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RESEARCH ARTICLE

Higher protein intake during resistance training does not potentiate strength, but modulates gut microbiota, in middle-aged adults: a randomized control trial

Colleen F. McKenna,[1] Amadeo F. Salvador,[2] Riley L. Hughes,[3] Susannah E. Scaroni,[1] Rafael A. Alamilla,[2] Andrew T. Askow,[2] Scott A. Paluska,[4] Anna C. Dilger,[5] Hannah D. Holscher,[1,2,3] Michael De Lisio,[6] Naiman A. Khan,[1,2,7] and Nicholas A. Burd[1,2]

[1]*Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois;* [2]*Division of Kinesiology and Community Health, University of Illinois at Urbana-Champaign, Urbana, Illinois;* [3]*Division of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, Illinois;* [4]*Division of Family Medicine, University of Illinois at Urbana-Champaign, Urbana, Illinois;* [5]*Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois;* [6]*School of Human Kinetics and Cellular and Molecular Medicine, University of Ottawa, Ontario, Canada; and* [7]*Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, Illinois*

Abstract

Protein intake above the recommended dietary allowance (RDA) and resistance training are known anabolic stimuli to support healthy aging. Specifically, protein supplementation after resistance exercise and nightly are strategies to maximize utilization of protein intake above the RDA in healthy adults. As such, the primary objective was to examine the efficacy of protein supplementation and nutritional counseling resulting in either moderate (MOD: ~1.0 g·kg^{-1}·day^{-1}) or higher (HIGH: ~1.6 g·kg^{-1}·day^{-1}) protein intake during resistance training on strength (one-repetition maximum, 1-RM; isokinetic and isometric peak torque) in healthy middle-aged adults. Exploratory analyses include diet-exercise effects on lean body mass (LBM), clinical biomarkers, gut microbiota, and diet composition. In all, 50 middle-aged adults (age: 50 ± 8 yr, BMI: 27.2 ± 4.1 kg/m^2) were randomized to either MOD or HIGH protein intake during a 10-wk resistance training program (3 × wk). Participants received dietary counseling and consumed either 15 g (MOD) or 30 g (HIGH) of protein from lean beef in the immediate postexercise period and each evening. Maximal strength (1-RM) for all upper and lower body exercises significantly increased with no effect of protein intake ($P < 0.050$). There was a main effect of time for LBM ($P < 0.005$). Cardiovascular, renal, or glycemic biomarkers were not affected by the intervention. Gut microbiota were associated with several health outcomes ($P < 0.050$). In conclusion, higher protein intake above moderate amounts does not potentiate resistance training adaptations in previously untrained middle-aged adults. This trial was registered at clinicaltrials.gov as NCT03029975.

NEW & NOTEWORTHY Our research evaluates the efficacy of higher in comparison with moderate animal-based protein intake on resistance exercise training-induced muscle strength, clinical biomarkers, and gut microbiota in middle-aged adults through a dietary counseling-controlled intervention. Higher protein intake did not potentiate training adaptations, nor did the intervention effect disease biomarkers. Both diet and exercise modified gut microbiota composition. Collectively, moderate amounts of high-quality, animal-based protein is sufficient to promote resistance exercise adaptations at the onset of aging.

gut microbiota; hypertrophy; insulin resistance; red meat

INTRODUCTION

Manipulation of dietary protein, especially when combined with regular resistance exercise, is an established strategy to promote healthy aging through skeletal muscle mass and strength maintenance and/or accretion (1). Past efforts have shown that the recommended dietary allowance (RDA) for protein intake of 0.8 g protein·kg body weight^{-1}·day^{-1} for adults may be inadequate to support muscle health with aging (2, 3). Evidence suggests that protein needs may be elevated above the RDA to ≥1.2 g·kg^{-1}·day^{-1} in healthy older adults (3). Moreover, loss of lean body mass with age is mitigated when older adults consume ≥1.2 g·kg^{-1}·day^{-1} (4). Protein needs for muscle health may further deviate from the current RDA within a physically active lifestyle, which includes the performance of regular resistance exercise. For example, resistance training is a crucial anabolic stimulus to prevent aging-related muscle mass loss (5), with current evidence suggesting that protein intake up to 1.6 g·kg^{-1}·day^{-1} may be required to maximize lean mass gain in healthy resistance-trained young adults (6). Hence, protein consumption may be a modifiable dietary factor to offset age-related

Correspondence: N. A. Burd (naburd@illinois.edu).
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loss of muscle mass and strength. Even though aging-related muscle mass and strength loss can manifest as early as the fourth to fifth decade of life (7), the optimal level of protein intake to enhance resistance training adaptations in middle-aged adults and help delay aging-associated muscle health declinations remains unclear.

The contribution of dietary protein to muscle health and function not only depends on total quantity but also food source and overall diet quality (8). Although protein foods with high biological availability (e.g., animal-based) strongly stimulate postprandial muscle protein synthesis rates, their impact on cardiometabolic outcomes remains controversial (9–11). Red meat receives the majority of the scrutiny. Still, minimally processed versions effectively stimulate postprandial muscle protein synthesis rates, as this source delivers target amounts of essential amino acids to skeletal muscle tissue (12) without compromising chronic disease risk (13). A commonly-implicated mediator of this diet-disease interaction is the gut microbiome, a collection of trillions of microorganisms in the gastrointestinal tract (14, 15). Although the primary fuel source of the gut microbiota is nondigestible carbohydrate, fermentation of undigested peptides and amino acids produces a diverse array of bioactive compounds that impact host health (16–20). Exercise impacts the gut microbiota (14); however, the bulk of this work focuses only on endurance exercise. Thus, it is of interest to explore the effects of a combined protein and resistance exercise intervention on the gut microbiota composition and associated health and performance effects.

Therefore, the primary purpose of this study was to compare resistance training-induced adaptations in middle-aged adults consuming moderate amounts of protein [MOD; ~1.0 $g \cdot kg^{-1} \cdot day^{-1}$; representing the general protein need of muscle with age (3, 4)] or higher protein amounts [HIGH; ~1.6 $g \cdot kg^{-1} \cdot day^{-1}$; representing the dose that may be required for maximum resistance training adaptations (6)]. Participants were counseled for adherence to the US-style healthy eating pattern, as it incorporates high-quality animal protein foods (21), and were provided with 15 g (MOD) or 30 g (HIGH) of protein from lean beef in the immediate postexercise period and each evening. For our primary outcome, we hypothesized that high protein intake would augment resistance training-induced changes in muscle strength when compared with moderate protein intake in healthy middle-aged men and women. Additional exploratory outcomes include changes in body composition, chronic disease biomarkers, diet composition, and gut microbiota composition to further understand the contribution of high protein diets to disease risk in the context of habitual resistance exercise in middle-aged adults.

METHODS

Participants

A total of 50 healthy overweight middle-aged men and women were enrolled to participate in this randomized, parallel-group trial. Self-reported health and exercise history questionnaires, anthropometrics, and blood pressure were evaluated for study inclusion. Eligible participants were individuals between 40–64 yr of age, as this range represents manifestation of early onset of muscle mass and strength loss (7). Those without chronic cardiometabolic diseases, BMI $\geq$ 18.5 and $<$35 kg/m^2, or not currently ($\geq$1 yr) participating in resistance exercise training were eligible. Individuals with uncontrolled hypertension, on confounding medication or dietary supplements (i.e., those known to affect strength, muscle mass, or other measured outcomes), current participation in an exercise program (resistance or other), exercise or dietary restrictions, musculoskeletal conditions or injuries sustained $\leq$1 yr, excessive alcohol intake (e.g., $>$10 drinks/wk), abnormal protein intake ($<$0.66 or $>$1.80 $g \cdot kg^{-1} \cdot day^{-1}$) or history of tobacco or marijuana use were excluded from participating in this study.

Ethical Approval

All participants were informed of the experimental procedures and potential risks before providing their written informed consent to participate during the screening visit. The study was approved by the University of Illinois Institutional Review Board and conformed to the standards for the use of human participants in research as outline in the *Declaration of Helsinki*. This trial was registered at ClinicalTrials.gov as NCT03029975 and is reported in accordance with CONSORT guidelines (22).

Experimental Design

An overview of the study timeline and frequency of measured outcomes is depicted in Fig. 1. Specifically, participants completed a 10-wk progressive strength training coupled with a dietary counseling-controlled intervention (detailed in METHODS: *Diet Counseling Control*). The screening visit included informed consent, self-reported questionnaires, blood pressure assessment, and anthropometrics to evaluate eligibility. An initial strength test session was performed during this appointment to familiarize participants with the protocol and equipment. Baseline and postintervention testing consisted of participants arriving to the laboratory after an overnight fast to measure body composition assessed by dual-energy X-ray absorptiometry (DXA), resting blood pressure, fasted blood collection, oral glucose tolerance test (OGTT), and muscle strength and performance (procedures detailed in respective METHODS subsections). Order of assessment was consistent between individuals and across time points. After completion of baseline measures, participants were randomized to consume either MOD (0.8–1.0 $g \cdot kg^{-1} \cdot day^{-1}$) or HIGH (1.6–1.8 $g \cdot kg^{-1} \cdot day^{-1}$) protein within a weight-maintenance diet during 10 wk of supervised resistance training. Randomization was achieved through an automated spreadsheet to generate a 1:1 ratio of those receiving the allocated intervention (i.e., still enrolled upon resistance training commencement). The spreadsheet stratified groups by age, sex, BMI, and baseline strength. Lead investigator/research dietitian (CFM) managed group allocation, whereas investigators performing data collection and sample processing were blinded to group allocation.

Protein Supplementation Intervention

The research team provided isocaloric meals during key anabolic windows (i.e., postexercise, before sleep) to facilitate the muscle adaptive response to resistance exercise (23,

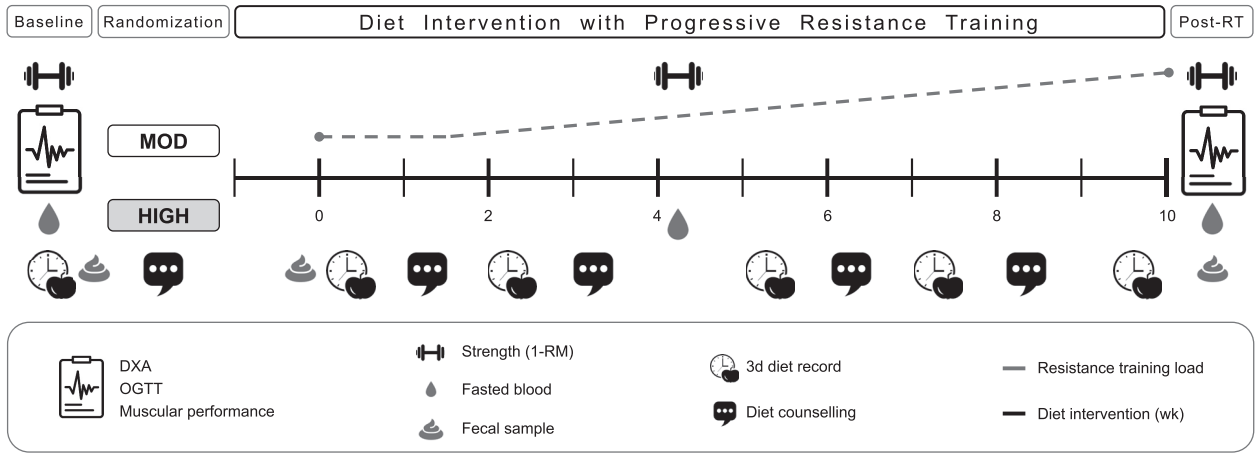

Figure 1. Experimental design. Baseline measures were tested before diet randomization. Diet intervention began before initiating the resistance exercise program at *wk −1* and resistance training began at *wk 0*. Postresistance training measures were assessed at *wk 10*. DXA, dual-energy x-ray absorptiometry; HIGH, higher protein intake; MOD, moderate protein intake; OGTT, oral glucose tolerance test; RT, resistance training; 1-RM, one-repetition maximum; 3d, 3-day.

24). Immediately after every exercise session (3 days/wk), participants consumed an isocaloric meal consisting of minimally processed beef (97.4% lean) and a carbohydrate beverage under research team supervision. Specifically, participants randomized to MOD or HIGH either consumed 3 oz (16 g protein) or 6 oz (32 g protein) of the minced beef steak, respectively. The beef was processed and individually packaged in respective portions by the University of Illinois Meat Science Laboratory after protein quantity was measured by nitrogen content using the combustion method (method 990.03; Association of Official Analytical Chemists International, 2000; TruMac; LECO Corporation). Each beef serving was freshly thawed and cooked until the inner temperature reached at least 65°C for immediate consumption after every exercise session. Postexercise energy intake was matched between protein conditions with additional beef tallow (2 g) added to the MOD 3 oz beef steak, and a dosed dextrose (i.e., MOD, 30 g; HIGH, 12 g) beverage. The participants were requested to not consume any additional energy-containing foods or drink for 2 h after the provided postexercise meal. In addition, participants were provided with nightly doses (7 day/wk) of beef isolate protein powder (True Nutrition, Vista, CA) to be consumed 1–2 h before sleep. Each beverage provided either 15 or 30 g of protein dissolved in water for MOD or HIGH, respectively. Nightly supplement beverages were isocaloric with MOD receiving an extra 15 g of maltodextrin for compensation. Compliance was assessed by nightly logs of supplement consumption returned to the research team weekly.

Diet Counseling Control

Dietary intake was assessed by 3-day (3d) diet records using the automated self-administered 24-h (ASA24) dietary assessment tool (version 2016, National Cancer Institute, Bethesda, MD). Research staff, trained and supervised by a registered dietitian, provided oral and written instructions on accurate recording (e.g., portion sizes, method of cooking) to the participants. These records were completed on 3 consecutive days, including 1 weekend day (i.e., Sunday–Tuesday). After baseline diet records were assessed for eligibility, each participant received dietary counseling and educational materials from the research dietitian. The participants received guidance on how to adhere to their randomized protein density goal. Specifically, they were counseled to preferentially consume high-quality, animal-based protein foods to achieve their daily protein targets. Weight-maintenance daily energy intake goal was calculated with the Institute of Medicine predictive energy equation (59). This was within the greater context of a healthy eating pattern that incorporates nutrient-dense options of typically consumed foods in appropriate portions as outlined in the 2015 Dietary Guidelines for Americans (DGA) (21). In addition, participants were asked to discontinue use of nutritional supplements, nonprescription medication, and alcohol for 4 wk before, and throughout, the 10-wk intervention. The diet intervention was initiated 1 wk before the resistance training program (−1 wk) to habituate individuals to their prescribed diet (Fig. 1). Additional 3d diet records were completed every other week during the resistance training program (1 weekend day + 1 training weekday + 1 nontraining weekday), with a follow-up counselling session occurring the next week to promote protein and overall diet adherence. Given the use of dietary counselling, participants were not blinded to their personal protein goal.

Resistance Training

Participants engaged in 10 wk of supervised progressive whole body resistance training during the diet intervention (Fig. 1). Exercise sessions occurred 3 days/wk with at least one rest day in between (e.g., Mondays, Wednesdays, and Fridays). Each session started with a 5 min warm-up on a cycle ergometer. Training consisted of a warm-up (2 sets × 10 repetitions at 30% and 75% of the working load) followed by training sets (3 × 10) for each of the five exercises. Leg press, leg curl, and leg extension exercises were preformed every session, with two upper body sets alternating between lower body exercises. Upper body exercises alternated each session either as push (chest and shoulder presses) or pull (seated row and bicep curls). All exercises were performed

on guided-motion machines with the exception of barbell bicep curls.

Training intensity was determined by lower body 1-repetition maximum (RM) and upper body 10-RM (detailed in METHODS: *Muscular Strength Assessments*). During the first two weeks (0–1 wk), participants performed training sets for each exercise at 65% 1-RM. After 2 wk of training habituation, the load was linearly progressed when participants successful completed 3×10 working sets while maintaining proper form and cadence. Sessions were supervised with continuous instruction on proper technique and form throughout the intervention.

Muscle Strength Assessments

Maximal strength was evaluated at baseline, midpoint (*wk 4*), and postintervention (Fig. 1). Participants completed a 1-RM familiarization test during the screening visit. Before the baseline maximal strength tests, participants refrained from moderate-to-vigorous physical activity for 72 h and caffeine for 24 h. Participants performed 1-RM for lower body exercises, and 10-RM for upper body exercises. 1-RM upper body strength was calculated from tested 10-RM to reduce injury risk in participants (25). Lower body 1-RM was performed on leg press, leg curl, and leg extension machines using the established procedures (26). Briefly, a repetition was deemed successful when the participant was able to move the weight through the full range of motion as judged by the research staff. The 1-RM was determined within three attempts, with a 3-min rest between attempts. Staff provided consistent verbal encouragement to promote maximal effort. Similar testing procedures were used for upper body 10-RM assessment with seated chest press, seated shoulder press, seated rows, and bicep curls as previously performed (25). All machine settings were recorded for each participant to ensure proper placement for strength testing and training sessions. A Biodex dynamometer (Biodex System 3, Shirley, NY) was used to assess muscle function by isometric maximal voluntary contraction (MVC) at 60° and isokinetic peak torque at 60°/s and 180°/s for the dominant knee with a familiarization test before data collection as previously described (27, 28). Gait speed (29) and handgrip strength of the dominant hand (Sammons Preston Rolyan, Bolingbrook, IL) were also measured.

Body Composition

DXA scans (Hologic QDR 4500 A, Bedford, MA) were performed at baseline testing and postintervention to evaluate changes in body composition and bone health. Participants were instructed to refrain from strenuous exercise for 72 h prior and arrived the laboratory in the morning after an overnight fast. Upon arrival, participants were asked to void their bladder, and metal and other personal effects that could interfere with the analysis were removed from the person before initiating the scan. Measures of body composition were determined as follows: total body fat mass directly quantified; lean body mass (LBM) quantified from lean soft tissue mass; skeletal muscle index (SMI, %) = extremity LBM $\times$ body weight$^{-1} \times 100\%$; lean index (kg/m^2) = LBM $\times$ height^{-2}; appendicular lean index (kg/m^2) = extremity LBM $\times$ height^{-2} (30).

Blood Collection and Analyses

Venous blood was collected after an overnight fast and 72 h of no moderate-to-vigorous physical activity at baseline, mid, and postintervention (Fig. 1). At baseline and postintervention, fasted blood collection (0 min) was immediately followed by an OGTT. In brief, A Teflon catheter was inserted into an antecubital vein for repeated blood sampling and remained patent by a 0.9% saline drip. Participants ingested a 75 g dextrose beverage with blood sampling at 15, 30, 45, 60, 90, and 120 min after dextrose. Immediately after collection, blood samples were centrifuged at 3,600 rpm for 10 min at 4°C. Aliquots of plasma were frozen and stored at −80°C until further analyses. Glucose tolerance outcomes were determined from EDTA-treated samples for whole blood glucose (YSI 2900, Yellow Springs, OH) and plasma insulin by commercial ELISA (80-INSHU-E01.1, Alpco, Salem, NH). HOMA-IR, Matsuda Index, and Insulinogenic Index were calculated as previously described (31–33). Metabolic and lipid panels were measured from lithium heparin-treated plasma (0 min) (Abaxis, Union City, CA). High-sensitivity C-reactive protein (CRP) (30-9710 s, Alpco, Salem, NH) was measured by commercial ELISAs from EDTA-treated plasma (0 min).

Diet Compositional Analysis

All diet records were analyzed by a registered dietitian and adherence to a healthy eating pattern was evaluated through the Healthy Eating Index-2015 (HEI). HEI total score, component scores, percent added sugar, and percent saturated fat were calculated by a publicly-available SAS code (HEI-2015 ASA24-2016 per person, National Cancer Institute, Bethesda, MD) using SAS University Edition. Dietary protein variables were extracted from ASA24 outputs and determined as follows: total protein foods serving density [ounce-equivalents (oz-eq)/1,000 kcal] includes total red meat, poultry, organ meat, cured meat, seafood, eggs, soy, legumes, and nuts and seeds reported serving intake relative to every 1,000 kcal consumed. Animal-based protein food serving density (oz-eq/1,000 kcal) includes only red meat, poultry, organ meat, cured meat, seafood, and eggs; plant-based protein foods serving density (oz-eq/1,000 kcal) includes only soy, nuts and seeds, and legumes; red meat protein foods serving density (oz-eq/1,000 kcal) refers to beef, veal, pork, lamb, and game meat and excludes organ meat and cured meat.

Gut Microbiota Analysis

An exploratory outcome of gut microbiota was added after trial commencement. Fecal samples were collected at baseline, intervention onset (i.e., after the 1-wk dietary habituation), and postintervention (i.e., after the 10-wk intervention). Upon collection, samples were homogenized, placed in aliquots, and stored at −80°C. Fecal DNA was isolated with the use of a PowerLyzer PowerSoil DNA Isolation Kit (MoBio Laboratories) according to the manufacturer's instructions. Bacterial (16S V4 region, 505f/806r) (34) genes were amplified on a Fluidigm Access Array then sequenced on a MiSeq with the use of v3 reagents (Illumina, Inc.) in the W. M. Keck Center for Biotechnology, University of Illinois, as previously described (35).

Sequence data were analyzed with QIIME 2 version 2019.10 (36). Forward reads were imported and demultiplexed before

Table 1. *Baseline participant characteristics*

Baseline Characteristics	MOD (n = 22)	HIGH (n = 28)
Demographics		
Sex, n	9 M, 13 F	14 M, 14 F
Age, yr	50 ± 8	49 ± 7
Body weight, kg	81.4 ± 16.4	81.3 ± 14.0
BMI, kg/m^2	27.5 ± 4.6	27.6 ± 4.0
Reported energy intake		
Total energy, kcal/day	1,960 ± 789	2,220 ± 564
Total carbohydrate, g/day	223 ± 106	249 ± 90
Total fat, g/day	79 ± 29	97 ± 39
Dietary protein intake		
Total protein, g/day	83 ± 33	91 ± 31
Relative protein, g·kg^{-1}·day^{-1}	1.04 ± 0.33	1.12 ± 0.36

Baseline data are presented as means ± SD. No differences between groups at baseline by independent Student's t test (P > 0.05). Relative protein intake to body weight. F, female; HIGH, higher protein intake; M, male; MOD, moderate protein intake. n = Participants analyzed per group.

denoising and sequences were classified using DADA2 (37) and SILVA (release 132), respectively (38). Comparisons of α-diversity (Faith's PD and Shannon) and β-diversity (Weighted and Unweighted UniFrac and DEICODE) between treatments and timepoints were performed in QIIME 2, using Kruskal–Wallis and PERMANOVA, respectively. To assess changes in specific taxa, data were exported and analyzed in R (v. 3.6.1). DESEq2 (v. 1.26.0) (39) was used to identify differentially abundant taxa between treatments and timepoints. Canonical correlation (40) was then utilized to determine correlations of significant DESeq2 taxa with linear combinations of health and performance variables, split into subsets of related outcomes. DESeq2 taxa showing large canonical coefficients (>|30|) were then assessed for significant correlations with individual health and performance outcomes using linear mixed-effects models with model terms as described below. Microbiota relative abundances greater than zero were log transformed to address outliers and an additional indicator term was added to the model indicating zero values. Values were not adjusted for multiple testing due to the preliminary nature of these secondary outcomes (41).

Statistical Analysis

A power analysis (42) was used to determine sufficient sample size to detect a difference in the 1-RM muscular strength in response to protein supplementation during resistance training. Previously published data from similar research (43) indicates that n = 18 is at β = 0.80 and α = 0.050. Considering a dropout rate of 20%, at least 20 participants were recruited per group.

Intent-to-treat (ITT) analysis of all randomized participants was performed on primary and secondary outcomes. Missing data was handled without ad hoc imputation (44, 45). Linear mixed-effects models were used to assess group (MOD, HIGH) × time (baseline, post) comparisons for the primary outcome and applicable secondary outcomes. Time, group, and group × time were fixed effects. Sex (M, F), a predefined categorical control variable, was also a fixed effect. Participant intercept was a random effect. Exploratory comparisons of energy × adherence (study goal, reported kcal/day), group × protein food source (animal-based, plant-based), and microbiome-related outputs were also assessed by linear mixed-effect models. Bonferroni adjustment was applied for post hoc multiple comparisons. Independent Student's t test was used for group comparisons: baseline characteristics, intervention diet composition and HEI, and total training volume (completed exercises × sets × repetitions). All analyses were performed with IBM SPSS Statistics (v. 23, IBM Corporation, Armonk, NY). The significant difference level was set as $P < 0.05$. Data are presented as means ± SD or mean difference (95% confidence interval).

RESULTS

Participants

Participant baseline characteristics are displayed in Table 1. From the 50 enrolled participants, nine dropped out before receiving allocated intervention; thus, only 41 initiated the diet intervention and training program. During the intervention, there were two injury-related dropouts (Fig. 1). Flow of participants through the intervention is depicted in Fig. 2. At baseline, there were no differences in age, body mass, or BMI. Diet recording compliance did not differ between MOD (92 ± 18%) and HIGH (97 ± 12%; P = 0.20). Exercise session attendance (MOD: 87 ± 9%; HIGH: 89 ± 6%; P = 0.48) and total training volume (MOD: 177,841 ± 67,740 kg; HIGH: 191,304 ± 55,109 kg; P = 0.50) were not different between groups.

Muscle Strength and Performance

There were no baseline differences in muscle strength or performance between the MOD and HIGH (Table 2). 1-RM for all upper and lower body exercises increased with no effect of protein amount after the intervention (all, P <

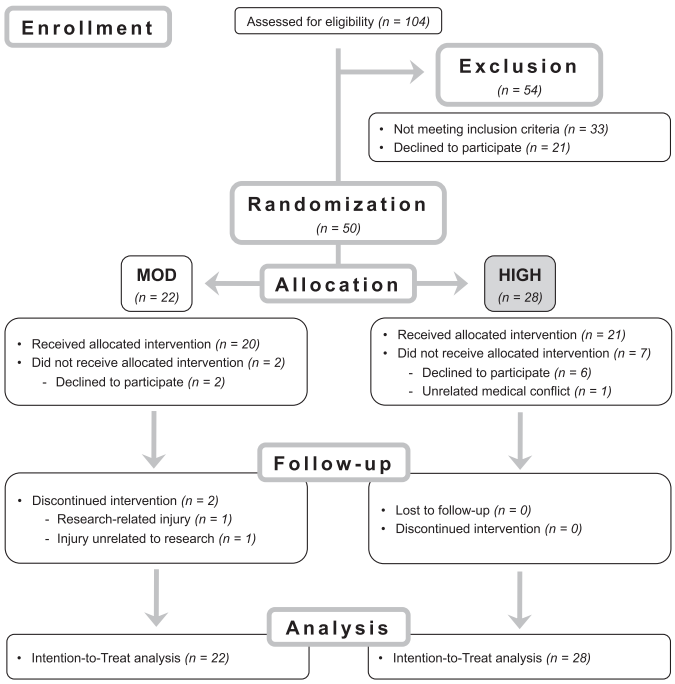

Figure 2. CONSORT flow diagram. 10-Wk resistance exercise intervention consuming either MOD (1.16 ± 0.19 g·kg^{-1}·day^{-1}) or HIGH (1.68 ± 0.26 g·kg^{-1}·day^{-1}) protein in middle-aged adults. HIGH, higher protein intake; MOD, moderate protein intake. n = Participants per parameter.

Table 2. *Muscular strength response to 10 wk of resistance training and dietary protein manipulation of moderate and high intake in middle-aged adults*

Muscular Strength	MOD (n = 22)		HIGH (n = 28)		Intervention Outcome	
	Baseline	Post Difference	Baseline	Post Difference	Group Difference	Interaction[1]
1-RM, kg						
Chest press	35 ± 18	9* (6, 13)	33 ± 15	12* (9, 15)	−2 (−4, 8)	0.249
Bicep curl	15 ± 6	5* (3, 6)	15 ± 6	7* (6, 9)	2 (−1, 4)	0.007*
Shoulder press	15 ± 10	8* (7, 10)	14 ± 8	10* (8, 11)	−1 (−2, 4)	0.201
Seated row	37 ± 15	9* (7, 12)	35 ± 13	12* (9, 14)	−2 (−3, 7)	0.187
Leg press	105 ± 40	71* (51, 92)	111 ± 39	54* (35, 73)	−16 (−10, 41)	0.218
Leg curl	58 ± 16	18* (13, 22)	65 ± 21	21* (17, 25)	8 (1, 16)	0.258
Leg extension	66 ± 17	33* (24, 42)	69 ± 17	35* (27, 44)	3 (−7, 13)	0.671
Isokinetic peak torque, N·m/kg						
Knee extension 60°/s	16.25 ± 3.48	−0.17 (−1.31, 0.97)	17.08 ± 3.36	0.43 (−0.63, 1.49)	1.32 (−0.78, 3.43)	0.438
Knee flexion 60°/s	10.32 ± 2.22	1.39* (0.49, 2.30)	10.91 ± 2.70	0.71* (−0.13, 1.55)	−0.15 (−1.60, 1.31)	0.271
Knee extension 180°/s	8.57 ± 3.49	0.53* (−0.78, 1.83)	9.56 ± 2.62	2.06* (0.82, 3.29)	2.42 (0.43, 4.41)	0.092
Knee flexion 180°/s	6.77 ± 2.10	1.05* (0.23, 1.88)	7.72 ± 1.70	0.96* (0.18, 1.74)	0.82 (−0.46, 2.10)	0.873
Isometric peak torque, N·m/kg						
Knee extension 60°	19.24 ± 4.11	−0.19 (−1.79, 1.42)	19.60 ± 3.75	−0.02 (−1.52, 1.47)	0.54 (−2.01, 3.09)	0.882
Knee flexion 60°	10.95 ± 2.64	0.83* (−0.19, 1.84)	10.87 ± 2.33	1.08* (0.11, 2.05)	0.00 (−1.61, 1.62)	0.716
Functional capacity						
Handgrip strength, kg	41.79 ± 13.34	−0.70 (−3.38, 1.98)	41.18 ± 11.68	1.47 (−0.98, 3.92)	−0.70 (−6.46, 5.05)	0.233
4-m gait speed, s	3.79 ± 0.70	−0.50* (−0.91, −0.10)	3.40 ± 0.71	−0.43* (−0.81, −0.05)	−0.25 (−0.66, 0.16)	0.791

Baseline data are presented as means ± SD. Within-group and between-group outcomes are mean differences (95% confidence interval). No differences between groups at baseline by independent Student's t test ($P > 0.05$). Intervention outcomes were assessed by the linear mixed model. *Main effect of time ($P < 0.05$). [1]Group × time fixed effect P value. HIGH, higher protein intake; MOD, moderate higher protein intake; 1-RM, one-repetition maximum; isokinetic and isometric peak torque are relative to dominant leg lean body mass. n = Participants analyzed per group.

0.001, Table 2). A group × time interaction was observed for bicep curl 1-RM (P = 0.024), with both groups increasing over time (both $P < 0.001$) but no difference between groups at either baseline (P = 0.58) or postintervention (P = 0.19). Likewise, isokinetic extension and flexion peak torque increased in contractions at 60°/s and 180°/s for both groups (main effect of time: $P < 0.01$), with the exception of 60°/s extension, which was unaltered by the intervention (Table 2). Isometric knee flexion at 60° improved over time (P = 0.009) with no effect of group, but no change in isometric knee extension was observed. Gait time was improved with the intervention regardless of the amount of protein consumed (P = 0.003, Table 2).

Anthropometrics and Body Composition

Body composition was not different between groups at baseline (Table 3). Total body mass increased to 1.3 (0.0, 2.6) kg in MOD and 1.5 (0.1, 1.0) kg in HIGH, with no difference between groups (main effect of time: P = 0.003). There was a main effect of time for measures of LBM (whole body, SMI, lean index, appendicular lean index, all $P < 0.001$, Table 3). Body adiposity and bone mineral density and content did not change in response to the intervention irrespective of protein intake (Table 3).

Health Biomarkers and Glycemic Control

At baseline, health status did not differ between groups (Table 4). Blood urea nitrogen (BUN) concentration and plasma creatinine (Cr) increased in response to the intervention (main effect of time: BUN P = 0.003, Cr P = 0.010), with no effect of protein amount. However, BUN/Cr was not altered by the intervention and values remained within normal limits. There were no changes in blood pressure, plasma lipids, or CRP in response to the intervention (Table 4). Fasting blood glucose concentrations did not change over time between the groups. Likewise, there was no differences in blood glucose concentrations 2 h after 75 g dextrose consumption before and after the 10-wk intervention regardless of group. Insulin resistance, peripheral insulin sensitivity, and β-cell function (as estimated by HOMA-IR, Matsuda index, and insulinogenic index, respectively) did not change in response to the intervention.

Diet Composition

At baseline, total dietary carbohydrate, fat, protein, and overall energy intake were not different between groups (Table 1). Throughout the intervention (i.e., *weeks 0*, *2*, *5*, *7*, and *9*), reported intakes (e.g., total energy, absolute carbohydrate, and fat) were not different within groups across time. As intended, absolute and relative protein intake was greater in HIGH throughout the intervention when compared with MOD (both $P < 0.001$, Supplemental Fig. S1; see https://doi.org/10.6084/m9.figshare.13230671.v1) Combined protein and dietary intake throughout the intervention are presented in Fig. 3 and Table 5, respectively. It is worth noting that despite continued counseling to eat within recommended protein goal ranges, the MOD group consumed a more protein-dense diet than intended. Despite a goal range of 0.8–1.0 g·kg^{-1}·day^{-1}, reported protein intake exceeded the upper threshold with a mean intake of 1.16 ± 0.19 g·kg^{-1}·day^{-1} during the intervention. Nevertheless, HIGH effectively consumed greater amounts of protein (1.68 ± 0.26 g·kg^{-1}·day^{-1}, $P < 0.001$; Fig. 3*A*) than MOD, with both groups consuming more animal-based than plant-based protein foods during the intervention (both, $P < 0.001$; Fig. 3*B*). Energy, macronutrient, and diet quality are presented in Table 5. There was a main effect of energy adherence (i.e., reported vs. study goal)

Table 3. *Body composition response to 10 wk of resistance training and dietary protein manipulation of moderate and high intake in middle-aged adults*

Body Composition	MOD (n = 22)		HIGH (n = 28)		Intervention Outcome	
	Baseline	Post Difference	Baseline	Post Difference	Group Difference	Interaction[1]
Lean body mass (LBM)						
Whole body LBM, kg	49.43 ± 12.79	1.86* (0.76, 2.97)	51.43 ± 11.55	0.94* (−0.11, 1.98)	−1.67 (−5.46, 2.13)	0.224
Whole body lean index, kg/m^2	16.75 ± 3.69	0.64* (0.28, 1.00)	17.39 ± 3.04	0.32* (−0.02, 0.66)	−0.57 (−1.83, 0.70)	0.208
Appendicular lean index, kg/m^2	7.18 ± 2.10	0.45* (0.26, 0.64)	7.75 ± 1.62	0.29* (0.11, 0.46)	−0.05 (−0.75, 0.66)	0.203
Skeletal muscle index, %	26.22 ± 4.37	1.19* (0.56, 1.82)	28.15 ± 4.55	0.59* (−0.01, 1.18)	0.29 (−1.26, 1.84)	0.167
Body adiposity						
Whole body fat mass, kg	28.22 ± 7.89	−0.28 (−1.28, 0.72)	27.17 ± 8.94	0.40 (−0.55, 1.34)	0.01 (−4.38, 4.40)	0.323
Body fat, %	35.07 ± 8.39	−0.76 (−1.79, 0.28)	33.28 ± 9.12	0.13 (−0.85, 1.10)	0.36 (−2.88, 3.59)	0.217
Waist:hip, ratio	0.98 ± 0.18	0.02 (−0.03, 0.06)	0.99 ± 0.17	−0.02 (−0.06, 0.02)	−0.06 (−0.17, 0.05)	0.190
Skeletal						
Bone mineral content, g	2,440 ± 545	−12 (−4, 46)	2,502 ± 439	−8 (−42, 47)	−55 (−276, 166)	0.800
Bone mineral density, g/cm^3	1.14 ± 0.13	0.00 (−0.01, 0.01)	1.16 ± 0.10	−0.01 (−0.02, 0.01)	0.00 (−0.07, 0.07)	0.601

Baseline data are presented as means ± SD. Within-group and between-group outcomes are mean differences (95% confidence interval). No differences between groups at baseline by independent Student's t test ($P > 0.05$). Intervention outcomes were assessed by the linear mixed model. *Main effect of time ($P < 0.05$). [1]Group × time fixed effect P value. Lean index = LBM × height^{-2}; appendicular lean index = extremity LBM × height^{-2}; skeletal muscle index = extremity LBM × body weight × 100%; percent body fat (%) = fat mass × body weight^{-1} × 100%. HIGH, higher protein intake; MOD, moderate protein intake. n = Participants analyzed per group.

regardless of protein group, with no difference in reported daily intake (P = 0.076) between protein groups. Reported dietary fat intake was different between groups (P = 0.005). There was no difference in total HEI score, nor component scores (all $P > 0.05$, Table 3), with the exception of Added Sugars component score being higher in HIGH when compared with MOD ($P < 0.001$). Total Protein Foods was the only component score where all participants met the DGA requirement with a maximum score of 5.0.

Gut Microbiota Composition

There was a difference between groups in Unweighted UniFrac β-diversity (PERMANOVA P = 0.007) but not in Weighted UniFrac or DEICODE metrics of β-diversity or in measures of α-diversity ($P > 0.05$). DESeq2 analysis showed that, after 1 wk of dietary habituation, participants in the HIGH protein group had decreased abundance of *Veillonellaceae* ($P < 0.001$), *Akkermansia* (P = 0.04), *Eggerthellaceae* ($P < 0.001$), and *Ruminococcaceae* (P = 0.01; Table 6). *Erysipelotrichaceae* decreased after the resistance training intervention in both groups ($P < 0.001$). In the HIGH protein group, the resistance training intervention increased the abundance of *Eggerthellaceae* ($P < 0.001$), *Veillonellaceae* ($P < 0.001$), and *Akkermansia* (P = 0.05). In the MOD protein group, only the increase in *Veillonellaceae* after resistance training was significant (P = 0.01). A full list of DESeq2 results is shown in

Table 4. *Chronic disease biomarkers response to 10 wk of resistance training and dietary protein manipulation of moderate and high intake in middle-aged adults*

Participant Characteristics	MOD (n = 22)		HIGH (n = 28)		Intervention Outcome	
	Baseline	Post Difference	Baseline	Post Difference	Group Difference	Interaction[1]
Cardiovascular						
Systolic BP, mmHg	131.0 ± 9.4	0.5 (−6.3, 7.2)	125.8 ± 11.9	−1.9 (−5.5, 1.7)	−8.0 (−16.9, 0.9)	0.530
Diastolic BP, mmHg	82.3 ± 7.2	−1.5 (−5.5, 2.5)	78.7 ± 8.5	−0.6 (−3.7, 2.4)	−3.2 (−9.1, 2.7)	0.722
Total cholesterol, mg/dL	187.4 ± 17.7	−2.9 (−12.1, 6.4)	195.4 ± 27.8	−5.7 (−13.1, 1.7)	1.8 (−16.2, 19.7)	0.626
LDL, mg/dL	110.9 ± 19.0	−4.6 (−11.3, 2.2)	120.2 ± 24.1	0.1 (−5.9, 6.2)	15.2 (1.5, 28.8)	0.299
HDL, mg/dL	59.1 ± 14.2	−2.4 (−6.0, 1.2)	53.4 ± 13.3	−2.0 (−5.1, 1.1)	−4.6 (−11.7, 2.6)	0.862
Triacylglycerol, mg/dL	102.6 ± 31.6	−7.8 (−23.3, 7.6)	111.4 ± 64.7	−8.1 (−21.2, 5.0)	16.6 (−12.8, 46.0)	0.977
Renal function						
BUN, mg/dL	12.53 ± 3.80	1.31* (−0.61, 3.23)	13.67 ± 3.12	2.70* (1.01, 4.40)	2.54 (0.27, 4.82)	0.077
Creatinine, mg/dL	0.81 ± 0.26	0.08* (−0.06, 0.21)	0.85 ± 0.18	0.16* (0.04, 0.28)	0.07 (−0.07, 0.21)	0.353
BUN:creatinine, ratio	15.32 ± 4.46	0.84 (−1.06, 2.74)	15.78 ± 4.47	−0.37 (−2.48, 1.74)	−0.02 (−2.94, 2.91)	0.391
Glycemic control						
Fasting glucose, mg/dL	77.3 ± 9.5	1.5 (−3.1, 6.0)	78.3 ± 10.05	2.1 (−2.7, 6.9)	1.5 (−4.4, 7.3)	0.848
2 h OGTT, mg/dL	95.0 ± 18.7	−9.1 (−22.2, 4.0)	91.3 ± 29.19	2.9 (−9.5, 15.2)	10.2 (−5.9, 26.4)	0.183
HOMA-IR	2.52 ± 2.25	0.19 (−0.21, 0.60)	3.32 ± 4.57	−0.07 (−0.44, 0.30)	0.58 (−1.71, 2.86)	0.335
Matsuda index	5.23 ± 2.72	0.02 (−1.07, 1.10)	6.33 ± 3.34	−0.71 (−1.73, 0.31)	0.63 (−1.45, 2.71)	0.329
Insulinogenic index	1.25 ± 1.11	−0.16 (−0.62, 0.29)	1.12 ± 1.02	−0.16 (−0.58, 0.25)	−0.07 (−0.62, 0.48)	0.996
Inflammation	2.08 ± 2.12	0.39 (−0.09, 0.88)	1.73 ± 1.53	0.07 (−0.27, 0.40)	−0.78 (−2.24, 0.69)	0.263
CRP, mg/L						

Baseline data are presented as means ± SD. Within-group and between-group outcomes are mean differences (95% confidence interval). No differences between groups at baseline by independent Student's t test ($P > 0.05$). Intervention outcomes were assessed by the linear mixed model. *Main effect of time ($P < 0.05$). [1]Group × time fixed effect P value. BP, blood pressure; BUN, blood urea nitrogen; CRP, C-reactive protein; HDL, high-density lipoprotein; HIGH, higher protein intake; HOMA-IR: homeostatic model assessment of insulin resistance; Matsuda index, measure of peripheral insulin sensitivity; MOD, moderate protein intake; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test (2 h after ingestion of 75 g dextrose). n = Participants analyzed per group.

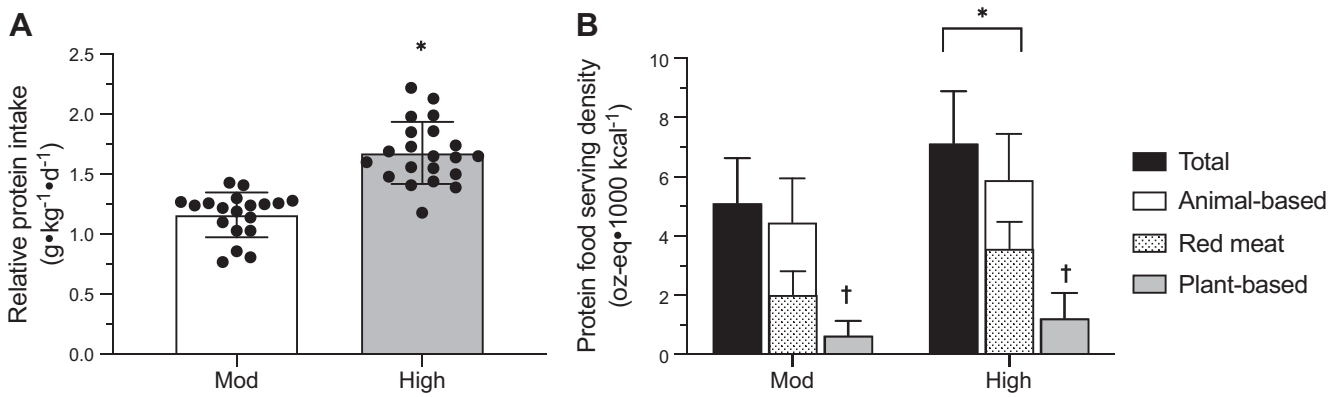

Figure 3. Daily dietary protein intake relative to body weight (*A*) and protein food source diet density (*B*) during the 10-wk resistance training program in middle-aged adults. *A*: assessed by independent Student's *t* test; *B*: assessed by linear mixed model. MOD $n = 20$, HIGH $n = 21$ participants. Data shown as means ± SD. *Significantly greater relative protein intake, animal-based protein serving density, and red meat serving density between groups (all $P < 0.001$). †Significantly greater serving density of animal-based than plant-based protein foods within each group (both groups, $P < 0.001$). High, higher protein intake; Mod, moderate protein intake.

Supplemental Table S1; see https://doi.org/10.6084/m9.figshare.13218923.v1. Canonical correlation revealed significant correlations between linear combinations of DESeq2 taxa and health and performance outcomes in the cohort (Supplemental Table S2; see https://doi.org/10.6084/m9.figshare.13230548.v1).

Table 5. *Diet composition during 10 wk of resistance training and dietary protein manipulation of moderate and high intake in middle-aged adults*

Diet Composition	MOD ($n = 20$)	HIGH ($n = 21$)
Energy intake, kcal/day		
Study goal	2,447 ± 502	2,495 ± 334
Reported	1,940 ± 566*	2,250 ± 506*
Carbohydrate intake		
Total carbohydrate, g/day	233 ± 80	220 ± 69
Dietary fiber, g/1,000 kcal	9 ± 2	9 ± 3
Added sugar, %/total kcal	13.1 ± 3.1	8.6 ± 3.4†
Fat intake		
Total fat, g/day	71 ± 23	92 ± 22†
Saturated fat, %/total kcal	10.5 ± 1.8	12.0 ± 2.9
Healthy eating index	61.2 ± 9.2	62.4 ± 11.7
Adequacy		
Total fruits[2]	3.2 ± 1.6	2.7 ± 1.6
Whole fruits[2]	3.9 ± 1.5	3.6 ± 1.7
Total vegetables[2]	3.8 ± 1.1	3.4 ± 1.2
Greens and beans[2]	3.3 ± 1.7	3.5 ± 1.9
Whole grains[1]	4.3 ± 2.0	3.3 ± 2.5
Dairy[1]	4.5 ± 2.6	5.5 ± 2.5
Total protein foods[2]	5.0 ± 0.0	5.0 ± 0.0
Seafood and plant proteins[2]	3.9 ± 1.5	4.5 ± 1.3
Fatty acids[1]	5.3 ± 2.3	5.3 ± 2.7
Moderation		
Refined grains[1]	8.6 ± 1.8	9.0 ± 1.5
Sodium[1]	2.2 ± 1.7	2.7 ± 2.5
Added sugars[1]	6.6 ± 1.6	8.6 ± 1.4†
Saturated fats[1]	6.8 ± 2.1	5.3 ± 2.6

Data are presented as means ± SD. The interaction of protein group and energy intake assessed by linear mixed model; all other comparisons assessed by independent Student's *t* test. *Main effect of energy adherence (study goal vs. reported, $P = 0.001$). †Significant difference between groups ($P < 0.050$). Healthy eating index: higher adequacy score indicates higher consumption; higher moderation score indicates lower consumption; [1]Maximum score of 10.0; [2]Maximum score of 5.0. HIGH, higher protein intake; MOD, moderate protein intake. n = Participants analyzed per group.

Linear mixed-effects models revealed significant associations between *Lactobacillaceae*, *Acidaminococcaceae*, and *Veillonellaceae* and appendicular lean body mass, gait performance, and blood pressure, respectively (Fig. 4).

DISCUSSION

Dietary protein intake above the RDA and resistance training are anabolic lifestyle strategies to support the maintenance of muscle mass and strength with advancing age (1). Our study shows that the fortification of an animal-based protein diet (1.16 g·kg^{-1}·day^{-1}) with more animal-based protein (1.68 g·kg^{-1}·day^{-1}) does not potentiate resistance training adaptations, such as increased muscle strength or lean body mass, in middle-aged adults. Thus, protein intake greater than the amount contained in a typical American diet (48) is not required to support resistance training-induced adaptations in previously untrained middle-aged adults when high biological value protein foods (i.e., animal-based) are consumed in moderate amounts.

Recent meta-analyses suggest the potential of higher daily protein intakes, up to 1.62 g·kg^{-1}·day^{-1} or higher, as optimal to maximize resistance training adaptations in healthy young adults (1, 6). Accordingly, our hypothesis adapted a similar assertion by evaluating the benefit of a higher intake of protein during resistance training for middle-aged adults. In accordance with previous findings in middle aged and older adults, our results indicate that excess protein intake did not further augment resistance training-induced lean mass accretion (49–51) nor muscle strength (49–54). Certainly, the impact of protein supplementation on supporting the skeletal muscle adaptive response to resistance training has been extensively investigated. However, few of these investigations have sought to control and monitor dietary habits outside of researcher-provided nutrition (50, 55). Our study administered 3d diet records every other week utilizing validated dietary assessment methods (56), with follow-up dietary counseling sessions to promote adherence to protein intake goals. These efforts allow for a more reliable evaluation of habitual protein consumption during the resistance training period; thus, a more accurate conclusion of dietary protein

Table 6. *DESeq2 within-group results following 1-wk dietary habituation and 10-wk resistance training in middle-aged adults*

log_2 FC	Δ	P'	P"	Family	Genus
			HIGH: baseline vs. onset[1]		
3.09	↓	0.036	1.000	*Akkermansiaceae*	*Akkermansia*
24.84	↓	<0.001	<0.001	*Eggerthellaceae*	Uncultured[2]
−25.00	↑	<0.001	<0.001	*Erysipelotrichaceae*	*Catenibacterium*
5.84	↓	0.011	0.405	*Ruminococcaceae*	*Ruminococcaceae UCG-010*
22.11	↓	<0.001	<0.001	*Veillonellaceae*	*Megasphaera*
21.90	↓	<0.001	<0.001	*Veillonellaceae*	*Veillonella*
			MOD: baseline vs. onset[1]		
−20.74	↑	<0.001	<0.001	*Acidaminococcaceae*	*Acidaminococcus*
−20.43	↑	<0.001	<0.001	*Erysipelotrichaceae*	*Holdemanella*
−23.25	↑	<0.001	<0.001	*Lachnospiraceae*	*Coprococcus*[2]
−0.36	↑	0.007	0.290	*Lachnospiraceae*	N/A[2]
1.31	↓	0.050	1.000	*Streptococcaceae*	*Streptococcus*
			HIGH: onset vs. post[3]		
−3.18	↑	0.047	1.000	*Akkermansiaceae*	*Akkermansia*
−22.39	↑	<0.001	<0.001	*Eggerthellaceae*	Uncultured[2]
21.07	↓	<0.001	0.010	*Erysipelotrichaceae*	*Catenibacterium*
−22.57	↑	<0.001	<0.001	*Veillonellaceae*	*Veillonella*
			MOD: onset vs. post[3]		
20.42	↓	<0.001	0.001	*Acidaminococcaceae*	*Acidaminococcus*
20.49	↓	<0.001	0.002	*Erysipelotrichaceae*	*Holdemanella*
23.72	↓	<0.001	<0.001	*Erysipelotrichaceae*	*Catenibacterium*
−5.28	↑	0.014	0.601	*Veillonellaceae*	*Veillonella*

Significant results of DESeq2 analysis. [1]Effect of 1-wk diet habituation (onset) within group; [2]uncultured or N/A, not available, not classified to genus level; [3]effect of 10-wk resistance exercise training within group. log_2FC, log_2 fold change effect size estimate as calculated by DESeq2 for the contrasts shown; Δ, direction of change with time whereby ↑ denotes increase and ↓ denotes decrease; *P*' and *P*" unadjusted and Benjamini–Hochberg adjusted *P* value, respectively. HIGH, higher protein intake; MOD, moderate protein intake. n = 29–37 participants per group per time point.

needs to support the demands of resistance exercise amidst aging.

In accordance with previous work (1), we observed that resistance training without intended weight loss does not significantly alter fat mass. Consequently, we observed an overall increase in body weight, namely due to lean mass gain, in both MOD and HIGH. In the absence of combined exercise training, body composition changes are also observed with dietary energy manipulations alone. Caloric surplus, with or without a protein-dense macronutrient distribution, increases fat mass (57, 58). Herein, we did not observe an increase in fat mass despite overall gain in body weight. The contribution of dietary energy balance to the observed changes in body composition is less discernable. Reported energy intake differed from predicted energy requirement for both groups (Table 5). These discrepancies, together with total weight gain, highlight the limitations of cross sectionally derived predictive energy equations for longitudinal application (59), as well as inherent error in dietary assessment methods (60).

The aging process not only increases risk of losses in muscle mass and strength (7), but also the risk of cardiometabolic diseases (61). To further complicate the elucidation of lifestyle recommendations that support healthy aging, dietary protein is indicated as protective against the loss of skeletal muscle mass and strength (2), yet it has also been paradoxically observed as either beneficial or detrimental to glycemic control, kidney function, and biomarkers and incidence of cardiovascular disease (9–11). We demonstrated that our 10-wk intervention did not influence insulin resistance or glucose tolerance estimations (Table 4). Our results contrast with other findings that suggest high intake of animal-based protein may contribute to an increased risk of pre- or type 2-diabetes (9, 62). Likewise, the capacity of resistance exercise training to improve glucoregulation is also equivocal. Although our combination of high-quality protein intake with resistance training did not synergistically alter measures of glycemic control, this lack of observation may partially be attributed to methodology (i.e., OGTT vs. gold-standard hyper insulinemic-euglycemic clamp) and participant health status (e.g., BMI, diabetes) (63, 64). We also showed no significant changes in plasma lipids, nor systolic and diastolic blood pressure. Our results differ from epidemiological observations of increased risk for the development of cardiovascular disease with red meat intake (65), yet remain consistent with randomized controlled trials that conclude no impairments in blood pressure or plasma concentrations of total cholesterol, LDL, HDL, and triacylglycerol (13). Indeed, the potential synergy of resistance exercise and dietary protein intake cannot be isolated within this current design. The design is nevertheless consistent with current recommendations that habitual participation in both healthy eating and activity patterns are required to support healthy aging (21, 66).

Past efforts have independently established a clear connection between the gut microbiome with metabolic health (67). Moreover, dietary protein is an important mediator of resistance training-induced strength adaptations for healthy aging (68). However, the interplay between the gut microbiome, dietary protein, and resistance exercise training induced strength adaptations are unknown. In agreement with previous findings of endurance exercise (69–72),

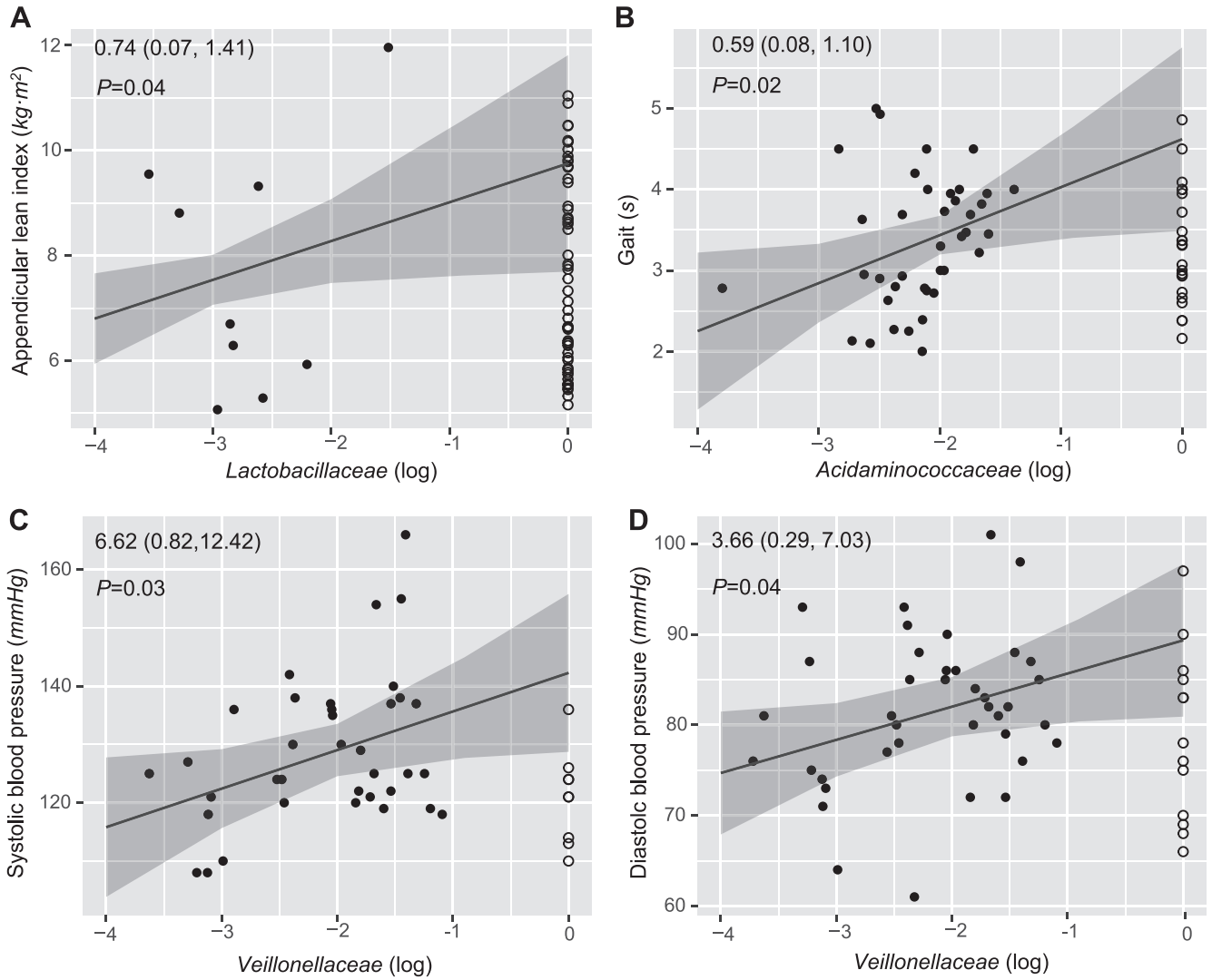

Figure 4. *A–D*: correlations between gut microbiota taxa and intervention outcomes. Groups collapsed, n_A = 64, n_B = 63, n_C = 45, and n_D = 52 participants. Results as mean (95% confidence interval). Relative abundances of taxa are log transformed, with zeros retained. A zero indicator was included in the linear mixed models as described in the METHODS. Appendicular lean index = extremity LBM × height^{-2}; Gait, time (s) to walk a distance of 4 m. LBM, lean body mass.

Erysipelotrichaceae abundance was decreased after resistance exercise training. Previous research has shown positive correlations between *Erysopelotrichaceae* and a high-fat diet, as well as obesity and colorectal cancer (73). This suggests that the resistance exercise-induced decrease in the abundance of this taxon observed in the current study may mediate some of the beneficial effects of exercise on metabolic health. Conversely, *Veillonellaceae* and *Akkermansia* abundance increased after the 10-wk intervention, though both taxa were decreased after the 1 wk dietary habituation without exercise in the HIGH protein group. These taxa have previously been positively associated with exercise and habitual physical activity level (46, 74–77) and have been associated with positive effects on health and endurance exercise performance (77, 78). Similarly, *Eggerthellaceae* was decreased in the HIGH protein group after the 1 wk dietary habituation but increased after the 10-wk resistance exercise training program. Evidence has shown that strains of *Eggerthella* are involved in bile acid metabolism and that primary bile acid formation by this taxon may reduce formation of secondary bile acids, such as deoxycholic acid, that have been linked to cancer (79). Therefore, the positive effect of resistance exercise on *Veillonellaceae*, *Akkermansia*, and *Eggerthellaceae* after an initial decrease in abundance with HIGH protein alone suggests that resistance exercise may "rescue" beneficial taxa that are negatively impacted by aspects of the HIGH protein diet (e.g., higher protein intake and higher total fat intake). The positive correlation between *Veillonellaceae* and blood pressure also echoes previous findings (80, 81). Overall, exercise modality (resistance vs. endurance exercise) may differentially influence the gut microbiota, potentially due to differences in activation of metabolic pathways and changes in splanchnic blood flow (47). However, implications for microbiota composition and exercise adaptations remain unclear. Our current findings provide a compass for future targeted evaluations on host microbiota mediation of resistance exercise training-induced performance and metabolic adaptations. Furthermore, our results indicate that

targeted exercise strategies may be an important compliment to a high protein diet to maintain gut health.

Although frequent diet recording throughout the intervention provides more reliable insight on the influence of dietary protein density on resistance training lean mass and strength gains, these results also highlight the translation of diet counselling for dietary changes and contextualize the interactions of diet, exercise, and disease risk. Despite continued dietary counselling, total HEI scores (Table 3) were far from the ideal 100 score, albeit numerically better than the national average of 58.3 (82). This disparity is consistent with the discrepancy between intended and reported dietary protein intake described above. These deviations from intended adherence reflect the obstacles to weight management practice, highlighting the importance of long-term, multicomponent interventions in the clinical setting (83). In terms of self-reported energy intake, absolute dietary fat intake was higher in HIGH, likely due to the greater intake of animal-based protein foods. Average fiber intake was lower than current recommendations of $\geq$14 g/1,000 kcal in both groups (84), which is consistent with the US intake ranges (85). Also, percent energy from added sugar was significantly higher with MOD, exceeding current recommendations of $\leq$10% kcal (21). Although these observations may be a consequence of study design and isocaloric postexercise meals containing a higher dose of dextrose than HIGH postexercise meals, this only explains one meal in three of the total 15 recording days throughout the 10-wk intervention. Nevertheless, despite these differential observations in key dietary components, cardiometabolic disease markers were unaltered with the intervention.

In conclusion, high daily protein intake does not further augment resistance training mediated muscle strength or lean mass gain when compared with moderate protein intake in middle-aged adults. Indeed, both MOD and HIGH conditions exceeded the minimum recommended protein food serving density of 2.0 oz-eq/1,000 kcal (21) and relative protein intakes were above current U.S. protein RDA of 0.8 $g \cdot kg^{-1} \cdot day^{-1}$. Both groups also consumed more animal-based than plant-based protein foods. Therefore, our results should be cautiously interpreted for situations of protein intake below current "aging" recommendations of $\sim$1.2 $g \cdot kg^{-1} \cdot day^{-1}$ or in the context of plant-based eating patterns (e.g., vegan). Our efforts nonetheless contextualize these strategies within a healthy lifestyle as high animal protein intake combined with resistance exercise training does not affect cardio-metabolic health markers. Moreover, higher dietary protein intake during resistance exercise training resulted in differential changes in gut microbiota composition, but there was no relationship to the measured health and performance outcomes within our intervention.

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DISCLOSURES

Beef Checkoff sponsor was only involved in financial support of the project, without involvement in design, data collection, and analysis, nor interpretation and dissemination of the report.

AUTHOR CONTIRBUTIONS

A.C.D., H.D.H., M.D.L., N.A.K., and N.A.B. conceived and designed research; C.F.M., A.F.S., R.L.H., S.E.S., R.A.A., S.A.P., A.C.D., and N.A.B. performed experiments; C.F.M., A.F.S., R.L.H., A.T.A., and A.C.D. analyzed data; C.F.M., A.F.S., R.L.H., S.E.S., R.A.A., A.T.A., S.A.P., A.C.D., H.D.H., M.D.L., N.A.K., and N.A.B. interpreted results of experiments; C.F.M., A.F.S., and R.L.H. prepared figures; C.F.M., A.F.S., R.L.H., and N.A.B. drafted manuscript; C.F.M., A.F.S., R.L.H., S.E.S., R.A.A., A.T.A., S.A.P., A.C.D., H.D.H., M.D.L., N.A.K., and N.A.B. edited and revised manuscript; C.F.M., A.F.S., R.L.H., S.E.S., R.A.A., A.T.A., S.A.P., A.C.D., H.D.H., M.D.L., N.A.K., and N.A.B. approved final version of manuscript.

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