weeks 3-13, period of dietary control and resistance exercise training intervention; Week 14, post-intervention measurements (dietary control and resistance training continued).

In previous protein intake and resistance training research in older people within our laboratory, group-specific differences in body composition of 0.0065 ± 0.0065 kg/L (mean ± SD) for body density (subjects consumed the RDA vs. 2xRDA for protein) (8), and 0.8 ± 0.7 kg for whole body protein-mineral mass (subjects consumed a vegetarian diet that contained the RDA for protein vs. an omnivorous diet that contained 125% of the RDA for protein) (24, 25) were detected. A sample size of n = 17 per group was deemed adequate to detect group-specific differences of this magnitude ($P<0.05$, 80% power) in the resistance training-induced changes in body composition (26-28).

Dietary Intervention

All participants were randomly assigned to one of two diet groups (men and women randomized separately). From study week 3-14, the subjects in the lower protein group (LP, 9 men, 9 women) were counseled to habitually consume a weight-maintenance diet that would provide 0.8 g protein $\cdot kg^{-1} \cdot d^{-1}$, the amount of protein currently recommended to adequately meet the dietary needs of virtually all healthy adults (29). The subjects in this group were counseled specifically to consume egg, striated tissue (beef, poultry, pork, fish), and dairy proteins as 5%, 25%, and 15% of their total protein intake, respectively. Subjects in the higher protein group (HP, 8 men, 10 women) were counseled to habitually consume an isocaloric diet that would provide 1.6 g protein $\cdot kg^{-1} \cdot d^{-1}$. The subjects in this group were counseled to consume egg, striated tissue, and dairy proteins as 25%, 20%, and 15% of their total protein intake, respectively. If body weight increased or decreased more than 1 kg during any week of the intervention dietary counseling was adjusted to prevent further changes. Food records were obtained on seven consecutive days at baseline (weeks 1-2), at the start of dietary control (weeks 3-4), middle of dietary control (weeks 9-10) and at the end of intervention (weeks 13-14), as described previously (18), and analyzed using Nutritionist Pro (N-squared computing, First DataBank version 1.3.36, San Bruno, CA) software.

Exercise Intervention

During weeks 3-14, all subjects participated in a supervised progressive resistance exercise training program three days per week, as previously described (18). Each exercise training session included warm-up and cool-down periods, and the performance of two sets of eight repetitions at 80% of pre-determined maximal strength for eight exercises and one set to voluntary fatigue (Keiser Sports Health Equipment Company, Fresno, CA, USA). At baseline and the fourth, eighth, and twelfth week of training, each subject's maximal strength was measured using a one-repetition maximum test (1RM) on five pieces of equipment (upper back, leg extension, chest press, leg curl, leg press) and the values summed to calculate a whole body strength summary.

Urine and blood collections and analyses

Two consecutive 24-hour urine collections were made at weeks 1, 5, 10 and 14, and urea nitrogen concentration measured (Cobas Mira Plus, Roche Diagnostic Systems, Indianapolis, IN). Fasting-state blood samples were obtained on two non-consecutive days at baseline and post-intervention from an antecubital vein after the subject had rested in a seated position for 15 minutes. A clinical chemistry profile was measured to assess normative values (blood urea nitrogen, albumin, complete blood count, electrolytes; analyzed by Mid America Clinical Laboratories, Indianapolis, IN). Total cholesterol (CHOL), high-density lipoprotein (HDL), and triacylglycerol (TG) concentrations were measured and low-density lipoprotein (LDL) concentration was estimated (LDL = CHOL – HDL – TG/5) (30) (COBAS Mira Plus, Roche Diagnostic Systems, Indianapolis, IN).

Body Composition

Body weight was measured immediately prior to each exercise session to aid subjects in maintaining body weight throughout the study. At baseline and post-intervention, regional and whole body bone mineral, fat tissue and lean tissue masses were measured via dual energy x-ray absorptiometry (DEXA, GE Lunar Prodigy with EnCORE software version 5.60, Madison, WI) (31). Fasting body weight was measured as well. Height was measured at the beginning of the study with a wall-mounted stadiometer (Holtain Ltd., Crymych, Wales).

Skeletal Muscle Fiber Cross-Sectional Area

Muscle biopsies from the vastus lateralis of the dominant leg were obtained following an overnight fast using a punch biopsy technique (32). Immediately following extraction, a piece of muscle was attached to a cork with tragacanth gum (ICN Biomedicals, Inc., Aurora, OH) with the fibers positioned perpendicularly to the surface of the cork. The samples were frozen in 2-methylbutane cooled in liquid nitrogen and later stored in plastic vials within a liquid-nitrogen-containing dewar. Serial 10- m cross-sections were cut using a Microm HM500 cryostat (Microm International, Waldorf, Germany) at a chamber temperature of -20°C and placed on microscope slides coated with 0.1% 3-aminopropyltriethoxysilan. Sections were then stained for myofibrillar adenosine triphosphatase (ATPase) in an acidic (pH 4.66, (33)) and basic (pH 10.4, (34)) solution and for succinate dehydrogenase (SDH) (35) to determine skeletal muscle fiber cross-sectional area (CSA). Three different images of each stained muscle section were captured using a Nikon DN100 digital camera (Nikon Corporation, Tokyo, Japan) mounted on a Nikon Labphot light microscope (Nikon Corporation, Tokyo Japan) and saved with Scion Image for Windows, release Beta 4.0.2 (Scion Corporation, Frederick, MD). Matching serial pictures were taken. Because samples were most distinctly stained with the acid ATPase stain, this stain was used for final analysis. Images were transferred to Adobe Photoshop 7.0 (Adobe Systems, Inc., San Hose, CA), printed and hand-traced onto tracing paper. The traced images were scanned and the area of each fiber was determined using Adobe Photoshop 7.0 (Adobe Systems, Inc., San Hose, CA). Fiber type was determined by staining intensity, and assessments were made for type I, IIa and IIx muscle fibers. All samples were traced by the same individual to avoid inter-individual variability. At baseline and post-intervention, 155±6 (range 86-229) and 154±5 (mean ± SEM, range 97-211) total fibers (types I, IIa and IIx combined) were assessed per sample.

Statistical Analyses

All data are reported as mean ± standard error of the mean (SEM). Baseline group and gender differences were assessed with 2-factor ANOVA. Change from baseline was calculated as: change from baseline = post value – baseline value. Main effects of resistance training, protein intake and gender, and their interactions on dependent variables were assessed via 3-factor ANOVA with time as a repeated measure. The effect of time was considered to be the effect of resistance training. The degree of linear association between parameters of interest was determined using the Pearson product-moment correlation. Statistical significance was assigned if $P < 0.05$. Data were not included in analysis if they were missing (i.e. unable to collect both pre and post muscle samples) or were outliers (± 3 standard deviations from the mean). Data were tested for similarity between data sets with the Kolmogorov-Smirnov test and for equal variances with the Levene tests. Data processing and statistical evaluations were completed using SPSS 12.0 for Windows (SPSS Inc, Chicago, IL).

Results

Unless noted, primary outcome measures did not differ between groups at baseline. Details regarding subjects, dietary intervention and exercise intervention have been reported previously (18).

Dietary intake

At baseline, among all subjects, protein intake was 1.1 ± 0.1 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1} and total energy intake was from 50% carbohydrate (includes 3% alcohol), 16% protein, and 34% fat. During the intervention, protein intakes were 0.9 ± 0.1 and 1.2 ± 0.0 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1} in LP and HP, respectively, with the HP group consuming more total, egg, and dairy protein (Table 1). Carbohydrate and fiber consumption increased in all subjects from baseline to the end of intervention, independent of group (Table 1, $P < 0.05$). Fat and alcohol consumption did not change from baseline and did not differ between groups (Table 1, $P < 0.05$). Subjects in HP consumed more dietary cholesterol than subjects in LP (Table 1, $P < 0.05$) during the intervention.

Table 1
Dietary Intakes

	Lower protein group (LP)		Higher protein group (HP)	
	Pre	Post	Pre	Post
Dietary Intake[a]				
Energy (kcal/d)	1973 ± 120	2079 ± 137	1956 ± 108	2099 ± 134
Total protein (g/d)	80 ± 5	64 ± 4[b,c]	82 ± 5	88 ± 5 [b,c]
Dairy (g/d)	17 ± 2	9 ± 1[d]	14 ± 2	13 ± 1[d]
Egg (g/d)	3 ± 1	2 ± 0 [b,c]	4 ± 1	22 ± 2 [b,c]
Meat (g/d)	28 ± 3	18 ± 3[d]	36 ± 3	21 ± 2[d]
Other (g/d)	32 ± 2	36 ± 3[d]	26 ± 2	31 ± 2[d]
Carbohydrate (g/d)	242 ± 18	274 ± 18[d]	228 ± 20	264 ± 23[d]
Fat (g/d)	71 ± 6	78 ± 7	80 ± 5	77 ± 6
Dietary Fiber (g/d)	21 ± 2	25 ± 2[d]	19 ± 2	22 ± 2[d]
Alcohol (g/d)	14 ± 5	13 ± 5	4 ± 3	4 ± 2
Cholesterol (mg/d)	250 ± 91	213 ± 21 [b,c]	281 ± 24	610 ± 105[b,c]

Values are mean ± SEM. Groups did not differ significantly at baseline, $P > 0.05$. a. LP 8 men, 8 women, n=16 (BMI 25.6 ± 0.8 kg/m2, range 20.1-31.5 kg/m2); HP 3 men, 9 women, n=12 (BMI 26.7 ± 0.9 kg/m2, range 20.6-35.1 kg/m2). Egg proteins include whole eggs, egg whites and egg products. Meat proteins include striated tissue (beef, poultry, pork, fish). Protein intake from 'Other' = protein from sources other than dairy, meat and egg. b. Significant time*group interaction, $P < 0.05$. c. Groups significantly different at 'post' $P < 0.05$. d. Significant effect of time, independent of group $P < 0.05$.

Consistent with a group difference in dietary protein intake, urinary urea nitrogen excretion was 42% higher in HP than in LP during intervention ($P < 0.05$, (18)).

Strength

Whole body strength increased 32 ± 8%, independent of group and gender. Lean mass was associated with strength at both baseline ($R=0.89$, $P<0.05$) and the end of the study ($R=0.88$, $P<0.05$). Changes in lean mass were not associated with changes in strength.

Body Composition and Skeletal Muscle Fiber Cross-Sectional Area

Body weight remained stable, lean mass increased and fat mass decreased with resistance training, independent of group and gender ($P < 0.05$, Figure 1 (18)). Regional lean and fat mass changes mirrored whole body changes, as lean mass increased in trunk, legs and arms, and fat mass decreased in these regions. Though total, trunk and leg bone mineral density remained unchanged, bone mineral density in the arms increased. Urinary creatinine excretion, a crude index of whole body muscle mass (36) increased over time comparably in both diet groups (18 increase from baseline, urinary creatinine excretion per body weight: LP 1%, HP 2% increase from baseline). The average cross-sectional area of muscle fibers was larger in men than women, was unchanged with resistance training, and was not differentially affected over time by the dietary interventions (Table 2).

Table 2

Average cross-sectional area (CSA) of vastus lateralis fibers

	Lower protein group (LP)		Higher protein group (HP)	
	Pre	**Post**	**Pre**	**Post**
All fiber types, CSA (μm²)	3882 ± 318	3689 ± 217	3769 ± 184	3895 ± 220
Type I, CSA (μm²)	3681 ± 238	3568 ± 174	3431 ± 172	3542 ± 163
Type IIa, CSA (μm²)	3806 ± 227	3731 ± 164	3381 ± 231	3555 ± 188
Type IIx, CSA (μm²)	2903 ± 174	3059 ± 221	2907 ± 240	2831 ± 144

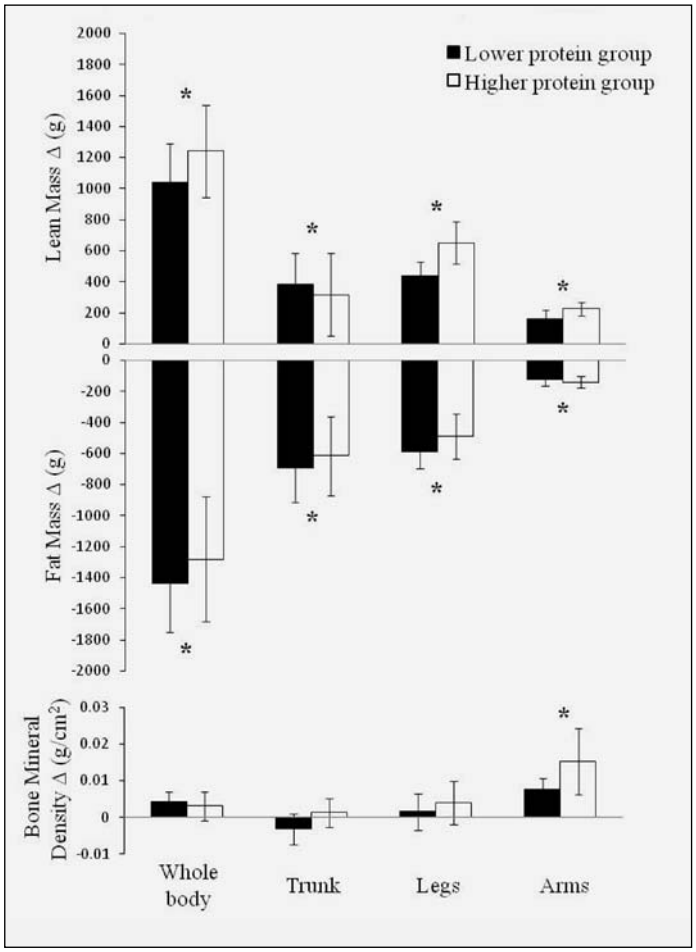
Values are mean ± SEM. Groups did not differ at baseline, $P > 0.05$. LP 9 men, 6 women, n=15; HP 7 men, 9 women, n = 16.

Blood chemistries

Blood urea nitrogen, albumin, complete blood count and electrolytes were within clinical normalcy at baseline and post-intervention (data not shown). Blood urea nitrogen decreased with intervention, independent of group and gender (18 ± 1 vs. 16 ± 1 mg/dL, pre vs. post all subjects combined, $P < 0.05$), and was not different between groups at the end of intervention (15 ± 1 vs. 17 ± 1 mg/dL, LP vs. HP). Independent of group and gender, serum total and LDL cholesterol decreased with resistance training (baseline and change: Total cholesterol (mg/dL): LP 190 ± 9, change -8 ± 4, HP 195 ± 7, change -3 ± 3; LDL: LP 117 ± 7, change -7 ± 3; HP 124 ± 6, change -4 ± 3 ($P < 0.05$) (18). HDL and triacylglycerol were unchanged (baseline and change: HDL (mg/dL): LP 51 ± 2, change 0 ± 1, HP 53 ± 3, change 0 ± 1; triacylglycerol: LP 111 ± 10, change -5 ± 6; HP 90 ± 7, change 6 ± 5 ($P < 0.05$) (18), as were LDL:HDL and total cholesterol:HDL (18, data not shown).

Figure 1

Resistance training improved whole body and regional composition. Values are mean ± SEM. Lower protein group n = 18, higher protein group n = 18. * – Significant effect of time (i.e. resistance training), all subjects combined $P < 0.05$



Correlations

Urinary creatinine + body composition: Urinary creatinine was correlated with lean mass at baseline ($R=0.67$, $P<0.05$) and at the end of intervention ($R=0.52$, $P<0.05$). Urinary creatinine was not correlated with fat mass at any time point ($P>0.05$). Changes in urinary creatinine excretion were not associated with changes in lean or fat mass.

Dietary and serum cholesterol + body composition: Dietary cholesterol intake (mg/d) was associated with lean mass at baseline ($R=0.67$, $P<0.05$). Dietary cholesterol intake was not associated with lean mass at the end of intervention, with fat mass at any point, or with changes in fat or lean mass. Serum cholesterol (mg/dL) was not associated with lean mass at any

time point. The ratios of LDL:HDL and total cholesterol:HDL were not associated with body composition or changes in body composition at any point.

Muscle fiber type characteristics + dietary and serum cholesterols, body composition, strength: CSA was not associated with total dietary or serum cholesterol at any time point. CSA was associated with lean mass at baseline ($R = 0.51$, $P<0.05$) and at the end of the study ($R = 0.50$, $P<0.05$). Changes in CSA and lean mass were not correlated. CSA and strength were correlated at baseline ($R=0.62$, $P<0.05$) and the end of intervention ($R=0.66$, $P<0.05$). Changes in CSA were not correlated with changes in strength.

Discussion

The results of this study indicate that the ingestion of higher quantities of animal-based foods, especially eggs, to achieve moderate increases in protein intake, does not enhance regional body composition changes (increase in lean body mass and decrease in fat mass) or influence skeletal muscle cross-sectional area in older adults who perform resistance training for 12 weeks. Similar resistance training-induced improvements in whole body composition have been reported previously (2, 5-12). Though regional body composition is not often reported, others have reported increases in regional lean mass following resistance training in men (arms, legs and trunk (37)) and women (arms (38), trunk (38, 39), legs (40). Reductions in regional fat mass have also been documented in men (arms and legs (37), trunk (37, 39)) and women (intra-adipose tissue (41)). Though results are not entirely consistent, this is likely due to differences in sample size, length of training and measurements taken. Collectively, these results support an increase in lean mass and decrease in fat mass caused by resistance training. The whole-body weight training program in the current study influenced body composition in all measured regions, as lean mass increased and fat mass decreased in arms, legs and trunk.

Consistent with some but not all (8) previous research, this improvement in body composition during resistance training was not influenced by dietary protein intake. This observation is generally consistent with the view that resistance training-induced changes in body composition, muscle strength and size, and physical functioning are not influenced by increased protein intake in older people who habitually consume adequate protein (moderately above the RDA) (42). The most dramatic differential response was observed when subjects consumed either lactoovovegetarian (0.8 g protein $\cdot kg^{-1} \cdot d^{-1}$) or omnivorous ($1.0$ g protein $\cdot kg^{-1} \cdot d^{-1}$) diets and completed 12 weeks of resistance training. Fat-free mass decreased by 0.8 kg among subjects who consumed lactoovovegetarian diets and increased 1.7 kg in those subjects who consumed omnivorous diets (time*group $P< 0.05$) during resistance training (8), suggesting that group differences were caused by either protein quantity or quality (i.e. lactoovovegetarian vs. omnivorous diets). The group differences in protein intake in the present (0.3 g protein $\cdot kg^{-1} \cdot d^{-1}$) and cited ($0.2$ g protein $\cdot kg^{-1} \cdot d^{-1}$) studies were similar, but groups in each study consumed different amounts of protein.

Resistance training did not cause a measurable increase in skeletal muscle fiber cross-sectional area. While unexpected, the lack of change in skeletal muscle fiber properties with resistance training has been previously documented (2, 25, 43-45). Though satellite cells in older people have an impaired ability to repair skeletal muscle tissue (46), most researchers have documented an increase in skeletal muscle fiber CSA with resistance training (2, 3, 13, 14). Other researchers have reported that response of CSA is limited or lacking in older women (2). The present study was powered to detect training or group difference, and was not powered to detect a gender specific difference. Therefore, a minimal response by women may have limited the ability to detect a training induced change in skeletal muscle fiber cross-sectional area. Similarly, the number of men within each group was not adequate to detect a response in men only.

Within the current study, dietary protein intake did not influence skeletal muscle fiber CSA. Similar results have been reported as skeletal muscle fiber CSA was unchanged in groups that consumed 0.8 and 1.6 g protein $\cdot kg^{-1} \cdot d^{-1}$ during 12 weeks of resistance training (8, 25). While type II fiber CSA increased after 12 weeks of resistance training after consumption of both lactoovovegetarian (0.8 g protein $\cdot kg^{-1} \cdot d^{-1}$) and omnivorous ($1.0$ g protein $\cdot kg^{-1} \cdot d^{-1}$) diets, protein intake did not influence this increase (8).

In a recent clinical trial, both dietary cholesterol intake and serum cholesterol concentration were associated with changes in lean mass in older men and women after a 12-week resistance training program (19). The authors suggested that this association was caused by the role of cholesterol in cellular signaling or its role in skeletal muscle fiber repair following resistance training (19). Therefore, a secondary, retrospective objective of this study was to assess the influence of dietary and serum cholesterol on resistance training induced changes in body composition and skeletal muscle fiber CSA. To assess the relationship between cholesterol and primary outcome measures, we correlated both dietary and serum cholesterol with FFM, strength and CSA. However, very few correlations were significant. In correlating absolute amounts of dietary or serum cholesterol with outcome variables (i.e. mg/d or mg/dL), only dietary cholesterol and FFM were correlated, and this apparent relationship was only evident at baseline. This association at baseline is not surprising, as these factors are not strictly independent of each other. Dietary intake, and therefore cholesterol intake is higher in heavier vs. lighter individuals, given similar activity levels. Similarly, heavier individuals generally have higher fat-free mass than lighter individuals. Therefore, an association between dietary cholesterol and FFM is likely.

To compare our results with others, we considered correcting dietary and serum cholesterol for fat-free mass (i.e. $mg \cdot dL^{-1} \cdot Kg\ FFM^{-1}$) (19). However, given that FFM was associated with both strength and CSA in the current study,

correlations of dietary or serum cholesterol per kg FFM with other outcome measures were not considered appropriate. The association with FFM may have driven the results. Furthermore, skeletal muscle fiber CSA was not associated with dietary cholesterol or serum cholesterol.

The use of dietary counseling and food records are potential limitations of this study. Dietary counseling lacks the control achieved when all foods are provided during a controlled feeding trial and may have contributed to subjects' inability to achieve the desired levels of protein intake. However, dietary counseling is a more common method of dietary modification and permits broader application of study results. The relatively small difference in protein intakes between groups may have limited the potential for diet specific responses. A larger difference in protein intakes between groups may be necessary to detect a differential response to resistance training, as other researchers have reported that protein supplements of 1.2 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1} plus habitual dietary intake caused greater lean body mass gain when compared to habitual dietary intake alone (6). Though food records are subject to misreporting (47), the difference in urinary urea nitrogen output between the groups confirms that groups consumed different amounts of protein (48, 49).

In summary, findings from this study confirm that resistance training improves whole- and regional-body composition and serum lipid-lipoprotein profile, and suggests that consumption of a diet that provides moderately higher protein does not influence these changes in older men and women.

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