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Randomized, Four-Arm, Dose-Response Clinical Trial to Optimize Resistance Exercise Training for Older Adults with Age-Related Muscle Atrophy

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Abstract

Purpose—The myriad consequences of age-related muscle atrophy include reduced muscular strength, power, and mobility; increased risk of falls, disability, and metabolic disease; and compromised immune function. At its root, aging muscle atrophy results from a loss of myofibers

Author Contributions

Conceptualization: MMB, ATM, DLM

Experimental Design: MMB, ATM, SCT, MJS, NAK, DLM

Data Acquisition: MJS, ATM, DLM, NAK, SCT, EKM, CJB, STW, LJD, BMR, KMV, IID, GMM, MMB

Data Analysis: MJS, ATM, BMR, IID, MMB Data

Interpretation: MJS, ATM, NAK, CSB, BMR, MMB

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and atrophy of the remaining type II myofibers. The purpose of this trial (NCT02442479) was to titrate the dose of resistance training (RT) in older adults in an effort to maximize muscle regrowth and gains in muscle function.

Methods—A randomized, four-arm efficacy trial in which four, distinct exercise prescriptions varying in intensity, frequency, and contraction mode/rate were evaluated: (1) high-resistance concentric-eccentric training (H) 3 d/wk (HHH); (2) H training 2 d/wk (HH); (3) 3 d/wk mixed model consisting of H training 2 d/wk separated by 1 bout of low-resistance, high-velocity, concentric only (L) training (HLH); and (4) 2 d/wk mixed model consisting of H training 1 d/wk and L training 1 d/wk (HL). Sixty-four randomized subjects (65.5 ± 3.6 y) completed the trial. All participants completed the same 4 wk of pre-training consisting of 3 d/wk followed by 30 wk of randomized RT.

Results—The HLH prescription maximized gains in thigh muscle mass (TMM, primary outcome) and total body lean mass. HLH also showed the greatest gains in knee extension maximum isometric strength, and reduced cardiorespiratory demand during steady-state walking. HHH was the only prescription that led to increased muscle expression of pro-inflammatory cytokine receptors and this was associated with a lesser gain in TMM and total body lean mass compared to HLH. The HL prescription induced minimal muscle regrowth and generally lesser gains in muscle performance vs. the other prescriptions.

Major Conclusions—The HLH prescription offers distinct advantages over the other doses, while the HL program is subpar. Although limited by a relatively small sample size, we conclude from this randomized dose-response trial that older adults benefit greatly from 2 d/wk high-intensity RT, and may further benefit from inserting an additional weekly bout of low-load, explosive RT.

Trial Registration—ClinicalTrials.gov NCT02442479

Keywords

Aging; exercise; muscle mass; muscle strength; muscle inflammation; exercise dose

1. INTRODUCTION

The progressive loss of muscle mass during normal aging is a major contributor to functional disability¹ and all-cause mortality², and the rate of atrophy is greatly accelerated beyond the fifth decade³. This loss of muscle mass during normal aging is primarily due to atrophy of type II myofibers and death of motor neurons (resulting in a loss of up to ~50% of total myofiber number by age 80) (reviewed in⁴). The functional consequences of age-related muscle atrophy include reduced muscle strength, power, mobility, and increased risk of falls⁵⁻⁸. Thus, it is imperative to develop effective intervention strategies to promote muscle regrowth in older, atrophied adults to improve quality of life and reduce mortality.

Various interventions have been tested (e.g. nutritional, pharmacologic, exercise training) (reviewed in⁹) and thus far resistance exercise training (RT) seems most effective; however, muscle regrowth results are generally suboptimal. For example, we¹⁰⁻¹³ and others¹⁴⁻¹⁷ have shown that RT induces meaningful increases in muscle strength, power, and functional

mobility, but the hypertrophic response is, on average, blunted in old compared to young^{10,11,13}, and more than one-third of older adults realize no myofiber hypertrophy in response to a 3 d/wk high-intensity prescription¹⁸. The age-related attenuation of RT-induced hypertrophy may be partially driven by a reduced protein synthesis response¹⁹, blunted ribosome biogenesis²⁰, attenuated myonuclear addition^{13,21,22}, and other unknown mechanisms. We speculate inflammation/cell stress may negatively affect the anabolic sensitivity to RT in older muscle, which is supported by muscle inflammation susceptibility we have noted in both resting muscle and in myotubes derived from older adults²³. Resistance exercise acutely elevates cell stress and inflammatory signaling in skeletal muscle^{24,25}, and if some of these pathways are already chronically elevated in resting muscles of older adults, they may negatively impact anabolic processes^{26,27} and blunt hypertrophy.

The purpose of this trial (NCT02442479) was to titrate the dose of RT in older adults in an effort to maximize muscle hypertrophy, with particular emphasis on the weight-bearing muscle groups of the thigh (*primary outcome = thigh muscle mass gained*), which are central to mobility and postural stability. The goal was to identify a prescription that sufficiently loaded muscle to promote myogenic processes, while allowing adequate recovery between bouts to limit muscle inflammation signaling. A few studies have manipulated RT intensity and frequency among older adults in an attempt to optimize gains in muscle strength, power, and functional ability²⁸⁻³⁰ but no dosing study to our knowledge has focused on treating muscle atrophy –i.e. “sarcopenia” as originally defined. Here we tested four, randomly assigned RT prescriptions with varying intensities, frequencies, and contraction modes. In brief, the four training prescriptions tested were: (1) high-resistance concentric-eccentric training (H) 3 d/wk (HHH); (2) H training 2 d/wk (HH); (3) 3 d/wk mixed model consisting of H training 2 d/wk separated by 1 bout of low-resistance, high-velocity, concentric only (L) training (HLH); and (4) 2 d/wk mixed model consisting of H training 1 d/wk and L training 1 d/wk (HL). We hypothesized that 2 d/wk of high-resistance loading (HH and HLH) would induce the greatest muscle regrowth – predicting this would optimize recovery time between high-resistance loading bouts, leading to enhanced muscle anabolism and minimal muscle inflammation – whereas HHH would not allow sufficient recovery between bouts, and HL would not provide a sufficiently frequent anabolic stimulus to induce hypertrophy. Secondly, we hypothesized that HLH would experience the greatest gains in muscle function due to the incorporation of 1 d/wk high-velocity L training as a supplement to 2 d/wk H training for hypertrophy.

2. METHODS

Subject recruitment began in January 2008 and final follow-up outcome measures were collected in May 2012 (tissue assays and data analyses concluded in 2015). An overview diagram of recruitment, informed consent, screening, randomization, follow-up, retention, and analysis/completion is shown in Figure 1.

2.1 Human Subjects

Older men and women (age 60-75y) were recruited from the greater Birmingham, Alabama catchment region via newspaper and e-news advertisements. Each subject passed a comprehensive physical examination performed by a geriatrician and diagnostic graded exercise stress test (GXT) with 12-lead electrocardiogram reviewed by a cardiologist prior to participation in the study. Exclusion criteria: neurological, musculoskeletal, or other disorder that would preclude one from completing resistance training and all performance tests; uncontrolled hypertension; unstable or exercise-induced angina pectoris or myocardial ischemia; diabetes mellitus; any other medical condition that would interfere with testing or increase one's risk of complications during exercise; inability to walk on a treadmill for 10 minutes; lidocaine allergy; prescription anti-coagulant (e.g., Coumadin) therapy; androgen (e.g., testosterone) or anabolic (e.g., GH, IGF-I) therapy; regular leg resistance exercise during the previous 3 years; food allergy to cow's milk; currently adherent to a weight reduction diet; obesity, or body mass index (BMI) ≥ 30 . All female participants were at least 5 years post-menopausal. Estrogen replacement therapy (ERT) was not an inclusion or exclusion criterion as ERT does not affect gains in muscle strength or lean mass during resistance training and does not alter single myofiber function. In a previous trial,¹¹ we found no differences in rates of myofiber hypertrophy, muscle mass gain, or strength gain between older women on and off of ERT. The study was approved by the Institutional Review Board (IRB) of the University of Alabama at Birmingham, and all subjects provided written informed consent prior to participation.

2.2 Overall Trial Design

The design was a randomized, four-arm efficacy trial (NCT02442479) in which four, distinct RT prescriptions varying in intensity, frequency, and contraction mode/rate (see details below) were evaluated to determine which dose(s) yielded the greatest muscle regrowth and gains in muscle function. In an effort to differentiate the effects of the four prescription doses, randomized RT was protracted to 30 weeks, and was preceded by 4 weeks of familiarization and pre-training (described below). Because thigh muscle mass (TMM) was the primary outcome, the DXA lean mass assessments defined the total number of subjects in the final data set ($n = 63$; 29F, 34M). For a variety of reasons leading to missing data, sample sizes for some secondary and tertiary outcomes were subsets of $n = 63$ as reported in results for each of these outcomes.

2.2.1 Adherence Plan—Because this was an efficacy trial (i.e. not “intent-to-treat”), adherence to the exercise regimen was imperative in order for the outcome data to be interpreted. Consequently, we established an IRB-approved incentives plan to ensure a minimum adherence rate, which included rewards for participation/adherence as well as a stepwise warning system and a plan for administrative withdrawal from the study if a subject failed to adhere per the established guidelines. A minimum adherence rate of 83.3% was required (i.e. 5 of every 6 consecutive sessions completed). Written warnings were issued to any subject who missed more than 1 of 6 consecutive sessions. We required that issuance of a warning be followed by 100% adherence during the subsequent 6 sessions in order for a subject to be considered in good standing and to avoid a second warning. A subject was removed from the study if he/she accumulated more than two warnings. However, special

allowances were made if a subject missed more than 1 of 6 sessions due to prearranged travel, illness, etc. For example, if a subject was scheduled to miss an entire week of exercise training due to pre-arranged travel, he/she was not penalized but the subject's overall duration of participation was extended 1 week to standardize the total weeks of exercise training.

2.2.2 Pre-Training Program—During the first four weeks of exercise training, all subjects were prescribed the same 3d/wk RT program. This pre-training program was designed to promote early – largely non-muscle mass-dependent – gains in neuromuscular performance to establish a true baseline from which the mechanisms of measurable muscle hypertrophy and functional outcomes could be studied in a tightly integrated fashion without bias in the subsequent dose-response trial. The 4 wk, 3 d/wk pre-training program included the same 10 movements prescribed for the subsequent 30 wk. The prescription recruited all major muscle groups, with an emphasis on the (biopsied) knee extensor muscles: squat, knee extension, leg press, heel raise, seated overhead press, chest press, seated cable row or pull-down, arm curl, and triceps push down on a combination of weight-stack (Body Masters, Life Fitness) and plate-loaded (Life Fitness) machines, and abdominal flexion on a floor mat. After familiarization, the exercise intensity for each set was ~10-15 repetitions to near failure, except for abdominal exercise, in which each set was performed to fatigue. During the first week, one set of each movement was performed; thereafter, two sets of each were completed with brief rest periods between sets.

2.2.3 Four-Arm, Randomized Dose-Response Resistance Training—In the week following the 4 wk pre-training period, subjects performed a battery of tests (see below). Subjects were then randomized into one of four training prescriptions for the next 30 wk, making the total study duration for each subject 35 wk plus pre- and post-testing. The four different training prescriptions were designed to elucidate which combination of varied exercise intensity, frequency, and mode of contraction was optimal for muscle regrowth and improvements in muscle function. Two of the training prescriptions involved only high-intensity, concentric-eccentric training (H) either 2d/wk (HH) or 3d/wk (HHH). H training bouts consisted of 3 sets $\times$ 8-12 repetitions to failure of the same exercises performed in the pre-training period plus three sets of the same abdominal flexion exercise. Continuous progression was achieved as described previously¹⁸. The remaining two exercise prescriptions involved mixed models combining H training with 1 d/wk low-resistance, high-velocity, concentric only training (L); either 2 d/wk (HL) or 3 d/wk (HLH). L training bouts consisted of 3 sets $\times$ 12 repetitions of the same exercises performed in the 4 wk pre-training period, but resistance loads were only two-thirds of the loads prescribed on H days. During L training, subjects were encouraged to complete the full concentric range of motion as quickly as possible. To ensure concentric-only contractions, a hydraulic braking system was used to lower the weights so no eccentric loading occurred. Again, three sets of abdominal flexion were also completed on these L training days. Overall, sixty-three subjects (65.5 ± 3.6 y) who were randomized to HH ($n = 15$; 65.0 ± 3.8 y; 9F, 6M), HHH ($n = 14$; 65.4 ± 3.8 y; 5F, 9M), HL ($n = 18$; 66.2 ± 3.8 y; 8F, 10M), or HLH ($n = 16$; 65.2 ± 3.2 y; 7F, 9M) are in the final data set for the primary outcome (Figure 1).

2.3 Whey Protein Supplement

Subjects consumed a purified whey protein supplement (WPC80; Agri-Mark, Inc., Onalaska WI, kindly provided by the US Dairy Export Council) twice per day $\times$ 3 d/wk throughout the 4 wk of pre-training and 30 wk of randomized training. The dose was standardized at 0.3 g whey protein kg body wt⁻¹ consumed twice per day for a total of 0.6 g whey protein kg body wt⁻¹ 3 d/wk (six total protein shakes per wk). Whey protein shakes were prepared and provided in the exercise facility, and consumed before and after each exercise session. For those randomly assigned to 2 d/wk prescriptions (HH and HL; Mondays and Fridays only) after the pre-training period, two whey protein doses were packaged with instructions for consumption at home on Wednesdays. Consumption at home was verified verbally and empty containers were returned each Friday.

2.4 Dietary Intake Monitoring

Prior to beginning RT, subjects met with a dietitian to learn how to complete accurate four-day diet records. Subjects were informed to consume ad libitum and to maintain a fairly consistent intake throughout the 35 wk trial. Diet record keeping was strongly encouraged at wk 0, 4, 20, and 35, with each four-day diet record being collected across two weekdays and two weekend days in succession to account for any weekend changes in dietary habits. Failure to complete these records was *not* grounds for administrative withdrawal. Consequently, only 42 subjects completed diet records. Total energy, macronutrient, and amino acid intakes were determined using Nutrition Data Systems for Research (NDSR) software v5.0 (Minneapolis, MN). Records were entered into the NDSR using established standards for food and beverages to ensure consistency.

2.5 Outcome Assessments

2.5.1 Muscle Mass and Body Composition—Dual energy X-ray absorptiometry (DXA, Lunar Prodigy Model #8743, GE Lunar Corporation, Madison, WI) was performed to determine body fat percentage, total body lean mass (LM) and bilateral sums of leg muscle mass, arm muscle mass (AMM), and our primary limb compartment of interest – thigh muscle mass (TMM) – which we normalized to femur length (cm). Scans were analyzed using enCORE 2002 software (version 6.10.029) according to manufacturer's instructions. Regarding precision of the Lunar Prodigy, published repeated measures data³¹ from 3 repeat scans on 253 adult humans – ranging 20-84 years of age and body mass index 15.7-52.0 kg/m² – yielded coefficients of variation (CV) of 2.0% for total body % fat and 1.2 – 4.4% for regional fat mass measurements; and intra-class correlation coefficients (ICCs) for total body and regional values ranged 0.990 – 0.997. DXA was performed at baseline (wk 0) and after completion of the dose-response trial (wk 35).

2.5.2 Muscle Strength and Power—All strength and power assessments were performed using our established methods^{6,10,12}. Dynamic, bilateral one-repetition maximum (1RM) strength was assessed for the leg press, knee extension, chest press, and overhead press at wk 0, 4, 12, 20, 28, and 35. 1RM was defined as the highest load lifted through a full range of motion prior to two failed attempts. 1RM strength testing was administered by a certified trainer who ensured a standard range of motion within each

subject over the course of training and testing. The initial wk 0 1RM tests were preceded by two familiarization sessions including practice 1RM tests. Unilateral knee extension isometric maximum voluntary contraction (MVC) strength of the dominant leg was assessed at 60° of knee flexion via a calibrated load cell attached to a fixed knee extension dynamometer. MVC was tested at wk 0, 4, 12, 20, 28, and 35.

Concentric, bilateral knee extension peak and mean power were assessed via two protocols: 1) using a relative load equal to 45% of that day's knee extension 1RM (i.e. relative power); and 2) using an absolute load equal to 60% of the subject's wk-0 knee extension 1RM (i.e. absolute power). To measure power, subjects completed three full repetitions of knee extension, with the concentric phase being performed as rapidly as possible, while the eccentric phase was mitigated by a hydraulic braking system. Knee angle was recorded at 500 Hz by electrogoniometry (Model SG150, Biometrics, Gwent, UK), and velocity was determined across the change in knee angle from 50° up to 20° of knee flexion. Power was determined as velocity $\times$ external load. Relative and absolute knee extension power were measured at wk 0, 4, 12, 20, 28, and 35.

2.5.3 Central Activation Ratio/Force Deficit—The central activation ratio (CAR) or force deficit was tested to determine the percentage of maximum muscle activation subjects achieved voluntarily during MVC by using our burst superimposition technique³². Stimulating electrodes were placed over motor points of the distal vastus medialis and proximal vastus lateralis. To familiarize participants to electrical stimulation (ES) of the quadriceps, several submaximal bursts of ES were performed with each burst increasing in intensity. After familiarization, participants were verbally encouraged during a 3-second MVC. After the first two seconds, a 100 Hz 12-pulse burst of at least 100 mA was delivered by an electrical stimulator (Digitimer Stimulator Model DS7AH, Hertfordshire, England). Any increase in peak isometric force due to ES indicates a failure to fully activate the quadriceps by voluntary effort. CAR was computed as MVC force/(MVC+ES) force. CAR was determined during three MVCs separated by one minute at wk 0, 4, 12, 20, 28, and 35, and the MVC yielding the highest force at each time point was selected for analysis.

2.5.4 Muscle Fatigability—Dynamic, voluntary muscle fatigue was assessed using our previously described methods³³. In brief, subjects performed 20 repetitions of bilateral knee extension using a relative load equal to 45% of that day's knee extension 1RM. Maximal concentric velocity was encouraged during each repetition, and eccentric loading was mitigated by a hydraulic braking system. The magnitude of fatigue was defined by the magnitude of decline in power from peak (repetitions 1-5) to final (repetitions 19-20). Knee extension muscle fatigue was assessed at wk 0, 4, 12, 20, 28, and 35.

2.5.5 Relative Motor Unit Activation—The magnitude of quadriceps motor unit activation (MUA) required during a controlled, three-repetition sit-to-stand task was assessed via surface electromyography (EMG) using our previously described methods^{6,12}. Ascent and descent were each completed in 2 s and standardized using an audiovisual metronome. Results were normalized to maximum MUA during MVC to yield the relative MUA demand during the sit-to-stand task. Sit-to-stand EMG data were analyzed at the knee angle equivalent to the knee angle during isometric MVC ($\approx 60^\circ$ below horizontal). Raw

EMG recordings (for both the three-repetition sit-to-stand and knee extension MVC) from each of the three superficial quadriceps muscles (vastus medialis, vastus lateralis, and rectus femoris) were full-wave rectified, converted to RMS using a 100ms sliding window, and averaged. The test was performed at wk 0, 4, 12, 20, 28, and 35.

2.5.6 Mobility Function and Flexibility—Prior to training (wk 0) and after completion of the dose-response trial (wk 35), mobility function was assessed by the 6-min walk test, time to stand up from the supine position on a floor mat, and chair sit-and-reach flexibility. All pre- and post-training mobility function assessments were conducted by the same trained member of the research team.

2.5.7 Steady State Exercise Cardiorespiratory Demand—Cardiorespiratory responses (e.g. VO_2 , respiratory exchange ratio, minute ventilation, % maximum heart rate) to steady-state treadmill walking and stair climbing were assessed using a calibrated Physiodyne II system (AEI Technologies, Inc., Bastrop, TX) to assess cardiorespiratory demand during typical daily tasks. Level treadmill walking was performed at a constant pace of 3.0 mph and stair climbing was performed on a computerized Stepmill (Stairmaster Inc., Vancouver, WA) at 31 steps/min (8" step height). Both walking and stair climbing consisted of five minutes of activity with steady-state data collected during minutes 4-5. Variables of interest included VO_2 (ml/kg/min), minute ventilation (V_E , ml/min), respiratory exchange ratio (RER), breathing frequency (F_B , breaths/min), and heart rate (HR, beats/min). Cardiorespiratory demand testing was performed prior to training (wk 0) and after completion of the dose-response trial (wk 35).

2.5.8 Muscle Biopsy and Analyses—Muscle samples were obtained in the fasted state at approximately 0800 am at wk 0, 4, and 35 under local anesthetic (1% lidocaine) from the vastus lateralis by percutaneous needle biopsy using established methods^{10,21,23,34}. To minimize the acute molecular effects of the most recent exercise bout, biopsies at wks 4 and 35 were performed 48-72 h post-exercise. All visible connective and adipose tissues were removed from the biopsy samples, a portion was mounted by cross-section for histology^{10,35}, and remaining tissue was snap frozen (~30 mg aliquots) in liquid nitrogen. All samples were stored at -80°C.

2.5.8.1 Histological Analysis: Myofiber type and type-specific cross-sectional areas were assessed via well-established myosin heavy chain (MHC) isoform immunofluorescence microscopy as described in detail previously^{10,18,35}. In brief, 6- μm muscle serial cross sections were fixed in 3% neutral-buffered formalin at room temperature for 45 min, washed in PBS, and blocked with 5% goat serum for 20 min. Anti-MHC type I (NCL-MHCs, NovoCastra Laboratories, 1:100), anti-MHC type IIa (University of Iowa Hybridoma Bank, 1:80), and anti-laminin (VP-L551, NovoCastra Laboratories, 1:80) primary mouse monoclonal antibodies were used to detect type I myofibers, type IIa myofibers, and basal lamina, respectively (type IIx fibers are the remaining, unstained fibers). Contiguous images of the entire mount were captured at 10X using an Olympus BX51 fluorescent microscope with an Olympus MagnaFire SP camera (S99810). Images were then digitally stitched into

one complete image of the sample cross-section and analyzed in coded fashion by a technician blinded to time point and randomization using Image-Pro Plus 6.0 software.

2.5.8.2 Target Gene Expression: For gene expression, snap frozen muscle samples – collected at baseline (wk 0), the conclusion of pre-training (wk 4), and the conclusion of the 30 wk randomized training period (wk 35) – were pulverized and total RNA was isolated using Tri-Reagent (Molecular Research Center, Cincinnati, OH) in accordance with the manufacturer's instructions. RNA quantity and quality were determined using a spectrophotometer (NanoDrop ND-1000, Thermo Scientific, Rockford, IL), and cDNA was synthesized via reverse transcription using the SuperScript VILO cDNA Synthesis kit (Invitrogen, Carlsbad, CA). Muscle expression of five pro-inflammatory genes was determined by quantitative PCR: tumor necrosis factor alpha (TNF α , Hs00174028_m1), TNF receptor 1 (TNFR1, Hs00533560_m1), TNF-like weak inducer of apoptosis (TWEAK, Hs00356411_m1), TWEAK receptor (TWEAK-R, aka Fn14, Hs0017993_m1), interleukin-6 receptor (IL-6R, Hs00794121_m1), and tenascin C (Hs01115665_m1). PCR was performed via the Taqman Gene Expression Assay (Applied Biosystems, Foster City, CA), with GAPDH expression (Hs02758991_g1) serving as an internal control. All samples were run in triplicate, and relative amounts of target mRNA (i.e. CT values) were determined using the comparative threshold cycle method via StepOne software version 2.2.2 (Applied Biosystems).

2.5.8.3 Protein Cell Signaling: For immunoblotting, snap frozen muscle samples were homogenized in 6 μ L/mg muscle of ice cold lysis buffer with protease and phosphatase inhibitors and then centrifuged at 15,000xg for 40 min at 4°C. Supernatant was stored at -80°C until assayed for protein content using the bicinchoninic acid technique with BSA as a standard. Twenty-five μ g of mixed muscle protein lysate were resolved on 4-12% SDS-PAGE gels and transferred to PVDF membranes. To assess inflammatory/proteolytic and anabolic signaling, antibodies for phosphorylated (Ser536) (CS-3033) and total NF κ B p65 (CS-8242), TNF receptor 1 (TNFR1; CS-3736), caspase-3 (CS-9662), phosphorylated (Ser421/Thr424) (CS-9204) and total p70S6K (CS-2708), ribosomal protein S6 (CS-2217) and eIF2B ϵ (CS-3595) were purchased from Cell Signaling Technologies (Danvers, MA). Primary antibodies were diluted 1:1000 in PBST and 5% goat serum (monoclonal antibodies) or 2% milk/2% BSA (polyclonal antibodies). HRP-conjugated secondary antibody (Pierce Thermo Scientific, Rockford, IL) was used at 1:50,000 (w/v) followed by chemiluminescent detection in a BioRad (Hercules, CA) ChemiDoc imaging system with band densitometry performed using BioRad Quantity One software (software package 4.5.1) as detailed previously³⁴.

2.6 Statistical Analysis

Paired t-tests were used to examine changes during pre-training (wk 0 to 4) across the entire cohort. For the four-arm, randomized trial (wk 5 to 35), prescription $\times$ time repeated measures analysis of variance (ANOVA) was used to test main effects of prescription and time, and prescription $\times$ time interaction effects. For all outcome measures repeat tested at the conclusion of the 4-wk pre-training period, these data served as the baseline in each prescription $\times$ time repeated measures ANOVA. For neuromuscular performance tests

conducted at wk 0, 4, 12, 20, 28, and 35, the randomized treatments were tested via 4 prescription $\times$ 5 time point (wk 4, 12, 20, 28, 35) repeated measures ANOVA. Muscle biopsies were collected at wk 0, 4, and 35; thus after pre-training, the randomized treatments were tested via 4 prescription $\times$ 2 time point (wk 4, 35) repeated measures ANOVA. Body composition (DXA), mobility function, and steady state exercise cardiorespiratory demand tests were performed only at wk 0 and wk 35; thus the wk 0 data were used as the baseline to test the randomized treatments via 4 prescription $\times$ 2 time point (wk 0, 35) repeated measures ANOVA. Consequently, comparisons among the randomized prescriptions for these tests include any potential influences of the standardized 4-wk pre-training period. For the primary outcome (TMM), follow-up repeated measures ANCOVA – covarying for sex – was also tested and confirmed no significant influence of sex on adaptations to the four prescriptions. A one-way ANOVA was used to determine if any dietary measures were different among the four prescriptions. Significant effects were further tested post hoc by Fisher's least significant difference tests. All data are reported as means $\pm$ SE. For all tests, $P \leq 0.05$ (two-sided) was considered statistically significant.

For the primary outcome (change in TMM), we conducted a 3-cluster K-means cluster analysis to classify subjects as: (i) poor responders, (ii) responders, or (iii) extreme responders, based on gains in TMM – enabling us to further explore the efficacy of each prescription at the individual level.

3. RESULTS

3.1 Participation and Retention

As summarized in Figure 1, 127 volunteers who met initial telephone screening criteria provided written, informed consent to participate. Of the 127, 18 were excluded on physical exam or GXT, 3 withdrew voluntarily, and 28 were administratively withdrawn for a variety of reasons (e.g., several missed appointments or other non-compliance issues); thus 78 participants completed the 4-wk pre-training program. At the conclusion of pre-training, four participants voluntarily withdrew; thus 74 were randomized to the four-arm clinical trial. Retention during the 30-wk randomized trial was 93% with 5 voluntary withdrawals. However, scheduling issues resulted in $n = 6$ missing DXA scans, leaving $n = 63$ in the final analysis for the primary outcome.

3.2 Adherence and Training Intensity during Randomized Trial

Adherence and training intensity results are summarized in Table 1. Overall adherence to prescribed exercise training averaged $\sim 90\%$. Adherence rates within each randomly assigned prescription ranged 87-93% (HL 93.3%, HH 91.3%, HLH 89.5%, HHH 86.9%). One-way ANOVA revealed significantly different adherence rates among groups, and post hoc testing indicated higher adherence for those training 2 d/wk (i.e. HL and HH) vs. 3 d/wk (i.e. HLH and HHH) ($92.4 \pm 0.8\%$ vs. $88.2 \pm 1.1\%$; $P < 0.01$).

The approach to progressive training was successful in maintaining relative training intensity and volume within the target ranges. After randomization, every effort was made to standardize relative training intensities on “H” days across the four prescriptions (as well as

“L” days in HL and HLH). There were no time effects or prescription $\times$ time interactions for H training intensity, demonstrating that relative H training intensity was maintained similarly among all four doses throughout the course of the study. For example, Table 1 displays average training intensities (% of 1RM) on H training days for the leg press and chest press across the treatment period. As shown, subjects within each prescription maintained a consistent relative training intensity throughout the course of the study for the leg press ($\sim 73\%$ 1RM) and chest press ($\sim 63\%$ 1RM). Further, the progression scheme based on a target of 8-12 repetitions per set resulted in no prescription differences in the average number of repetitions per set (~ 10.5 repetitions).

3.3 Dietary Intake Analysis

Average daily dietary intake by randomized exercise prescription is summarized in Table 2. Intake values do not include the 0.6 g/kg whey protein consumed by all subjects three days per week (Monday, Wednesday, Friday). Total energy, macronutrient, and amino acid (including BCAA, leucine) intakes did not differ between groups, indicating diet did not influence differential adaptations among the exercise prescriptions.

3.4 Adaptations to Pre-training (wk 0 to 4) Program

The 4 wk familiarization and pre-training program induced gains in knee extension peak power (relative power +29%, absolute power +23%), modest 1RM strength gains for upper and lower body exercises (up to 16%), and a 12% increase in isometric knee extension MVC ($P < 0.01$ for all). For the 20-repetition knee extension fatigue test, total power output increased substantially, but the rate of fatigue (determined by the decline in velocity across 20 repetitions at 45% 1RM) was unchanged. Reductions were noted in the relative quadriceps motor unit activation (MUA) required to both stand from and sit into a chair [concentric (sit-to-stand) MUA: 63% to 51%; eccentric (stand-to-sit) MUA: 58% to 47%; $P < 0.001$ for both]. These performance adaptations were accompanied by a significant increase in type II myofiber cross-sectional area (+18%; $P < 0.01$) and a significant IIX-to-IIa fiber type shift (type IIa, 48% to 57%; type IIX, 14% to 7%; $P < 0.001$ for both).

3.5 Results of the Randomized, Dose-Response Trial

3.5.1 Lean Mass, Thigh Muscle Mass, and Myofiber Size—Overall, progressive resistance training resulted in significant gains in LM, TMM, and AMM as assessed by DXA (time effect, $P < 0.001$ for all) (Figure 2). Significant prescription $\times$ time interactions were found for LM and AMM ($P < 0.05$) and an interaction trend was noted for TMM ($P = 0.117$). Post hoc tests revealed that HHH, HLH, and HH significantly increased LM, TMM, and AMM ($P < 0.05$ for all) with training, while HL did not (although a trend ($P = 0.071$) was noted for TMM in the HL prescription). In response to the HLH prescription, LM and TMM gains essentially doubled those noted in HHH and HH. For example, regarding total body LM, HLH gained nearly 2 kg (+1.89 kg, $P < 0.001$) compared to ~ 1 kg in HHH (+1.05 kg, $P < 0.01$) and HH (+0.95 kg, $P < 0.01$) (Figure 2A). Similarly, HLH gained 2x more TMM than HH and HHH (Figure 2B). On the other hand, HHH gained the most AMM (Figure 2C). There was a main time effect for body fat percentage, and post-hoc analysis

revealed that only HH (-1.1%), HLH (-1.5%), and HHH (-1.2%) significantly decreased body fat percentage following training ($P < 0.05$ for all; data not shown).

Results of K-means cluster analysis for the primary outcome (TMM), shown in Figure 3, demonstrate a remarkable range of changes in TMM across individuals in each prescription. The center and range of each cluster were as follows: (i) *poor responders* ($n = 25$; center = -93 g; range -570 to +187 g), (ii) *responders* ($n = 33$; center = +521 g; range +264 to +908 g), and (iii) *extreme responders* ($n = 5$; center = +1349 g; range +1015 to +1734 g).

While substantial inter-individual heterogeneity was seen in response to each of the four RT prescriptions, it is noteworthy that all but two of the HLH participants (i.e. 88% of HLH) responded positively (range 280 to 1734 g), including two extreme responders. Such a high responder rate was not the case in any of the other three prescriptions. Over 50% of HHH recipients were poor responders; four lost TMM (range -37 to -203 g) and four realized little to no gain (range 7 to 187 g). Consequently only six of 14 HHH subjects adapted positively with TMM hypertrophy (gains in HHH responders ranged 448 to 1015 g). For HH, six of 15 were poor responders [four lost TMM (-65 to -326 g) and two showed little change (+13 and +81 g)]. The remaining nine (60% of HH) were responders, gaining 256 to 1166 g of TMM. Interestingly, 50% (9/18) responded to HL (range 299 to 1294 g) but the responders were counterbalanced by eight who lost TMM (range -31 to -570 g) and one with minimal gain (178 g).

Due to limited biopsy samples, myofiber size and type distribution were assessed on a subset of subjects ($n = 30$) after randomization. Resistance training from wk 4 to wk 35 had no significant effect on type I myofiber CSA, but did result in further type II myofiber hypertrophy (time effect, $P < 0.05$) beyond the initial hypertrophy noted after the first 4 wk of pre-training (Figure 4). Although there were no significant interaction effects, HH and HLH appeared to experience the greatest type II hypertrophy from wk 4 to wk 35 (+22% and +21%), followed by HHH (+13%), while HL did not induce any gains in type II CSA. Regarding myofiber type distribution, the IIX to IIA shift that began during the 4 wk pre-training program continued during randomized training, as a further IIX to IIA shift was significant among all training prescriptions except HL (no prescription $\times$ time interaction), resulting in very few type IIX myofibers post-training ($P < 0.01$) (Figure 5).

3.5.2 Muscle Strength and Central Activation Ratio—All training prescriptions increased 1RM leg press (HHH +29%, HLH +29%, HH +27%, HL +13%) and knee extension (HHH +26%, HLH +36%, HH +26%, HL +21%) from wk 4 to wk 35 (Figure 6A-B). However, a significant prescription $\times$ time interaction showed that the HHH, HLH, and HH groups increased leg press strength as early as 8 wk into randomized training ($P < 0.01$), while the HL group did not realize further gains in leg press strength until wk 24 of randomized training ($P < 0.05$). Only HLH increased unilateral knee extension MVC beyond pre-training (i.e. from wk 4 to 35) (+20%, $P < 0.001$) (Figure 6C). Using the burst superimposition technique³², we found no changes in the central activation ratio (CAR) over the course of the training program, as CAR averaged >95% at all time points from pre-testing to post-testing. Upper body strength further increased from wk 4 to wk 35 among all prescriptions, as assessed by 1RM chest press (HHH +25%, HLH +16%, HH +11%, HL

+17%), overhead press (HHH +14%, HLH +14%, HH +7%, HL +10%), and arm curl (HHH +55%, HLH +36%, HH +17%, HL +30%) ($P < 0.01$ for all). The only prescription $\times$ time interaction found for upper body strength from wk 4 to wk 35 was in the arm curl ($P < 0.01$), with HHH gaining the most strength (Figure 6D).

3.5.3 Muscle Power—Knee extension relative peak power and mean power significantly increased overall (+20% and +21%, respectively) from wk 4 to wk 35 (time effect; $P < 0.0001$ for both), with post-hoc analysis showing all prescriptions significantly increased power except for HH (Figure 7A-B). Similarly, knee extension absolute peak and mean power increased overall (+10% and +11%, respectively) (time effect; $P < 0.01$ for both). Relative peak and mean power appeared to reach a plateau at wk 20, with no further gains occurring from wk 20 to wk 35 (data not shown).

3.5.4 Muscle Fatigability—While peak knee extension power increased in all groups, dynamic fatigability (i.e. rate of decline in concentric power across 20 repetitions) remained unchanged from wk 4 to wk 35. This resulted in an upward shift in the power generated across the 20-repetition fatigue test with no change in the slope (not shown). There was no prescription $\times$ time interaction.

3.5.5 Sit-to-Stand Motor Unit Activation—Resistance training following the 4 wk pre-training period did not further reduce MUA during the concentric sit-to-stand phase in any of the training groups; however, MUA during the eccentric phase was further reduced from wk 4 to wk 35 (47% at wk 4 to 40% at wk 35, $P < 0.01$) with no effect of exercise prescription (not shown).

3.5.6 Mobility Function and Flexibility—Overall, 35 wk of resistance training significantly increased distance walked during the 6MWT and reduced the amount of time to stand up from the floor ($P < 0.0001$ for both) (Table 3). The HHH and HH groups had the most notable gains in distance walked during the 6-minute walk test (+32m and +42m, respectively); while the HLH, HL, had HHH groups had the greatest reductions in time to stand up from the floor (-0.47s, -0.45s, and -0.37s). Training did not improve performance on the chair sit-and-reach flexibility test in any of the groups (not shown).

3.5.7 Cardiorespiratory Responses to Steady-State Exercise—The effects of 35 wk of RT on cardiorespiratory demand during steady state walking (3.0 mph, level grade) and stair climbing (31 steps/min) are summarized in Table 3. Steady-state Walking: Overall, RT improved metabolic economy from an average VO_2 demand prior to training of 15.0 mL/kg/min to 14.2 mL/kg/min at wk 35 (time effect; $P < 0.05$), accompanied by reduced minute ventilation (V_E) ($P < 0.01$). Similarly, steady state heart rate fell from 103 to 98 bpm (time effect; $P < 0.05$), indicating reduced cardiac workload. Via post-hoc analyses, HLH was the only training prescription yielding within-group reductions in steady-state VO_2 (-8%) and heart rate (-8%) from wk 0 to wk 35 ($P < 0.05$). Steady-state Stair-climbing: While there was no effect of RT on stair climbing metabolic economy (~18 mL/kg/min overall), the metabolic demand was met with a lower HR requirement (113 to 110 bpm, time effect; $P < 0.05$) and a lower RER (time effect; $P < 0.01$) post-training; both indicators that the absolute task was accomplished at a lower relative intensity.

3.5.8 Muscle Gene Expression and Cell Signaling—PCR and immunoblotting were performed on a subset of individuals ($n = 38$ and $n = 54$, respectively) based on tissue availability. The 4 wk pre-training program had no influence on muscle expression of the five pro-inflammatory target genes. On the other hand, differential responses to long-term randomized training were noted. As shown in Figure 8, elevated expression ($P < 0.05$) of TNFR1 (2.35-fold) and TWEAK-R (2.36-fold) was found after HHH training only, suggesting the HHH prescription induced an inflammatory burden in the quadriceps. No significant changes were noted in expression levels of IL-6R, TWEAK, or tenascin-C during 30 wk of randomized training (Supplemental Table 1).

3.5.8.1: Supplemental Figure 1 depicts representative immunoblots for proteins involved in pro-inflammatory (Figure S1A) and pro-anabolic (Figure S1B) signaling. There were no significant main or interaction effects for phospho-NF κ B p65 (Ser536), TNFR1, or caspase-3. At wk 35, levels of total NF κ B p65 protein were lower overall ($P < 0.05$), and post hoc analysis revealed a within-groups effect in HLH only (-16%, $P < 0.05$). We noted modest overall reductions in total eIF2 β and total p70S6K protein content from wk 0 to wk 35 (-10%, and -15%, respectively) (time effect, $P < 0.05$ for each). A group $\times$ time interaction ($P < 0.05$) revealed that only HHH and HLH had lower levels of phospho-p70S6K (Thr421/Ser424) at wk 35 vs. wk 0 (-64% and -48%, respectively; $P < 0.05$ for each). RPS6 protein levels did not change in any group. Data are provided in Supplemental Table 2.

4. DISCUSSION

This is the first randomized, dose-response RT clinical trial in which multiple frequencies and intensities were tested with the aim of identifying an RT prescription that maximizes muscle regrowth in atrophied older adults. The primary finding was that the HLH prescription maximized muscle regrowth based on whole body lean mass and TMM, which supports our central hypothesis. While the hypertrophic adaptation to RT exerts substantial inter-individual heterogeneity as we have shown repeatedly,^{36,37} results of the current trial demonstrate the dose of RT can be titrated to achieve significantly different outcomes on average (i.e. across all subjects exposed to a specific prescription), which is a major step toward defining a prescription that may best fit the needs of most older individuals.

No prescription induces homogeneous adaptations among recipients, and studies of inter-individual response heterogeneity by us^{18,21,37,38} and others (reviewed in³⁹) have yielded valuable insight. The 3-cluster K-means cluster analysis of the primary outcome (change in TMM) – summarized in Figure 3 – revealed clinically meaningful findings, as a remarkable 88% of HLH recipients were clustered as *positive responders* including two extreme responders. This is in stark contrast to the high proportions of *poor responders* noted in all other prescriptions (HHH 57%, HL 50%, HH 40%). Sex had no major influence on overall responsiveness to the four prescriptions in sum, as 38% of females and 41% of males were poor responders. On the other hand, within prescriptions, it was interesting to note that 6 of 9 males responded poorly to HHH compared to only 2 of 5 females; while 6 of 9 females responded well to HH compared to only 3 of 6 males. Regarding HLH, 6 of 7 females and 8 of 9 males responded very well with robust TMM hypertrophy.

It is clear that a large majority of older adults (>60y) are in need of significant muscle regrowth⁴⁰; however, the dose of RT that maximizes muscle regrowth in this population has not yet been established. We have previously shown that a typical 3 d/wk high-intensity RT program induces robust myofiber hypertrophy in naïve young adults, but the hypertrophic response to this same RT prescription among naïve older adults is generally blunted¹³ and highly variable.^{18,37}

Impaired recovery/regenerative capacity is well-established in aging skeletal muscle⁴¹⁻⁴⁴ and, as we have reported, this may be linked in part to muscle inflammation susceptibility at rest²³. Applying this to mechanical load-induced muscle hypertrophy, we hypothesized a traditional, high-intensity RT program performed 3 d/wk may not allow sufficient recovery time between exercise bouts in older adults, as aberrant muscle inflammation may blunt RT-induced protein synthesis and hypertrophy. Indeed, we noted that only the HHH prescription led to elevations in muscle gene expression of the pro-inflammatory cytokine receptors TNFR1 and TWEAK-R (Figure 8). By contrast, substituting the mid-week “H” bout with a bout of low-resistance, high-velocity, concentric only training (i.e. HLH) augmented the hypertrophic response without impacting cytokine receptor expression. While this observation is undoubtedly limited by the number and timing of muscle biopsies, it provides some insight into potential molecular processes driving differential responses to two different 3 d/wk prescriptions varying in mechanical stress only once per week.

Beyond this comparison of our two 3 d/wk prescriptions, several prior studies have shown successful muscle regrowth in older adults dosed with essentially the 2 d/wk HH program used here.⁴⁵⁻⁴⁸ In fact, we hypothesized that HLH and HH would yield similar muscle hypertrophy, and this was the case at the myofiber level (i.e. vastus lateralis type II myofiber CSA). On the other hand, assessments of total body lean mass and TMM obviously capture more muscle mass and the results favor HLH, indicating the added midweek “L” exercise bout somehow augmented muscle regrowth. Still, the benefits of the HH prescription should not be overlooked, as meaningful gains in muscle performance were noted, and 60% responded with TMM hypertrophy. It could be argued that – for many older adults – HH offers a reasonable balance of efficacy and practicality, requiring only 2 d/wk.

We fully expected the HL prescription would be insufficient for hypertrophy based on the low frequency (only 1 d/wk) of “H” loading and, again, this is supported by failed vastus lateralis type II myofiber hypertrophy, no significant change in DXA-derived total body lean mass, and a 50% TMM non-responder rate after 30 wk of HL. Fewer than two “H” stimuli per week was clearly not effective for eliciting myofiber hypertrophy, as HL was only sufficient to retain the type II hypertrophy seen in the pre-training period, which corroborates our earlier trial of 1 d/wk “H” for 32 wk of maintenance training following a progressive RT prescription.¹¹

Secondary to gains in muscle mass, we sought to examine which of the four training prescriptions could provide the greatest improvements in muscle function. Overall, significant gains in 1RM strength were seen among all training prescriptions, which is in accordance with previous literature showing that RT programs of varying intensity, volume, and recovery periods can all induce significant gains in muscle strength/power, and

functional performance in older adults²⁸⁻³⁰. However, it is important to note that the HHH, HLH, and HH groups further increased strength (e.g., leg press) as early as 8 wk after the 4 wk pre-training period, while the HL group did not realize additional gains until 24 wk after the pre-training period. Additionally, the HLH group was the only group to demonstrate significant gains in isometric knee extension MVC strength from wk 4 to wk 35. These findings support the idea that differential gains in muscle mass, at least in part, account for differential gains in strength. It did appear, however, that gains in muscle power beyond pre-training were less dependent on gains in muscle mass (Figure 6); a finding noted previously.⁴⁹

Perhaps one of the most important outcomes of an RT program for older adults is its efficacy to induce improvements in functional mobility. Overall, RT increased the distance walked during the 6MWT, even among these participants who were not mobility impaired (i.e. 6MWT > 600 m) and considered high functioning older adults. However, the HLH group was the only group to significantly decrease walking steady-state VO_2 and steady-state heart rate following training. This may have important implications on activities of daily living, as a reduced effort during walking may make daily tasks easier, leading to greater voluntary activity.

We recognize some limitations in this efficacy trial. First, the total sample size of completed participants ($n = 63$), combined with missing data for some outcomes limited the power to test prescription $\times$ time interactions for some key outcomes (e.g., number of muscle biopsy specimens vs. total number of subjects with DXA scans). Second, findings at the molecular level (both mRNA and protein cell signaling) are limited by the number and timing of serial muscle biopsies within subjects. We fully recognize the collection of specimens 48-72 hours post-exercise limits the investigation of early responses to each exercise dose; on the other hand, we consider the prolonged changes in pro-inflammatory gene expression useful in differentiating exercise doses. Finally, we specifically recruited relatively healthy older adults with limited disease risks. Thus, without additional study we do not know whether the results would be applicable to older adults with comorbid conditions including mobility limitations.

4.1 Conclusion

In conclusion, we used a four-arm, dose-response approach in an attempt to define an optimal RT program for muscle regrowth, as well as gains in muscle and mobility function among atrophied older adults. Because thigh muscle mass was our primary outcome, prior to randomization we implemented a standardized 4-wk pre-training period to account for the well-known, early RT adaptations that are largely independent of muscle mass gains. Considering the results in total, it appears the HLH prescription offers some distinct advantages over the other doses, while the HL program is subpar for many of the outcomes studied here. Although limited by a relatively small sample size, we can comfortably conclude from this randomized dose-response trial that older adults benefit greatly from 2 d/wk high-intensity RT, and may further benefit from inserting an additional weekly bout of low-load, explosive RT.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- HLH maximized gains in thigh muscle mass and total body lean mass.
- HLH induced the greatest gains in knee extension maximum isometric strength.
- HLH reduced cardiorespiratory demand during steady-state walking.
- HHH led to increased muscle expression of pro-inflammatory cytokine receptors.
- The HL prescription induced minimal muscle regrowth and lesser gains in performance.

Figure 1.
Subject recruitment, randomization, and adherence to the four exercise prescriptions.

Figure 2.

Effects of the dose-response RT trial on gains in total body lean mass (A), bilateral thigh muscle mass (adjusted to femur length) (B), and bilateral arm muscle mass (C) from wk0 to wk35. *Significant change from pre-training, $P < 0.05$. †Magnitude of change different from other groups: In (A), HLH is different from HL and HH ($P < 0.05$) and tends to be different from HHH ($P = 0.1$). In (B), HLH is different from HL ($P < 0.05$), and tends to be different from HH ($P = 0.07$) and HHH ($P = 0.09$). In (C), HHH is different from HL ($P < 0.05$).

Figure 3.

K-means cluster analysis of individual changes in the primary outcome, bilateral thigh muscle mass (TMM) demonstrate a remarkable range of changes in TMM across individuals in each prescription. The center and range of each cluster were as follows: (i) **poor responders** (n = 25; center = -93 g; range -570 to +187 g), (ii) **responders** (n = 33; center = +521 g; range +264 to +908 g), and (iii) **extreme responders** (n = 5; center = +1349 g; range +1015 to +1734 g). While substantial inter-individual heterogeneity was seen, it is noteworthy that all but two of the HLH participants (i.e. 88% of HLH) responded positively (range 280 to 1734 g), including two extreme responders. Such a high responder rate was not the case in any of the other three prescriptions.

Figure 4. Effects of the dose-response RT trial on type I (A) and type II (B) myofiber cross-sectional area. *Significant change from previous time point (main time effect; $P < 0.05$).

Figure 5. Effects of the dose-response RT trial on myofiber type distribution. *Significant change from previous time point (main time effect; $P < 0.05$).

Figure 6. Effects of the dose-response RT trial on lower body (A-C) and upper body (D) strength gains. *Significant change from previous time point, $P < 0.05$. †Significantly different than HL and HH at respective time point ($P < 0.05$).

Figure 7.
Effects of the dose-response RT trial on relative mean (A) and peak (B) knee extension power. *Significant change from previous time point, $P < 0.05$.

Figure 8. Effects of the dose-response RT trial on pro-inflammatory gene expression in the primary muscle group of interest (quadriceps). A) TNF-R gene expression. B) TWEAK-R gene expression. *Significant main time effect from previous time point, $P < 0.05$. †Post-hoc analysis showed only HHH significantly increased gene expression from wk 4 to wk 35 ($P < 0.01$).

Table 1

Mean adherence and training intensity among prescriptions across 30 wk of randomized training.

Exercise Prescription	HL	HH	HLH	HHH
Adherence* (% sessions completed)	93.3 ± 1.0	91.3 ± 1.1	89.5 ± 1.4	86.9 ± 1.7
Leg Press Intensity (% 1RM during “H” sessions)	72.6 ± 3.3	74.4 ± 3.1	71.7 ± 3.0	71.7 ± 3.1
Chest Press Intensity (% 1RM during “H” sessions)	61.4 ± 3.3	64.6 ± 2.4	61.1 ± 3.7	64.3 ± 4.2

Adherence data are based on the percentage of total sessions completed during the 30 wk. Total possible sessions were 60 for groups prescribed 2 d/wk training (HH, HL) and 90 for 3 d/wk training (HHH, HLH). Intensity data are provided for representative lower body (leg press) and upper body (chest press) movements based on the results of serial 1RM testing at multiple time points during training. To ensure progression was appropriate and unbiased, after each 1RM test, the resistance loads prescribed during the “H” training session immediately preceding 1RM testing were normalized to that 1RM (i.e. % 1RM), and then averaged across the 30 wk of randomized training. “H” = high-intensity concentric-eccentric resistance training in the 8-12 RM range. 1RM = one-repetition maximum. *Difference in adherence between 2 d/wk training (HH, HL) and 3 d/wk training (HHH, HLH), $P < 0.05$. Values are mean ± SE.

Table 2

Daily energy and nutrient intakes of subjects in the four resistance training prescriptions reported on four-day diet records.

	HL	HH	HLH	HHH
Energy, kcal/d *	1835 ± 159	1887 ± 185	1863 ± 97	1832 ± 99
Protein, g/d *	79.0 ± 6.1	73.6 ± 4.1	82.8 ± 3.9	85.2 ± 5.3
Protein, g/kg/d *	0.99 ± 0.06	0.95 ± 0.08	1.00 ± 0.07	1.05 ± 0.09
Carbohydrate, g/d	208 ± 19	236 ± 30	213 ± 13	205 ± 16
Fat, g/d	74.9 ± 8.1	70.5 ± 9.1	74.8 ± 5.6	74.0 ± 5.2
BCAA, g/d	13.8 ± 1.1	12.6 ± 0.7	14.1 ± 0.7	14.7 ± 0.9
Leucine, g/d	6.13 ± 0.49	5.64 ± 0.33	6.28 ± 0.32	6.49 ± 0.40

BCAA = branched chain amino acids. No significant differences in any dietary measures between training groups. Values are mean ± SE.

* Intake values do not include the 0.6 g/kg whey protein consumed three days per week (Monday, Wednesday, Friday).

Table 3

Effects of the four resistance training prescriptions on mobility function.

	HL		HH		HLH		HHH	
	wk0	wk35	wk0	wk35	wk0	wk35	wk0	wk35
6-minute walk test, m [*]	656.7 ± 20.7	681.4 ± 18.4	609.9 ± 16.7	651.6 ± 14.7 [†]	638.2 ± 16.8	656.1 ± 15.4	595.0 ± 11.1	626.5 ± 18.2 [†]
Time to stand up from the floor, s [*]	2.10 ± 0.19	1.65 ± 0.18 [†]	2.64 ± 0.19	2.34 ± 0.16	2.51 ± 0.27	2.04 ± 0.17 [†]	2.55 ± 0.21	2.18 ± 0.20 [†]

^{*} Main time effect from wk 0 to wk 35 ($P < 0.05$).

[†] Significantly different from wk 0 within prescription ($P < 0.05$). Values are mean ± SE.

Table 4

Effects of the four resistance training prescriptions on cardiorespiratory responses to steady-state exercise.

	HL			HH			HLH			HHH		
	Wk 0	Wk 35	Wk 0	Wk 35	Wk 0	Wk 35	Wk 0	Wk 35	Wk 0	Wk 35	Wk 0	Wk 35
Steady-state Walking (3.0 mph)												
VO ₂ , mL/kg/min *	14.1 ± 0.5	13.4 ± 0.5	14.6 ± 0.5	14.0 ± 0.4	15.4 ± 0.5	14.2 ± 0.5 [†]	15.7 ± 0.8	15.3 ± 0.6	15.7 ± 0.8	15.3 ± 0.6	15.7 ± 0.8	15.3 ± 0.6
HR, bpm *	100 ± 5	94 ± 3	104 ± 3	100 ± 3	102 ± 4	94 ± 3 [†]	106 ± 4	104 ± 4	106 ± 4	104 ± 4	106 ± 4	104 ± 4
V _E , L/min *	29.9 ± 1.5	28.6 ± 1.8	34.6 ± 2.2	32.3 ± 1.9	33.1 ± 2.0	30.7 ± 1.6	34.9 ± 2.2	33.7 ± 2.2	34.9 ± 2.2	33.7 ± 2.2	34.9 ± 2.2	33.7 ± 2.2
RER, VCO ₂ /VO ₂	0.78 ± 0.01	0.79 ± 0.01	0.83 ± 0.01	0.81 ± 0.01	0.81 ± 0.01	0.79 ± 0.01	0.81 ± 0.02	0.79 ± 0.01	0.81 ± 0.02	0.79 ± 0.01	0.81 ± 0.02	0.79 ± 0.01
Steady-state Stair-climbing (31 steps/min)												
VO ₂ , mL/kg/min	18.1 ± 0.3	18.0 ± 0.4	18.3 ± 0.4	17.8 ± 0.4	18.3 ± 0.4	18.6 ± 0.4	18.3 ± 0.4	18.8 ± 0.4	18.3 ± 0.4	18.8 ± 0.4	18.3 ± 0.4	18.8 ± 0.4
HR, bpm *	110 ± 4	109 ± 5	116 ± 4	111 ± 4 [†]	113 ± 4	109 ± 4	114 ± 4	111 ± 5	114 ± 4	111 ± 5	114 ± 4	111 ± 5
V _E , L/min *	36.3 ± 1.8	35.8 ± 1.9	41.2 ± 2.2	38.6 ± 1.7	39.9 ± 2.6	39.5 ± 2.3	41.8 ± 2.7	43.1 ± 2.8	41.8 ± 2.7	43.1 ± 2.8	41.8 ± 2.7	43.1 ± 2.8
RER, VCO ₂ /VO ₂ *	0.82 ± 0.01	0.82 ± 0.01	0.89 ± 0.01	0.85 ± 0.01 [†]	0.86 ± 0.02	0.85 ± 0.01	0.87 ± 0.02	0.84 ± 0.02	0.87 ± 0.02	0.84 ± 0.02	0.87 ± 0.02	0.84 ± 0.02

HR = heart rate, RER = respiratory exchange ratio, VE = minute ventilation.

* Main time effect from wk 0 to wk 35 (P < 0.05).

[†] Significantly different from wk 0 within prescription (P < 0.05). Values are mean ± SE.