Resistance training and dietary protein: effects on glucose tolerance and contents of skeletal muscle insulin signaling proteins in older persons[1–3]

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ABSTRACT

Background: Resistance training (RT) and dietary protein independently influence indexes of whole-body glucose control, though their synergistic effects have not yet been documented.

Objective: This study assessed the influence of dietary protein intake on RT-induced changes in systemic glucose tolerance and the contents of skeletal muscle insulin signaling proteins in healthy older persons.

Design: Thirty-six older men and women (age: 61 ± 1 y) performed RT (3 times/wk for 12 wk) and consumed either 0.9 g protein · kg^{-1} · d^{-1} [lower-protein (LP) group; ≈112% of the Recommended Dietary Allowance (RDA)] or 1.2 g protein · kg^{-1} · d^{-1} [higher-protein (HP) group; ≈150% of the RDA]; the HP group consumed more total, egg, and dairy proteins.

Results: After RT, body weight was unchanged; whole-body protein and water masses increased, and fat mass decreased with no significantly different responses observed between the LP and HP groups. The RT-induced improvement in oral glucose tolerance (decreased area under the curve, AUC) was not significantly different between the groups (LP: −28%; HP: −25%). The insulin (−21%) and C-peptide (−14%) AUCs decreased in the LP group but did not change significantly in the HP group. Skeletal muscle insulin receptor, insulin receptor substrate-1, and Akt contents were unchanged, and the amount of atypical protein kinase C ζ/λ (aPKC ζ/λ), a protein involved with insulin signaling, increased 56% with RT, independent of protein intake.

Conclusion: These results support the hypothesis that older persons who consume adequate or moderately high amounts of dietary protein can use RT to improve body composition, oral glucose tolerance, and skeletal muscle aPKC ζ/λ content without a change in body weight. *Am J Clin Nutr* 2007;85:1005–13.

KEY WORDS Protein, diet, resistance training, skeletal muscle insulin signaling, elderly, glucose tolerance

INTRODUCTION

Even in healthy older adults, glucose tolerance decreases with age (1). This age-associated decrease in glucose tolerance may be caused by altered body composition (2, 3), decreased physical activity (3, 4), decreased tissue sensitivity to insulin (5), or a combination of these factors. Although 7% of the general American population has diabetes, 21% of Americans (10.3 million people) aged ≥60 y have diabetes (6). Safe and effective therapies are needed to improve glucose tolerance and decrease the risk of developing type 2 diabetes mellitus in older persons.

Most (7–11), but not all (12, 13), researchers report that resistance training improves glucose tolerance and insulin action in older persons. Resistance training decreases fat mass and increases fat-free mass (14, 15); both are associated with positive changes in the control of blood glucose (16). Although chronic resistance training often improves both body composition and glucose control (10, 11), an acute bout of resistance training also improves glucose control (17). This indicates that factors other than body composition influence the resistance training–induced improvement in glucose tolerance (4). Skeletal muscle insulin receptor and Akt contents increased with single leg resistance training in both healthy and diabetic older men (18), suggesting that resistance training positively alters the skeletal muscle insulin signaling pathway. To our knowledge, the influence of whole-body resistance training on these variables has not been assessed in healthy older men and women.

During a period of resistance training, appropriate energy and macronutrient intakes are necessary to promote performance and body-composition changes (15). Dietary protein requirements may increase with physical activity (19), though this has been documented primarily in athletes. The preferred protein intake during a resistance training program is currently undefined in older people, though it would permit maximal fat-free mass gains during resistance training. Some (20, 21), but not all (15), researchers have reported that protein intake influences resistance training–induced improvement in body composition. A higher protein intake during resistance training may enhance improvements in whole-body glucose tolerance and metabolism by optimizing gains in muscle mass, the primary target of insulin-stimulated glucose uptake (22). Chronic high protein intake in subjects with type 2 diabetes improved glucose tolerance (23) but is associated with worsened glucose control in healthy subjects

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(24, 25). Acute protein intake is associated with a minimal rise in blood glucose concentration (26), though when consumed together, protein increases the insulin response to glucose in individuals with type 2 diabetes (27). The disparate results indicate that this topic should be further studied.

The objective of the present study was to determine the influence of resistance training combined with a lower or higher dietary protein intake on improvements in glucose tolerance in older people. We hypothesized that resistance training would increase the content of skeletal muscle insulin signaling proteins and that when compared with consumption of a lower protein diet, resistance training and a higher protein diet would further improve glucose tolerance.

SUBJECTS AND METHODS

Subjects

Because older persons are at greater risk for developing impaired glucose metabolism and tolerance than are young persons (1), we chose to study healthy, older adults. We selected nondiabetic subjects rather than diabetic subjects because we wanted to focus on prevention or delay of problems in glucose control.

Fifty men and women from the greater Lafayette, IN, area were recruited to participate in the study. Recruitment criteria included the following: *1*) age range: 50–80 y; *2*) body mass index (BMI; in kg/m^2) of 20 to 35 (28); *3*) nondiabetic and not on insulin replacement therapy; *4*) clinically normal kidney, liver, and cardiac functions; *5*) not currently using antiinflammatory steroid medications; *6*) no hip replacement; *7*) no habitual resistance training in the past 6 mo; and *8*) women were ≥2 y postmenopausal. Potential subjects completed a screening evaluation that included a medical history, resting electrocardiogram, resting blood pressure, and routine blood and urine chemistries. The subjects were approved by the study physician before starting the protocol. After both written and verbal explanations were provided, the subjects signed an informed consent agreement. The Purdue University Institutional Review Board reviewed and approved the informed consent agreement and the study protocol. A monetary stipend was provided to the subjects.

Thirty-six of the fifty subjects completed the study protocol. The timing of dropout and reason for leaving varied among the subjects. During baseline measurements, subjects left the study due to the time commitment [$n = 3$ in the higher-protein (HP) group], a fear of needles [$n = 1$ in the lower-protein (LP) group], and unknown reasons ($n = 1$ in the LP group and 1 in the HP group). During the intervention period, subjects left the study due to the time commitment ($n = 2$ in the LP group and 1 in the HP group), a dislike of the study diet ($n = 1$ in the LP group and 1 in the HP group), and health reasons unrelated to the study ($n = 2$ in the LP group and 1 in the HP group).

Experimental design

The 14-wk protocol was conducted on the Purdue University campus. The subjects were randomly assigned to 1 of 2 diet groups based on entry date into the study. Preintervention measurements were taken during weeks 1 and 2, while the subjects continued habitual diet and activity. Dietary protein intake and resistance training intervention followed in weeks 3–14, and postintervention measurements were taken during week 14 (dietary control and resistance training continued). The subjects were asked to continue habitual medication and supplement use during the study and agreed not to change this routine or add medications, drugs, or supplements. The subjects were also instructed to maintain habitual activity levels unrelated to the resistance training intervention.

At the time of the study design, we were unaware of any published research study that had evaluated the combined effects of dietary protein and resistance training on glucose tolerance. We determined that 36 total subjects—18 subjects in each of the 2 diet groups—was sufficient to test the main effect of time (ie, resistance training) and the group-by-time interaction (ie, differential response of protein intake during resistance training) (7, 29).

Exercise intervention

The subjects were familiarized with the resistance training equipment and procedures before the intervention. All resistance training sessions were completed in the Exercise Research Facility at Purdue University with the use of pneumatic resistance exercise equipment (Keiser Sports Health Equipment Company, Fresno, CA). The subjects trained 3 d/wk during weeks 3–14. Before each exercise session, the subjects completed a preexercise questionnaire designed to assess their current health and ability to safely complete a resistance training session. Each training session lasted ≈1.25 h and included warm-up and cool-down periods and 2 sets of 8 repetitions plus a third set to voluntary failure at 80% of the measured 1-repetition maximum for 8 exercises. An upper back seated row, leg extension, chest press, leg curl, latissimus dorsi pull down, and double leg press were performed during each training session. The shoulder raise and seated calf press were alternated with hip adductor and hip abductor exercises and were performed during every other training session, so that subjects completed 6 primary exercises and 2 additional exercises for a total of 8 exercises per training session. All exercises were performed in a slow uniform fashion, giving equal time to the concentric and eccentric portions (6–8 s per repetition). The subjects rested for 1 min between sets. On the third set of each exercise, the subjects exercised until voluntary fatigue, with a maximum of 12 repetitions performed. If the subjects reached 12 repetitions, the resistance was increased by 5% for that exercise at the next training session.

The training stimulus was periodically adjusted to maintain an intensity of 80% of maximum strength, as assessed by the 1-repetition maximum test taken at baseline, twice during intervention, and at the end of the study. Maximum strength was assessed on 5 core pieces of equipment (upper back seated row, leg extension, chest press, leg curl, and leg press). A whole-body strength summary was calculated as the sum of the 1-repetition maximum results for these 5 exercises. A moderate resistance training program was used in concurrence with current recommendations for the inclusion of resistance training for healthy living (8, 30).

Energy expenditure of physical activity

The Yale Physical Activity Questionnaire was used to assess the subjects' average energy expenditure and habitual activity at baseline and the end of the study (31). As calculated in accordance with this questionnaire, habitual activity is reported in units per week and reflects a weighted composite of vigorous activity, walking, moving, sitting, and standing (31).

Dietary intervention

The subjects were instructed to maintain body weight and were counseled to habitually consume 1 of 2 diets throughout the intervention period. The LP group was counseled to consume 0.8 g protein · kg^{-1} · d^{-1}, the Recommended Dietary Allowance (RDA) for protein, with egg, striated tissue (beef, poultry, pork, or fish), and dairy proteins as 5%, 25%, and 15% of total protein intake, respectively. The subjects in the HP group were counseled to consume 1.6 g protein · kg^{-1} · d^{-1}, with egg, striated tissue, and dairy proteins as 25%, 20%, and 15% of total protein intake, respectively. The higher total protein intake of the HP group was achieved via the consumption of larger quantities of egg, striated tissue, and dairy proteins than those the subjects in LP group consumed. The subjects in the HP group were counseled to consume a higher percentage of protein from egg than from other proteins because eggs are a readily available high-quality protein source with a true digestibility score ≥97%. Each individualized diet was designed to provide 1.5 times the basal energy requirement, as calculated by sex-specific Harris-Benedict equations (32). All dietary counseling (verbal and written) was based on the American Diabetes Association–American Dietetic Association Exchange Lists for Weight Management (33). Written instructions, detailing the number of food exchanges each subject was to consume daily, a tracking sheet, guidelines on how to appropriately measure food selections, a list of common serving sizes, and a scale to weigh portion sizes were provided.

Food records collected for 7 consecutive d 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) were analyzed for energy and macronutrient content (Nutritionist Pro, N-squared computing; First DataBank version 1.3.36, San Bruno, CA). Food records that were not returned to the study site or were incomplete were excluded from analysis. Food records were deemed incomplete if the average energy intake over 7 d was not greater than or equal to the subject's Harris-Benedict predicted basal energy requirement. A total of 56 food records (78% of a possible 72) were included in analysis of dietary change from baseline, and a total of 100 food records (69% of a possible 144) were included in analysis of all food records.

Urine analyses

The subjects completed 2 consecutive 24-h urine collections in wk 1, 5, 10, and 14. Total volume was calculated as mass of the entire 24-h sample divided by the average of 3 measurements of specific gravity (Digital Probe Refractometer; Misco Products Division, Cleveland, OH). Samples were processed, stored frozen at −20 °C, and later analyzed for urinary urea nitrogen (the primary nitrogenous component of urine) to help assess dietary protein intake before, during, and at the end of the period of intervention and for measurement of urinary creatinine, as a marker of muscle mass (Cobas Mira Plus; Roche Diagnostic Systems, Indianapolis, IN).

Blood analyses

Fasting-state blood samples were obtained on 2 nonconsecutive days at baseline and the end of intervention. Poststudy collection occurred 1–2 d after a resistance training session (12–48 h). Immediately after collection, vials were inverted 7 times, maintained at room temperature for 45 min to allow clotting, and then kept on ice until they were centrifuged at 4 °C for 10 min at 3000 × *g* (collected in vials containing serum separator and silica clot activator; BD Vacutainer Brand; Benton, Dickinson and Co, Franklin Lakes, NJ). Serum samples were separated into plastic storage vials and stored frozen at −80 °C until analysis. To assess if a diet high in animal proteins altered the lipid profile (34), total cholesterol concentrations, HDL-cholesterol concentrations, and triacylglycerol concentrations were measured, and LDL was calculated by COBAS MIRA PLUS as LDL cholesterol = total cholesterol – HDL + 0.2 × triacylglycerol (analyzed on COBAS Mira Plus; Roche Diagnostic Systems, Indianapolis, IN). A standard clinical chemistry profile was measured on one day to assess normative values (blood urea nitrogen, albumin, complete blood count, and electrolytes; analyzed by Mid America Clinical Laboratories, Indianapolis, IN).

Body-composition assessment

To monitor body weight stability, clothed body weight was measured immediately before each exercise session (3 times/wk). If body weight fluctuated by >2 kg from baseline on ≥3 consecutive sessions, the subject's diet was adjusted accordingly to encourage a return to and maintenance of baseline body weight. At baseline and the end of the study, whole-body composition was assessed in the fasting state with a 4-compartment model (fat mass, water mass, protein mass, and mineral mass) (35). All measurements were completed within the same morning. Total body water was measured via the deuterium oxide dilution technique (deuterium, 99.9%; Cambridge Isotope Laboratory, Woburn, MA) (36). All urine was collected before oral consumption of a preweighed amount of deuterium oxide, and 3 timed collections were taken at 2, 3, and 4 h after consumption. The total volume of each sample was recorded, and aliquots were stored frozen at −20 °C for duplicate analysis of deuterium oxide concentration with the use of an infrared spectrophotometer (AVATAR 360 ESP FT-IR Spectrometer System; Thermo Nicolet Corporation, Madison, WI). Whole-body bone mineral, fat, and lean tissue masses were measured via dual-energy X-ray absorptiometry (DXA; GE Lunar Prodigy with EnCORE software version 5.60, Madison, WI) (37). The subjects wore loose metal free clothing and remained in a supine position while scanning was completed. Body weight and whole-body density were assessed via whole-body plethysmography (BodPod; Life Measurement Instruments Inc, Concord, CA). The subjects wore minimal tight-fitting metal-free clothing and completed triplicate tests. Tidal lung volume was measured within each test. Body circumference measurements were taken at the natural waist, umbilicus, and hip (widest girth below natural waist and umbilicus) with a fiberglass spring tape measure. Height was measured at the beginning of the study with a wall-mounted stadiometer (Holtain Ltd, Crymych, Wales, United Kingdom).

Glucose tolerance assessment

The subjects completed an oral-glucose-tolerance test (OGTT) before and after intervention to measure blood glucose, insulin, and C-peptide responses to drinking a sugar solution that contained 75 g dextrose. The poststudy OGTT occurred one (n = 4 in the LP group and 4 in the HP group) to 2 (n = 13 in the LP group and 15 in the HP group) d after a resistance training session. The day of testing did not affect the results ($P > 0.05$).

Blood samples were collected at 0, 15, 30, 45, 60, 90, and 120 min (BD Vacutainer Brand; Becton Dickinson and Co). Collection vials contained either lithium heparin (for future glucose and

insulin analyses) or EDTA and aprotinin (for C-peptide analysis; 85 μL aprotinin was added to reduce cleaving, Sigma A-6012 aprotinin from bovine lung; Sigma-Aldrich Corp, St Louis, MO). Samples were inverted 7 times and kept on ice until they were centrifuged at 4 °C for 10 min at 3000 × *g*. The plasma was separated into plastic storage vials and stored frozen at −80 °C until further analysis. Samples were analyzed for glucose (oxidase method; COBAS Mira Plus; Roche Diagnostic Systems) (9), insulin (EIA; ALPCO diagnostics 1–2-3 Human Insulin EIA, Windham, NH), and C-peptide (EIA; ALPCO diagnostics C-peptide EIA) concentrations. The 120-min integrated AUC for glucose, insulin, and C-peptide was determined by using the trapezoidal method (38). Hepatic insulin sensitivity and whole-body (composite) insulin sensitivity were calculated as follows (39):

$$\text{ISI(HOMA)} = k/(\text{FPI} \times \text{FPG}) \quad (1)$$

$$\text{ISI (composite)} = 10\,000/\sqrt{(\text{FPG} \times \text{FPI}) \times (\bar{G} \times \bar{I})} \quad (2)$$

where ISI is the insulin sensitivity index, HOMA is the homeostatic model assessment, $k = 22.5 \times 18$, FPI is fasting plasma insulin (in μU/mL), FPG is fasting plasma glucose (in mg/dL), $\bar{G}$ is the mean plasma glucose concentration during the OGTT (mg/dL), and $\bar{I}$ is the mean plasma insulin concentration during the OGTT (in μU/mL).

Fasting blood samples were analyzed for fructosamine (COBAS Mira Plus; Roche Diagnostic Systems) and glycated hemoglobin (Hb A_{1c}), which are markers of shorter (2–3 wk) and longer-term (2–3 mo) glycemic control, respectively (40).

Skeletal muscle insulin signaling proteins

At baseline and postintervention, a muscle biopsy sample was obtained in the fasting state from the vastus lateralis of the dominant leg by using a punch biopsy technique (41). Each biopsy sample was divided into 30–50-mg pieces and stored in liquid-nitrogen for later analysis. Muscle was analyzed for changes in content of the proteins involved in the insulin signaling pathway, including insulin receptor (IR), insulin receptor substrate 1 (IRS-1), Akt, and atypical protein kinase C ζ/λ (aPKC ζ/λ) protein content with homogenization and Western blotting (42) (all antibodies purchased from Santa Cruz Biotechnology, Santa Cruz, CA). Because IR and IRS-1 have not previously been shown to be influenced by resistance training, a subset of these samples were analyzed ($n = 20$ and 15, respectively) to assess changes. Protein contents of IR or IRS-1 did not significantly change in this subset so further analysis of these proteins was not completed because of limited sample size. Western blot images were scanned and band densitometry was assessed with Gel Pro Analyzer software (Media Cybernetics, Silver Spring, MD). Band density is in arbitrary units. A sample from each group was run on each blot, and blots were made relative by using a blot average technique, which divides each band density by the average density of all the bands on the blot.

Statistical analyses

All data are reported as means ± SEMs. Baseline group and sex differences were assessed with 2-factor analysis of variance (ANOVA) and the main effects of time, protein intake and sex, and their interactions on dependent variables were assessed via 3-factor ANOVA with time as a repeated measure. The main effect of time was considered to be the effect of resistance training. Although the study was not powered to test sex differences, certain data are presented within both group and sex. The change from baseline was defined as the postintervention value minus the baseline value. The degree of linear association between the variables of interest was determined by using either the Pearson product-moment correlation or the Spearman rho nonparametric ranked correlation, as appropriate. Statistical significance was assigned if $P < 0.05$. Data were not included in analysis if they were missing (ie, unable to obtain anthropometric measurements on one subject), outliers (±3 SDs), or physiologically improbable (ie, total body water measurement was far too low for 1 subject). Data processing and statistical evaluations were completed by using SPSS version 12.0 for WINDOWS (SPSS Inc, Chicago, IL).

RESULTS

Subjects

At baseline, subjects were between 50 and 80 y old (range: 50–80 y for the LP group, 50–75 y for the HP group) and BMI ranged from 20 to 32 in the LP group and 21 to 35 in the HP group. Primary outcome measurements were not significantly different between the groups at baseline (**Table 1**).

Exercise intervention

With intervention, composite maximum strength increased significantly ($P < 0.05$) in both groups (LP group: 28 ± 4%; HP group: 34 ± 5%; Table 1). Both habitual activity and average energy expenditure outside of the laboratory were not significantly ($P > 0.05$) changed from baseline (change from baseline: −3 ± 5 units/wk, 1215 ± 812 kcal/wk, ≈174 kcal/d in the LP group; −6 ± 4 units/wk, −1262 ± 1466 kcal/wk, ≈−180 kcal/d in the HP group).

Dietary intervention

At baseline, as a percentage of average total daily energy intake, the subjects' diets were composed of 34% fat, 50% carbohydrate (includes 3% alcohol), and 16% protein. The macronutrient content of average daily intake was not significantly different between the groups. At baseline and throughout the intervention, the men consumed more energy, protein, carbohydrates, and fat than did the women (data not shown). Protein intake changed over time, and the subjects in the HP group consumed more protein than did those in the LP group at the end of intervention (**Table 2**).

The average daily protein intake at baseline was 1.1 ± 0.1 g protein · kg^{-1} · d^{-1}. An analysis of food records completed during the last week of intervention indicated that the subjects in the LP group consumed 0.9 ± 0.1 g protein · kg^{-1} · d^{-1} and the subjects in the HP group consumed 1.2 ± 0.0 g protein · kg^{-1} · d^{-1} (significant group × time interaction, $P <$ 0.05). Total, egg, and dairy protein intakes were different between the groups during the intervention (**Figure 1**). Total meat intake did not differ significantly between the groups. Urinary urea nitrogen excretion (UUN) during the intervention was higher in the HP group than in the LP group, consistent with the group difference in dietary protein intake (significant group difference at postintervention and significant group × resistance

TABLE 1
Characteristics of the subjects in the lower-protein (LP) and higher-protein (HP) groups[1]

	LP group		HP group	
	Baseline	After intervention	Baseline	After intervention
Subjects				
Age (y)[2]	62 ± 2		61 ± 2	
BMI (kg/m^2)[2]	25.6 ± 0.8	25.6 ± 0.8	26.7 ± 0.9	26.8 ± 0.9
Exercise intervention				
Composite 1RM (kg)[3]	397 ± 40	500 ± 45[4]	409 ± 37	531 ± 41[4]
Energy expenditure of physical activity (kcal/d)[5]	958 ± 109	1132 ± 155	1412 ± 189	1232 ± 134
Habitual activity (U)[5]	57 ± 7	54 ± 6	55 ± 7	50 ± 6
Serum and urine analyses				
HDL (mg/dL)[6]	51 ± 2	51 ± 2	53 ± 3	53 ± 4
LDL (mg/dL)[6]	117 ± 7	111 ± 7[4]	124 ± 6	120 ± 7[4]
Triacylglycerol (mg/dL)[6]	111 ± 10	106 ± 11	90 ± 7	96 ± 6
Total cholesterol (mg/dL)[6]	190 ± 9	182 ± 8[4]	195 ± 7	192 ± 8[4]
Urinary creatinine/body weight (mg · kg^{-1} · d^{-1})[2]	10.4 ± 0.6	11.0 ± 0.6[4]	10.6 ± 0.8	12.5 ± 0.8[4]
Urinary urea nitrogen/body weight (mg · kg^{-1} · d^{-1})[2]	87.1 ± 5.2	74.0 ± 4.3[7]	88.6 ± 7.4	104.8 ± 7.3[7,8]
Body composition				
Body fat (kg)[6]	26.4 ± 2.2	24.6 ± 2.5[4]	27.0 ± 1.8	25.0 ± 1.9[4]
Body weight (kg)[2]	74.8 ± 3.6	74.7 ± 3.6	78.2 ± 3.1	78.6 ± 3.2
Hip circumference (cm)[9]	100.3 ± 1.7	100.0 ± 1.8	101.9 ± 1.6	101.9 ± 1.5
Mineral mass (kg)[6]	3.4 ± 0.2	3.4 ± 0.2	3.8 ± 0.2[8]	3.8 ± 0.2[8]
Protein mass (kg)[6]	7.6 ± 0.7	7.8 ± 0.7[4]	8.3 ± 0.6	9.0 ± 0.8[4]
Total body water (L)[6]	36.4 ± 2.4	37.9 ± 1.9[4]	39.2 ± 2.0	40.1 ± 2.0[4,8]
Waist circumference (cm)[9]	84.8 ± 2.9	83.5 ± 3.0[4]	84.8 ± 3.1	83.5 ± 2.9[4]
Waist:hip[9]	0.84 ± 0.02	0.83 ± 0.02[4]	0.83 ± 0.03	0.82 ± 0.02[4]

[1] All values are $\bar{x}$ ± SEM. Mineral mass differed significantly between groups at baseline, $P < 0.05$. No other significant group differences were observed at baseline, $P > 0.05$.
[2] LP: $n = 9$ men and 9 women; HP: $n = 8$ men and 10 women.
[3] LP: $n = 7$ men and 7 women; HP: $n = 8$ men and 10 women.
[4] Significant effect of resistance training, independent of group ($P < 0.05$).
[5] LP: $n = 9$ men and 9 women; HP: $n = 8$ men and 9 women.
[6] LP: $n = 8$ men and 9 women; HP: $n = 8$ men and 10 women.
[7] Significant time × group interaction, $P < 0.05$.
[8] Significantly different from LP group at same time point, $P < 0.05$.
[9] LP: $n = 9$ men and 8 women; HP: $n = 7$ men and 9 women.

training interactions, $P < 0.05$ for both; Table 1). Changes in protein intake and UUN were positively correlated ($r = 0.428$, $P < 0.05$).

Blood lipid-lipoprotein profile

Total and LDL cholesterol decreased (−5 ± 3 mg/dL and −6 ± 2 mg/dL, respectively; $P < 0.05$, all subjects combined) and HDL cholesterol and triacylglycerol were not significantly changed (0 ± 1 mg/dL and 0 ± 4 mg/dL, respectively; $P > 0.05$) with intervention, independent of diet and sex (Table 1).

Body composition

Body weight remained stable, total body water and protein mass increased, and fat mass decreased with resistance training,

TABLE 2
Dietary intakes in the lower-protein (LP) and higher-protein (HP) groups[1]

	LP group		HP group	
	Baseline	After intervention	Baseline	After intervention
Energy (kcal/d)	1973 ± 120	2079 ± 137	1956 ± 108	2099 ± 134
Protein (g/d)	80 ± 5	64 ± 4[2]	82 ± 5	88 ± 5[2,3]
Carbohydrate (g/d)	242 ± 18	274 ± 18[4]	228 ± 20	264 ± 23[4]
Fat (g/d)	71 ± 6	78 ± 7	80 ± 5	77 ± 6
Alcohol (g/d)	14 ± 5	13 ± 5	4 ± 3	4 ± 2
Dietary fiber (g/d)	21 ± 2	25 ± 2[4]	19 ± 2	22 ± 2[4]

[1] All values are $\bar{x}$ ± SEM. LP: $n = 8$ men and 8 women; HP: $n = 3$ men and 9 women. The groups did not differ significantly at baseline, $P > 0.05$.
[2] Significant time × group interaction, $P < 0.05$.
[3] Significantly different from LP group at the same time point, $P < 0.05$.
[4] Significant effect of time, independent of group ($P < 0.05$).

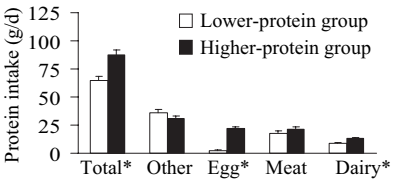

FIGURE 1. Mean (±SEM) protein intake from targeted foods during intervention in the lower-protein group ($n = 16$) and in the higher-protein group ($n = 12$). "Other" represents protein from all sources except egg, meat, and dairy products. *Significant group difference, $P < 0.05$.

independent of group and sex (**Figure 2**). Urinary creatinine excretion increased significantly ($P < 0.05$) with intervention (change from baseline: 0.6 ± 0.2 mg Ucreatinine $\cdot$ kg^{-1} $\cdot$ d^{-1} in the LP group compared with 1.9 ± 0.2 mg Ucreatinine $\cdot$ kg^{-1} $\cdot$ d^{-1} in the HP group; Table 1).

Glucose tolerance

All OGTTs were completed within 48 h of a resistance training session, and the timing of the OGTT (ie, within 24 or 48 h) did not significantly influence the results. The glucose AUC in response to an oral glucose challenge decreased with the intervention, independent of group and sex. Insulin and C-peptide AUC responses were influenced by protein intake during resistance training (significant group × resistance training interactions, $P < 0.05$; **Figure 3**). Both insulin and C-peptide AUCs decreased in the LP group ($P < 0.05$) but did not significantly change in the HP group ($P > 0.05$). Fasting plasma glucose, insulin, and C-peptide did not significantly change with resistance training and were not influenced by protein intake (Figure 3). No significant changes were observed in fructosamine, HbA1c, HOMA, or composite insulin sensitivity with resistance training or due to diet ($P < 0.05$; **Table 3**).

Skeletal muscle insulin signaling proteins

Analysis of a subset of samples indicated no significant change in IR or IRS-1 content with the intervention ($n = 15$ and 20, respectively). An analysis of all samples ($n = 32$) indicated that Akt was not significantly changed and that aPKC ζ/λ increased with intervention, independent of group and sex (significant effect of resistance training, $P < 0.05$; **Table 4**).

Correlations

Changes in body composition were not correlated with changes in glucose, insulin, or C-peptide AUCs during the OGTT or to changes in aPKC ζ/λ (data not shown).

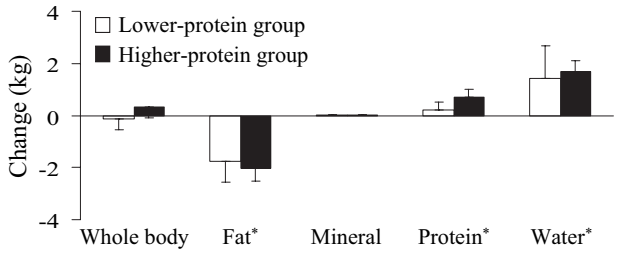

FIGURE 2. Changes in body weight and composition during intervention in the lower-protein group ($n = 17$) and in the higher-protein group ($n = 18$). *Significant effect of time (ie, resistance training), $P < 0.05$.

DISCUSSION

The 25–28% decrease in plasma glucose AUC during the OGTT observed after the 12-week period of resistance training supports the effectiveness of this mode of exercise to positively impact oral glucose tolerance in older, weight-stable people. This robust response contrasts with previous research that documented that although glucose metabolism improves with resistance training (8), the glucose response to an OGTT is not improved (9–11, 38, 43). The resistance training–induced decrease in glucose AUC during the OGTT in the present study may be caused by several factors, including an exercise-associated increase in glucose clearance (44) or an improvement in body composition (16). Because all OGTTs were conducted within 48 h of the last exercise session, it is possible that the decrease in glucose AUC and alterations in insulin and C-peptide AUC were caused by the recent exercise session and not by chronic training (8, 44, 45).

Fasting plasma glucose (9), insulin (7, 10, 11), and C-peptide and insulin response to an OGTT (7, 9–11) have been documented to decrease with chronic resistance training, although improvements were not documented with a shorter intervention period (4 wk compared with 12 wk). In resistance training programs similar to that of the current study, insulin response to an oral glucose challenge decreased by 19% and 33% (9, 43), whereas glucose (10, 38, 43) and C-peptide (9) responses were unchanged after resistance training in older subjects.

The synergistic effect of protein intake and resistance training on glucose tolerance has not been previously studied. Chronic higher protein intake has been associated with improved glucose metabolism in type 2 diabetics (46) and increased glucose stimulated insulin secretion in nondiabetic individuals (25). Other researchers have suggested that diet is not independently associated with insulin sensitivity or secretion in older women (47). The present findings that insulin and C-peptide responses to the oral glucose stimulus decreased after resistance training only in the LP group implicates diet as an important factor influencing the hormonal control of oral glucose tolerance in conjunction with resistance training. How or why this occurred was not measured within the current study. This difference does not appear to be caused by either body-composition or skeletal muscle insulin signaling protein contents, because the responses in the LP and HP groups were not significantly different. Perhaps the change in habitual protein intake (change from baseline: -0.2 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1} in the LP group compared with 0.1 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1} in the HP group) in addition to the actual level of protein intake influenced glucose tolerance (48), though this cannot be assessed within the confines of the current project. Although confirmed by both diet records and UUN (49, 50), the relatively small change in protein intake from baseline may have limited the ability to detect group-specific differences in response. Interestingly, despite the group difference in insulin and C-peptide responses, insulin sensitivity as estimated from the OGTT was not significantly changed. Because average plasma insulin during the OGTT is the only variable of 4 included in the composite insulin sensitivity index (39) that was differentially affected by diet, the overall alteration in insulin sensitivity was likely too small to detect. The lack of difference in insulin AUC responses between the groups at the end of the intervention

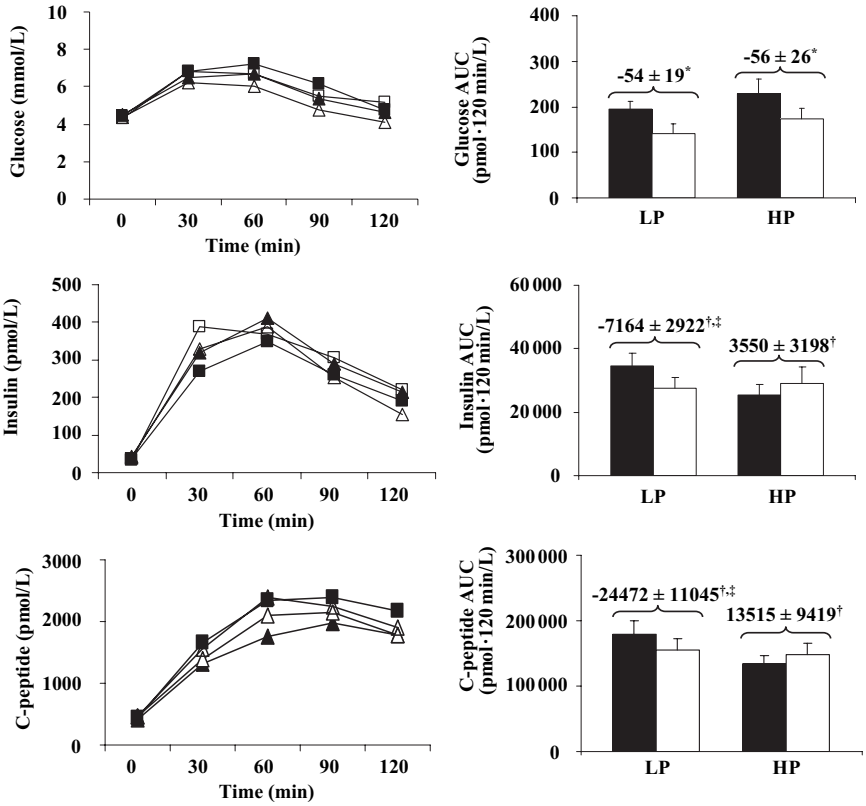

FIGURE 3. Glucose, insulin, and C-peptide concentrations and areas under the curve (AUCs) in response to a 75-g oral glucose challenge before and at the end of intervention in the lower-protein (LP; $n = 8$ men and 7 women) and higher-protein (HP; $n = 8$ men and 9 women) groups. Numbers above brackets indicate the mean (±SEM) change within each group from baseline measurements. ■, LP group at baseline; □, LP group after intervention; ▲, HP group at baseline; △, HP group after intervention. *Significant effect of time (ie, resistance training, RT) for both groups combined, $P < 0.05$. †Significant resistance training × group interaction, $P < 0.05$. ‡Significant effect of time within a group, $P < 0.05$.

period provides further caution as to whether the modest differences in protein intake achieved in the present study differentially affected the insulin-mediated control of oral glucose tolerance.

Although the improvements in body composition were not significantly associated with improvements in glucose tolerance, they may have influenced these changes, because body fat has been shown to predict insulin sensitivity in older people (2). The decrease in fat mass combined with increases in total body water and protein mass during weight maintenance suggest that skeletal muscle mass increased during the study (35). The increase in urinary creatinine excretion further supports an increase in skeletal muscle mass with resistance training (51). Given that skeletal muscle is the primary site of glucose uptake (22), this increase in skeletal muscle mass likely contributed to the improvement in glucose tolerance (4). The minimal change in fat-free mass (1.2 kg) but large decrease in insulin during the OGTT (1.9% increase in fat-free mass compared with 35% decrease in plasma insulin

TABLE 3
Changes in glycemic control and insulin sensitivity indexes in the lower-protein (LP) and higher-protein (HP) groups after 12 wk of progressive resistance training[1]

	LP group		HP group	
	Baseline	Change	Baseline	Change
Fructosamine (μmol/L)[2]	222 ± 4	−5 ± 3	231 ± 2	−1 ± 3
Hb A_{1c} (%)[3]	5.1 ± 0.1	0.0 ± 0.0	5.3 ± 0.1	0.0 ± 0.0
ISI composite (dL^2/mg^2)[3,4]	8.9 ± 2.0	1.1 ± 0.7	9.8 ± 1.6	−0.2 ± 1.3
HOMA (dL^2/mg^2)[3,5]	1.2 ± 0.2	0.0 ± 0.1	1.4 ± 0.2	0.2 ± 0.1

[1] All values are $\bar{x}$ ± SEM. The groups did not differ significantly at baseline, $P > 0.05$. ISI, insulin sensitivity index; Hb A_{1c}, glycated hemoglobin; HOMA, homeostatic model assessment.
[2] LP: $n = 7$ men and 7 women; HP: $n = 8$ men and 9 women.
[3] LP: $n = 8$ men and 7 women; HP: $n = 8$ men and 9 women.
[4] Whole-body insulin sensitivity.
[5] Hepatic insulin sensitivity.

TABLE 4
Skeletal muscle insulin signaling protein content in the lower-protein (LP) and higher-protein (HP) groups[1]

	LP group		HP group	
	Baseline	After intervention	Baseline	After intervention
IR[2]	0.90 ± 0.19	1.08 ± 0.21	1.09 ± 0.14	0.97 ± 0.18
IRS-1[3]	1.10 ± 0.27	1.32 ± 0.19	0.97 ± 0.18	0.77 ± 0.19
Akt[4]	0.90 ± 0.09	0.92 ± 0.08	0.92 ± 0.07	1.09 ± 0.09
aPKC[4]	0.69 ± 0.09	0.98 ± 0.11[5]	0.73 ± 0.11	1.23 ± 0.15[5]

[1] All values are $\bar{x}$ ± SEM. IR, insulin receptor; IRS-1, IR substrate 1; aPKC, atypical protein kinase C zeta/lambda (ζ/λ). The groups did not differ significantly at baseline, $P > 0.05$.
[2] LP: $n = 5$ men and 4 women; HP: $n = 5$ men and 6 women.
[3] LP: $n = 3$ men and 4 women; HP: $n = 4$ men and 4 women.
[4] LP: $n = 7$ men and 7 women; HP: $n = 7$ men and 8 women.
[5] Significant effect of time (ie, resistance training), independent of group ($P < 0.05$).

during the OGTT) previously documented after 16 wk of resistance training (10) suggests that it is not just a mass effect, but that skeletal muscle is more insulin sensitive after resistance training.

Resistance training improves insulin signaling in muscle from rodent models but has not been investigated in human models (52). Because we performed OGTT measurements, we were unable to measure the stimulation of the insulin signaling cascade; however, we did measure the skeletal muscle content of IR, IRS-1, Akt, and aPKC ζ/λ, which play important roles in the insulin activation of glucose transport. Previous research has indicated that aerobic (53, 54) and resistance (18, 55) training increase the skeletal muscle content of insulin signaling proteins, though research in older persons is limited. To our knowledge, only one study has documented an increase in skeletal muscle insulin signaling proteins (IR, Akt α/β, glycogen synthase, and glycogen synthase activity) with resistance training in older persons, though resistance training was limited to one-legged exercise and the subjects were all men (18). One recent study in both diabetic and nondiabetic individuals found that endurance training caused an increase in GLUT4 protein content and Akt expression in both diabetic and nondiabetic individuals but that IRS-1 and PI3-kinase were unaffected (54). The authors suggested that exercise improves insulin-stimulated glucose disposal by increasing skeletal muscle GLUT4 expression and does not influence the proximal signaling pathway (54). Similarly, in the subset of samples analyzed for IR and IRS-1, we found no significant change with resistance training, suggesting that resistance training does not influence this portion of the insulin signaling pathway. However, we did find a robust increase in aPKC ζ/λ content after resistance training, which has not been shown before. Atypical PKC ζ/λ has recently emerged as an integral component of insulin signaling to glucose transport (56). Furthermore, aPKC ζ/λ content is reduced in skeletal muscle of humans with obesity and type 2 diabetes (57). An increase in aPKC ζ/λ protein content appears to occur only after chronic resistance training; a previous study showed no difference in aPKC ζ/λ content between endurance trained and sedentary individuals (58). Interestingly, aPKC ζ/λ may also play a role in protein synthesis pathways, because it has been shown to associate with p70S6k, a key protein in muscle hypertrophy (59).

In conclusion, these data indicate that 12 wk of progressive resistance training is an effective stimulus for older, weight-stable persons to achieve an improvement in oral glucose tolerance. The unchanged compared with decreased insulin and C-peptide responses in the HP and LP groups, respectively, after the ingestion of glucose suggests that dietary protein intake influences how the improvement in glucose control was achieved. Results from the present study also showed that resistance training increases skeletal muscle aPKC ζ/λ content, a newly emerging component of the insulin signaling pathway.

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HBI and WWC conceived and designed the experiment. HBI, JWA, and WWC conducted the clinical portion of this study. HBI, JPT, JWA, and WWC participated in sample analysis and data processing. HBI, JPT, and WWC were involved in data interpretation. HBI, JPT, and WWC wrote the manuscript. None of the authors had any personal or financial conflicts of interest.

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