Effects of High-Volume versus High-Load Resistance Training on Skeletal Muscle Growth and Molecular Adaptations

Christopher G. Vann^{1,2}, Casey L. Sexton¹, Shelby C. Osburn¹, Morgan A. Smith¹, Cody T. Haun³, Melissa N. Rumbley¹, Petey W. Mumford⁴, Brian K. Ferguson¹, Nathan T. Montgomery¹, Carlton D. Fox¹, Bradley A. Rupple¹, James McKendry⁵, Jonathan Mcleod⁵, Adil Bashir⁶, Ronald J. Beyers⁶, Matthew S. Brook⁷, Kenneth Smith⁷, Philip J Atherton⁷, Darren T. Beck^{1,8}, James R. McDonald¹, Kaelin C. Young^{1,8}, Stuart M. Phillips⁵, Michael D. Roberts^{1,8*}

Affiliations: ¹School of Kinesiology, Auburn University, Auburn AL, USA, ²Duke Molecular Physiology Institute, Duke University School of Medicine, Duke University, Durham, NC, USA; ³Fitomics, LLC, Pelham, AL, USA, ⁴Department of Kinesiology, Lindenwood University, St. Charles MO, USA, ⁵Department of Kinesiology, McMaster University, Hamilton ON, CAN; ⁶MRI Research Center, Auburn University, Auburn AL, USA; ⁷MRC-ARUK Centre of Excellence for Musculoskeletal Ageing Research, Clinical, Metabolic, and Molecular Physiology, University of Nottingham, Derby, UK; ⁸Edward Via College of Osteopathic Medicine - Auburn Campus, Auburn AL, USA

*address correspondence to:

Michael D. Roberts, Ph. D.

Associate Professor, School of Kinesiology

Director, Molecular and Applied Sciences Laboratory

Affiliate Research Professor, Edward Via College of Osteopathic Medicine – Auburn Campus
Auburn University

301 Wire Road, Office 286

Auburn, AL 36849

Phone: 334 – 844 – 1925

Fax: 334 – 844 – 1467

E-mail: mdr0024@auburn.edu

Running title: Higher volume versus higher load training adaptations

ABSTRACT

Aim: We evaluated the effects of higher-load (HL) versus (lower-load) higher-volume (HV) resistance training on skeletal muscle hypertrophy, strength, and muscle-level molecular markers. **Methods:** Trained men (n=15, age: 23±3 y; training experience: 7±3 y) performed unilateral lower body training for 6 weeks (3x weekly), where single legs were assigned to HV and HL paradigms. Vastus lateralis (VL) biopsies were obtained prior to study initiation (PRE) as well as 3 days (POST) and 10 days following the last bout (POSTPR). Body composition and strength tests were performed at each testing session, and biochemical assays were performed on muscle tissue after study completion. Two-way within subjects repeated measures ANOVAs were performed on all dependent variables except tracer data, which was compared using dependent samples t-tests. **Results:** A significant ($p<0.05$) interaction existed for unilateral leg extension 1RM (HV<HL at POST and POSTPR). Six-week integrated sarcoplasmic protein synthesis (iSarcoPS) rates were higher in the HV versus HL leg, while no difference between legs existed for integrated myofibrillar protein synthesis rates. Main time effects existed for unilateral leg press strength (PRE<POST and POSTPR), knee extensor peak torque (PRE and POST<POSTPR), dual-energy x-ray absorptiometry (DXA)-derived upper leg lean mass (PRE<POST and POSTPR), ultrasound-derived VL thickness (PRE and POSTPR<POST), sarcoplasmic protein concentrations (POST and POSTPR<PRE), and tropomyosin and troponin protein abundances (POST and POSTPR<PRE). **Conclusions:** With the exception of differences in leg extensor strength and iSarcoPS between legs, our data suggest that short-term (6 weeks) HV and HL training elicit similar hypertrophic, strength, and molecular-level adaptations.

55 **Keywords:** Higher-load resistance training; higher-volume resistance training; muscle
56 hypertrophy; sarcoplasmic protein; myofibrillar protein

1. INTRODUCTION

Skeletal muscle hypertrophy has been defined as an increase in the weight or cross-sectional area of muscle^{1,2}, with the increased volume of muscle coming from an enlargement of each muscle fiber³⁻⁵. It is generally recognized that resistance training results in skeletal muscle growth through proportional increases in myofibrillar and sarcoplasmic protein content⁶⁻⁹. Myofibril proteins are defined herein as the proteins that make up the rigid structure of muscle (e.g., dystrophin, actinin, titin, nebulin, etc.) as well as contractile proteins (e.g., actin and myosin isoforms). In contrast, sarcoplasmic proteins are involved with signal transduction, energy synthesis, energy breakdown, and other metabolic processes¹⁰.

Recently, there has been interest regarding whether or not higher-load (HL) versus higher-volume (HV) resistance training elicits differential training adaptations at the macroscopic, molecular, and functional levels. Historically, research has typically suggested that HL training elicits superior increases in strength and muscle fiber hypertrophy compared to lower-load HV training¹¹. However, Mitchell and colleagues reported that 10 weeks of HL or HV resistance training led to similar increases in muscle hypertrophy as assessed through MRI and fiber histology¹². Subsequent literature indicates that both HL and HV training can: i) elicit similar changes in skeletal muscle hypertrophy (assessed through either ultrasound or MRI)¹³⁻¹⁷, and ii) elicit similar strength adaptations^{13,18}, although equivocal evidence exists suggesting HL training elicits superior strength adaptations^{14,15,17}. Reasons for similar outcomes between HL and HV training could be due to total volume lifted being comparable between paradigms. However, few HV versus HL studies have sought to modulate training loads with the intent of accumulating more training volume during HV conditions.

Our laboratory recently reported that six weeks of extremely HV resistance training decreased the relative abundance of myosin heavy chain and actin protein content per milligram of dry tissue¹⁹. Our findings, as well as those of others who have reported moderate-to-higher volume resistance training elicits similar molecular adaptations²⁰⁻²², led us to postulate that a disproportionate increase in the sarcoplasmic space relative to myofibril protein accretion may be a training adaptation to HV resistance training²³. More recently, our laboratory demonstrated that lower volume, higher load resistance training (3-5 sets of 2-6 repetitions at 65-90% 1RM) resulted in a maintenance of type I muscle fiber cross-sectional area (fCSA) while increasing type II fCSA. Additionally, no changes in sarcoplasmic protein concentrations were observed despite a modest but significant decrease in actin protein concentrations^{24,25}. While preliminary, these two studies from our laboratory suggest that HV resistance training may facilitate a more robust expansion of non-contractile proteins in myofibers, whereas HL training may promote a proportional increase in myofibril protein accretion with muscle growth. However, no single study to date has examined whether HV versus HL training elicit differential molecular adaptations related to the aforementioned findings.

The purpose of the present study was to elucidate whether six weeks of unilateral HV versus HL lower-body resistance training differentially affected metrics of skeletal muscle hypertrophy, strength, and/or molecular variables assessed from skeletal muscle biopsies sampled from the vastus lateralis (VL). The intent of our study was to ensure HV training achieved more training volume relative to HL training. We hypothesized no differences would exist between HV and HL training when examining changes in VL muscle area assessed via magnetic resonance imaging (MRI), or VL thickness assessed via ultrasound. Additionally, we hypothesized that HL training would elicit superior increases in various indices of strength.

However, we posited HV training would result in increased sarcoplasmic protein concentrations and a concomitant decrease in the relative abundances of contractile proteins, whereas HL training would result in no changes in these markers. Additionally, we hypothesized that the integrated sarcoplasmic protein synthesis (iSarcoPS) rates would be greater in HV versus HL training, whereas integrated myofibrillar protein synthesis (iMyoPS) rates would be greater in HL versus HV training.

2. RESULTS

2.1 *Participant characteristics*

Baseline participant characteristics can be found in Table 1. Briefly, 15 college-age males (23 ± 3 years) with an average training age of 7 ± 3 years volunteered for this study. At PRE, participants weighed 89.5 ± 11.6 kg with 69.1 ± 7.4 kg being lean soft tissue mass (LSTM) and 17.3 ± 7.5 kg being fat mass, on average. Additionally, participants had an average relative squat to body mass ratio of $1.9 \times$ body mass (167 ± 34 kg).

INSERT TABLE 1

2.2 *Training Volume and Strength Metrics*

Training volumes and strength metrics are presented in Figure 1. Data for 14 of 15 participants is presented for unilateral leg press and unilateral leg extension one repetition maximums (1RM) due to one participant feeling lower extremity discomfort at POST with these exercises.

There was a condition $\times$ time interaction observed for lower body training volume ($p < 0.001$, $\eta_p^2 = 0.914$; Fig. 1a). Additionally, there was a main effect of condition ($p = 0.003$, $\eta_p^2 = 0.467$) where the HV condition completed more volume than the HL condition (8100 ± 480

kg versus 7296 ± 421 kg, respectively). Lower body training volume changed over time ($p < 0.001$, $\eta_p^2 = 0.955$, Fig. 1a) and within each condition over time (HV: $p < 0.001$, $\eta_p^2 = 0.952$; HL: $p < 0.001$, $\eta_p^2 = 0.954$, Fig. 1a). Post hoc analysis revealed lower training volumes at weeks 1 and 2 in the HV leg compared to the HL leg ($p < 0.001$), no differences between conditions at week 3, and higher training volumes in the HV leg at weeks 4-6 as compared to the HL leg ($p < 0.001$).

A condition $\times$ time interaction ($p = 0.512$, $\eta_p^2 = 0.050$, Fig. 1b) was not observed for estimated unilateral leg press 1RM. Additionally, no main effect of condition ($p = 0.475$, $\eta_p^2 = 0.040$, Fig. 1b) was observed. There was a main effect of time ($p < 0.001$, $\eta_p^2 = 0.818$, Fig. 1b) where estimated unilateral leg press 1RM at POST ($p < 0.001$) and POSTPR ($p < 0.001$) were greater than PRE. A condition $\times$ time interaction was observed for estimated unilateral leg extension 1RM ($p = 0.032$, $\eta_p^2 = 0.265$, Fig. 1c). A main effect of condition ($p = 0.026$, $\eta_p^2 = 0.328$, Fig. 1c) was also observed where the HL condition (grand mean = 113 ± 5 kg) estimated unilateral leg extension 1RM was higher than the HV condition (grand mean = 109 ± 5 kg). Estimated unilateral leg extension 1RM also changed over time ($p < 0.001$, $\eta_p^2 = 0.885$, Fig. 1c) and within each condition over time (HV: $p < 0.001$, $\eta_p^2 = 0.858$; HL: $p < 0.001$, $\eta_p^2 = 0.884$). Post hoc analysis revealed no differences in estimated unilateral leg extension 1RM at PRE; however, the HL condition had higher values at POST and POSTPR compared to the HV condition ($p < 0.05$).

There was no condition $\times$ time interaction observed for knee-extensor peak torque at $60^\circ/\text{sec}$ ($p = 0.995$, $\eta_p^2 < 0.001$, Fig. 1d) and 120° ($p = 0.366$, $\eta_p^2 = 0.069$, Fig. 1e), or knee-extensor mean torque at $120^\circ/\text{sec}$ ($p = 0.681$, $\eta_p^2 = 0.027$, Fig. 1g). Additionally, there were no main effects of condition or time observed for the aforementioned variables. Knee-extensor mean torque at $60^\circ/\text{sec}$ changed over time ($p = 0.041$, $\eta_p^2 = 0.204$, Fig. 1f) where knee-extensor mean torque at

60°/sec was higher at POSTPR than at PRE ($p=0.029$) and POST ($p=0.043$). There were no differences observed between PRE and POST ($p=0.805$).

INSERT FIGURE 1

2.3 *Body Composition*

PRE, POST, and POSTPR whole-body composition changes for all participants are presented in Table 2; notably, these data were derived from dual-energy x-ray absorptiometry (DXA) scans. Total body mass increased over time ($p<0.001$, $\eta_p^2=0.435$), where POST ($p=0.001$) and POSTPR ($p=0.012$) body masses were greater than PRE. However, no differences were observed between POST and POSTPR body masses ($p=0.119$). Lean soft tissue mass (LSTM) increased over time ($p=0.003$, $\eta_p^2=0.338$) where POST LSTM was greater than PRE ($p=0.002$) and POSTPR ($p=0.014$). No significant differences in LSTM were observed between PRE and POSTPR ($p=0.286$). No differences were observed for DXA measured whole body fat mass ($p=0.097$).

INSERT TABLE 2

2.4 *Segmental Upper Leg Composition*

There were no condition×time interactions observed for DXA-derived upper leg mass ($p=0.069$, $\eta_p^2=0.173$, Fig. 2a), upper leg LSTM ($p=0.174$, $\eta_p^2=0.117$, Fig. 2b), or upper leg fat mass ($p=0.959$, $\eta_p^2=0.003$, Fig. 2c). A main effect of time was observed for upper leg mass ($p=0.001$, $\eta_p^2=0.392$, Fig. 2a) where POST ($p=0.002$) and POSTPR ($p=0.013$) were higher than PRE. No differences were observed between POST and POSTPR ($p=0.240$). A main effect of time was observed for DXA upper leg LSTM ($p=0.001$, $\eta_p^2=0.418$, Fig. 2b) where POST ($p<0.001$) and POSTPR ($p=0.002$) were higher than PRE. No differences were observed between POST and

POSTPR ($p=0.148$). No main effects of condition ($p=0.102$) or time ($p=0.595$) were observed for DXA upper leg fat mass.

INSERT FIGURE 2

2.5 *Vastus Lateralis Muscle Morphology*

A condition×time interaction was observed for magnetic resonance image (MRI)-derived VL cross-sectional area ($p=0.046$, $\eta_p^2=0.211$, Fig. 3a); however, no main effects of condition ($p=0.490$, $\eta_p^2=0.037$) or time ($p=0.351$, $\eta_p^2=0.077$) were observed. Post hoc analysis revealed no differences between conditions at PRE ($p=0.246$), POST ($p=0.673$), or POSTPR ($p=0.247$). There was no condition×time interaction ($p=0.338$, $\eta_p^2=0.075$, Fig. 3d) or main effect of condition ($p=0.457$, $\eta_p^2=0.040$) observed for ultrasound measured VL thickness. VL thickness changed over time ($p=0.035$, $\eta_p^2=0.241$) where POST values were greater than PRE ($p=0.026$) and POSTPR ($p=0.003$). No differences were observed between PRE and POSTPR ($p=0.614$). There were no interactions observed for muscle pennation angle of the VL ($p=0.393$, $\eta_p^2=0.064$, Fig. 3f) or estimated VL muscle fiber length ($p=0.602$, $\eta_p^2=0.036$, Fig. 3c). Additionally, there were no main effects of condition or time for the aforementioned variables ($p>0.05$).

INSERT FIGURE 3

2.6 *Muscle Protein Adaptations*

There was no condition×time interaction observed for sarcoplasmic protein concentrations per mg of wet tissue weight ($p=0.112$, $\eta_p^2=0.159$, Fig. 4a). There was a main effect of condition ($p=0.002$, $\eta_p^2=0.497$, Fig. 4a) where sarcoplasmic protein concentrations in the HV group were higher than the HL group (44.8 ± 1.6 versus 42.6 ± 1.3 respectively). Additionally, there was a main effect of time ($p=0.022$, $\eta_p^2=0.239$, Fig. 4a) where PRE sarcoplasmic protein concentrations were higher than POST ($p=0.038$) and POSTPR ($p=0.032$).

No differences in sarcoplasmic protein concentrations were observed between POST and POSTPR ($p=0.524$)

There were no condition×time interactions observed for myosin heavy chain (MyHC) protein abundance per mg of wet tissue weight ($p=0.668$, $\eta_p^2=0.028$, Fig. 4b) or actin protein abundance per mg of wet tissue weight ($p=0.254$, $\eta_p^2=0.093$, Fig. 4c). Additionally, no main effects of condition or time were observed for these variables ($p>0.05$). There was no condition×time interaction ($p=0.180$, $\eta_p^2=0.115$, Fig. 4d) or main effect of condition ($p=0.762$, $\eta_p^2=0.007$, Fig. 4d) observed for tropomyosin protein abundance per mg wet tissue weight. However, a main effect of time was observed for this variable ($p=0.008$, $\eta_p^2=0.294$, Fig. 4d) where PRE was greater than POST ($p=0.009$) and POSTPR ($p=0.010$). No differences were observed between POST and POSTPR ($p=0.704$). There was no condition×time interaction ($p=0.112$, $\eta_p^2=0.145$, Fig. 4e) or a main effect of condition ($p=0.912$, $\eta_p^2=0.001$, Fig. 4e) observed for troponin protein abundance per mg wet tissue weight. A main effect of time was observed for this variable ($p<0.001$, $\eta_p^2=0.431$, Fig. 4e) where PRE was greater than POST ($p<0.001$) and POSTPR ($p=0.005$). No differences were observed between POST and POSTPR ($p=0.867$).

INSERT FIGURE 4

2.7 Six-week Integrated Myofibrillar and Sarcoplasmic Protein Synthesis

No difference was observed in integrated myofibrillar protein synthesis (iMyoPS) between the HV and HL conditions ($p=0.687$; $d=-0.106$; Figure 5a). A significant difference was observed for integrated sarcoplasmic protein synthesis (iSarcoPS) where the HV condition exhibited a higher iSarcoPS (%/day) than the HL condition ($p=0.0018$; $d=0.693$; Figure 5b).

INSERT FIGURE 5

3. DISCUSSION

Chief findings from the current study include: i) a condition×time interaction for MRI-derived VL mCSA although, when the model was statistically decomposed, no differences were found between conditions or over time, ii) an interaction for estimated unilateral leg extension 1RM where values were higher in the HL condition at POST and POSTPR when compared to the HV condition at the same time points, and iii) no significant difference in iMyoPS rates, albeit iSarcoPS was greater in the HV versus HL condition over the duration of the study. The relevance of these as well as other findings are discussed below.

The literature regarding macro-level muscle tissue changes following high- and low-load training are sparse. Furthermore, limitations in the available literature exist due to lack of congruency between loading paradigms and discrepancies on what constitutes low-load/high-volume and high-load/low-volume training. Nonetheless, there is prior literature that has interrogated differences between such training paradigms. Holm and colleagues²⁶ reported that high-load (~70% 1RM) versus very low-load (~15.5% 1RM) leg extensor training increased quadriceps CSA; however, the change in the high-load condition was greater than the change in the low-load condition. Chestnut and Docherty²⁷ reported similar increases in muscle CSA of the upper arm following 10 weeks of upper body resistance training using ~85% of 1RM for 6 sets of 4 repetitions versus ~70% for 3 sets of 10 repetitions. The aforementioned study by Mitchell and colleagues reported that performing three sets of knee-extensor training to fatigue at 30% or 80% of 1RM resulted in similar increases in quadriceps volume measured by MRI. Both modalities yielded greater quadriceps hypertrophy than performing one set at 80% 1RM to voluntary failure¹². Furthermore, a systematic review and meta-analysis conducted by Schoenfeld and colleagues concluded that similar skeletal muscle growth can be realized across a

variety of loading ranges²⁸. As mentioned previously, while we observed a condition×time interaction for VL mCSA, decomposition of the model yielded no significant differences between HL and HV training. This is in agreement with previous literature showing that (on average), when loading exceeds 30% 1RM and training is executed near or at volitional fatigue, similar increases in whole-muscle hypertrophy can be realized.

Unilateral leg press and leg extension strength metrics increased over time, albeit there was no interaction observed for leg extensor peak torque or leg press strength changes. However, an interaction was observed for leg extension strength changes, where values increased more in the HL versus HV condition. Several studies have examined changes in strength between different loading paradigms. Campos and colleagues reported high-load resistance training (3-5RM) over an 8-week period yielded greater leg extension strength increases compared to high-volume resistance training (20-28RM)²⁹; however, no differences in strength adaptations were reported between the 3-5RM group and a third group which performed training using 9-11RM loads. Additionally, Jenkins et al. published two studies comparing 30% 1RM versus 80% 1RM leg extensor training^{14,15}. Results from both studies suggest that higher-load training elicited greater strength increases due to neural factors. Jessee and colleagues³⁰ reported that unilateral training (4 sets to volitional failure) over an 8-week period resulted in greater strength adaptations for HL training (70% 1RM/no blood flow restriction) than low load conditions with or without blood flow restriction. Furthermore, Schoenfeld and colleagues reported increased barbell back squat strength with lower (30-50% 1RM) and higher-load (70-80% 1RM) training, with higher-load training resulting in greater strength adaptations¹⁷. When considering our findings in the context of these studies, it seems plausible that training at 60-90% 1RM over shorter-term periods may elicit similar strength changes. Collectively, our findings in the

context of prior literature suggest that, while training at 30% 1RM seemingly yields similar increases in hypertrophy compared to training at higher loads, 30% 1RM training may not optimize strength gains due to differential neural adaptations.

A novel aspect of the current study was to compare how HL versus HV training affected molecular markers from muscle biopsies. This interrogation was prompted by select literature suggesting that a disproportionate increase in non-contractile proteins in myofibers may occur following high-volume resistance training. However, as reviewed by Jorgenson and colleagues, it should be noted that a large portion of the literature has shown that mechanical-load induced skeletal muscle hypertrophy is largely attributed to proportional increases in the contractile and non-contractile elements of the myofiber³¹. Nonetheless, a handful of studies exist showing that a disproportionate increase in non-contractile proteins may occur following months to years of resistance training^{20,21,32,33}. Recently, our laboratory has reported increases in sarcoplasmic protein concentrations with concomitant decreases in the relative abundances of myosin heavy chain and actin protein abundances per mg of dry tissue weight following 6 weeks of extremely high-volume resistance training in previously-trained college-aged men¹⁹. We concluded at the time that “sarcoplasmic hypertrophy” may have occurred and that both of these molecular observations were reflective of this phenomenon. Our laboratory subsequently reported that sarcoplasmic protein concentrations were maintained while minor decrements occurred in actin protein abundance in previously-trained college-aged males that partook in a 10-week low-volume, high-load training paradigm²⁴. When considering the findings from both studies, we hypothesized that HV training might facilitate sarcoplasmic hypertrophy, whereas HL training may facilitate proportional accretion of contractile and sarcoplasmic proteins with whole-muscle hypertrophy (i.e., conventional hypertrophy). In the current study, we observed decreased

285 sarcoplasmic protein concentrations for both conditions at POST. Additionally, no significant
286 changes in the relative abundances of actin and myosin heavy chain proteins were observed in
287 either condition. Although these data disagree with prior findings from Haun et al., it is
288 important to note the key differences that exist between the high-volume components of each
289 study. First, Haun et al. utilized a bilateral exercise paradigm using the barbell back squat as the
290 primary knee-extensor exercise, whereas the current study was a unilateral study design using a
291 combination of unilateral leg press and leg extension to overload the knee-extensor muscles. To
292 this end, it could be posited that training stress on the legs was much greater in the Haun et al.
293 study versus the current study. Second, Haun et al. used a 6-week intervention starting at 10 sets
294 of 10 repetitions per week (for each exercise) and finishing with 32 sets of 10 repetitions per
295 week where loads were standardized at 60% 1RM³⁴. The current study started the HV leg at 10
296 sets of 10 repetitions per week (split between two exercises) at week 1 and finished with 20 sets
297 of 10 repetitions per week at week 6 where loads were standardized at 60% 1RM. Thus,
298 although the HV leg was exposed to more training volume compared to the HL leg herein, the
299 HV leg did not experience nearly the amount of volume as both legs incurred in the study by
300 Haun et al. Moreover, the total training volume data in Figure 1 indicates that the HV leg was
301 only exposed to ~11% more volume compared to the HL leg. We speculate that similar
302 molecular adaptations between legs may have been due a relatively small difference in total
303 training volume between legs throughout the duration of the study. Alternatively stated, a higher
304 total training volume may have been needed to yield data suggestive of sarcoplasmic versus
305 myofibrillar expansion in the HV condition. In spite of our null findings, however, it is
306 intriguing that HV training increased iSarcoPS versus HL training. This partially supports the

notion that HV training may affect the sarcoplasmic protein pool to a greater extent than HL training, but this warrants continued research.

As with many studies examining the effects of training interventions, the present study is limited due to a small sample size. The procurement of skeletal muscle tissue via percutaneous muscle biopsy inherently has a finite tissue yield. Due to this limitation, we lacked sufficient tissue to perform histology and assess fCSA values in each leg over time, and these assays would have been greatly beneficial for this study. While protein synthesis rates were measured herein, it is notable that muscle protein breakdown rates were not assessed and could have influenced outcomes. Additionally, previous literature has shown the ability to measure gross changes in skeletal muscle growth after 3-6 weeks of resistance training in untrained to recreationally trained men³⁴⁻³⁶. In this regard, we posit that the training status of the cohort in the current study may have precluded our ability to detect any meaningful training adaptations over the 6-week training period.

In conclusion, similar changes in muscle volume, morphology, and protein composition appeared with 6 weeks of HV versus HL training. However, HL training resulted in a greater increase in leg extension strength and higher 6-week iSarcoPS rates. Additionally, the current data challenge our prior muscle-molecular findings given: i) the lack of change in sarcoplasmic protein concentrations observed in the HV condition, and ii) no alterations being observed in myosin heavy chain and actin protein abundances following training. However, the current iSarcoPS findings suggest some muscle-molecular differences exist between HV and HL training, and warrant further research.

4. MATERIALS AND METHODS

4.1 *Ethical approval and Pre-screening*

Prior to study initiation, this protocol was reviewed and approved by the Auburn University Institutional Review Board and was conducted in accordance to the standards set by the latest revision of the Declaration of Helsinki (IRB approval #: 19-245 MR 1907).

College-aged resistance-trained men from the local community were solicited to participate in this study and were screened 4-7 days prior to the start of the study. Participants had to be free of cardio-metabolic diseases (e.g., morbid obesity, type II diabetes, severe hypertension), or any conditions that preclude the collection of a skeletal muscle biopsy. Additionally, training status for participants was determined by two criteria: i) self-reported resistance training >1 year at least 3 times weekly and; ii) a tested barbell back squat of $\geq 1.5 \times$ bodyweight (estimated from a 3 repetition maximum [3RM] test) in accordance to standards designated by the National Strength and Conditioning Association³⁷. At the conclusion of the screening visit, participants were asked to maintain their current nutritional practices and to cease all training outside of the study.

4.2 *Study design*

A schematic of the study design is provided in Figure 6a. Briefly, participants performed a battery of testing prior to the start of training (PRE), 72 hours following the last bout of training after 6 weeks of unilateral lower body resistance training (POST), and 10 days following the last bout of training (POSTPR). The battery of tests performed are detailed further below, following a description of the training intervention and tracer methodologies.

4.3 *Resistance Training*

Participants performed overloading unilateral lower body resistance training (i.e., single-leg leg press and single-leg leg extension) 3 days per week in conjunction with compound upper body exercises (i.e., barbell bench press, pronated grip barbell row, barbell stiff-leg deadlift). Notably, participants were randomly assigned to lower body training conditions prior to the start of the study, where some participants performed HV training on the left leg and HL training on the right leg or vice versa. All upper body exercises were performed for 3 sets of 10 repetitions at 70% of tested 1RM. Progression for the lower body training can be found in Figure 6b.

INSERT FIGURE 6 HERE

4.4 Isotope Tracer Protocol

Deuterium oxide (D₂O) (Cambridge Isotope Laboratories, Inc.; Andover, MA, USA) was provided to the participants three days prior to and over the first 6 weeks of the study at 1 mL•kg⁻¹ of lean body mass. For rapid enrichment of deuterium (²H) participants were instructed to orally consume 6 doses of D₂O over an eight-hour period, three days prior to the first data collection (PRE), and were instructed to consume a top-up dose daily thereafter consisting of one dose of D₂O until data collection was performed at the conclusion of week 6 of the study (POST). Saliva samples were taken utilizing sterile salivettes (SARSTEDT AG & Co, Nümbrecht, Germany). Briefly, participants were instructed to chew on the cotton swab for 1 min and place the swab back into the top compartment of the salivette. This process was completed daily for the first 10 days of the study and every Monday, Wednesday, and Friday thereafter. Participants were instructed to place salivettes in their home freezers on days when saliva was donated outside of the laboratory. Samples were stored at -20°C until further processing as described below.

4.5 Testing sessions

Urine specific gravity testing for adequate hydration. Upon arrival to each testing session participants submitted a urine sample (~5mL) for urine specific gravity (USG) assessment. Measurements were performed using a handheld refractometer (ATAGO; Bellevue, WA, USA). USG levels in all participants were ≤ 1.020 indicative of a euhydrated state³⁸ and thus were considered adequately hydrated for further testing.

Body composition testing. Following hydration testing, participants underwent height and weight testing utilizing a digital column scale (Seca 769; Hanover, MD, USA) with body mass collected to the nearest 0.1 kg and height to the nearest 0.5 cm. Participants were then subjected to a full body dual energy x-ray absorptiometry (DXA) scan (Lunar Prodigy; GE Corporation, Fairfield CT, USA). Our laboratory³⁹ has previously shown same day reliability of the DXA during test-calibrate-retest on 10 participants to yield an intra-class correlation coefficient (ICC) of 0.998 for total body lean mass.

Measurements of muscle morphology. Following body composition testing, participants were tested for VL muscle thickness and muscle pennation angle via ultrasound. VL thickness of both legs were assessed by placing a 3 to 12 MHz multi-frequency linear phase array transducer (Logiq S7 R2 Expert; General Electric, Fairfield, CT, USA) midway between the iliac crest and lateral epicondyle of the femur. Measurements were taken from a standing position and participants were instructed to bear the majority of their weight on the leg contralateral to the leg being measured. VL pennation angles were taken immediately following thickness measures by placing the transducer longitudinally at the same site mentioned above. VL thickness was measured as the distance between the superficial and deep aponeurosis while VL pennation angle was measured as the angle of the deep aponeurosis as it relates to the individual fascicles.

Estimated fiber length was calculated using methods similar to those described by Fukunaga and colleagues⁴⁰.

$$est. fiber length = \frac{a}{\cos(90^\circ - \theta)}$$

Whereas a is equal to the distance between the superficial fascia and the deep aponeurosis and θ is equal to the angle of pennation. Importantly – to minimize variability in measurements as suggested in previous studies^{41,42} – all measures were taken by the same investigator (S.C.O.) whom in a test-retest validation on 11 participants has been found to have an ICC of 0.983. Critically, this investigator was not privy to the (HV or HL) condition for each image. Moreover, the location of measurements were marked, using anatomical landmarks, by the investigator so that the subsequent MRI scans and muscle biopsies could be obtained from the same plane of measurement.

MRI for muscle cross-sectional area. Following ultrasound assessments, participants were shuttled to the Auburn University MRI Research Center to perform dual- leg mid-thigh MRI scans. All measurements were performed on a 3T VARIO system (Siemens, Erlangen, Germany). Briefly, upon arrival participants were placed in a supine position for 10 minutes to allow for body fluid stabilization to occur. Volume coil was used for RF transmit and body and spine array coils placed around the legs were used for signal receive. 3D gradient echo sequence (3D fast low angle shot) was used to acquire fat suppressed images with the following parameters: TR/TE = 10/4.92 ms; flip angle = 10°; bandwidth = 510 Hz/pixel, in-plane resolution 1mm × 1mm and slice thickness = 2.2mm. An axial 3D 35.2mm thick slab (a6 partitions) was placed to image both thighs with the thickness dimension carefully centered on the participant biopsy marking. Following the conclusion of the study, MRI scans were digitized offline using Osirix MD software (Pixmeo, Geneva, CHE), and software tools were used to

manually trace the border of the VL yielding mCSA values. All MRI scans and image analyses were performed by the same investigators (R.J.B. and M.A.S., respectively), without knowledge of the (HV or HL) condition.

Collection of muscle tissue. Following MRI scans, right and left leg VL muscle biopsies were collected using a 5-gauge needle under local anesthesia as previously described^{43,44}. Immediately following tissue procurement, tissue was teased of blood and connective tissue, wrapped in pre-labelled foils, flash frozen in liquid nitrogen, and subsequently stored at -80°C for molecular analyses described below.

Strength Testing. Following muscle skeletal muscle biopsies, participants underwent isokinetic dynamometry (Biodex System 4; Biodex Medical Systems, Inc., Shirley, NY, USA) for leg extensor peak torque and 3RM testing. For right and left leg extensor peak torque testing, participants were fastened to the isokinetic dynamometer. Each participant's lateral epicondyle was aligned with the axis of the dynamometer, and seat height was adjusted to ensure the hip angle was approximately 90°. Prior to torque assessment, each participant performed a warm-up consisting of submaximal to maximal isokinetic knee extensions. Participants then completed five maximal voluntary isokinetic knee extension actions at 1.05 rad/s (60°/s) and 2.09 rad/s (120°/s). Participants were provided verbal encouragement during each contraction. The isokinetic contraction resulting in the greatest value was used for analyses. Following isokinetic dynamometry participants performed maximum strength testing for the exercises utilized over the duration of the study (single-leg leg press, single-leg leg extension, barbell bench press, pronated grip barbell row, and barbell stiff-leg deadlift). Briefly, participants performed 3 warm-up sets starting at ~50% of their self-selected opening weight for 10 repetitions, then 75% of their self-selected opening weight for 5 repetitions, and 90% of their self-selected opening

weight for 3 repetitions. Following warm-ups, participants executed their opening attempt for 3 repetitions with 5-10% increases being made from there on until a 3RM was achieved. All strength testing was performed by investigators holding the NSCA certified strength and conditioning specialist credential (C.G.V. and C.L.S.). This process was completed for all exercises at PRE while only the single-leg exercises were tested at POST and POSTPR.

4.6 Biochemical assays

Sarcoplasmic and myofibrillar protein isolation. Isolation of protein fractions was performed using the proteomic validated “MIST” or “myofibrillar isolation and solubilization technique”⁴⁵. 1.7 mL polypropylene tubes were pre-filled with ice-cold buffer (300 µL; Buffer 1: 25 mM Tris, pH 7.2, 0.5% Triton X-100, protease inhibitors) and placed on ice. Skeletal muscle foils were removed from -80°C, placed on a liquid nitrogen-cooled ceramic mortar and pestle, and tissue was pulverized into 2-4 mm³ chunks. Chunks (~20 mg) were weighed using a scale with a sensitivity of 0.0001 g (Mettler-Toledo; Columbus, OH, USA) and placed into 1.7 mL polypropylene tubes with buffer and placed on ice. Samples were homogenized using tight-fitting pestles and centrifuged at 1,500 g for 10 minutes at 4°C. Supernatants (sarcoplasmic fraction) were collected and placed in new 1.7 mL polypropylene tubes on ice. As a wash step, the resultant myofibrillar pellet was resuspended in 300 µL of Buffer 1 and centrifuged at 1,500 g for 10 minutes at 4°C. The supernatant was discarded and the myofibrillar pellet was solubilized in 300 µL of ice-cold resuspension buffer (20 mM Tris-HCl, pH 7.2, 100 mM KCl, 20% glycerol, 1 mM DTT, 50 mM spermidine, protease inhibitors). Protein concentrations for the sarcoplasmic fraction were determined the same day as protein isolations to minimize freeze-thaw artifact, and the myofibrillar fraction was prepared for actin and myosin heavy chain

protein abundance analyses and stored at -80°C until analysis occurred. The methodologies used for the above are described in further detail below.

Determination of sarcoplasmic protein concentrations. Sarcoplasmic protein resuspensions were batch-assayed for determination of protein concentration using a commercially available bicinchoninic acid (BCA) kit (Thermo Fisher Scientific; Waltham, MA, USA). Samples were assayed in duplicate (sarcoplasmic protein) using a microplate assay protocol where a small volume of sample was assayed (20 µL of 5x diluted sample + 200 µL Reagent A + B). The average duplicate coefficient of variation for sarcoplasmic protein concentration was 2.27%.

SDS-PAGE and Coomassie staining for relative contractile protein abundance. Determination of contractile protein abundances per mg wet tissue were performed as previously described by our laboratory and others^{19,44,46,47}. Briefly, SDS-PAGE sample preps were made using 10 µL resuspended myofibrils, 65 µL distilled water (diH₂O), and 25 µL 4x Laemmli buffer. Samples (5 µL) were then loaded on precast gradients (4-15%) SDS-polyacrylamide gels in duplicate (Bio-Rad Laboratories) and subjected to electrophoresis at 180 V for 40 minutes using pre-made 1x SDS-PAGE running buffer (Ameresco). Following electrophoresis, gels were rinsed in diH₂O for 15 minutes and immersed in Coomassie stain (LabSafe GEL Blue; G-Biosciences; St. Louis, MO, USA) for 2 hours. Gels were then destained in diH₂O for 60 minutes, and band densitometry was performed with a gel documentation system and associated software (ChemiDoc; Bio-Rad Laboratories, Hercules, CA, USA). Given that a standardized volume from all samples was loaded onto gels, myosin heavy chain and actin band densities were normalized to input muscle weights to derive arbitrary density units (ADU) per mg wet muscle. All values were then divided by the mean of the PRE time point to depict myosin heavy

chain, actin, tropomyosin, and troponin abundances. Our laboratory has reported that this method yields exceptional sensitivity in detecting 5-25% increases in actin and myosin content⁴⁴. Average duplicate coefficients of variation for relative actin, myosin, tropomyosin, and troponin protein concentrations herein were 1.95%, 1.90%, 2.22%, and 3.54% respectively.

Six-week integrated myofibrillar and sarcoplasmic protein synthesis rates. Protein isolations were performed using ~30 mg of tissue utilizing the MIST method as described above. Prior to preparation for tracer analysis the sarcoplasmic protein fraction was lyophilized and precipitated in 1 mL of 1M perchloric acid to form a pellet. The myofibrillar pellet was purified by adding 500 μ L of DDH₂O followed by vortexing for 5 s and centrifugation at 1500 rpm at 4°C for 10 minutes. Following centrifugation, 1 mL of 0.3 M NaOH was added to the sample and then vortexed for 5 s followed by being placed in a heat block at 50°C for 30 minutes of which samples were vortexed for 5 s every 10 minutes. Samples then underwent centrifugation at 10,000 rpm at 4°C for 10 minutes. The supernatant (sarcoplasmic or myofibrillar fraction) was transferred into a 4 mL glass screw-top tube. 1 M perchloric acid was then added to tubes and centrifuged at 2500 rpm at 4°C for 10 minutes. The supernatant was removed, and the remaining pellet was washed in 70% ethanol and centrifuged at 2500 rpm at 4°C for 10 minutes twice. Amino acids were extricated through the addition of 1 mL of 1 Dowex resin (50WX8-200 resin; Sigma Aldrich) and 1 mL of 1 M HCl prior to heating at 110°C for 72 hours. Cation exchange columns were used to isolate the free amino acids after which the amino acids were analyzed for deuterated-alanine content (²H-alanine) using a gas chromatography pyrolysis isotope ratio mass spectrometer. The amino acids were derivatized as their *n*-methoxycarbonyl methyl esters. Dried samples were suspended in 60 μ L distilled water and 32 μ L methanol, and following vortex, 10 μ L of pyridine and 8 μ L of methylchloroformate were added. Samples were vortexed for 30 s

and left to react at room temperature for 5 min. The newly formed *n*-methoxycarbonyl methyl esters of amino acids were then extracted into 100 µl of chloroform. A molecular sieve was added to each sample for ~20 s before being transferred to a clean glass Gas Chromatography insert. Incorporation of deuterium into protein bound alanine was determined by gas chromatography–pyrolysis–isotope ratio mass spectrometry (Delta V Advantage) alongside a standard curve of known l-alanine-2,3,3-d₄ enrichment to validate measurement accuracy of the instrument⁴⁸.

The water phase of saliva was injected 6 times with the average of the last 3 injections being used for data analysis. The ²H isotope enrichments for both muscle and saliva were initially expressed as δ²H‰ and then converted to atom percent excess using standard equations as reported by Wilkinson et al⁴⁸. Fractional synthetic rate for the myofibrillar and sarcoplasmic protein fractions were calculated using the standard precursor-product method as described by other laboratories⁴⁹⁻⁵¹.

$$FSR (\%day^{-1}) = \left[\frac{(E_{Ala2} - E_{Ala1})}{E_{BW} \times t} \right] \times 3.7 \times 100$$

Whereas E_{Ala1} and E_{Ala2} represent ²H enrichment at PRE and POST respectively (in atom percent excess) from skeletal muscle biopsies. E_{BW} is the average ²H enrichment (in atom percent excess) of total body water between time-points and *t* is time in the number of days D₂O was ingested. Multiplying by 3.7 adjusts for average ²H atoms that can be bound to alanine and multiplying by 100 converts this to a percentage per day^{48,52}.

4.7 Statistical Analyses

Statistical analyses were performed using SPSS (Version 26; IBM SPSS Statistics Software, Chicago, IL, USA), open-source software JASP (Version 0.11.0; JASP Team; 2019),

and RStudio (Version 1.1.463, R Foundation for Statistical Computing, Vienna, AT). Prior to analysis, assumptions testing for normality was performed using Shapiro-Wilk's test for all dependent variables. If the assumption of heteroscedasticity was violated for repeated measures, a Greenhouse-Geisser correction factor was applied. Dependent variables were analyzed using multi-factorial repeated measures ANOVAs, and LSD *post hoc* tests were used to assess differences in dependent variables for leg or time. Statistical significance for null hypothesis testing was set at $p < 0.05$. Data are presented throughout as mean $\pm$ standard deviation (bar graphs) or box and whiskers plots including median (central horizontal line), 25th and 75th percentile (box), minimum and maximum values (vertical lines) and mean values (cross).

AUTHOR CONTRIBUTIONS

CGV and MDR designed the study and primarily drafted the manuscript. PWM and CGV ran statistical analyses. CGV designed the training program and coordinated the study. All other authors assisted with testing, assays, or other aspects of the studies.

CONFLICTS OF INTEREST

None of the authors declares conflicts of interest exist in relation to these data.

SUPPORT

Funding for assays and participant compensation was provided through discretionary laboratory funds from M.D.R. Funding for MRI imaging was provided through discretionary laboratory funds from K.C.Y. Funding for deuterium oxide was provided through discretionary

lab funds from S.M.P. Additionally, a portion of C.G. Vann's effort was funded through the National Institutes of Health (R01AG054840). The data that support the findings of this study are available from the corresponding author upon reasonable request.

ACKNOWLEDGEMENTS

The authors would like to thank the participants for their dedication to executing this study. We would also like to thank Johnathon Moore, Samantha Slaughter, Andy Cao, Max Coleman, Max Michel, Megan Edwards, and Sullivan Clement for their assistance in collecting data.

567 REFERENCES

- 568 1. Tesch PA, Larsson L. Muscle hypertrophy in bodybuilders. *Eur J Appl Physiol Occup*
569 *Physiol.* 1982;49(3):301-306.
- 570 2. Folland JP, Williams AG. The adaptations to strength training : morphological and
571 neurological contributions to increased strength. *Sports Med.* 2007;37(2):145-168.
- 572 3. Goldberg AL, Etlinger JD, Goldspink DF, Jablecki C. Mechanism of work-induced
573 hypertrophy of skeletal muscle. *Med Sci Sports.* 1975;7(3):185-198.
- 574 4. Gollnick PD, Piehl K, Saubert CWt, Armstrong RB, Saltin B. Diet, exercise, and
575 glycogen changes in human muscle fibers. *J Appl Physiol.* 1972;33(4):421-425.
- 576 5. Morpurgo B. Ueber Activitiits-Hypertrophie der willktirlichen Muskeln. *Arch path Anat*
577 *Physiol.* 1897;1S0:522-554.
- 578 6. Goldspink G. The combined effects of exercise and reduced food intake on skeletal
579 muscle fibers. *J Cell Comp Physiol.* 1964;63:209-216.
- 580 7. Gordon EE, Kowalski K, Fritts M. Adaptations of muscle to various exercises. Studies in
581 rats. *Jama.* 1967;199(2):103-108.
- 582 8. Helander EA. Influence of exercise and restricted activity on the protein composition of
583 skeletal muscle. *Biochem J.* 1961;78(3):478-482.
- 584 9. Seiden D. Quantitative analysis of muscle cell changes in compensatory hypertrophy and
585 work-induced hypertrophy. *Am J Anat.* 1976;145(4):459-465.
- 586 10. Haun CT, Vann CG, Roberts BM, Vigotsky AD, Schoenfeld BJ, Roberts MD. A Critical
587 Evaluation of the Biological Construct Skeletal Muscle Hypertrophy: Size Matters but So
588 Does the Measurement. *Front Physiol.* 2019;10:247.
- 589 11. Fry AC. The role of resistance exercise intensity on muscle fibre adaptations. *Sports Med.*
590 2004;34(10):663-679.
- 591 12. Mitchell CJ, Churchward-Venne TA, West DW, et al. Resistance exercise load does not
592 determine training-mediated hypertrophic gains in young men. *J Appl Physiol (1985).*
593 2012;113(1):71-77.
- 594 13. Ikezoe T, Kobayashi T, Nakamura M, Ichihashi N. Effects of low-load, higher-repetition
595 versus high-load, lower-repetition resistance training not performed to failure on muscle
596 strength, mass, and echo intensity in healthy young men: a time-course study. *J Strength*
597 *Cond Res.* 2017.
- 598 14. Jenkins ND, Housh TJ, Buckner SL, et al. Neuromuscular Adaptations After 2 and 4
599 Weeks of 80% Versus 30% 1 Repetition Maximum Resistance Training to Failure. *J*
600 *Strength Cond Res.* 2016;30(8):2174-2185.
- 601 15. Jenkins NDM, Miramonti AA, Hill EC, et al. Greater Neural Adaptations following
602 High- vs. Low-Load Resistance Training. *Front Physiol.* 2017;8:331.
- 603 16. Morton RW, Oikawa SY, Wavell CG, et al. Neither load nor systemic hormones
604 determine resistance training-mediated hypertrophy or strength gains in resistance-trained
605 young men. *J Appl Physiol (1985).* 2016;121(1):129-138.
- 606 17. Schoenfeld BJ, Peterson MD, Ogborn D, Contreras B, Sonmez GT. Effects of Low- vs.
607 High-Load Resistance Training on Muscle Strength and Hypertrophy in Well-Trained
608 Men. *J Strength Cond Res.* 2015;29(10):2954-2963.
- 609 18. Dinyer TK, Byrd MT, Garver MJ, et al. Low-Load vs. High-Load Resistance Training to
610 Failure on One Repetition Maximum Strength and Body Composition in Untrained
611 Women. *J Strength Cond Res.* 2019;33(7):1737-1744.

19. Haun CT, Vann CG, Osburn SC, et al. Muscle fiber hypertrophy in response to 6 weeks of high-volume resistance training in trained young men is largely attributed to sarcoplasmic hypertrophy. *PLoS One*. 2019;14(6):e0215267.
20. MacDougall JD, Sale DG, Elder GC, Sutton JR. Muscle ultrastructural characteristics of elite powerlifters and bodybuilders. *Eur J Appl Physiol Occup Physiol*. 1982;48(1):117-126.
21. Meijer JP, Jaspers RT, Rittweger J, et al. Single muscle fibre contractile properties differ between body-builders, power athletes and control subjects. *Exp Physiol*. 2015;100(11):1331-1341.
22. Roth SM, Martel GF, Ivey FM, et al. Ultrastructural muscle damage in young vs. older men after high-volume, heavy-resistance strength training. *J Appl Physiol* (1985). 1999;86(6):1833-1840.
23. Roberts MD, Haun CT, Vann CG, Osburn SC, Young KC. Sarcoplasmic Hypertrophy in Skeletal Muscle: A Scientific "Unicorn" or Resistance Training Adaptation? *Front Physiol*. 2020;11:816.
24. Vann CG, Osburn SC, Mumford PW, et al. Skeletal Muscle Protein Composition Adaptations to 10 Weeks of High-Load Resistance Training in Previously-Trained Males. *Front Physiol*. 2020;11:259.
25. Vann CG, Osburn SC, Mumford PW, et al. Skeletal Muscle Protein Composition Adaptations to 10 Weeks of High-Load Resistance Training in Previously-Trained Males. *Frontiers in Physiology*. 2020;11(259).
26. Holm L, Reitelseder S, Pedersen TG, et al. Changes in muscle size and MHC composition in response to resistance exercise with heavy and light loading intensity. *J Appl Physiol* (1985). 2008;105(5):1454-1461.
27. CHESTNUT JL, DOCHERTY D. The Effects of 4 and 10 Repetition Maximum Weight-Training Protocols on Neuromuscular Adaptations in Untrained Men. *The Journal of Strength & Conditioning Research*. 1999;13(4):353-359.
28. Schoenfeld BJ, Grgic J, Ogborn D, Krieger JW. Strength and Hypertrophy Adaptations Between Low- vs. High-Load Resistance Training: A Systematic Review and Meta-analysis. *J Strength Cond Res*. 2017;31(12):3508-3523.
29. Campos GE, Luecke TJ, Wendeln HK, et al. Muscular adaptations in response to three different resistance-training regimens: specificity of repetition maximum training zones. *Eur J Appl Physiol*. 2002;88(1-2):50-60.
30. Jessee MB, Buckner SL, Mouser JG, et al. Muscle Adaptations to High-Load Training and Very Low-Load Training With and Without Blood Flow Restriction. *Front Physiol*. 2018;9:1448.
31. Jorgenson KW, Phillips SM, Hornberger TA. Identifying the Structural Adaptations that Drive the Mechanical Load-Induced Growth of Skeletal Muscle: A Scoping Review. *Cells*. 2020;9(7):1658.
32. Penman KA. Ultrastructural changes in human striated muscle using three methods of training. *Res Q*. 1969;40(4):764-772.
33. Toth MJ, Miller MS, VanBuren P, et al. Resistance training alters skeletal muscle structure and function in human heart failure: effects at the tissue, cellular and molecular levels. *J Physiol*. 2012;590(5):1243-1259.
34. Haun CT, Vann CG, Mobley CB, et al. Effects of Graded Whey Supplementation During Extreme-Volume Resistance Training. *Front Nutr*. 2018;5:84.

35. DeFreitas JM, Beck TW, Stock MS, Dillon MA, Kasishke PR, 2nd. An examination of the time course of training-induced skeletal muscle hypertrophy. *Eur J Appl Physiol*. 2011;111(11):2785-2790.
36. Seynnes OR, de Boer M, Narici MV. Early skeletal muscle hypertrophy and architectural changes in response to high-intensity resistance training. *J Appl Physiol* (1985). 2007;102(1):368-373.
37. Haff G, Triplett NT, National S, Conditioning A. *Essentials of strength training and conditioning*. 2016.
38. American College of Sports M, Sawka MN, Burke LM, et al. American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc*. 2007;39(2):377-390.
39. Kephart WC, Wachs TD, Thompson RM, et al. Ten weeks of branched-chain amino acid supplementation improves select performance and immunological variables in trained cyclists. *Amino Acids*. 2016;48(3):779-789.
40. Fukunaga T, Ichinose Y, Ito M, Kawakami Y, Fukashiro S. Determination of fascicle length and pennation in a contracting human muscle in vivo. *J Appl Physiol* (1985). 1997;82(1):354-358.
41. Lockwood CM, Roberts MD, Dalbo VJ, et al. Effects of Hydrolyzed Whey versus Other Whey Protein Supplements on the Physiological Response to 8 Weeks of Resistance Exercise in College-Aged Males. *J Am Coll Nutr*. 2017;36(1):16-27.
42. Lohman M, Tallroth K, Kettunen JA, Marttinen MT. Reproducibility of dual-energy x-ray absorptiometry total and regional body composition measurements using different scanning positions and definitions of regions. *Metabolism*. 2009;58(11):1663-1668.
43. Roberson PA, Romero MA, Osburn SC, et al. Skeletal muscle LINE-1 ORF1 mRNA is higher in older humans but decreases with endurance exercise and is negatively associated with higher physical activity. *J Appl Physiol* (1985). 2019;127(4):895-904.
44. Roberts MD, Romero MA, Mobley CB, et al. Skeletal muscle mitochondrial volume and myozenin-1 protein differences exist between high versus low anabolic responders to resistance training. *PeerJ*. 2018;6:e5338.
45. Roberts MD, Young KC, Fox CD, et al. An optimized procedure for isolation of rodent and human skeletal muscle sarcoplasmic and myofibrillar proteins. *J Biol Methods*. 2020;7(1):e127.
46. Cohen S, Brault JJ, Gygi SP, et al. During muscle atrophy, thick, but not thin, filament components are degraded by MuRF1-dependent ubiquitylation. *J Cell Biol*. 2009;185(6):1083-1095.
47. Dowling P, Zweyer M, Swandulla D, Ohlendieck K. Characterization of Contractile Proteins from Skeletal Muscle Using Gel-Based Top-Down Proteomics. *Proteomes*. 2019;7(2).
48. Wilkinson DJ, Franchi MV, Brook MS, et al. A validation of the application of D(2)O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans. *Am J Physiol Endocrinol Metab*. 2014;306(5):E571-579.
49. Bell KE, Brook MS, Snijders T, et al. Integrated Myofibrillar Protein Synthesis in Recovery From Unaccustomed and Accustomed Resistance Exercise With and Without Multi-ingredient Supplementation in Overweight Older Men. *Frontiers in nutrition*. 2019;6:40-40.

- 704 50. Chinkes DL, Rosenblatt J, Wolfe RR. Assessment of the mathematical issues involved in
705 measuring the fractional synthesis rate of protein using the flooding dose technique.
706 *Clinical Science*. 1993;84(2):177-183.
- 707 51. McKendry J, Shad BJ, Smeuninx B, et al. Comparable Rates of Integrated Myofibrillar
708 Protein Synthesis Between Endurance-Trained Master Athletes and Untrained Older
709 Individuals. *Front Physiol*. 2019;10:1084.
- 710 52. MacDonald AJ, Small AC, Greig CA, et al. A novel oral tracer procedure for
711 measurement of habitual myofibrillar protein synthesis. *Rapid Commun Mass Spectrom*.
712 2013;27(15):1769-1777.

FIGURE CAPTIONS AND LEGENDS

Figure 1. Training Volume and Strength Metrics

Legend: Data are presented as box and whiskers plots including median (central horizontal line), 25th and 75th percentile (box), minimum and maximum values (vertical lines) and mean values (cross) for training volume load (panel a), unilateral leg press (panel b), unilateral leg extension (panel c), knee extension peak torque at 60°/s (panel d), knee extension peak torque at 120°/s (panel e), knee extension mean torque at 60°/s (panel f), and knee extension mean torque at 120°/s (panel g). Abbreviations: HV, high-volume; HL, high-load. *, indicates a condition×time interaction whereas POST and POSTPR were greater than PRE.

Figure 2. Segmental Upper Leg Composition

Legend: Data are presented as box and whiskers plots including median (central horizontal line), 25th and 75th percentile (box), minimum and maximum values (vertical lines) and mean values (cross) for DXA upper leg total mass (panel a), DXA upper lean mass (panel b), and DXA upper leg fat mass (panel c). Abbreviations: HV, high-volume; HL, high-load; DXA, dual energy x-ray absorptiometry.

Figure 3. Vastus Lateralis Muscle Morphology

Legend: Data are presented as box and whiskers plots including median (central horizontal line), 25th and 75th percentile (box), minimum and maximum values (vertical lines) and mean values (cross) for VL mCSA (panel a), Est. VL fiber length (panel c), VL thickness (panel d), and VL muscle pennation angle (panel f). Representative images: Dual leg MRI for VL mCSA (panel b), ultrasound cross-section for VL thickness (panel e), ultrasound cross-section for pennation

angle (panel g). No significance was observed following decomposition of condition×time interaction for VL mCSA. Abbreviations: HV, high-volume; HL, high-load; VL, vastus lateralis; mCSA, muscle cross-sectional area, Est., estimated.

Figure 4. Muscle Protein Adaptations

Legend: Data are presented as box and whiskers plots including median (central horizontal line), 25th and 75th percentile (box), minimum and maximum values (vertical lines) and mean values (cross) for sarcoplasmic protein concentrations (panel a), MyHC protein abundance (panel b), actin protein abundance (panel c), tropomyosin protein abundance (panel d), and troponin protein abundance (panel e). Representative image: Coomassie blue stained poly-acrylamide gel for protein abundance. Abbreviations: HV, high-volume; HL, high-load; VL; MyHC, myosin heavy chain; ADU, arbitrary density units; kD, kilodalton.

Figure 5. Six-week Integrated Myofibrillar and Sarcoplasmic Protein Synthetic Rates

Data are presented as box and whiskers plots including median (central horizontal line), 25th and 75th percentile (box), minimum and maximum values (vertical lines) and mean values (cross) for iMyoPS (panel a) and iSarcoPS (panel b). No significant differences were observed for iMyoPS between conditions. iSarcoPS was significantly lower in the HL condition as compared to the HV condition. Abbreviations: HV, high-volume; HL, high-load; MyoPS, myofibrillar protein synthesis; SarcoPS, sarcoplasmic protein synthesis.

Figure 6. Study Design

770 Legend: Panel a provides an overview of testing, training, D₂O administration, and saliva
771 collection times. Panel b provides a schematic of training by day and total training for each
772 week. Abbreviations: wk, week; D₂O, deuterium oxide.

TABLES

Table 1. Participant characteristics

Variable	Mean $\pm$ SD
Age (years)	23 $\pm$ 3
Height (cm)	182 $\pm$ 8
Weight (kg)	89.5 $\pm$ 11.6
Lean soft tissue mass (kg)	69.1 $\pm$ 7.4
Fat tissue mass (kg)	17.3 $\pm$ 7.5
Fat-free mass index	20.9 $\pm$ 2.2
Est. 1RM Squat (kg)	167 $\pm$ 34
Squat relative to body weight	1.9 $\pm$ 0.4

N=15 participants. Abbreviations: Est. 1RM, estimated 1 repetition maximum. All measures taken prior to onset of training intervention.

Table 2. Body composition changes during training

Variable	PRE	POST	POSTPR	ANOVA
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	p-value
Total Body Mass (kg)	89.3 $\pm$ 11.5	90.8 $\pm$ 11.9	90.4 $\pm$ 12.2	<0.001 [†]
DXA Whole Body LSTM (kg)	69.1 $\pm$ 7.4	70.2 $\pm$ 7.5	69.5 $\pm$ 7.5	0.003 *
DXA Whole Body Fat Mass (kg)	17.3 $\pm$ 7.5	17.4 $\pm$ 7.8	17.7 $\pm$ 7.8	0.097

N=15 participants. Abbreviations: DXA, dual energy x-ray absorptiometry; LSTM, lean soft tissue mass. [†], indicates measurement was higher at POST and POSTPR than PRE ($p<0.05$); *, indicates measurement was higher at POST than at PRE and POSTPR ($p<0.05$).

a

Condition = 0.003 (HL<HV)
 Time < 0.001 (all weeks sig.)
 C*T < 0.001

b

Condition = 0.475
 Time < 0.001 (PRE<POST&POSTPR)
 C*T = 0.512

c

Condition = 0.026 (HV<HL)
 Time < 0.001 (PRE<POST<POSTPR)
 C*T = 0.032

d

Condition = 0.854
 Time = 0.073
 C*T = 0.995

e

Condition = 0.310
 Time = 0.225
 C*T = 0.366

f

Condition = 0.359
 Time = 0.041 (PRE&POST<POSTPR)
 C*T = 0.960

g

Condition = 0.245
 Time = 0.067
 C*T = 0.681

a

Condition = 0.211

Time = 0.001 (PRE<POST&POSTPR)

C*T = 0.069

b

Condition = 0.407

Time = 0.001 (PRE<POST&POSTPR)

C*T = 0.174

c

Condition = 0.102

Time = 0.595

C*T = 0.959

a

b

[illegible]

[†] Participants arrived in an overnight fasted state for testing consisting of: USG, height, weight, DXA, US, MRI, blood draw, VL muscle biopsy, isokinetic dynamometry, strength testing.

Training Protocol*

[illegible]

*Participants also completed BB bench press, pronated grip BB row, and BB SLDL for 9x10 @ 70% of estimated 1RM. All sets and reps are shown as weekly prescriptions.