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Molecular Differences in Skeletal Muscle After 1 Week of Active vs. Passive Recovery From High-Volume Resistance Training

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Abstract

Vann, CG, Haun, CT, Osburn, SC, Romero, MA, Roberson, PA, Mumford, PW, Mobley, CB, Holmes, HM, Fox, CD, Young, KC, and Roberts, MD. Molecular differences in skeletal muscle after 1 week of active vs. passive recovery from high-volume resistance training. *J Strength Cond Res* 35(8): 2102–2113, 2021—Numerous studies have evaluated how deloading after resistance training (RT) affects strength and power outcomes. However, the molecular adaptations that occur after deload periods remain under-studied. Trained, college-aged men ($n = 30$) performed 6 weeks of whole-body RT starting at 10 sets of 10 repetitions per exercise per week and finishing at 32 sets of 10 repetitions per exercise per week. After this period, subjects performed either active (AR; $n = 16$) or passive recovery (PR; $n = 14$) for 1 week where AR completed ~15% of the week 6 training volume and PR ceased training. Variables related to body composition and recovery examined before RT (PRE), after 6 weeks of RT (POST), and after the 1-week recovery period (DL). Vastus lateralis (VL) muscle biopsies and blood samples were collected at each timepoint, and various biochemical and histological assays were performed. Group × time interactions ($p < 0.05$) existed for skeletal muscle myosin heavy chain (MHC)-IIa mRNA (AR > PR at POST and DL) and 20S proteasome activity (post-hoc tests revealed no significance in groups over time). Time effects ($P < 0.05$) existed for total mood disturbance and serum creatine kinase and mechano growth factor mRNA (POST > PRE &D L), VL pressure to pain threshold and MHC-IIx mRNA (PRE&DL > POST), Atrogin-1 and MuRF-1 mRNA (PRE < POST < DL), MHC-I mRNA (PRE < POST & DL), myostatin mRNA (PRE & POST < DL), and mechanistic target of rapamycin (PRE > POST & DL). No interactions or time effects were observed for barbell squat velocity, various hormones, histological metrics, polyubiquitinated proteins, or phosphorylated/pan protein levels of 4E-BP1, p70S6k, and AMPK. One week of AR after a high-volume training block instigates marginal molecular differences in skeletal muscle relative to PR. From a practical standpoint, however, both paradigms elicited largely similar responses.

Key Words: deload, skeletal muscle hypertrophy, skeletal muscle proteolysis

Introduction

Performance during resistance training (RT) has been suggested to be influenced by fatigue that is accumulated over the time course of training. One commonly used method to dissipate fatigue are periods of deloading or tapering where lower volumes or lower intensities are used to facilitate recovery. A taper is defined as a reduction in training stimulus through a reduction in training frequency and training volume to reduce fatigue with the intent of maximizing performance. Deloading is a practical term that refers to a reduction of training volume load with similar aims to tapering generally accomplished through a reduction in training volume and training intensity. Conceptually, both strategies involve the manipulation of variables related to training with the specific aim of mitigating fatigue and subsequently improving training outcomes. Deload paradigms are commonly practiced in periodized training

to reduce fatigue and increase preparedness for successive training cycles or competition (12). Using lower volumes and/or intensities allows for the recovery or regeneration of the underlying physiological systems that support performance (3).

Historically, much of the research performed on recovery from RT has focused on performance-based outcomes (e.g., repetition maxes, force outputs, speed, etc.) or to mitigate injuries over longer duration training studies (30). Reductions in fatigue resulting from a taper or deload have been shown to result in increased muscle size, strength, power, and speed (8). When evaluating strength, researchers have shown that 2 weeks of tapering increased 1 repetition maximum (1RM) of the barbell back squat and barbell bench press (26). Importantly, although studies have examined the interaction of recovery protocols on muscle size and strength using tapering protocols—which would normally lead into competitive endeavors—little has been performed evaluating different deloading paradigms.

Stone et al. (33) suggested that the 2 primary outcomes of hypertrophy training are as follows: (a) positive changes in body composition and (b) increases in short-term muscular endurance.

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Although training execution is critical for skeletal muscle hypertrophy, recovery tactics also play an integral role. Notably, several studies have shown that allowing periods of time to recover after strenuous training may help maximize the hypertrophic response and promote increases in strength while also reducing the potential for stagnation and overtraining during the training cycle (23,29,34,35). In addition, periods of detraining (i.e., lighter load and volume or no training) in resistance-trained men have been shown to increase serum testosterone and decrease serum cortisol levels (21), which may be beneficial for skeletal muscle hypertrophy. However, beyond the evidence presented above, little work has been performed to evaluate physiological or molecular changes from different deloading or taper structures following periods of RT.

The aim of the current study was to evaluate the effect of an active recovery (AR) and a passive recovery (PR) paradigm on body composition, serum markers, muscle fiber cross-sectional area (fCSA), and the expression of protein and mRNA targets in skeletal muscle. We hypothesized that 1 week of AR vs. PR would lead to similar alterations in body composition and fCSA. However, because of the exploratory nature of this study, we did not propose a hypothesis for serum markers or the molecular targets in skeletal muscle.

Methods

Experimental Approach to the Problem

Before the start of the study, subjects were randomized into an active or passive recovery condition. Subjects then underwent 6 weeks of whole-body high-volume RT before completing their respective recovery paradigm. Subjects assigned to the AR group completed ~15% of volume completed during week 6, whereas subjects in the PR group ceased training for a period of 7 days. Intervention design, testing procedures, molecular analyses, and imaging methodologies are described in further detail below.

Subjects

Previously trained, college-aged men ($n = 30$) were recruited for this study and randomly assigned to an AR ($n = 16$) or PR ($n = 14$ mean ± SD) condition of which was executed after 6 weeks of high-volume RT. Subjects were required to have a self-reported training age of 1 or more years defined as RT greater than 2 days weekly over the course of a year. In addition, for subjects to qualify to engage in the protocol, they had to squat equal to or greater than $1.5\times$ body mass. Notably, 1 subject was removed ex post facto because of a lack of adherence to the study protocol. Subject baseline characteristics can be found in Table 1. The age range of subjects was 19–27 years old. This study was approved by the Institutional Review Board at Auburn University (Protocol #17-425 MR 1710) and conformed to the most recent standards set by the latest revision of the Declaration of Helsinki. Before any data being collected, subjects provided verbal and written consent which was conducted after an informational briefing where the design, rationale, and purpose of the study were covered.

Procedures

Resistance Training and Deload Protocol. Before beginning the training program, subjects were screened and familiarized with the study design and technical parameters of each exercise (barbell [BB] back squat, BB bench press, BB overhead press, BB stiff-legged deadlift, and supinated grip machine lat pull-down). Screening and testing of 3 repetition maximum (3RM) and a familiarization session were conducted 3–7 days before the start of the study and occurred under the direct supervision of research staff holding the National Strength and Conditioning Association's Certified Strength and Conditioning Specialist credential. The 3RM was used to calculate estimated 1RM which was used to dictate loads for the subjects to use over the duration of the study.

Readers can find a more thorough description of the training intervention in Haun et al. (10). In brief, subjects engaged in 6 weeks of voluminous RT starting at 10 sets of 10 repetitions per week per exercise, accumulating sets over a 6-week period, and ending at 32 sets of 10 repetitions per exercise, standardized at 60% of estimated 1RM. Exercises were performed in a compound set of which the BB back squat was performed first, followed by the BB pressing movement (BB bench press or BB overhead press), the BB stiff-legged deadlift, and finally the supinated grip lat pull-down. Rest between each compound set was standardized to 2 minutes; however—if the subject felt prepared to execute the next compound set—they were allowed to do so. Importantly, the standardization of intensity at 60% of estimated 1RM was made in concert with data collected during a pilot of the study design ($n = 2$) and a review published by Poortmans and Carpentier (24), suggesting a robust increase in myofibrillar protein synthesis when training is completed at ~60% of 1RM.

Training occurred on M/W/F of each week. Importantly, the BB bench press was substituted with BB overhead press on Wednesday training sessions. After the 6-week RT intervention, subjects performed either an AR or PR paradigm for a period of 1 week (termed "deload"; DL). The AR paradigm consisted of training sessions on M/W/F with 2 sets of 10 repetitions being completed on Monday and Friday and 1 set of 10 repetitions being completed on Wednesday of which were standardized to 60% of estimated 1RM. The PR paradigm ceased training for a period of 1 week. A schematic of the RT and recovery paradigm can be found in Figure 1 below. Calculation of total volume load was completed by multiplying the mass used for each exercise (in kilograms) and the total number of repetitions completed. The value generated for each exercise were summed together for a total training volume load.

Barbell velocity for the back squat was measured with a linear position transducer (TENDO unit; TENDO Sports Machines, Trencin, Slovak Republic) before the first session, before the last training session, and on the Friday of the deload paradigm, similar to methods used by Zourdos et al. (36).

PRE, POST, and DL Testing Sessions. Subjects reported for testing in an overnight-fasted state on Saturday from 0630 to 1400 before

Table 1

Baseline characteristics.*†

Variable	AR ($n = 16$)	PR ($n = 14$)	Total ($n = 30$)
Age (y)	24 ± 2	24 ± 2	24 ± 2
Training age (y)	5 ± 3	6 ± 2	5 ± 3
Height (cm)	181 ± 7	178 ± 9	180 ± 8
Body mass (kg)	82.1 ± 10.5	83.8 ± 12.8	82.9 ± 11.5
BIS-measured FFM (kg)	67.9 ± 8.4	68.4 ± 10.9	68.1 ± 9.5
BIS-measured fat Mass (kg)	14.2 ± 3.9	15.5 ± 5.6	14.8 ± 4.7
Est. 1RM back squat (kg)	145 ± 21	140 ± 8	143 ± 19
Squat relative to body mass	1.78 ± 0.25	1.69 ± 0.21	1.74 ± 0.23

*AR = active recovery; PR = passive recovery; BIS = bioelectrical impedance spectroscopy; FFM = fat-free mass; 1RM = 1 repetition maximum.

†Data are presented in mean ± SD.

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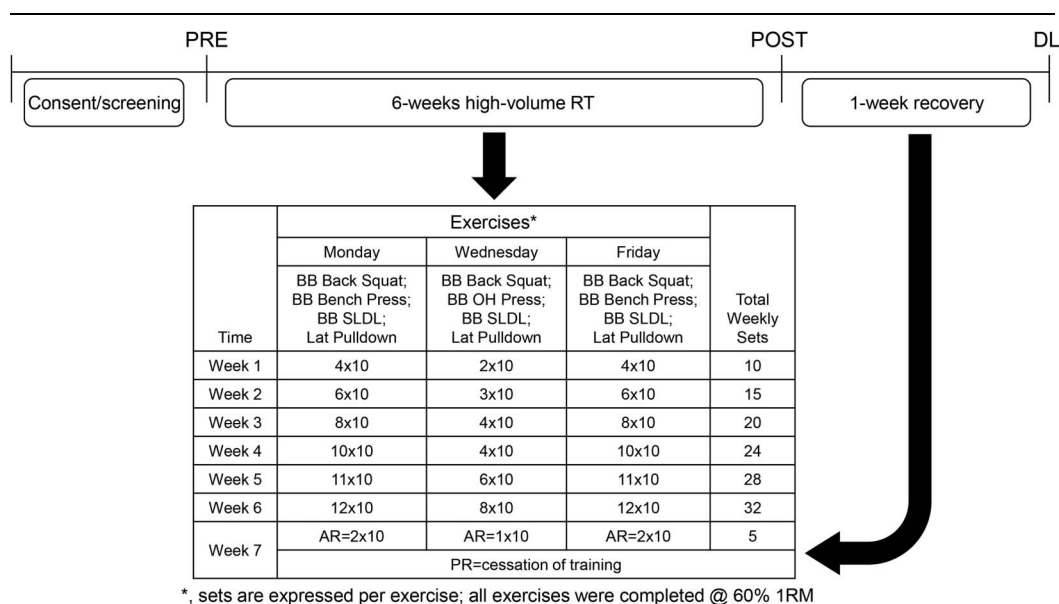*J*ournal of *S*trength and *C*onditioning *R*esearch™

Figure 1. Study design. AR = active recovery; PR = passive recovery; DL = deload; RT = resistance training; BB = barbell; SLDL = stiff-legged deadlift. "*" denotes that all exercises were completed at a standard intensity of 60% of estimated 1 repetition maximum derived from the testing of 3 repetition maximums for each exercise.

beginning the study (PRE), 24 hours after the last training bout on the sixth week of training (POST), and the Saturday after a 1-week deload (DL). Although many tests were completed in conjunction with these testing batteries and are described elsewhere (10), tests specific to this data set are described below.

Hydration, Profile of Mood State, and Algometry On arrival to each testing session, subjects were instructed to submit a urine sample (~5 ml) to assess normal hydration-specific gravity levels (<1.020 ppm) using a handheld refractometer (ATAGO, Bellevue, WA). Subjects having a urine specific gravity ≥1.020 were instructed to consume ~400 ml of tap water and were retested after 20 minutes. Subjects were also instructed to fill out the profile of mood state (POMS) questionnaire published by Grove and Prapavessis (7) to assess total mood disturbance (TMD). Calculation of TMD was completed by summing negative emotion scores and subtracting positive emotion scores to determine an aggregate score. After completion of the POMS survey, subjects underwent pressure-to-pain threshold (PPT) testing on the outer portion of the right upper thigh using a handheld algometer (Force Ten FDX; Wagner Instruments, Greenwich, CT) using methods described by our laboratory (9). In brief, the proximal, medial, and distal portions of the vastus lateralis (VL) were marked for accuracy after which, focal pressure was applied to the respective markings at a rate of ~5 N·s^{-1} until the subject audibly indicated the sensation of pain was felt. Notably, algometry measures were taken before biopsy samples being collected, and the medial VL algometry measure was taken ~2 cm from previous biopsy sampling points. The reading on the algometer was then recorded and repeated until 3 measures were taken at each locus with ~30 seconds between each measure. The average of the triplicate measures for each locus was taken, and these were averaged for total PPT.

Ultrasound for Muscle Thickness and Bioelectrical Impedance Spectroscopy for Body Composition After hydration testing and completion of the POMS questionnaire, subjects' height and body mass were measured using a digital column scale (Seca 769; Seca, Hanover, MD) with body masses and heights collected to the nearest 0.1 kg and 0.5 cm, respectively. Subjects then underwent ultrasound assessment to determine muscle thickness of the right VL using a 3–12 MHz multifrequency linear phase array transducer (Logiq S7 R2 Expert; General Electric, Fairfield, CT). These measurements were standardized by using the midway point between the iliac crest and patella of the right leg while standing upright and bearing a majority of their body mass on the left leg. All ultrasound measurements were taken by the same investigator (P.W.M.) to minimize variability of measurement as suggested in Lohman et al., and Lockwood et al. (15,16). Notably, test-retest reliability was performed on 33 subjects at PRE which yielded an intraclass correlation coefficient (ICC) of 0.994. After ultrasound assessment, subjects underwent body composition testing using bioelectrical impedance spectroscopy (BIS) as measured with the SFB7 (ImpediMed, Ltd., Queensland, Australia). Subjects were in a supine position for ~10 minutes, whereas height, body mass, sex, and age were entered. Electrical frequencies ranging from 4 to 1,000 kHz were sent through a series of 4 electrodes (2 on the dorsal surface of the right hand/wrist and 2 on the right ankle/foot) in accordance with the manufacturer's instructions. Proprietary models were then used to derive total body water (TBW), intracellular fluid (ICF), and extracellular fluid (ECF) (20). Resultant values were used to extrapolate fat free mass (FFM) by dividing the TBW by the assumed hydration constant of 0.732 (FFM = TBW/0.732) and fat mass (FM) by subtracting FFM from total body mass (FM = TBM − FFM) as suggested in Birzniece et al. (2). All BIS measurements were supervised by the same investigator (K.C.Y.), and test-retest reliability was performed on 24 subjects at PRE with an ICC of 0.999.

Venipuncture and Collection of Muscle Tissue After ultrasound and BIS measures, blood samples were extracted from the antecubital vein using a 5 ml serum separator tube (BD Vacutainer, Franklin Lakes, NJ). Blood samples were centrifuged at 3,500g for 5 minutes at room temperature, and serum was aliquoted into 1.7 ml polypropylene tubes and stored at −80° C

until processing. Skeletal muscle biopsy collections were obtained from the right VL using a 5-gauge needle as described in Mobley et al. (19). After biopsy collection, ~20–40 mg of tissue was teased of connective tissue and blood then embedded in cryomolds containing optimal cutting temperature (OCT) media (Tissue-Tek; Sakura Finetek, Inc., Torrence, CA). Tissue was embedded in a nonstretched manner for perpendicular slicing before rapid freezing using liquid nitrogen-cooled isopentane before being wrapped in foil and stored at −80° C. The remaining portion of the sample was placed in prelabeled foils and flash frozen by liquid nitrogen and stored at −80° C until processing. Notably, all biopsies were collected by the same investigators (M.D.R. and C.T.H.) and were spaced ~2 cm apart at approximately the same depth at each testing session.

Immunohistochemistry for Fiber Cross-Sectional Area and Myonuclear Quantity

Methodology for immunohistochemistry as it pertains to this investigation can be found in Haun et al. (10). In brief, OCT-preserved sections of tissue were cut at 8 μm using a cryotome (Leica Biosystems, Buffalo Grove, IL) and adhered to positively charged histology slides, and stored at −80° C until all samples were sectioned. Samples were air-dried at room temperature for up to 10 minutes, followed by permeabilization in a phosphate-buffered saline (PBS) solution containing 0.5% Triton X-100 for 10 minutes. After permeabilization, samples were blocked with Pierce Super Blocker (Thermo Fisher Scientific, Waltham, MA) for 10 minutes before being washed for 2 minutes in PBS. Samples were then incubated for 10 minutes with a commercially available, prediluted rabbit antidystrophin IgG antibody solution (catalog #: GTX15277; GeneTex, Inc., Irvine, CA) and spiked in mouse antimyosin I IgG (catalog #: A4.951 supernatant; Hybridoma Bank, Iowa City, IA; 40 μl per 1 ml of dystrophin antibody solution). Sections were washed for 2 minutes in PBS followed by a 15-minute incubation in the dark with a secondary antibody solution consisting of Texas-Red conjugated antirabbit IgG (catalog #: TI-1000; Vector Laboratories, Burlingame, CA) and Alexa-Fluor 488-conjugated antimouse IgG (catalog #: A-11001; Thermo Fisher Scientific; ~6.6 μl of all secondary antibodies per 1 ml of blocking solution). Sections were then washed for 2 minutes in PBS and air-dried followed by mounting with fluorescent media containing 4,6-diamidino- 2-phenylindole (DAPI; catalog #: GTX16206; GeneTex, Inc.). Sections were stored in the dark at 4° C until immunofluorescent imaging was conducted.

Digital images were captured using a fluorescence microscope (Nikon Instruments, Melville, NY) using a 10× objective. Approximate exposure times were 400 milliseconds for TRITC and FITC imaging, and 80 milliseconds for DAPI. This staining method allowed for the identification of cell membranes (detected by the TRITC filter), type I fiber green cell bodies (detected by the FITC filter), and myonuclei (detected by the DAPI filter). Quantification of myonuclei, type I, and type II fibers and measurement of the aforementioned fibers was completed with the open-source software CellProfiler (5) using the protocol described in Haun et al. (10), where the number of pixels within the border of each fiber were counted and converted to a total area (in squared micrometers). In addition, the total number of myonuclei was quantified using CellProfiler. Of importance, all sections were cut and imaged by investigators blinded to the interventions (H.M.H. and C.D.F.).

Muscle Tissue Processing

Muscle tissue foils were removed from −80° C and crushed using a liquid nitrogen cooled mortar and pestle. Approximately 30 mg of powdered muscle tissue was placed in a 1.7 ml microcentrifuge tube along with 500 μl of ice-cold general cell lysis buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, and 1%Triton; Cell Signaling, Danvers, MA, prestocked with protease and Tyr/Ser/Thr phosphatase inhibitors). Micropestles were used to manually homogenize tissue samples followed by centrifugation at 500*g* for 5 minutes to remove insoluble proteins. The sample lysates that were obtained were stored at −80° C before Western blotting and the 20S proteasome activity assay described below.

Western Blotting

Muscle tissue lysate samples were batch processed for total protein content using a BCA Protein Assay Kit (Thermo Fisher Scientific). Lysates were then prepared for Western blotting with 4× Laemmli buffer at 1 μg/μl. After sample preparation, 15 μl of each sample were loaded onto a 4–15% SDS-polyacrylamide gel (Bio-Rad Laboratories, Hercules, CA) followed by electrophoresis (180 V for 60 minutes) using premade 1× SDS-PAGE running buffer (Ameresco, Farmingdale, MA). Proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories), at 200 mA for 2 hours followed by Ponceau S staining and imaging to ensure equal protein loading between lanes. A blocking solution made of 5% nonfat milk powder and Tris-buffered saline with 0.1% Tween-20 (TBST; Ameresco) was applied to the membranes for a period of 1 hour at room temperature. Rabbit antihuman phospho-mechanistic target of rapamycin (mTOR) (Ser 2448) (1:1,000; catalog #: 2971; Cell Signaling), rabbit antihuman pan mTOR (1:1,000; catalog #: 2972; Cell Signaling), rabbit antihuman phospho-AMPKα (Thr 172) (1:1,000; catalog #: 2535; Cell Signaling), rabbit antihuman pan AMPKα (1:1,000; catalog #: 2532; Cell Signaling), rabbit antihuman phospho-4E-BP1 (Thr 37/46) (1:1,000; catalog #: 2855; Cell Signaling), rabbit antihuman pan 4E-BP1 (1:1,000; catalog #: 9644; Cell Signaling), rabbit antihuman phospho-p70 s6k (Thr 389) (1:1,000; catalog #: 9234; Cell Signaling), rabbit antihuman pan p70 s6k (1:1,000; catalog #: 2708; Cell Signaling), and rabbit antihuman ubiquitin (1:1,000; catalog #: 3933; Cell Signaling) were incubated with membranes for 24 hours at 4° C in TBST with 5% bovine serum albumin (BSA). After a 24-hour incubation, samples underwent a 1-hour incubation in horseradish peroxidase–conjugated antirabbit (1:2,000) (catalog #: 7074; Cell Signaling) in TBST with 5% BSA at room temperature. Membranes were developed using an enhanced chemiluminescent reagent (Luminata Forte HRP substrate; EMD Millipore, Billerica, MA), with band densitometry performed with a gel documentation system and associated densitometry software (UVP, Upland, CA). Densitometry values for phosphorylated Western blot targets were normalized to their respective pan densitometry values similar to the methods reported by Roberson et al. (27).

Real-Time Polymerase Chain Reaction

RNA was reverse transcribed into cDNA for quantitative polymerase chain reaction (qPCR) analysis using 2 μg of RNA with cDNA synthesis reagents (Quanta Biosciences, Gaithersburg, MD) in accordance with the manufacturer's recommendations. Quantitative PCR was performed using SYBR green chemistry (Quanta Biosciences) and gene-specific primers which can be found in Table 2. Calculations for qPCR were performed as previously described in Romero et al. (28).

Serum Assays for Creatine Kinase Activity, Testosterone, and Cortisol

Serum creatine kinase (CK) levels were determined using a commercially available activity assay (Bioo Scientific, Austin, TX). Values were not analyzed if the sample was missing

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or if the standard curve indicated that serum levels were negative. Commercially available ELISA kits (ALPCO Diagnostics, Salem, NH) were used to measure serum testosterone and cortisol. All kits were performed in accordance with manufacturer's specifications, and plates were read using a 96-well spectrophotometer (BioTek, Winooski, VT). The mean coefficient of variation (CV) of duplicate values for each assay was 1.4% for CK, 6.9% for testosterone, and 2.8% for cortisol.

20S Proteasome Activity Assay Muscle lysates (described above) were batch processed for 20S proteasome activity using commercially available fluorometric kits (catalog #: APT280; Millipore Sigma, Burlington, MA) in accordance with the manufacturer's instructions. Values from the assay are expressed as relative fluorometric units. The mean CV for all duplicates was 8.7%.

Statistical Analysis

Statistical analyses were performed in SPSS (version 24; IBM SPSS Statistics Software, Chicago, IL) and Google Sheets. Before analysis, assumptions testing was performed for all dependent variables consisting of (a) Shapiro-Wilk's tests for normality, (b) Mauchly's test for sphericity, and (c) Levene's test of homogeneity of variance. A Greenhouse-Geisser correction was applied if the assumption of homogeneity of variance and/or sphericity was violated. Gross and molecular markers of skeletal muscle hypertrophy, variables related to RT performance, and biochemical assays were analyzed using repeated measures analysis of covariance where a mean-centered covariate for PRE measures was created and implemented within the model. Western blot and qPCR data were analyzed using repeated measures analysis of variance. LSD post-hoc tests were used to assess differences in dependent variables for group or time. Statistical significance was set at $p \leq 0.05$. Data are presented throughout as mean $\pm$ SD.

Results

Training and Deload Volume Load

Training volume (Figure 2) exhibited a significant effect of time ($p < 0.001$; $\eta_p^2 = 0.975$). Combined training volume for weeks 1–6 were higher than DL training volume ($p < 0.001$; confidence interval [CI]$_{wks1-6}$ = 329,114.53–374,995.58; CI$_{DL}$ = 5,538.36–7,356.84). There was no group × time interaction or main effect of group observed. Independent samples t-tests were run on combined training volume for weeks 1–6 and DL training

volume to demonstrate similarities in intervention training volume and to show differences in DL training volume. There were no differences observed in combined (weeks 1–6) training volume ($p = 0.423$); however, as expected, training volume at DL was different between AR and PR conditions ($p < 0.001$).

Total Mood Disturbance, Barbell Velocity, and Soreness

Barbell velocity (Figure 3A) did not exhibit a group × time interaction ($p = 0.761$; $\eta_p^2 = 0.011$). In addition, there was no main effect of group ($p = 0.652$; $\eta_p^2 = 0.013$; CI$_{AR}$ = 0.66–0.76; CI$_{PR}$ = 0.64–0.75) or time ($p = 0.075$; $\eta_p^2 = 0.166$; CI$_{PRE}$ = 0.74–0.74; CI$_{POST}$ = 0.65–0.75, CI$_{DL}$ = 0.61–0.74). Total mood disturbance (Figure 3B) did not exhibit a group × time interaction ($p = 0.294$; $\eta_p^2 = 0.052$) or main effect of group ($p = 0.592$; $\eta_p^2 = 0.013$; CI$_{AR}$ = 85.98–91.37; CI$_{PR}$ = 84.72–90.54). TMD did, however, exhibit a main effect of time ($p = 0.006$; $\eta_p^2 = 0.200$; CI$_{PRE}$ = 87.73–87.73; CI$_{POST}$ = 87.54–95.80; CI$_{DL}$ = 81.71–88.41) where POST TMD was greater than DL ($p = 0.007$). No differences were observed between PRE and POST TMD. Pressure to pain threshold (Figure 3C) did not exhibit a group × time interaction ($p = 0.542$; $\eta_p^2 = 0.021$) or main effect of group ($p = 0.363$; $\eta_p^2 = 0.033$; CI$_{AR}$ = 58.95–67.41; CI$_{PR}$ = 51.53–70.68). There was, however, a main effect of time observed for PPT ($p < 0.001$; $\eta_p^2 = 0.307$; CI$_{PRE}$ = 70.19–70.19; CI$_{POST}$ = 87.54–95.80; CI$_{DL}$ = 81.71–88.41) where PPT was lower at POST than at PRE ($p < 0.001$) and DL ($p = 0.016$).

Body Composition and Vastus Lateralis Thickness

Body composition and VL muscle thickness values are presented in Table 3. Total body mass did not exhibit a group × time interaction ($p = 0.855$; $\eta_p^2 = 0.003$) or main effect of group ($p = 0.788$; $\eta_p^2 = 0.003$; CI$_{AR}$ = 82.75–84.33; CI$_{PR}$ = 82.87–84.51). Total body mass did exhibit a main effect of time ($p = 0.002$; $\eta_p^2 = 0.258$; CI$_{PRE}$ = 82.776–82.776; CI$_{POST}$ = 83.50–85.15; CI$_{DL}$ = 82.82–84.67) where total body mass at POST was greater than PRE ($p < 0.001$) and DL ($p = 0.041$). Bioelectrical impedance spectroscopy measured fat-free mass did not exhibit a group × time interaction ($p = 0.491$; $\eta_p^2 = 0.024$) or main effect of group ($p = 0.916$; $\eta_p^2 < 0.001$; CI$_{AR}$ = 68.29–70.09; CI$_{PR}$ = 68.33–70.19). There was, however, a main effect of time observed for BIS fat-free mass ($p < 0.001$; $\eta_p^2 = 0.383$; CI$_{PRE}$ = 67.95–67.95; CI$_{POST}$ = 69.50–71.42; CI$_{DL}$ = 68.20–70.36) where fat-free mass at POST was greater than at PRE ($p < 0.001$) and DL ($p = 0.018$). Furthermore, BIS measured fat mass did not exhibit a

Table 2

Quantitative polymerase chain reaction primer sequences.*

	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
Gene		
MGF	5′-CGAAGTCTCAGAGAAGGAAAGG-3′	5′-ACAGGTAACTCGTGCAGAGC-3′
MSTN	5′-GACCAGGAGAAGATGGGCTGAATCCGTT-3′	5′-CTCATCACAGTCAAGACCAAAATCCCTT-3′
Atrogin-1	5′-ATGTGCGTGTATCGGATGG-3′	5′-AAGGCAGGTCAGTGAAGC-3′
MuRF-1	5′-GCCTTCTTCGCCTTCTCC-3′	5′-AGCTCATACAGACTCAGTTCC-3′
MHC I	5′-GTATGAGGAGTCGCAGTCGG-3′	5′-AGGGACTCCTCATAGGCGTT-3′
MHC IIa	5′-GAACACCCAAGGCATCCTCA-3	5′-GCTGTTCCTTCAGGTCCTCC-3′
MHC IIx	5′-CTGGTGGACAAACTGCAAGC-3′	5′-CCTGCGGAATTTGGAGAGGT-3′
Housekeeping genes		
B2M	5′- ATGAGTATGCCTGCCGTGTGA-3′	5′-GGCATCTTCAAACCTCCATG-3′
PPIA	5′-CGATGTCTCAGAGCACGAAA-3′	5′-CCCACCTGTTTCTTCGACAT-3′

*MGF = mechano growth factor; MSTN = myostatin; MuRF-1 = muscle ring finger-1; MHC-I = myosin heavy chain-1; MHC-IIa = myosin heavy chain-IIa; MHC-IIx = myosin heavy chain-IIx; B2M = beta-2-microglobulin; PPIA = cyclophilin.
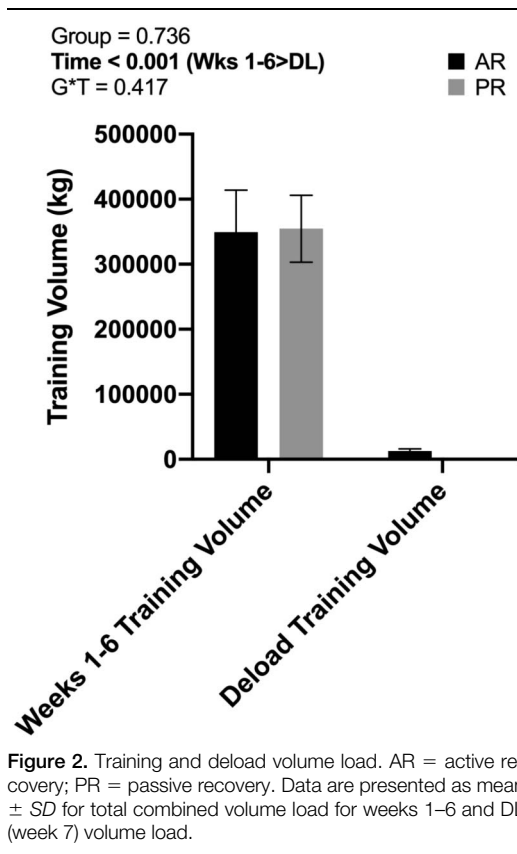
Figure 2. Training and deload volume load. AR = active recovery; PR = passive recovery. Data are presented as mean ± *SD* for total combined volume load for weeks 1–6 and DL (week 7) volume load.

group × time interaction ($p = 0.174$; $\eta_p^2 = 0.065$) or main effect of group ($p = 0.906$; $\eta_p^2 = 0.001$; $CI_{AR} = 13.84–14.89$; $CI_{PR} = 13.86–14.96$). A main effect of time observed for BIS fat mass ($p = 0.005$; $\eta_p^2 = 0.187$; $CI_{PRE} = 14.83–14.83$; $CI_{POST} = 13.22–14.51$; $CI_{DL} = 13.87–15.06$) where fat mass at PRE and DL were greater than at POST ($p = 0.005$ and $p = 0.019$, respectively).

Vastus lateralis thickness measured by ultrasound did not exhibit a group × time interaction ($p = 0.769$; $\eta_p^2 = 0.010$). In addition, there was no effect of group ($p = 0.691$; $\eta_p^2 = 0.006$; $CI_{AR} = 2.94–3.14$; $CI_{PR} = 2.96–3.18$) or time ($p = 0.064$; $\eta_p^2 = 0.100$; $CI_{PRE} = 2.98–3.11$; $CI_{POST} = 3.01–3.20$; $CI_{DL} = 2.91–3.12$).

There was no group × time interaction observed ($p = 0.440$; $\eta_p^2 = 0.030$) for TBW. In addition, there was no main effect of group ($p = 0.988$; $\eta_p^2 < 0.001$; $CI_{AR} = 48.12–49.24$; $CI_{PR} = $

48.08–49.29). Total body water did, however, exhibit a main effect of time ($p < 0.001$; $\eta_p^2 = 0.376$; $CI_{PRE} = 47.88–47.88$; $CI_{POST} = 48.79–50.03$; $CI_{DL} = 48.07–49.42$) where TBW at POST was greater than PRE ($p < 0.001$) and DL ($p = 0.002$). In addition, TBW at DL was greater than at PRE ($p = 0.014$). Intracellular water (ICW) did not exhibit a group × time interaction ($p = 0.474$; $\eta_p^2 = 0.027$) or main effect of group ($p = 0.865$; $\eta_p^2 = 0.002$; $CI_{AR} = 29.35–30.10$; $CI_{PR} = 29.36–30.17$). There was a main effect of time observed for ICW ($p < 0.001$; $\eta_p^2 = 0.317$; $CI_{PRE} = 29.23–29.23$; $CI_{POST} = 29.62–30.55$; $CI_{DL} = 29.52–30.33$) where ICW at PRE was lower than at POST ($p < 0.001$) and DL ($p = 0.002$). Extracellular water (ECW) did not exhibit a group × time interaction ($p = 0.737$; $\eta_p^2 = 0.027$) or main effect of group ($p = 0.865$; $\eta_p^2 = 0.002$; $CI_{AR} = 18.67–19.25$; $CI_{PR} = 29.36–30.17$). There was, however, a main effect of time observed for ECW ($p < 0.001$; $\eta_p^2 = 0.277$; $CI_{PRE} = 18.65–18.65$; $CI_{POST} = 29.62–30.55$; $CI_{DL} = 29.52–30.33$) where ECW at POST was greater than at PRE ($p < 0.001$) and DL ($p = 0.001$).

Testosterone and Cortisol

Serum testosterone (Figure 4A) did not exhibit a group × time interaction ($p = 0.359$; $\eta_p^2 = 0.044$), main effect of group ($p = 0.178$; $\eta_p^2 = 0.078$; $CI_{AR} = 14.75–16.92$; $CI_{PR} = 13.56–15.91$), or main effect of time ($p = 0.436$; $\eta_p^2 = 0.035$; $CI_{PRE} = 15.52–15.52$; $CI_{POST} = 13.61–15.98$; $CI_{DL} = 14.05–17.02$). In addition, serum cortisol (Figure 4B) did not exhibit a group × time interaction ($p = 0.530$; $\eta_p^2 = 0.027$), main effect of group ($p = 0.843$; $\eta_p^2 = 0.002$; $CI_{AR} = 331.62–400.29$; $CI_{PR} = 333.77–407.95$), or main effect of time ($p = 0.943$; $\eta_p^2 = 0.003$; $CI_{PRE} = 371.01–371.01$; $CI_{POST} = 319.55–409.76$; $CI_{DL} = 330.74–408.33$). Finally, serum testosterone to cortisol ratio (T:C ratio; Figure 4C) did not exhibit a group × time interaction ($p = 0.245$; $\eta_p^2 = 0.060$), main effect of group ($p = 0.403$; $\eta_p^2 = 0.031$; $CI_{AR} = 0.04–0.05$; $CI_{PR} = 0.04–0.05$), or main effect of time ($p = 0.910$; $\eta_p^2 = 0.004$; $CI_{PRE} = 0.04–0.04$; $CI_{POST} = 0.04–0.05$; $CI_{DL} = 0.04–0.05$).

Fiber Cross Sectional Area and Myonuclear Quantity

Mean fCSA (Figure 5A) did not exhibit a group × time interaction ($p = 0.853$; $\eta_p^2 = 0.006$), main effect of group ($p = 0.846$; $\eta_p^2 = 0.001$; $CI_{AR} = 3,895.98–4,287.27$; $CI_{PR} = 2,862.07–4,267.24$), or main effect of time ($p = 0.376$; $\eta_p^2 = 0.037$; $CI_{PRE} = 4,035.09–4,035.09$; $CI_{POST} = 3,931.66–4,451.79$; $CI_{DL} = 3,730.39–4,284.83$). Type I mean fCSA (Figure 5B) did not
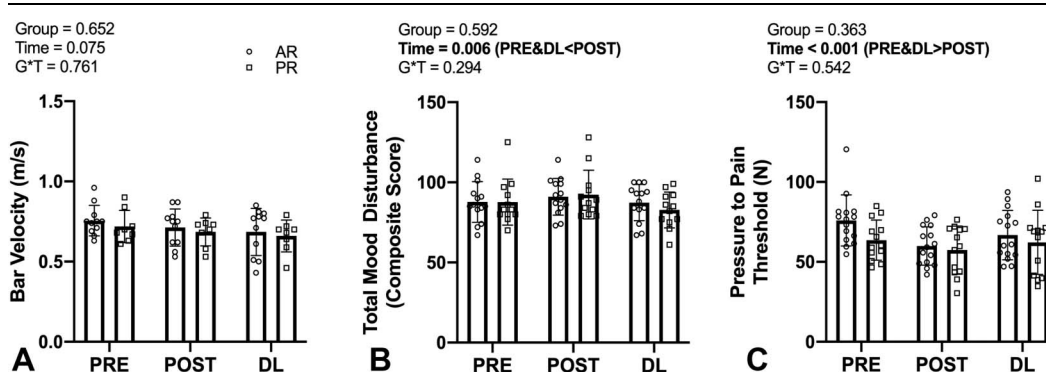

Figure 3. Barbell velocity, total mood disturbance, and soreness. AR = active recovery; PR = passive recovery. Data are presented as mean ± *SD* for barbell velocity (panel A), total mood disturbance (panel B), and soreness (panel C).

Table 3

Body composition.*

Variable	PRE Mean ± SD	POST Mean ± SD	DL Mean ± SD	p Time	p Group	p Group × time
Total mass (kg)						
AR (n = 15)	81.80 ± 10.77	83.24 ± 10.75	82.72 ± 10.06	0.002†	0.788	0.855
PR (n = 14)	83.82 ± 12.82	85.48 ± 12.53	84.84 ± 13.03			
BIS-measured FFM (kg)						
AR (n = 15)	67.56 ± 8.61	70.28 ± 8.74	68.62 ± 7.16	<0.001†	0.916	0.491
PR (n = 14)	68.36 ± 10.93	70.67 ± 11.13	69.96 ± 11.49			
BIS-measured FM (kg)						
AR (n = 15)	14.24 ± 3.99	12.96 ± 4.43	14.10 ± 4.57	0.005‡	0.906	0.174
PR (n = 14)	15.46 ± 5.61	14.81 ± 5.90	14.87 ± 5.75			
VL thickness (cm)						
AR (n = 15)	2.97 ± 0.54	3.02 ± 0.41	2.96 ± 0.45	0.064	0.691	0.769
PR (n = 14)	3.13 ± 0.52	3.19 ± 0.46	3.06 ± 0.37			
Total body water (L)						
AR (n = 15)	47.66 ± 5.45	49.37 ± 5.40	48.36 ± 4.44	<0.001‡	0.988	0.440
PR (n = 13)	48.13 ± 6.98	49.50 ± 7.31	49.16 ± 7.43			
Intracellular water (L)						
AR (n = 15)	29.28 ± 3.36	30.21 ± 3.15	29.83 ± 2.68	<0.001‡	0.865	0.474
PR (n = 13)	29.17 ± 4.03	29.95 ± 4.46	30.02 ± 4.32			
Extracellular water (L)						
AR (n = 15)	18.38 ± 2.23	19.16 ± 2.52	18.53 ± 2.00	<0.001‡	0.830	0.737
PR (n = 13)	18.97 ± 3.10	19.54 ± 3.05	19.14 ± 3.33			

*FFM = fat-free mass; FM = fat mass; AR = active recovery; PR = passive recovery; BIS = bioelectrical impedance spectroscopy; VL = vastus lateralis.
†Measurement was higher at POST and DL ($p < 0.05$).
‡Measurement was higher at POST ($p < 0.05$).

exhibit a group × time interaction ($p = 0.869$; $\eta_p^2 = 0.005$), main effect of group ($p = 0.647$; $\eta_p^2 = 0.008$; $CI_{AR} = 3{,}571.54$–$3{,}983.52$; $CI_{PR} = 3{,}631.35$–$4{,}057.86$), or main effect of time ($p = 0.731$; $\eta_p^2 = 0.012$; $CI_{PRE} = 3{,}819.69$–$3{,}819.69$; $CI_{POST} = 3{,}567.40$–$4{,}160.53$; $CI_{DL} = 3{,}490.17$–$4{,}008.93$). Type II mean fCSA (Figure 5C) did not exhibit a group × time interaction ($p = 0.605$; $\eta_p^2 = 0.019$), main effect of group ($p = 0.787$; $\eta_p^2 = 0.003$; $CI_{AR} = 3{,}930.77$–$4{,}427.04$; $CI_{PR} = 3{,}874.51$–$4{,}388.30$), or main effect of time ($p = 0.163$, $\eta_p^2 = 0.067$; $CI_{PRE} = 4{,}076.04$–$4{,}076.01$; $CI_{POST} = 4{,}019.99$–$4{,}604.48$; $CI_{DL} = 3{,}769.28$–$4{,}385.15$). The mean myonuclei per muscle fiber (Figure 5D) did not exhibit a group × time interaction ($p = 0.192$; $\eta_p^2 = 0.061$) or main effect of time ($p = 0.747$; $\eta_p^2 = 0.011$; $CI_{PRE} = 2.57$–2.57; $CI_{POST} = 2.42$–2.96; $CI_{DL} = 2.30$–2.99). The mean myonuclei per muscle fiber did, however, have a main effect of group ($p = 0.037$; $\eta_p^2 = 0.157$; $CI_{AR} = 2.59$–3.02; $CI_{PR} = 2.25$–2.69) where the AR group had more myonuclei per muscle fiber than the PR group ($p = 0.037$).

Markers of Skeletal Muscle Proteolysis

A significant group × time interaction was observed for 20S proteasome activity ($p = 0.049$; $\eta_p^2 = 0.122$; Figure 6A); however, post-hoc tests revealed no statistically significant differences between conditions at PRE ($p = 0.833$), POST ($p = 0.298$), and DL ($p = 0.078$). 20S proteasome activity did not exhibit a main effect of group ($p = 0.540$; $\eta_p^2 = 0.015$; $CI_{AR} = 68.05$–79.08; $CI_{PR} = 65.46$–76.88) or time ($p = 0.889$; $\eta_p^2 = 0.005$; $CI_{PRE} = 70.98$–70.98; $CI_{POST} = 65.46$–81.09; $CI_{DL} = 63.23$–82.47). Fold change for muscle polyubiquitinated proteins (Figure 6B) did not exhibit a group × time interaction ($p = 0.631$; $\eta_p^2 = 0.013$), main effect of group ($p = 0.633$; $\eta_p^2 = 0.009$; $CI_{AR} = 0.91$–1.18; $CI_{PR} = 0.83$–1.12), or main effect of time ($p = 0.212$; $\eta_p^2 = 0.059$; $CI_{PRE} = 1.00$–1.00; $CI_{POST} = 0.81$–1.07; $CI_{DL} = 0.90$–1.28). Atrogin-1 mRNA expression (Figure 6D) did not exhibit a group × time interaction ($p = 0.971$; $\eta_p^2 = 0.001$) or main effect of group ($p = 0.838$; $\eta_p^2 = 0.002$; $CI_{AR} = 2.10$–5.29;
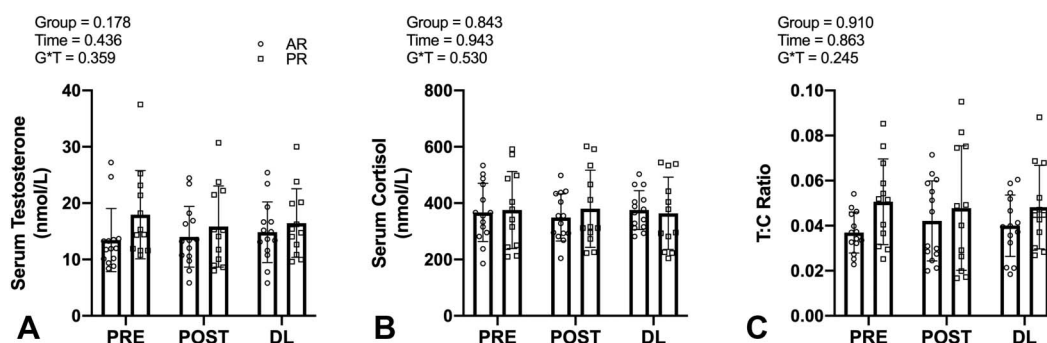

Figure 4. Testosterone and cortisol. AR = active recovery; PR = passive recovery; T:C ratio = testosterone to cortisol ratio. Data are presented as mean ± SD for testosterone (panel A), cortisol (panel A), and T:C ratio (panel C).
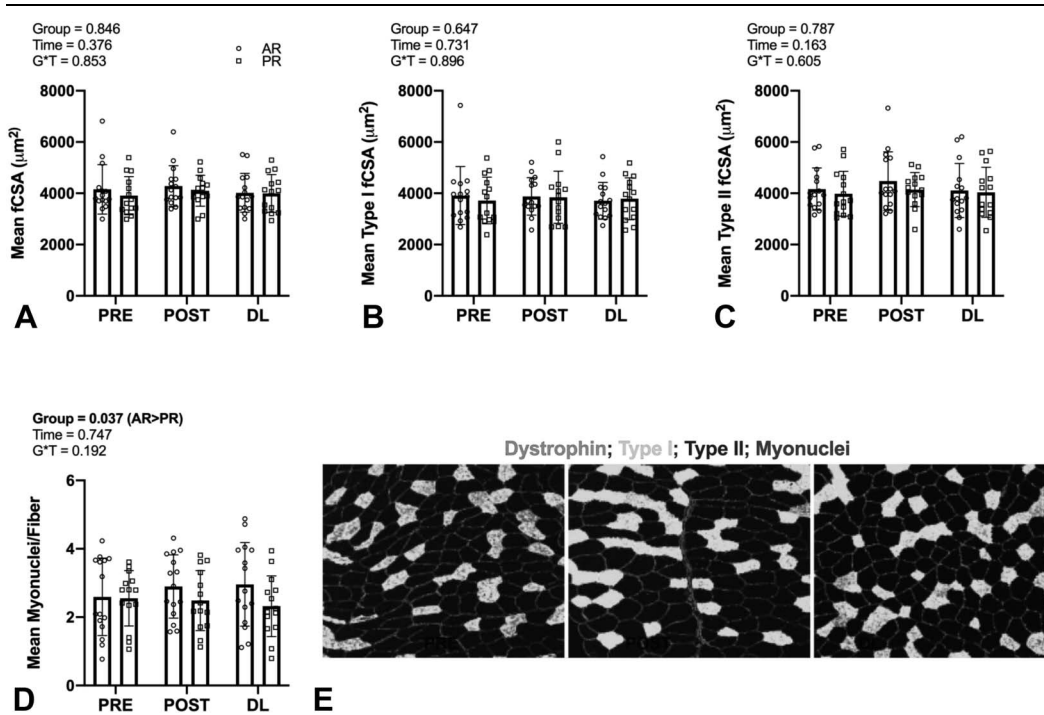
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Figure 5. Fiber cross sectional area and myonuclear quantity. AR = active recovery; PR = passive recovery; fCSA = fiber cross-sectional area. Data are presented as mean ± *SD* for mean fCSA (panel A), type I fCSA (panel B), type II fCSA (panel C), and mean myonuclei per fiber (panel D). Panel E is a representative histology image for *n* = 1 subject at PRE, POST, and DL.
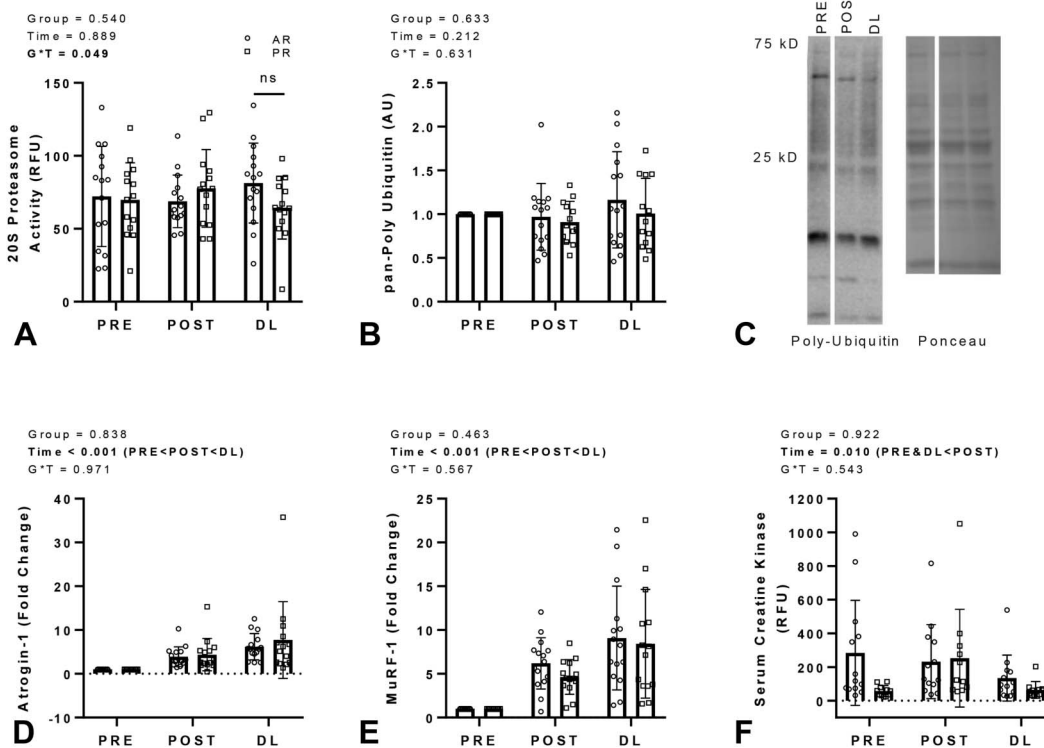

Figure 6. Markers of skeletal muscle proteolysis. AR = active recovery; PR = passive recovery. Data are presented as mean ± *SD* for 20S proteasome activity (panel A), pan-polyubiquitin (panel B), atrogin-1 mRNA (panel D), MuRF-1 mRNA (panel E), and serum creatine kinase (panel F). Panel C is a representative Western blot (*n* = 1) of pan-polyubiquitin. "ns" indicates an interaction effect for the 20S proteasome at DL where AR values trended higher than PR values (*p* = 0.078).

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CI_{PR} = 2.71–6.01). Atrogin-1 mRNA expression did, however, exhibit a main effect of time ($p < 0.001$; $\eta_p^2 = 0.791$; CI_{PRE} = 1.00–1.00; CI_{POST} = 2.98–5.29; CI_{DL} = 4.49–9.41), where atrogin-1 mRNA expression was greater at DL than at PRE and POST ($p < 0.001$). In addition, atrogin-1 mRNA expression was greater at POST than at PRE ($p < 0.001$). MuRF-1 mRNA expression (Figure 6E) did not exhibit a group × time interaction ($p = 0.567$; $\eta_p^2 = 0.015$) or main effect of group ($p = 0.463$; $\eta_p^2 = 0.020$; CI_{AR} = 4.01–6.84; CI_{PR} = 3.22–6.15). MuRF-1 mRNA expression did, however, exhibit a main effect of time ($p < 0.001$; $\eta_p^2 = 0.598$; CI_{PRE} = 1.00–1.00; CI_{POST} = 4.46–6.37; CI_{DL} = 6.44–11.06) where MuRF-1 mRNA expression was greater at DL than at PRE and POST ($p < 0.001$). In addition, MuRF-1 mRNA expression was greater at POST than at PRE ($p < 0.001$). Serum CK (Figure 6F) did not exhibit a group × time interaction ($p = 0.543$; $\eta_p^2 = 0.022$) or main effect of group ($p = 0.922$; $\eta_p^2 < 0.001$; CI_{AR} = 114.25–239.36; CI_{PR} = 103.48–240.79). There was, however, a main effect of time ($p = 0.010$; $\eta_p^2 = 0.197$; CI_{PRE} = 180.12–180.12; CI_{POST} = 132.549–353.85; CI_{DL} = 54.10–146.12) for serum CK where this measure was lower at DL than at PRE ($p = 0.002$) and POST ($p = 0.011$).

mRNA Markers of Skeletal Muscle Anabolism

Myosin heavy chain (MHC) I mRNA expression (Figure 7A) did not exhibit a group × time interaction ($p = 0.136$; $\eta_p^2 = 0.071$) or main effect of group ($p = 0.106$; $\eta_p^2 = 0.094$; CI_{AR} = 6.84–11.79; CI_{PR} = 3.86–8.97). However, a main effect of time was observed for MHC-I mRNA expression ($p < 0.001$; $\eta_p^2 = 0.586$; CI_{PRE} = 1.00–1.00; CI_{POST} = 8.87–15.27; CI_{DL} = 7.81–13.20) where

MHC-I mRNA expression at PRE was lower than at POST and DL ($p < 0.001$). There was a group × time interaction ($p = 0.002$; $\eta_p^2 = 0.213$) for MHC-IIa mRNA expression (Figure 7B). Post-hoc analysis revealed MHC-IIa mRNA expression to be higher in AR than PR at POST ($p = 0.034$) and at DL ($p = 0.001$). In addition, there was a main effect of group ($p = 0.003$; $\eta_p^2 = 0.294$; CI_{AR} = 6.80–12.50; CI_{PR} = 2.51–8.42) where AR had a higher expression of MHC-IIa mRNA than PR ($p = 0.003$). Furthermore, there was a main effect of time for MHC-IIa mRNA expression ($p < 0.001$; $\eta_p^2 = 0.908$; CI_{PRE} = 1.00–1.00; CI_{POST} = 7.39–13.66; CI_{DL} = 7.92–14.38) where mRNA expression of MHC-IIa was greater at POST and DL than at PRE ($p < 0.001$). Myosin heavy chain IIx mRNA expression (Figure 7C) did not exhibit a group × time interaction ($p = 0.280$; $\eta_p^2 = 0.046$) or main effect of group ($p = 0.690$; $\eta_p^2 = 0.006$; CI_{AR} = 0.40–1.12; CI_{PR} = 0.55–1.29). Myosin heavy chain IIx did, however, exhibit a main effect of time ($p < 0.001$; $\eta_p^2 = 0.611$; CI_{PRE} = 1.00–1.00; CI_{POST} = 0.12–0.51; CI_{DL} = 0.55–1.87) where expression was greater at PRE than at POST ($p < 0.001$) and DL ($p = 0.013$). In addition, MHC-IIx mRNA expression was found to be greater at DL than at POST ($p < 0.001$). Mechano growth factor (MGF) mRNA expression (Figure 7D) did not exhibit a group × time interaction ($p = 0.590$; $\eta_p^2 = 0.021$) or main effect of group ($p = 0.926$; $\eta_p^2 < 0.001$; CI_{AR} = 0.76–2.03; CI_{PR} = 1.01–2.32). Mechano growth factor mRNA expression did, however, exhibit a main effect of time ($p = 0.033$; $\eta_p^2 = 0.128$; CI_{PRE} = 1.00–1.00; CI_{POST} = 1.28–2.92; CI_{DL} = 0.85–2.14) where MGF mRNA expression was greater at POST than at PRE ($p = 0.028$) and DL ($p = 0.040$). Finally, myostatin (MSTN) mRNA expression (Figure 7E) did not exhibit a group × time interaction ($p = 0.920$;
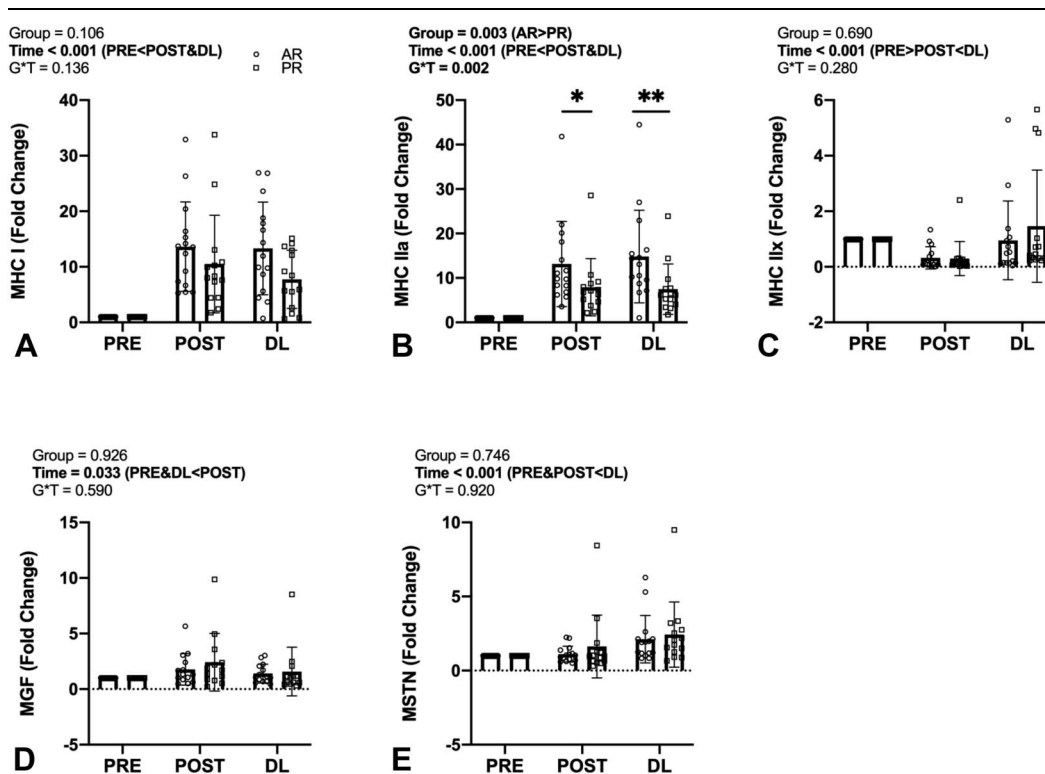

Figure 7. mRNA markers of skeletal muscle anabolism. AR = active recovery; PR = passive recovery; MHC = myosin heavy chain; MGF = mechano growth factor; MSTN = myostatin. Data are presented as mean ± *SD* for MHC I (panel A), MHC IIa (panel B), MHC IIx (panel C), MGF (panel D), and MSTN (panel E). "*" indicates MHC-IIa fold change was higher in the AR group at POST than PRE ($p = 0.034$); "**" indicates MHC-IIa fold change was higher in the AR group at DL than PRE ($p = 0.001$).
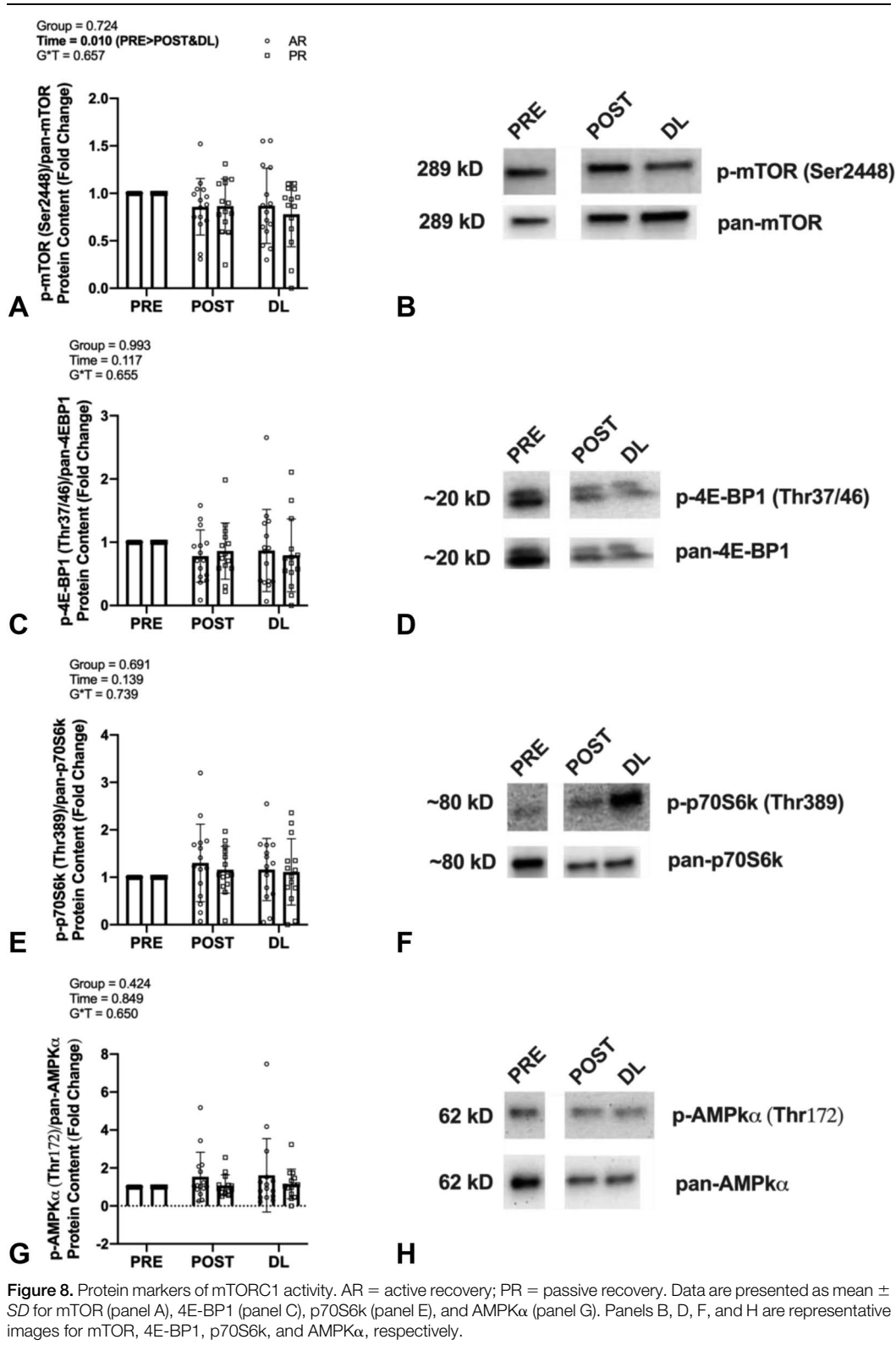
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Figure 8. Protein markers of mTORC1 activity. AR = active recovery; PR = passive recovery. Data are presented as mean ± SD for mTOR (panel A), 4E-BP1 (panel C), p70S6k (panel E), and AMPKα (panel G). Panels B, D, F, and H are representative images for mTOR, 4E-BP1, p70S6k, and AMPKα, respectively.

$\eta_p^2 = 0.003$) or main effect of group ($p = 0.746$; $\eta_p^2 = 0.004$; $\text{CI}_{AR} = 0.83–1.99$; $\text{CI}_{PR} = 1.09–2.29$). Myostatin mRNA expression did exhibit a main effect of time ($p < 0.001$; $\eta_p^2 = 0.405$; $\text{CI}_{PRE} = 1.00–1.00$; $\text{CI}_{POST} = 0.79–1.95$; $\text{CI}_{DL} = 1.55–3.00$) where MSTN mRNA expression was greater at DL than at PRE and POST ($p < 0.001$).

Protein Markers of Mechanistic Target of Rapamycin Complex 1 Activity

Mechanistic target of rapamycin (Figure 8A) did not exhibit a group × time interaction ($p = 0.657$; $\eta_p^2 = 0.015$) or a main effect of group ($p = 0.724$; $\eta_p^2 = 0.005$; $\text{CI}_{AR} = 0.80–1.02$; $\text{CI}_{PR} = 0.77–0.99$). Mechanistic target of rapamycin did, however, exhibit

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a main effect of time ($p = 0.010$; $\eta_p^2 = 0.156$; $CI_{PRE} = 1.00–1.00$; $CI_{POST} = 0.75–0.97$; $CI_{DL} = 0.68–0.97$) where values were greater at PRE than at POST ($p = 0.017$) and DL ($p = 0.017$). 4EBP1 (Figure 8C) did not exhibit a group × time interaction ($p = 0.655$; $\eta_p^2 = 0.013$), main effect of group ($p = 0.993$; $\eta_p^2 < 0.001$; $CI_{AR} = 0.71–1.06$; $CI_{PR} = 0.71–1.06$), or main effect of time ($p = 0.117$; $\eta_p^2 = 0.008$; $CI_{PRE} = 1.00–1.00$; $CI_{POST} = 0.66–0.98$; $CI_{DL} = 0.60–1.07$). p70S6K (Figure 8E) did not exhibit a group × time interaction ($p = 0.739$; $\eta_p^2 = 0.008$), main effect of group ($p = 0.691$; $\eta_p^2 = 0.006$; $CI_{AR} = 0.93–1.39$; $CI_{PR} = 0.85–1.33$), or main effect of time ($p = 0.139$; $\eta_p^2 = 0.074$; $CI_{PRE} = 1.00–1.00$; $CI_{POST} = 0.97–1.49$; $CI_{DL} = 0.88–1.40$). AMPK (Figure 8G) did not exhibit a group × time interaction ($p = 0.650$; $\eta_p^2 = 0.008$), main effect of group ($p = 0.424$; $\eta_p^2 = 0.025$; $CI_{AR} = 1.16–1.61$; $CI_{PR} = 0.85–1.31$), or main effect of time ($p = 0.849$; $\eta_p^2 = 0.002$; $CI_{PRE} = 1.00–1.00$; $CI_{POST} = 0.92–1.69$; $CI_{DL} = 0.81–1.95$).

Discussion

This study is one of the first to investigate some of the underlying physiological adaptations that result from different deloading paradigms after high-volume RT. Novel findings include the interactions observed for 20S proteasome activity and MHC IIa mRNA expression, which are discussed in more detail below. However, a majority of our data suggest 1 week of active vs. passive recovery results in similar effects on self-reported mood state, barbell squat velocity, body composition, and molecular as well as histological variables related to skeletal muscle hypertrophy. Notably, the lack of meaningful observations for BB velocity should be interpreted with caution as previous research has shown variability in the reliability of this measure (6,32).

Although it is well known that atrogin-1 mRNA, MuRF-1 mRNA, and the 20S and 26S proteasomes are associated with muscle atrophy, these markers have also been implicated in the remodeling processes associated with skeletal muscle hypertrophy (1,22). After 6 weeks of RT and 1 week of deloading, atrogin-1 mRNA and MuRF-1 mRNA were elevated in both groups, whereas 20S proteasome activity remained high in the AR group but decreased in the PR group at DL. Stefanetti and colleagues observed an increase in atrogin-1 mRNA, but not MuRF-1 mRNA, in human subjects after a 10-week progressively overloaded RT intervention (31). In addition, Léger et al. (14) found atrogin-1 mRNA, MuRF-1 mRNA, and 20S proteasome activity to be elevated after 8 weeks of RT, and levels decreased to baseline levels after a cessation of training for 8 weeks. This study adds to the current body of literature given that no other human study has examined changes in these markers with RT followed by 1 week of deloading. Furthermore, we report that localized VL hypertrophy did not seem to occur and whole-body hypertrophy was similar between groups; therefore, making it difficult to interpret the relevance of differences in 20S proteasome data between DL paradigms.

As with proteolysis measures, currently available literature on the mRNA expression of the various MHC isoforms in relation to chronic RT interventions is lacking. Assuming alterations in MHC mRNAs lead to changes in MHC protein levels, the main effects observed herein suggest that high volume training promotes more of an oxidative phenotype (i.e., increases in MHC I and IIa protein) while decreasing the expression of MHC-IIx. Interestingly, increases in MGF and MSTN mRNA coincided with training, and this is likely a reflection of the increased remodeling that occurred during the high-volume training intervention. However, the implications of these data are limited given that (a) only main effects of time were observed for most observed variables and (b) localized VL muscle growth did not seem to occur.

Aside from these molecular differences, the most profound findings herein are the lack of differences between active vs. passive deload. Changes in self-reported mood disturbance and localized pain (assessed objectively with an algometer) were similar after both forms of deload. Notably, whole-body fat-free mass increased in both groups despite no changes being observed in VL thickness and fCSA or barbell squat velocity in either group. Anabolic signaling has been shown to increase as a result of an acute low-intensity and high-intensity training bout in untrained and previously trained males (4,9). In addition, skeletal muscle growth has been shown to result from chronic low-volume and high-volume RT (13,18); however, the instrument used to measure this adaptation may be of critical importance. Of interest, Haun and colleagues (11) published a review discussing measurements commonly used for skeletal muscle hypertrophy reporting low measurement agreement between dual-energy x-ray absorptiometry, ultrasound-based measures, and histological interrogation such as fCSA analysis. Recent commentaries have posited that active deloading may be better than passive deloading for maintaining skeletal muscle hypertrophy and strength; however, the data are mixed (17,25). Although this may hold true for long deload periods (e.g., greater than 1 week), our data as well as other recent studies on tapering and cessation of training suggest that a week of inactivity does not tend to lead to losses in skeletal muscle size or performance. Thus, future studies are needed to examine how much time of inactivity is needed to observe decrements in these variables and how specific forms and doses of AR may exert different effects.

Biopsy and blood samples taken at POST and DL were sampled with all subjects having finished POST training 24 hours before sampling, and the AR group finishing the deload training 24 hours before DL sampling. The time at which samples were obtained have the possibility of impacting some of the biomarkers that were interrogated, which is unfortunately an unresolved logistical limitation. In addition, the authors note a lack of discrimination between type IIa and IIx fibers histologically to be a limitation. Finally, the authors also recognize that a lack of postintervention and postrecovery strength testing is a limitation particularly regarding deloading or tapering protocols.

Practical Applications

These data suggest that molecular markers related to skeletal muscle hypertrophy are similar after a short period of active vs. passive recovery (i.e., 1 week). Although gross measurements of skeletal muscle hypertrophy did not differ between groups, interesting molecular findings suggest that differing recovery paradigms may affect the overall physiology of skeletal muscle in the long term. Importantly, these data are limited, and more research is needed on short-term and long-term deloading paradigms to distinguish their differential effects on skeletal muscle physiology.

Acknowledgments

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