Effects of high-volume and high-intensity resistance training on skeletal muscle ultrastructure

By

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ABSTRACT

Background: Recently there has been enthusiasm surrounding the differential training adaptations that result from higher-load (HL) versus higher-volume (HV) resistance training. The purpose of this study was to evaluate the effects of HL versus HV training on markers of skeletal muscle hypertrophy and strength as well as a broad spectrum of molecular markers obtained from skeletal muscle biopsies. **Methods:** Trained college-age males (n=15, age: 23±3 years old; training age: 7±3 years; squat 1RM relative to body mass: 1.9) performed unilateral lower body training three days per week over 6 weeks, with one leg being randomly assigned to HV training and the other to HL training. Vastus lateralis (VL) biopsies were obtained prior to the start of the study (PRE), 72 hours following the last exercise bout (POST), and following 10 days of passive recovery (POSTDL). Body composition and strength tests were also performed at each testing session, and various biochemical assays were performed on muscle tissue. **RESULTS:** Condition×time interactions were observed for unilateral leg extension 1RM (HL>HV at POST and POSTDL) and VL muscle cross-sectional area (post hoc tests indicated no significance between conditions over time). A main effect of condition was observed for unilateral leg extension 1RM (HL>HV) and sarcoplasmic protein concentrations (HV>HL). A main effect of time was observed for unilateral leg press 1RM (PRE<POST and POSTDL), unilateral leg extension 1RM (PRE<POST<POSTDL), knee extensor peak torque at 60°/sec (PRE and POST<POSTDL), dual-energy x-ray absorptiometry (DXA)-derived upper leg total and lean mass values (PRE<POST and POSTDL), ultrasound-derived VL thickness (PRE and POSTDL<POST), sarcoplasmic protein concentrations (POST and POSTDL<PRE), and tropomyosin and troponin protein

abundances (PRE>POST and POSTDL). **CONCLUSION:** In summary, our data suggest that short-term HV and HL training elicit similar hypertrophic, strength, and molecular adaptations.

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List of Abbreviations

mCSA	muscle cross sectional area
fCSA	fiber cross sectional area
g	gram/s
kg	kilogram/s
cm	centimeters
PRE	Pre-testing (T1)
POST	Post-testing (T2)
POSTDL	Post deload testing (T3)
RT	resistance training
D_2O	deuterium oxide
FSR	fractional synthesis rate
RT	Resistance training
TEM	transmission electron microscopy
ULLS	unilateral limb suspension
VL	vastus lateralis
MPS	muscle protein synthesis
MPB	muscle protein breakdown
LO	low responders
HI	high responders
MF-FSR	myofibrillar fractional synthesis rate
YT	young trained

YU	young untrained
OU	old untrained
HV	high-volume
HL	high-load
SR	sarcoplasmic reticulum
MHC-I	myosin heavy chain I
MHC-II	myosin heavy chain II
RIR	repetitions in reserve
RPE	rate of perceived exertion
TVL	total volume load

Chapter I

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 individual fiber [3-5]. It is generally recognized that resistance training results in skeletal muscle growth and has been suggested to result in proportional increases in myofibrillar and sarcoplasmic protein content [6-9]. Myofibril proteins are defined herein as proteins that make up the rigid structure of muscle (i.e., 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 processes (e.g., sarcoplasmic reticulum, enzymes, etc.) [10].

While evidence of disproportional increases in the sarcoplasmic protein fraction exists, these data are sparse. Penman reported increases in space between myofilaments with a concomitant decrease in the concentration of myosin fibers [11]. MacDougall and colleagues provided further evidence of disproportionate sarcoplasmic expansion in subjects following 6 months of resistance training. Specifically, using transmission electron microscopy (TEM), the authors found a decrease in space occupied by myofibrils despite an increase in type II fiber cross-sectional area (fCSA) [12]. Meijer and colleagues [13] reported lower specific tension values in vastus lateralis fibers in trained bodybuilders as compared to their untrained counterparts. These studies suggest a dilution of contractile proteins occurred with resistance training despite increases in muscle size.

Contrary to the evidence above, several studies have reported a proportional or disproportional increase in myofibrillar protein content in conjunction with muscle growth. In

1970 Penman conducted a study utilizing leg extensions and stair running, again using TEM to evaluate contractile protein density and spacing. The author reported an increase in packing density suggestive of myofibrillar hypertrophy [14]. Lüthi showed increases in muscle cross-sectional area (mCSA) with no measurable change in fCSA or myofibril area, as measured by TEM, following 6 weeks of whole-body resistance training [15], which may be related to the expansion in both the myofibrillar and sarcoplasmic fractions. Woolstenhulme and colleagues examined changes in fCSA and select protein markers following a single bout of resistance training and eight weeks of resistance training [16]. The authors reported that type I fCSA did not change between one bout and 8 weeks of training. However, type II fCSA increased following 8 weeks of RT, and this change was accompanied by an increase in the scaffolding protein desmin and no changes in total protein concentrations, actin protein expression, or dystrophin protein expression. Following 6 weeks of unilateral training, Brook and colleagues reported an increase in myofibrillar fractional synthetic rates (MF-FSR) with no increases in total protein concentrations despite increases in DXA lean leg mass and ultrasound VL thickness [17]. Reidy et al. [18] reported that following 12 weeks of RT in untrained men, there was an increase in fCSA and muscle protein synthesis (MPS) with a decrease in muscle protein breakdown (MPB). However, total protein concentrations from pre and post-intervention skeletal muscle biopsies were similar. This study also reported increases in muscle thickness and volume with an increase in muscle water content. Roberts et al. [19], performed a responder (classified based on change score for fCSA) analysis in untrained men following 12 weeks of resistance training, and found high responders increased in fCSA PRE to POST while low responders had similar values PRE to POST despite neither group showing increases in actin and myosin content per milligram wet tissue. With exception to the 1970 report by Penman which suggests myofibril packing occurred during resistance training, the

aforementioned findings suggest myofibril protein accretion occurred in proportion to increases in muscle mass and/or fiber size.

The type of resistance training may be an important factor in dictating whether sarcoplasmic or myofibrillar hypertrophy occurs. In previously-trained college-aged males performing 6 weeks of resistance training starting at 10 sets of 10 repetitions a week and finishing at 32 sets of 10 repetitions per week, all at 60% of 1 repetition maximum (1RM), Haun and colleagues showed increases in type I and type II fCSA accompanied by increased sarcoplasmic protein content and a decrease in actin and myosin protein concentrations per mg dry tissue [20]. Conversely, Vann and colleagues more recently demonstrated that, following lower volume, higher intensity resistance training (3-5 sets of 2-6 repetitions at 65-90% 1RM), type I fCSA remained the same while type II fCSA increased despite a decrease in actin protein concentrations and no change in sarcoplasmic protein concentrations [21]. While preliminary, these two studies from our laboratory suggest that higher volume resistance training may facilitate sarcoplasmic hypertrophy, while higher load training may promote a proportional increase in myofibril protein accretion with hypertrophy.

The above-mentioned research has led to questions concerning whether a proportional increase in myofibrillar and sarcoplasmic protein fractions occurs with different training paradigms. Therefore, the purpose of the present study was to elucidate whether high-volume resistance training and high-load resistance training differentially affect select markers of hypertrophy following 6 weeks of unilateral training.

Specific aims of this proposal

1. Examine segmental upper leg lean soft tissue mass (LSTM) differences between high volume (HV) and high load (HL) legs prior to the 6-week training intervention (PRE), 72 hours following the last exercise bout (POST), and 10 days following the last exercise bout (POSTDL).
2. Examine fiber cross-sectional area (fCSA) differences between HV and HL legs at PRE, POST, and POSTDL.
3. Examine sarcoplasmic, actin, and myosin protein concentration alterations that occur following HV and HL resistance training at PRE, POST, and POSTDL.
4. Examine the 6-week integrated myofibrillar protein synthesis (MyoPS) and sarcoplasmic protein synthesis (SarcoPS) rate responses that occur following HV and HL resistance training at PRE and POST.

Hypotheses

1. HV and HL training will result in similar increases in DXA-measured upper leg LSTM, ultrasound-measured vastus lateralis (VL) thickness, and MRI-measured VL area.
2. HV training will lead to increased type I and type IIa fCSA, while HL training will result in increased type IIa and type IIx fCSA.
3. HV training will lead to increased sarcoplasmic protein concentrations and decreased actin and myosin concentrations (all per milligram wet tissue), while HL training will result in no changes in the concentrations of these markers. Furthermore, these observations will be potentially explained through the MyoPS and SarcoPS data.

Chapter II

Literature Review

In this literature review, I first provide an overview of muscle cell structure. I then give an overview of muscle hypertrophy through the underlying mechanisms important to resistance training. I then discuss select studies that have evaluated sarcoplasmic and myofibrillar protein synthesis. Lastly, I discuss deuterium oxide and its use as a tracer in the evaluation of muscle protein synthesis.

Muscle Cell Structure and Properties

Skeletal muscle is a plastic tissue and can be categorized into the following levels: i) whole muscle which is covered in connective tissue known as the fascia (epimysium); ii) fascicles of muscle fibers which are sheathed by peri- and endomysium, respectively; iii) myofibrils within each individual muscle fiber; iv) sarcomeres which are the smallest functional unit of muscle; and v) protein structures within each individual sarcomere.

At the whole muscle level, tissue is covered in connective tissue known as the fascia, which is made primarily of collagen. This matrix consists of ~55-70% water, and when dehydrated, 60-85% of the extracellular matrix is collagen protein. The extracellular matrix possesses at least three major roles: i) mechanical support for vessels and nervous tissue; ii) the plastic response of muscle; and iii) the transmission of force from muscle to tendon and ultimately bone for locomotion [22]. Researchers have shown that the underlying connective tissue structures are highly organized and may not deform in a uniform fashion as sarcomeres increase length, yielding a load-bearing network that transmits shear forces for locomotion through the endomysium [23, 24]. The manner in which skeletal muscle and connective tissue are organized has also been

suggested to be determined by the torque of the muscle and how it affects adjacent fibers [25]. The extracellular matrix further segments the muscle into fascicles and individual fibers and is comprised mostly of connective tissue and vasculature. This matrix occupies ~20% of whole tissue space [22].

The individual muscle fiber contains myofibrils, sarcoplasmic reticulum, transverse tubules, mitochondria, intramuscular triglycerides, and glycogen [26-28]. While most cells are singly nucleated, skeletal muscle is unique in that it is one of the few cell types that is multinucleated. Myonuclei have been suggested to have a domain that regulates a finite space within muscle fibers, and myonuclear domains may play a role in how muscle responds to various exercise stimuli [29]. It has been suggested that satellite cells play an integral role in the continued response to exercise training by donating their nuclei to muscle cells. While satellite cell-mediated myonuclear accretion generates more myonuclear domains, satellite cells have also been suggested to have a role in muscle fiber remodeling and regulation of the extracellular matrix [30, 31]. Training-induced muscle growth may be predicated on myonuclear accretion through the donation of nuclei from satellite cells. Goh and colleagues reported an increased in myonuclear quantity following 2, 4, and 8 weeks of high-intensity interval training in mice with muscle growth in the quadriceps muscle occurring between weeks 4 and 8 [32]. Petrella et al. [29] showed that following 16 weeks of resistance training, irrespective of age or sex, participants showing more robust increases in markers of hypertrophy additionally had larger pools of satellite cells. Furthermore, Egner and colleagues [33] showed robust synergist ablation-induced increases in plantaris fCSA in vehicle-treated Pax7-DTA mice who possessed satellite cells; however, fCSA did not change in the absence of satellite cells in Pax7-DTA mice treated with tamoxifen.

The sarcoplasmic reticulum is thought to have been initially discovered in the late 1800s [34], and plays a role in the regulation of contraction and relaxation through regulation of intracellular calcium (Ca^{2+}). The sarcoplasmic reticulum has two regions known as the non-junctional sarcoplasmic reticulum and the junctional sarcoplasmic reticulum [35]. The junctional sarcoplasmic reticulum (terminal cisternae that faces the transverse tubule) stores and releases Ca^{2+} through interactions with the transverse tubules through the ryanodine receptor [36]. The transverse tubule network thought to be discovered in the early 1900s [37], is highly organized consisting of a portion that interacts with the terminal cisternae [38]. Transverse tubule junctions have high quantities of dihydropyridine receptors that facilitate interaction with the terminal cisternae through voltage-regulated interactions with ryanodine receptors located on the terminal cisternae [39].

Skeletal muscle fibers have a striated appearance with isotropic bands (I band) appearing light and anisotropic (A band) appearing dark. Myofibrils and the cross-striated appearance of muscle fibers were initially discovered in the late 1600s [40]. One of the first studies to formally examine this striated appearance was performed in 1897 when black India ink was injected into cardiac muscle tissue of various species, and varying light intensities were observed through light microscopy [41].

Several studies have evaluated muscle at the protein level and range from evaluations of occupied space to percent of muscle constituted by certain protein pools. Spatially, ~85% of skeletal muscle is occupied by myofibrils with ~15% of space being intermyofibrillar, containing mitochondria (~5% of space), ribosomes, glycogen, lipid droplets, and other macromolecules [42]. In a recent reappraisal of the skeletal muscle proteome, Gonzalez-Freire [43] used mass spectrometry to estimate most proteins within skeletal muscle are related to metabolic processes,

with ~40% being enzymes and only ~10% being related to muscle contraction. Additionally, about 20% of the proteins were related to the mitochondria, possibly serving in oxidative metabolism. Of the myofibrillar fraction, it has been estimated that ~50% is myosin, ~20% is actin, ~10% is titin, while nebulin, troponin, and tropomyosin each make up ~5% of the fraction by concentration [44-46]. A Recent study by Vann and colleagues using proteomic analysis suggests the myofibrillar protein fraction of muscle tissue occupies ~14% of the volume with ~6% being sarcoplasmic protein and the rest being fluid and other constituents in trained young men. Within the myofibrillar protein fraction it was estimated that ~60% is made of myosin (sum of all isoforms), ~11% actin (sum of all isoforms), ~5% troponin (sum of all isoforms), ~4% titin, ~3% tropomyosin and alpha-actinin (sum of all isoforms), and ~1% nebulin with the remainder being made of other proteins. The sarcoplasmic fraction is predominantly composed of non-contractile proteins. In young, healthy trained men this fraction is made up of ~27% glycolytic enzymes, ~11% creatine kinase (sum of all isoforms), ~6% myoglobin, ~5% Krebs cycle enzymes, ~4% SERCA 1/2, ~3% electron transport chain proteins, ~1% beta-oxidation enzymes, with the remainder being proteins that were not assigned to one of the aforementioned processes [47]. This is contradictory to the dogma of ~60-70% of skeletal muscle proteins being classified as myofibrillar, ~20-30% being classified as being involved in metabolism (sarcoplasmic), and ~10% being mitochondrial proteins [48]. There are some noteworthy differences between type I and II fibers that have been found in animal tissues. Type I fibers have a greater abundance of mitochondria [49], type II fibers have larger sarcoplasmic reticulum vesicles around myofibrils [50]. Additionally, the myofibril diameter may be larger, sarcomeres may be slightly longer, and Z lines may be slightly thicker in type II fibers [49].

Skeletal muscle composition is likely conserved evolutionarily across species as proteins with sequence identities as low as 30% retain a similar structure [51]. *Homo sapiens*, however, are distinctly different from other species, and this may be a result of evolving to the specific environmental demands resulting in a metabolic divergence in skeletal muscle [52, 53]. Interestingly, when evaluating myosin heavy chain isoforms, our closest living relative on a phylogenetic tree (chimpanzees), as well as macaques, exhibit higher numbers of myosin heavy chain type II positive fibers and a vastly greater normalized strength on pushing and pulling exercises [52, 53]. Said differently, since the emergence of the *homo sapiens* species ~7-8 million years ago, it is likely myosin heavy chain type I fibers became more prevalent over time due to the demands of walking and other prolonged forms of exercise that were important for survival. Historically, the survival of a species was predicated on the ability to adapt to environmental conditions (i.e., energetic demands) in order to find adequate safety, food and shelter [54].

Muscle Hypertrophy

Skeletal muscle hypertrophy is a well-known adaptation to resistance training. The legend of Milo of Croton (6^{th} century BCE) whom was said to carry a calf daily until it became a full-grown ox, alludes to the concept of training becoming progressively more difficult to further adaptation [55].

Training-induced muscle growth is known to be a result of progressive overload. Resistance training is commonly thought to have three prominent variables that contribute to overload: i) training frequency, ii) training intensity, and iii) training volume. Pertinent to this literature review, training frequency will be defined as the number of sessions per week, training intensity will be discussed as a percent of maximum strength (1RM) or perceived exertion, and

training volume will be discussed as the total weight lifted yielded from the total number of repetitions per week multiplied by the weight utilized per repetition. To an extent, rest periods can be used as another variable when applying overload. However, studies have shown that optimal hypertrophic and strength outcomes come from resting at least 3 minutes between sets [56, 57]. Thus, rest periods will not be discussed herein.

Training frequency, as mentioned above, is the number of days a muscle is trained over the course of one week. In 2016, Schoenfeld and colleagues reviewed the effects of training frequency on muscle growth, and concluded that training muscles twice per week yielded greater growth responses than once per week [58]. Colquhoun and colleagues evaluated alterations in muscle size and indices of strength over a 6-week time frame with groups of participants training 6 times weekly or 3 times weekly and found no differences in strength or muscle size [59].

Training intensity from a research perspective has most commonly been reported as a percentage of one repetition maximum (%1RM). A 1RM can be defined as the maximal amount of weight an individual can perform one repetition with for a given exercise. In recent research, it has also been expressed as the rating of perceived exertion using a modified Borg scale (RPE; modified to use a 10 point scale with 0 being no effort and 10 being maximal effort) or repetitions in reserve (RIR; the numbers of repetitions a participant could still reasonably complete) [60-62]. Historically, heavy training loads dosed at specific set and repetition schemes have been touted as the best way to optimize muscle growth [63, 64]. Interestingly, the muscle protein synthetic response has been found to be negligible when utilizing intensities 20-40% of 1RM, yet increase when using 70-90% of 1RM when workloads are matched [65]. Historically, loads less than or equal to 40% of 1RM have been considered light, whereas loads at 60% of 1RM or greater have been deemed heavy. However, current evidence suggests training with lighter loads can yield

slightly greater short-term growth responses [66, 67]. When evaluating training at lower versus higher intensities, a single bout taken to momentary volitional failure leads to similar increases in anabolic signaling [66, 68]. Similarly, chronic training at low and higher intensities – to volitional failure – lead to similar hypertrophic outcomes [67, 69]. Notwithstanding, a review by Poortmans [70] suggests that training at intensities in excess of 50% 1RM elicit a more robust increase in MPS.

Training volume has emerged as a critical variable that influences muscle growth. Notably, when calculating the total training volume-load, it inherently encompasses training intensity and training frequency. Training volume has been said to possibly have a dose-response relationship with muscle growth, meaning that the more training volume accumulated over a week (to a point), the greater the muscle growth response [71]. In a literature review, Wernbom et al. concluded that 10 or more sets for the quadriceps femoris muscle group yielded greater growth responses than 4-6 sets [72]. In a systematic review and meta-analysis, Schoenfeld and colleagues showed that performing <5 sets on an exercise yields an ~5.4% increase in size; performing 5-9 sets yields an ~6.6% increase in size; and performing >9 sets yields an ~8.2% increase in muscle size [73]. The lead author also suggested in a separate commentary that performing >10 sets may be required to maximize the potential for muscle growth [74].

The individual response to resistance training can be inherently different depending on training status [75]. Previously trained individuals have been shown to have differences in select markers associated with transcriptional, translational, and adaptive responses to resistance training [76]. Additionally, the dampened response to exercise (endurance and resistance exercise) in trained individuals is suggested to stem from diminished stress mediated through an increase in heat shock proteins, which aid in the maintenance of homeostasis [77]. Hence it is likely trained

individuals need to train at higher volumes (e.g., increased intensity and set/rep quantity) to facilitate continued adaptation [78].

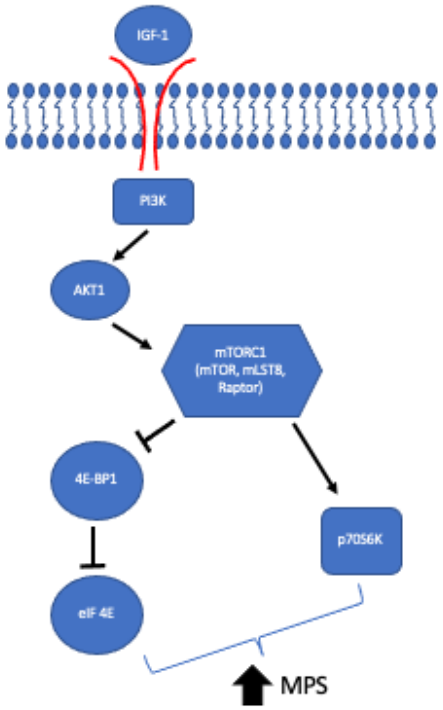
Regulation of hypertrophy

It is widely accepted that the mechanistic target of rapamycin (mTOR) is a regulator of muscle growth via increased MPS. Drummond and colleagues [79] showed that blocking of mTOR with rapamycin blunts resistance exercise-induced MPS. Notably, there are two separate complexes that can be formed with mTOR: i) mTORC1 which phosphorylates downstream substrates and leads to the formation of translation-competent ribosomes while also playing a role in ribosome biogenesis, and ii) mTORC2 which seemingly plays a role in mitochondrial function [80]. Pathways associated with mTORC1 will be discussed herein. A schematic of the mTORC1 pathway can be found in Figure 2.1.

Activation of mTORC1 is seemingly necessary for load-induced muscle growth [81]. Mechanotransduction is the transmission of mechanical energy into chemical energy, which can affect MPS and MPB [82]. One important pathway for skeletal muscle growth is the phosphatidylinositol-3-kinase/Akt/mTORC1 pathway. This pathway is post-translationally regulated via the serine/threonine kinase Akt [83]. Akt is largely accepted as a regulator of mTORC1 [84] having three isoforms (Akt1, Akt2, and Akt3) of which only Akt1 and Akt 2 are expressed in skeletal muscle [85]. Briefly, Akt can be activated by PI3K through growth factor receptor binding (e.g., mechano growth factor; MGF). Akt activates mTORC1 which is comprised of 3 major components (mTOR, Raptor, mLST8) [86, 87]. mTORC1 promotes increases in protein synthesis as a result of downstream phosphorylation of 4E-BP1 and p70s6k [88-91]. p70s6k is directly phosphorylated by mTORC1, allowing p70s6k to phosphorylate downstream substrates

leading to increased translational efficiency [92, 93]. 4E-BP1 is a substrate of mTORC1 that inhibits translation through binding and sequestering eIF4E to prevent assembly of the eIF4E complex [92-94]. The phosphorylation of 4E-BP1 via mTORC1 results in a dissociation from eIF4E, allowing mRNA translation to occur [95]. It should be noted that many factors governing the regulation of mTORC1 are still being elucidated. For instance, Spangenburg and colleagues [96] have shown that IGF-1 activation of mTORC1 is not required for muscle growth leading to questions about the requirement of the abovementioned pathway for muscle growth. Furthermore, phosphorylation of Akt is not a requirement for activation of mTORC1 [97]. Finally, it has been shown that the activation of mTORC1 can occur via overload-induced stimulation from phosphatidic acid in an Akt-independent manner [98].

Figure 2.1. ***mTORC1 Pathway***



Legend: Mechanistic target of rapamycin complex 1 pathway. It is well accepted that resistance exercise results in the upregulation of this pathway resulting in increased muscle protein synthesis.

Ribosome biogenesis is also thought to play an important role in muscle growth due to the function ribosomes carry out in translating mRNA to protein. The biogenesis of ribosomes is a complicated and energetically costly process that requires the transcription of the 28S, 18S, 5S, 5.8S ribosomal RNAs in addition to the translation of ~80 ribosomal proteins [99]. Protein synthesis can be affected by translational capacity (total ribosome content) and translational efficiency (rate of translation per ribosome) with the former potentially increasing the likelihood of translation [100, 101]. Select ribosomal protein mRNAs have been found to be elevated using transcriptome profiling in response to resistance training where higher levels exist in participants deemed to be high or extreme responders [102]. Likewise, data from our laboratory and others suggest high responders to resistance training show the greatest increases in ribosome biogenesis [103, 104].

Little is known about specific genes that regulate skeletal muscle hypertrophy. Myostatin is a known inhibitor of muscle growth and muscle differentiation [105]. In cell culture models, treatment of myotubes with myostatin has been found to blunt activity of Akt/mTORC1/p70s6k [106]. Conversely, follistatin has been suggested as a promoter of muscle growth [107]. Follistatin is an inhibitor of myostatin and has been reported to activate the Akt/mTOR/p70s6k/S6 pathway independently of mechanisms related to myostatin supportive of a disconnect in mTOR and myostatin signaling [108]. A recent study evaluating muscle cross-sectional area and strength following 8 weeks of resistance training in an elderly population showed increases in muscle size and strength with decreased myostatin expression and increased follistatin expression [109]. Said differently, decreased myostatin expression with increased follistatin expression potentially

resulted in increased leg muscle growth and strength. Select polymorphisms have also been identified as being associated with lean mass; however, more work is needed in this field in order to elucidate genes strongly associated with muscle size. Zillikens and colleagues [110] associated lean body mass to single nucleotide polymorphisms through a genome-wide association and identified five gene candidates (HSD17B11, VCAN, ADAMTSL3, IRS1, and FTO). Little is known about ADAMTSL3 (a disintegrin-like and metalloprotease domain with thrombospondin type I motifs-like 3), but it has been commonly associated with individuals of larger stature in *homo sapien* samples [111]. Hydroxysteroid (17-beta) dehydrogenase 11 (HSD17B11) has been found to have an effect of glucocorticoids and is highly expressed in human skeletal muscle [112]. Versican (VCAN) has been found to play a role in connecting muscle cells with the extracellular matrix [113]. Various alleles within the FTO gene have been extensively studied in regards to obesity predisposition; however, Livshits and colleagues [114] have shown that different FTO polymorphisms are associated with DXA lean and fat mass. Insulin receptor substrate 1 (IRS1) is a part of the insulin signaling pathway, playing roles in growth hormone and adipocytokine signaling [110]. Little is known about the role of IRS1 in skeletal muscle, but it is highly expressed [115].

It has also been suggested that DNA methylation status and epigenetics may be involved in training-induced muscle growth. A study evaluating high-level athletes and sedentary controls found polymorphisms are thought to exist in several enzymes implicated in DNA methylation, which may have positive effects on regulation of muscle growth [116]. This is suggestive of training increasing or decreasing methylation status allowing for transcription to occur. Additionally, it has also been suggested that human skeletal muscle may possess an epigenetic memory to recent resistance training bouts, and that re-engaging in training following a de-load

period may result in more rapid muscle growth due to prolonged DNA methylation adaptations that occur at the onset of training[117].

Studies suggestive of sarcoplasmic hypertrophy

Sarcoplasmic hypertrophy is an increase in muscle fiber or tissue growth through an expansion of the sarcoplasm and an up-regulation in non-contractile proteins, including those involved in signal transduction, energy synthesis and breakdown, and other metabolic pathways. One of the first studies to examine skeletal muscle hypertrophy was conducted by Morpurgo in 1897, in which he evaluated hypertrophy in the sartorius muscle of dogs following 2 months of run training. Morpurgo reported increases in fiber diameter, and suggested these were a result of sarcoplasmic expansion [5]. The first study to find evidence of sarcoplasmic hypertrophy in humans was conducted in 1969 by Penman who used TEM to evaluate contractile protein density and spacing in subjects following endurance or resistance training. The author reported a decrease in the concentration of myosin filaments as well as an increase in myofilament spacing following 8 weeks of isotonic leg extension, isometric contraction (performed at 135°), or stair running [11]. This finding could be a result of increased sarcoplasm expansion and/or sarcoplasmic protein content. A subsequent study by MacDougall and colleagues compared biopsies from bodybuilders and untrained males matched for similar size. Notably, the untrained participants were from previous studies [12, 118] and underwent 5-6 weeks of resistance training performing 3-5 sets of 8-10 repetitions on the bench press and vertical dip. Histology and TEM were used to evaluate fCSA and space occupied by myofibrils, respectively. The untrained men had similar fCSAs to their well-trained counterparts following resistance training. Furthermore, following training, the untrained men showed a decrease in space occupied by myofibrils with an increase in cytoplasmic

area similar to their well-trained counterparts [12]. Alternatively stated, chronic resistance training led to an increase in fCSA through increased sarcoplasmic volume. Toth et al. [119] examined muscle ultrastructure with TEM in 10 heart failure patients and 14 controls following 18 weeks of resistance training. The author reported a decrease in space occupied by myofibrils in both groups with no changes in mitochondrial volume. Alternatively stated, the alterations in muscle morphology may be a result of increased sarcoplasmic space and potentially sarcoplasmic protein concentration. Our laboratory has shown that following six weeks of high-volume resistance training, there is an increase in type I and type II fCSA accompanied by an increase in sarcoplasmic protein concentrations. Notably, despite an increase in fCSA, there was a decrease in actin and myosin protein concentrations per mg of dry tissue [20]. Moreover, we used phalloidin staining to demonstrate that the density of actin per fiber decreased as training ensued.

Studies suggestive of proportional hypertrophy or myofibrillar hypertrophy

The increase in myofibrillar protein content (i.e., myofibril accretion) is thought to be a driver of muscle hypertrophy. Goldspink was one of the first investigators to report myofibrillar hypertrophy – or an accretion of myofibril protein prior to fCSA increases – using a pulley-based resistance training design in female mice. Specifically, this author reported that the mice housed with pulley systems had larger biceps brachii musculature and less sarcoplasmic space as evaluated with TEM than their control counterparts [6]. This finding is suggestive of increased myofibril packing density. One of the first studies reporting similar findings in humans was conducted by Penman in 1970 [14] using TEM to evaluate contractile protein density and spacing. The author reported an increase in packing density suggestive of myofibrillar hypertrophy. Numerous studies have reported increases in fCSA with concomitant increases in myofibrillar protein content

following resistance training [120-123]. Following 6 weeks of unilateral training, Brook and colleagues reported an increase in myofibrillar fractional synthetic rates (MF-FSR) with no increases in total protein content despite increases in DXA lean leg mass and ultrasound VL thickness [17]. Reidy et al. [18] reported that, following 12 weeks of resistance training in untrained men, there was an increase in fCSA, increases in muscle protein synthesis (MPS), and decreases in muscle protein breakdown (MPB). However, total protein concentrations from pre and post-intervention skeletal muscle biopsies were similar. This study also reported an increase in muscle thickness and volume, as well as an increase in muscle water content. Roberts et al. [19] performed a responder analysis in untrained men following 12 weeks of resistance training and found high hypertrophic responders increased in fCSA PRE to POST while low responders had similar values PRE to POST despite neither group showing increases in actin and myosin content.

Deuterium Oxide

Deuterium oxide (D_2O) is a type of water that contains a larger than normal amount of the hydrogen isotope deuterium. When consumed or administered through other means, it equilibrates into different tissues at different rates. For the purpose of this review, the use of D_2O as a tracer for tracking fractional protein synthetic rates will be briefly discussed.

Wilkinson and colleagues [124] discussed the use of isotope tracers is discussed in relation to research. Specifically, these authors noted the following:

"There are two forms of isotopic tracers: radio-isotope tracers (e.g., ^{14}C and ^{3}H (tritium)) are radioactive and therefore potentially harmful; their use is now largely restricted to animal rather than human experiments. Stable isotope tracers (e.g., ^{18}O, ^{15}N, ^{13}C and ^{2}H) in contrast, are non-radioactive, making them ideal for use in human research. Crucially, the chemistry of these

elements and the compounds into which they are incorporated is essentially the same as that of the endogenous ones. As such, they can be used to 'trace' metabolic flux in pathways of interest and the fate of the tracer can be monitored (via its mass difference), providing vital information regarding rates and extent of its metabolism."

When using stable isotope tracers, researchers can study the protein synthetic response to an acute bout of exercise or over the duration of an intervention. Herath and colleagues [125] divulged some of the dynamics of using D_2O to examine the proteome and stated:

"The labeling of a proteolytic peptide reflects (i) the rate of protein synthesis, (ii) the number of hydrogens (n) that are stably incorporated into the respective protein-bound amino acids, (iii) temporal changes in the ^2H labeling of an amino acid in vivo… Provided that the ^2H labeling of free amino acids is constant, one can determine relative changes in protein synthesis by comparing the ^2H labeling in a proteolytic peptide with that in body water"

Specifically, labeling of proteins occurs by ^2H being covalently incorporated into C—H bonds of free alanine (Ala) through intermediary metabolic reactions; specifically, through Ala tRNA, into Ala residues of newly synthesized proteins [126].

Integrative protein synthetic response to resistance exercise

In a review by Simmons et al., [127] it was posited that, through the recent advances in tracer methodologies (i.e., D_2O), a wide range of capabilities become available to researchers some of which may help elucidate changes that may result from training. Specifically, Simmons stated:

"Deuterium methodologies will enable scientists to better capture measures of protein synthesis with dynamic alterations to the precursor pool (as occurs with supplements and/or nutritional intake). These methods also will allow for assessments to occur over longer periods of time and

thus permit the direct measure of protein synthesis following interventions, with or without exercise."

In human research, various methods have been used to show increases in MPS occurs in response to resistance exercise or training. Franchi and colleagues [128] showed that increases in muscle thickness are related to increased MyoPS and may be a result of early-structural remodeling. Said differently, training induced increases in muscle thickness using concentric leg extensor training and eccentric leg extensor training could be the result of increases in myofibrillar protein accretion given that MyoPS increases were associated with hypertrophic outcomes. To this point, many studies have used stable isotope tracers to measure MPS or MyoPS. However, prior to the advent of D_2O administration, this method required indwelling catheters and participants to remain ambulatory for a period of time. Conversely, the use of D_2O allows participants to carry out normal daily functions. The use of D_2O as a tracer to evaluate the protein synthetic response to exercise is a relatively new method. In 2004, Previs and colleagues used D_2O with end-stage renal disease patients and found that: i) it was safe for long-term use in evaluating long-term protein synthetic rates, and ii) a rapid exchange of hydrogens occurred between alanine and body water [129]. Importantly, this method has been validated in rodents and humans when compared to stable isotopes of phenylalanine. In 2009, Gasier performed an acute study in rodents evaluating the use of labeled phenylalanine through a flood injection, and D_2O administered 24 hours prior to one bout of resistance exercise and found that both methodologies produced qualitatively similar results in measuring protein synthetic rates [130]. Wilkinson and colleagues sought to further validate D_2O by performing a study evaluating day-to-day changes in the fractional synthetic rate of muscle protein subfractions (i.e., myofibrillar, sarcoplasmic, and collagen). The authors compared their findings to those found using labeled-phenylalanine and reported that following a

single dose of D_2O, differences were detectable within 48 hours and comparable to labeled-phenylalanine [131].

Using D_2O in conjunction with an endurance training model in elderly participants with an age-matched young sedentary population, Robinson found muscle protein synthesis to be elevated over a 6-week period in the exercise group as compared to their sedentary age-matched controls. Importantly, this study had a supplemental element in conjunction with exercise with one group receiving a carbohydrate supplement while the other received a protein supplement, and no differences were found in muscle protein synthesis between groups [132]. Gasier and colleagues used a unilateral training model and reported that mixed muscle protein synthesis rates were similar between the control leg and exercise leg, but rates were increased following resistance exercise for ~24 hours [133]. Bell and colleagues evaluated MyoPS rates in older healthy males following resistance training and high-intensity interval training (HIIT) [134]. The authors reported increased MyoPS rates occurred following both modalities at the 24- and 48-hour post-exercise time points, with MyoPS rates in the resistance trained group being higher than HIIT. Interestingly, this study also reported that sarcoplasmic protein synthesis (SarcoPS) rates were only elevated in the HIIT group at 24 hours post-exercise. Brook et al. used D_2O over the course of a 6-week unilateral training protocol and found that, as expected, the untrained leg did not realize an increase in MyoPS rates while the trained leg exhibited an increase in MyoPS rates from weeks 1-3 but not weeks 3-6 [17]. The authors posited that remodeling of muscle tissue might have been indicative of the initial increase in MyoPS rates, which also coincides with Franchi's data mentioned above [128]. Doering et al. [135] took muscle biopsies prior to the onset of three days of exercise testing (PRE) and 72 hours following PRE in masters-level triathletes (mean age 57 ± 2 yrs.) and younger triathletes (27 ± 2 yrs.). Exercise consisted of a 30-minute downhill run

followed by a 20 km cycling time trial on day 1, and a 20 km cycling time trial on days 2 and 3. The authors reported MyoPS rates were elevated to a greater extent in the younger cohort at the 72 hours post-exercise time point. Camera et al. [136] evaluated SarcoPS and MyoPS rates using dynamic proteome profiling in participants consuming a high-fat low carbohydrate diet that performed three resistance exercise bouts over a 9-day period compared to participants on a high fat low carbohydrate diet with no exercise. These authors found no associations existed between protein synthetic rates and relative protein abundances for each fraction. Additionally, MyoPS rates were elevated in those that performed exercise, whereas SarcoPS rates were equal in both groups. McKendry and colleagues examined integrated myofibrillar protein synthesis (iMyoPS) over a 48-hour period following an unaccustomed bout of resistance exercise in older controls (n=8) and endurance training in masters-athletes (n=7). iMyoPS was similar between groups at rest, while both forms of exercise similarly increased iMyoPS [137].

The use of D_2O as a tracer in the muscle protein synthetic response has allowed for studies to be designed using a wide variety of interventions. Indeed, the validation of this technique makes it an intriguing option to researchers studying muscle physiology in healthy and diseased states. The utilization of D_2O has been found to be safe for short and long-term studies. Furthermore, studies utilizing D_2O as a tracer will give further insight into muscle adaptation in acute and chronic interventions.

Purpose Statement

It has been suggested that completing higher volumes of resistance training results in a greater growth response in skeletal muscle [73]. Haun and colleagues have shown that, with 32 sets of 10 repetitions per week, there is a robust increase in whole-body skeletal muscle mass as

extrapolated through dual-energy x-ray absorptiometry [138]. However, when equating for extracellular water, the differences between performing 20 sets of 10 repetitions per week and performing 32 sets of 10 repetitions may be negligible. A follow-up analysis from these participants revealed an increase in sarcoplasmic protein concentrations per milligram dry tissue with a decrease in actin and myosin abundances per milligram dry tissue following the six-week high volume training protocol [20]. Thus, the hypertrophic response that was observed may have been due to either edema or sarcoplasmic hypertrophy. More recently, Vann and colleagues have shown that, with moderate volume high-load resistance training, there was no change in sarcoplasmic protein concentrations and a more modest decrease in actin and myosin concentrations per milligram wet tissue [21]. When considering findings from both studies, it is possible that higher volume training may increase sarcoplasmic hypertrophy more so than high load training. However, no study to date has definitely demonstrated this phenomenon. Thus, the purpose of this study was to examine the effects of high-volume versus high-load unilateral leg resistance training on: i) muscle thickness and cross-sectional area, ii) muscle pennation angle, iii) fiber cross-sectional area, iv) alterations in actin, myosin, and sarcoplasmic protein concentrations per milligram wet muscle, and v) the integrated MyoPS and SarcoPS rates throughout the entirety of training. Notably, participants were previously-trained men, and each person served as his own control with each leg being randomly assigned to the high volume or high load training protocol.

Chapter III

Methods

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).

Young 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 $\geq$ 1.5x bodyweight (estimated from a 3 repetition maximum [3RM] test) in accordance to standards designated by the National Strength and Conditioning Association [139]. At the conclusion of the screening visit, participants were asked to keep their current nutritional practices and to cease all training outside of the study.

Study design

A schematic of the study design is provided in Figure 3.1. 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 (POSTDL). The battery of tests performed are detailed further below, following a description of the training intervention and tracer methodologies.

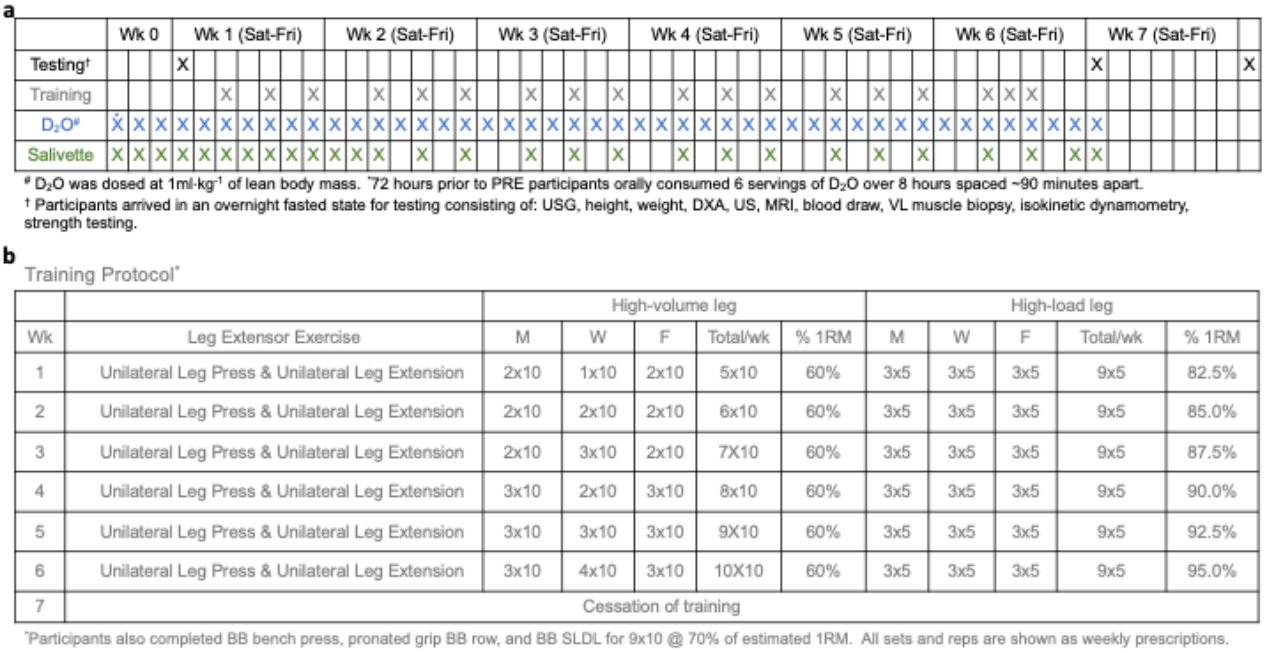
Resistance Training

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 high-volume training on the left leg and high-load 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 3.1.

Isotope Tracer Protocol

Deuterium oxide (D_2O) (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 $mL \bullet kg^{-1}$ of lean body mass. For rapid enrichment of deuterium (^2H) participants were instructed to orally consume 6 doses of D_2O 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 D_2O 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, Nümbrect, 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.

Figure 3.1. ***Study Design***



a

	Wk 0			Wk 1 (Sat-Fri)						Wk 2 (Sat-Fri)						Wk 3 (Sat-Fri)						Wk 4 (Sat-Fri)						Wk 5 (Sat-Fri)						Wk 6 (Sat-Fri)						Wk 7 (Sat-Fri)						
Testing†				X																																										X						X
Training						X		X		X			X		X		X			X		X			X		X			X		X			X		X	X	X										
D_2O#	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Salivette	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X			X		X			X		X			X		X			X		X		X		X		X		X	X			

D_2O was dosed at 1ml·kg^{-1} of lean body mass. ˆ72 hours prior to PRE participants orally consumed 6 servings of D_2O over 8 hours spaced ~90 minutes apart.
† 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.

b

Training Protocol*

		High-volume leg					High-load leg				
Wk	Leg Extensor Exercise	M	W	F	Total/wk	% 1RM	M	W	F	Total/wk	% 1RM
1	Unilateral Leg Press & Unilateral Leg Extension	2x10	1x10	2x10	5x10	60%	3x5	3x5	3x5	9x5	82.5%
2	Unilateral Leg Press & Unilateral Leg Extension	2x10	2x10	2x10	6x10	60%	3x5	3x5	3x5	9x5	85.0%
3	Unilateral Leg Press & Unilateral Leg Extension	2x10	3x10	2x10	7X10	60%	3x5	3x5	3x5	9x5	87.5%
4	Unilateral Leg Press & Unilateral Leg Extension	3x10	2x10	3x10	8x10	60%	3x5	3x5	3x5	9x5	90.0%
5	Unilateral Leg Press & Unilateral Leg Extension	3x10	3x10	3x10	9X10	60%	3x5	3x5	3x5	9x5	92.5%
6	Unilateral Leg Press & Unilateral Leg Extension	3x10	4x10	3x10	10X10	60%	3x5	3x5	3x5	9x5	95.0%
7	Cessation of training										

*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.

Abbreviations: Wk, week; D_2O, deuterium oxide. Panel a provides an overview of testing, training, D_2O administration, and saliva collection times. Panel b provides a schematic of training by day and total training for each week.

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 state [140] 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 bioelectrical impedance spectroscopy (BIS) test for total body water (TBW), intracellular fluid (ICF), and extracellular fluid (ECF) using the SOZO device (ImpediMed Limited, Queensland, AU) according to the methodologies described in Esco et al, [141]. Briefly, SOZO measurements were taken with participants in a standing position making contact with eight electrodes (2 on each foot and 2 on each hand) in accordance with the manufacturer's specifications. Frequencies ranging from 5-500kHz are passed through the electrodes into the body and impedance is measured to give an estimation of body water. Following BIS testing, participants underwent full body dual energy x-ray absorptiometry (DXA) testing (Lunar Prodigy; GE Corporation, Fairfield CT, USA). Our laboratory [142] 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 vastus lateralis (VL) muscle thickness and muscle pennation angle. 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. Importantly, all measurements were taken by the same investigator (S.C.O.) in order to minimize variability in measures as suggested in previous studies [143, 144].

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 1 mm × 1 mm and slice thickness = 2.2 mm. An axial 3D 35.2mm thick slab (16 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 analyzed 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). Again, neither investigator was privy to which leg from each participant was assigned to 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 [19, 145]. Immediately following tissue procurement, ~20-40 mg of tissue was placed in optimal cutting temperature (OCT) media (Tissue-Tek, Sakura Finetek Inc; Torrence, CA, USA) on cork at a resting length and were then frozen using liquid nitrogen-cooled 2-methylbutane and subsequently stored at -80ºC until histology was performed. The remaining tissue was teased of blood and connective tissue, wrapped in pre-labelled foils, flash frozen in liquid nitrogen, and subsequently stored at -80ºC for other molecular analyses described below.

Strength Testing. Following muscle skeletal muscle biopsies, participants underwent isokinetic dynamometry (Biodex System 4; Biodex Medical Systems, Inc., Shirly, 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 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 POSTDL.

Biochemical assays

Sarcoplasmic and myofibrillar protein isolation. Isolation of these protein fractions was performed using our recently published and proteomic validated "MIST" or "myofibrillar isolation and solubilization technique" [146]. 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 -80ºC, placed on a liquid nitrogen-cooled ceramic mortar and pestle, and tissue was pulverized into 2-4 mm^3 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 (MHC) protein abundance and stored at -80°C until analysis occurred. The methodologies used for the above are described in further detail below.

Determination of protein concentration. 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 coefficients of variation for sarcoplasmic protein concentrations 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, 20, 147, 148]. 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 and actin abundances. Our laboratory has reported that this method yields exceptional sensitivity in detecting 5-25% increases in actin and myosin content [19]. 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.

Statistical Analyses

Statistical analyses were performed in SPSS (Version 26; IBM SPSS Statistics Software, Chicago, IL, USA), and open source softwares 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 was applied. Variables were analyzed using a within-within multi-factorial ANOVA. If significance was found, a paired samples t-test was used to assess differences in leg or time. Statistical significance for null hypothesis testing was set at $p<0.05$. Data are presented throughout as mean ± standard deviation (SD) values.

Chapter 4

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

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KEYWORDS: Muscle hypertrophy; high-volume; high-load; sarcoplasmic hypertrophy

ABSTRACT

Background: Recently there has been enthusiasm surrounding the differential training adaptations that result from higher-load (HL) versus higher-volume (HV) resistance training. The purpose of this study was to evaluate the effects of HL versus HV training on markers of skeletal muscle hypertrophy and strength as well as a broad spectrum of molecular markers obtained from skeletal muscle biopsies. **Methods**: Trained college-age males (n=15, age: 23±3 years old; training age: 7±3 years; squat 1RM relative to body mass: 1.9) performed unilateral lower body training three days per week over 6 weeks, with one leg being randomly assigned to HV training and the other to HL training. Vastus lateralis (VL) biopsies were obtained prior to the start of the study (PRE), 72 hours following the last exercise bout (POST), and following 10 days of passive recovery (POSTDL). Body composition and strength tests were also performed at each testing session, and various biochemical assays were performed on muscle tissue. **RESULTS**: Condition×time interactions were observed for unilateral leg extension 1RM (HL>HV at POST and POSTDL) and VL muscle cross-sectional area (post hoc tests indicated no significance between conditions over time). A main effect of condition was observed for unilateral leg extension 1RM (HL>HV) and sarcoplasmic protein concentrations (HV>HL). A main effect of time was observed for unilateral leg press 1RM (PRE<POST and POSTDL), unilateral leg extension 1RM (PRE<POST<POSTDL), knee extensor peak torque at 60°/sec (PRE and POST<POSTDL), dual-energy x-ray absorptiometry (DXA)-derived upper leg total and lean mass values (PRE<POST and POSTDL), ultrasound-derived VL thickness (PRE and POSTDL<POST), sarcoplasmic protein concentrations (POST and POSTDL<PRE), and tropomyosin and troponin protein

abundances (PRE>POST and POSTDL). **CONCLUSION**: In summary, our data suggest that short-term HV and HL training elicit similar hypertrophic, strength, and molecular adaptations.

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 individual muscle fiber [3-5]. It is generally recognized that resistance training results in skeletal muscle growth through proportional increases in myofibrillar and sarcoplasmic protein content [6-9]. Myofibril proteins are defined herein as 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 processes (e.g., sarcoplasmic reticulum, enzymes, etc.) [10].

Recently, there has been growing 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 training [11]. However, Mitchell and colleagues reported that 10 weeks of HL and HV resistance training led to similar increases in muscle area measured by MRI [12]. Subsequent literature in this area has indicated both HL and HV training: 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 [14, 15, 17]. 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 tissue [19]. Our findings, as well as the findings of others who have reported higher-volume resistance training elicits similar molecular adaptations [20-22], has led us to postulate that sarcoplasmic hypertrophy – or a

disproportionate increase in the sarcoplasmic space relative to myofibril protein accretion – may be a training adaptation to HV resistance training. More recently, our laboratory has demonstrated that lower volume, higher intensity 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 [23, 24]. While preliminary, these two studies from our laboratory suggest that HV resistance training may facilitate sarcoplasmic hypertrophy, whereas HL training may promote a proportional increase in myofibril protein accretion with muscle growth. However, no research to date has examined whether these phenomena occur. The purpose of the present study was to elucidate whether six weeks of HV versus HL resistance training differentially affected metrics of skeletal muscle hypertrophy, strength, and/or molecular variables assessed from skeletal muscle biopsies. Trained college-age males performed unilateral lower body exercise three days per week, with one leg being assigned to HV training and the other to HL training over the six-week period. Vastus lateralis (VL) biopsies were obtained prior to the start of the study (PRE), 72 hours following the last exercise bout (POST), and following 10 days of passive recovery (POSTDL). Biopsy samples were used to assess: i) sarcoplasmic protein concentrations relative to wet muscle weights, and ii) concentrations of major contractile and cytoskeletal proteins. Mid-thigh MRI and ultrasound images were obtained at each time point, and strength testing of each leg at each time point was also performed. Based on the aforementioned data obtained from our laboratory, we hypothesized that HV resistance training would result in increased sarcoplasmic protein concentrations with a concomitant decrease in contractile protein concentrations, whereas HL training would result in no changes in these markers. Additionally, we hypothesized that there would be no differences in

VL area assessed through MRI, or VL thickness assessed through ultrasound. Finally, we hypothesized that HL training would elicit superior increases in various indices of strength.

RESULTS

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±7.4kg being lean soft tissue mass (LSTM) and 17.3±7.5kg being fat mass. Additionally, participants had an average relative squat of 1.9× bodyweight (167±34 kg).

Table 4.1. ***Baseline Characteristics***

Variable	Mean ± SD
Age (years)	23 ± 3
Training age (years)	7 ± 3
Height (cm)	182 ± 8
Weight (kg)	89.5 ± 11.6
Lean soft tissue mass (kg)	69.1 ± 7.4
Fat tissue mass (kg)	17.3 ± 7.5
Fat-free mass index	20.9 ± 2.2
Est. 1RM Squat (kg)	167 ± 34
Squat relative to body weight	1.9 ± 0.4

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

Training Volume and Strength Metrics

Training volumes and strength metrics are presented in Figure 1. Importantly, data for 14 of 15 participants is presented for unilateral leg press and unilateral leg extension one repetition

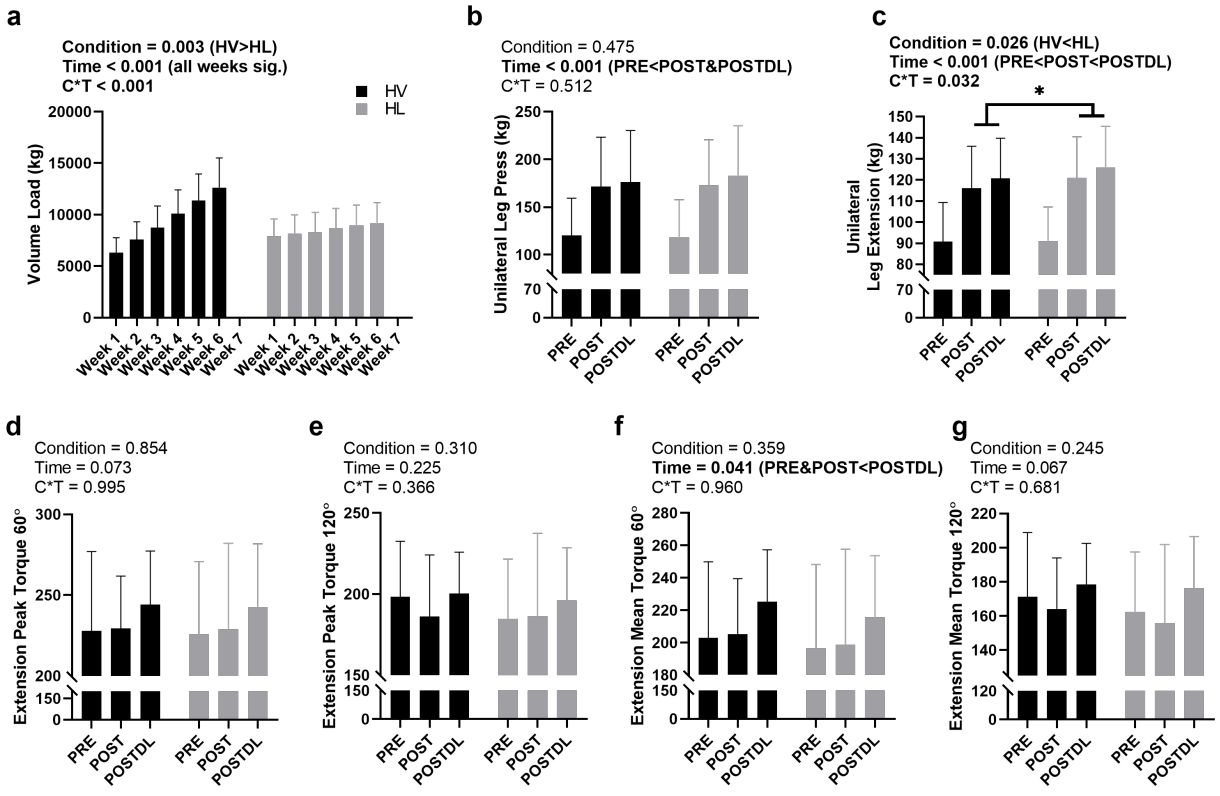
maximums (1RM) due to one participant feeling lower extremity discomfort at POST with these exercises.

There was a condition×time interaction observed for lower body training volume (p<0.001, η_p^2=0.914; Fig. 1a). Additionally, there was a main effect of condition (p=0.003, η_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, η_p^2=0.955, Fig. 1a) and within each condition over time (HV: p<0.001, η_p^2=0.952; HL: p<0.001, η_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×time interaction (p=0.512, η_p^2=0.050, Fig. 1b) was not observed for estimated unilateral leg press 1RM. Additionally, no main effect of condition (p >0.475, η_p^2=0.040, Fig. 1b) was observed. There was a main effect of time (p<0.001, η_p^2=0.818, Fig. 1b) where estimated unilateral leg press 1RM at POST (p<0.001) and POSTDL (p<0.001) were greater than PRE. A condition×time interaction was observed for estimated unilateral leg extension 1RM (p=0.032, η_P^2=0.265, Fig. 1c). A main effect of condition (p=0.026, η_p^2=0.328, Fig. 1c) was also observed whereas the HL condition (M=113±5) estimated unilateral leg extension 1RM was higher than the HV condition (M=109±5). Estimated unilateral leg extension 1RM also changed over time (p<0.001, η_p^2=0.885, Fig. 1c) and within each condition over time (HV: p<0.001, η_p^2=0.858; HL: p<0.001, η_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 (p=0.007) and POSTDL (p=0.009).

There were no condition×time interactions observed for knee extensor peak torque at 60°/sec (p=0.995, η_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°/sec (p=0.681, η_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°/sec changed over time (p=0.041, η_p^2=0.204, Fig. 1f) where knee extensor mean torque at 60°/sec was higher at POSTDL than at PRE (p=0.029) and POST (p=0.043). There were no differences observed between PRE and POST (p=0.805)

Figure 4.1. ***Training Volume and Strength Metrics***



Abbreviations: HV. high-volume; HL, high-load. Legend: Data are presented as mean±SD 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). * indicates a condition×time interaction whereas POST and POSTDL were greater than PRE.

Body Composition

PRE, POST, and POSTDL whole-body composition changes for all participants are presented in Table 1; notably, these data were derived from dual-energy x-ray absorptiometry (DXA) scans. Total body mass increased over time (p<0.001, η_p^2=0.435), whereas POST (p=0.001) and POSTDL (p=0.012) body masses were greater than PRE. However, no differences were observed between POST and POSTDL body masses (p=0.119). Lean soft tissue mass (LSTM) increased over time (p=0.003, η_p^2=0.338) where POST LSTM was greater than PRE (p=0.002) and POSTDL (p=0.014). No differences in LSTM were observed between PRE and POSTDL (p=0.286). No differences were observed for DXA measured whole body fat mass (p=0.097).

Table 4.2. ***Body Composition***

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

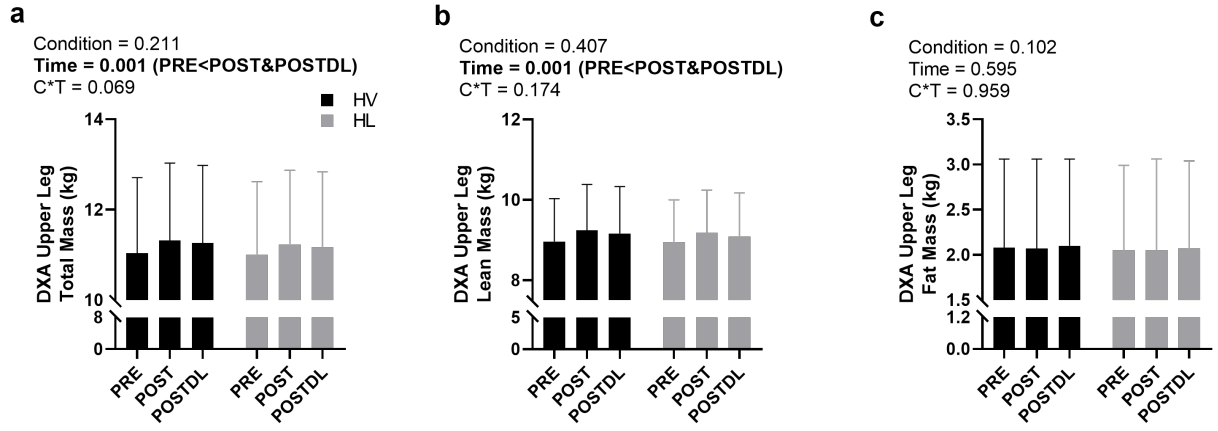
Variable	PRE Mean ± SD	POST Mean ± SD	POSTDL Mean ± SD	ANOVA p-value
Total Body Mass (kg)	89.32 ± 11.54	90.75 ± 11.94	90.40 ± 12.15	**<0.001**†
DXA Whole Body LSTM (kg)	69.13 ± 7.43	70.18 ± 7.49	69.49 ± 7.49	**0.003***
DXA Whole Body Fat Mass (kg)	17.26 ± 7.49	17.40 ± 7.75	17.73 ± 7.76	0.097

Segmental Upper Leg Composition

There were no condition×time interactions observed for DXA-derived upper leg mass (p= 0.069, η_p^2=0.173, Fig. 2a), upper leg LSTM (p=0.174, η_p^2=0.117, Fig. 2b), or upper leg fat mass (p=0.959, η_p^2=0.003, Fig. 2c). A main effect of time was observed for upper leg mass (p=0.001,

η_p^2=0.392, Fig. 2a) where POST (p=0.002) and POSTDL (p=0.013) were higher than PRE. No differences were observed between POST and POSTDL (p=0.240). A main effect of time was observed for DXA upper leg LSTM (p=0.001, η_p^2=0.418, Fig. 2b) where POST (p<0.001) and POSTDL (p=0.002) were higher than PRE. No differences were observed between POST and POSTDL (p=0.148). No main effects of condition (p=0.102) or time (p=0.595) were observed for DXA upper leg fat mass.

Figure 4.2. ***Segmental Upper Leg Composition***



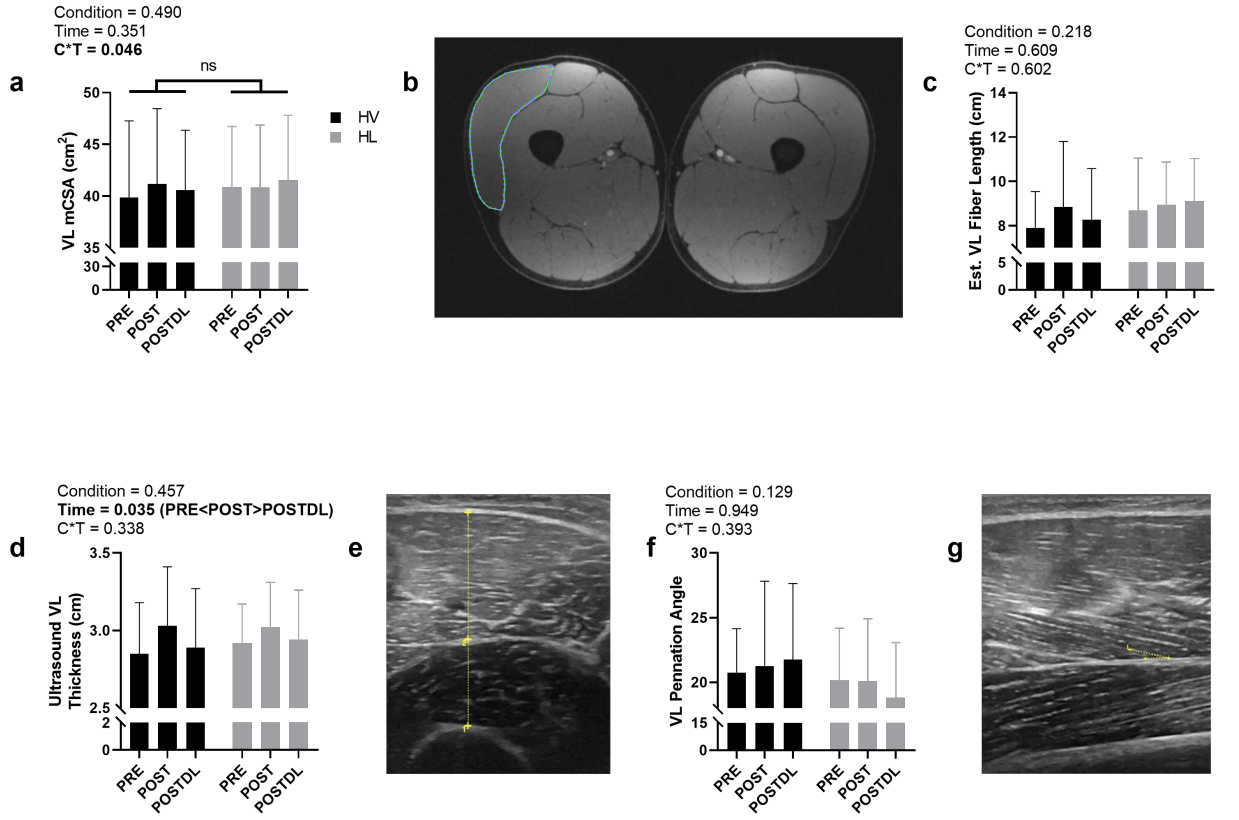
Abbreviations: HV, high-volume; HL, high-load; DXA, dual energy x-ray absorptiometry. Legend: Data are presented as mean±SD for DXA upper leg total mass (panel a), DXA upper lean mass (panel b), and DXA upper leg fat mass (panel c).

Vastus Lateralis Muscle Morphology

A condition×time interaction was observed for magnetic resonance image (MRI)-derived VL cross-sectional area (p=0.046, η_p^2=0.211, Fig. 3a); however, no main effects of condition (p=0.490, η_p^2=0.037, Fig. 3a) or time (p=0.351, η_p^2=0.077, Fig. 3a) were observed. Post hoc analysis revealed no differences between conditions at PRE (p=0.246), POST (p=0.673), or POSTDL (p=0.247). There was no condition×time interaction (p=0.338, η_p^2=0.075, Fig. 3b) or main effect of condition (p=0.457, η_p^2=0.040, Fig. 3b) observed for ultrasound measured VL

thickness. VL thickness changed over time (p=0.035, η_p^2=0.241, Fig. 3b) where POST values were greater than PRE (p=0.026) and POSTDL (p=0.003). No differences were observed between PRE and POSTDL (p=0.614). There were no interactions observed for muscle pennation angle of the VL (p=0.393, η_p^2=0.064, Fig. 3c) or estimated VL muscle fiber length (p=0.602, η_p^2=0.036, Fig. 3d). Additionally, there were no main effects of condition or time for the aforementioned variables (p>0.05).

Figure 4.3. ***Vastus Lateralis Muscle Morphology***



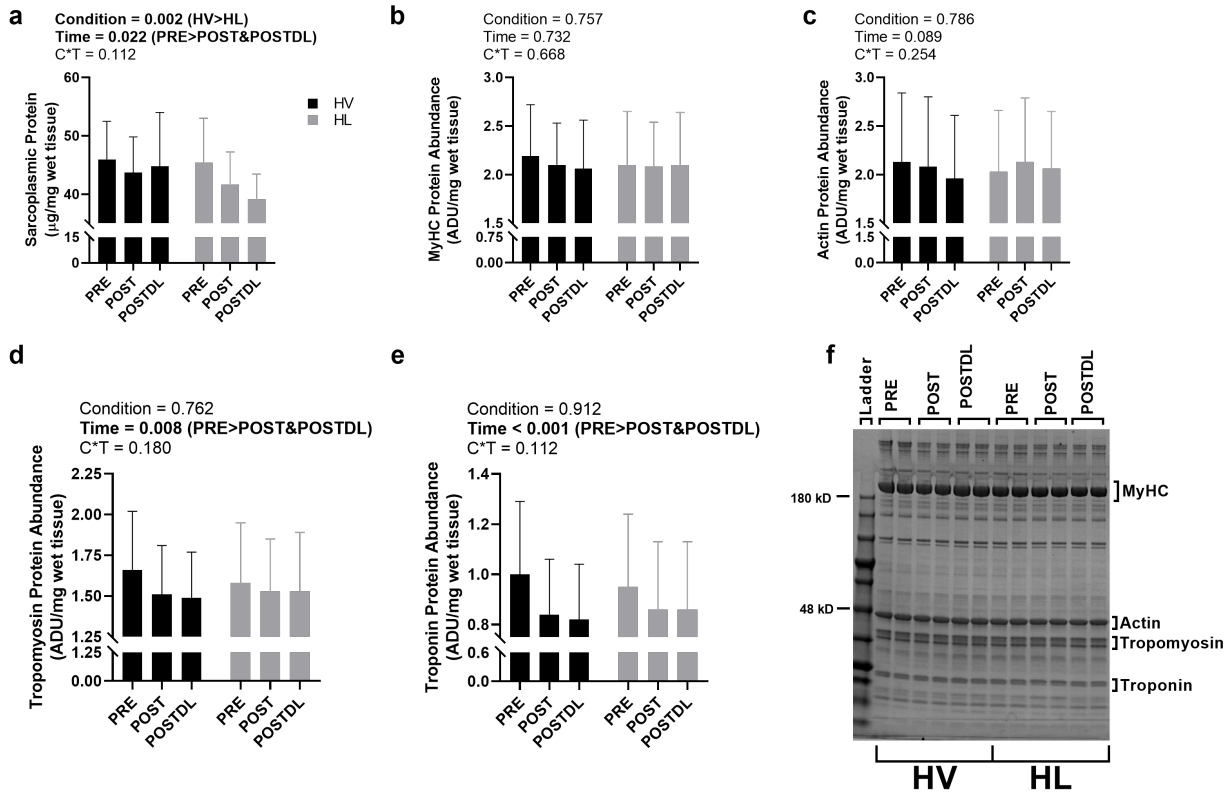
Abbreviations: HV, high-volume; HL, high-load; VL, vastus lateralis; mCSA, muscle cross-sectional area, Est., estimated. Legend: Data are presented as mean±SD for VL mCSA (panel a), Est. VL fiber length (panel c), VL thickness (panel d), and VL muscle pennation angle (panel f). Representative images: 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.

Muscle Protein Adaptations

There was no condition×time interaction observed for sarcoplasmic protein concentrations per mg of wet tissue weight (p=0.112, η_p^2=0.159, Fig. 4a). There was a main effect of condition (p=0.002, η_p^2=0.497, Fig. 4a) where sarcoplasmic protein concentrations in the HV group were higher than the HL group (44.808±1.559 versus 42.057±1.275 respectively). Additionally, there was a main effect of time (p= 0.022, η_p^2=0.239, Fig. 4a) where PRE sarcoplasmic protein concentrations were higher than POST (p=0.038) and POSTDL (p=0.032). No differences in sarcoplasmic protein concentrations were observed between POST and POSTDL (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, η_p^2=0.028, Fig. 4b) or actin protein abundance per mg of wet tissue weight (p=0.254, η_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, η_p^2=0.115, Fig. 4d) or main effect of condition (p=0.762, η_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, η_p^2=0.294, Fig. 4d) where PRE was greater than POST (p=0.009) and POSTDL (p=0.010). No differences were observed between POST and POSTDL (p=0.704). There was no condition×time interaction (p=0.112, η_p^2=0.145, Fig. 4e) or a main effect of condition (p=0.912, η_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, η_p^2=0.431, Fig. 4e) where PRE was greater than POST (p<0.001) and POSTDL (p=0.005). No differences were observed between POST and POSTDL (p=0.867).

Figure 4.4. ***Muscle Protein Adaptations***



Abbreviations: HV, high-volume; HL, high-load; VL; MyHC, myosin heavy chain; ADU, arbitrary density units; kD, kilodalton. Legend: Data are presented as mean±SD 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.

DISCUSSION

The purpose of the present study was to examine the effects of HL versus HV training on strength adaptations as well as gross and molecular markers of skeletal muscle physiology in previously trained young men. The main findings of the current study include the following: i) there was a condition×time interaction for VL mCSA measured via MRI; however, when the model was

decomposed, no differences were found between conditions or over time; ii) an interaction was observed for estimated unilateral leg extension 1RM where values were higher in the HL condition at POST and POSTDL than the HV condition; iii) there was a main effect of time for unilateral leg press and knee extensor mean torque at 60°/sec where these measures were higher at POST and POSTDL than at PRE; iv) while there was not an interaction observed for sarcoplasmic protein concentrations, there was a main effect of time where sarcoplasmic protein content decreased over time, and there was a main effect of condition where the HL condition had a lower sarcoplasmic protein concentration than the HV condition, and v) there were no interactions observed for the relative abundances of the major contractile proteins.

The literature regarding macro-level changes in the vastus lateralis 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 which has interrogated differences between such training paradigms. Chestnut and Docherty [25] 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. Mitchell et al. reported that performing 3 sets of knee extensor training to fatigue at 30% or 80% of 1RM resulted in similar increases in quadriceps volume measured by MRI, and both modalities yielded greater quadriceps hypertrophy than performing one set at 80% 1RM to voluntary failure [12]. 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 the findings of Mitchell and colleagues as well as the finding of Chestnut and Docherty whom showed no differences in loading paradigms. Conversely, Holm and colleagues

[26] reported that high-load (~70% 1RM) versus 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. These findings in conjunction with our current data suggest that at least 30% 1RM training to volitional fatigue is needed to optimize whole-muscle hypertrophy. However, there (on average) appears to be no differences in whole-muscle hypertrophy when loading exceeds 30% 1RM.

Interestingly, while both unilateral leg press and unilateral leg extension estimated 1RM increased over time, there was no interaction observed for leg press strength changes, whereas there was an interaction for leg extension strength changes. Moreover, data obtained from isokinetic dynamometer testing for knee extensor peak torque indicated that no differences over time occurred in either leg. Several studies have examined changes in strength between different load-volume 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) [27]; 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 which compared 30% 1RM versus 80% 1RM leg extensor training [14, 15]. Results from both studies suggest that higher-load training elicited more optimal strength increases due to neural factors. Jessee and colleagues [28] 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 adaptations [17]. 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. Conversely, while training at 30% 1RM seemingly yields similar increases in hypertrophy compared to higher-load training when carried to volitional fatigue, this training stimulus may not yield optimal increases in strength due to suboptimal neuromuscular adaptations.

A novel aspect of the current study was to compare how HL versus HV training affected concentrations of major contractile proteins as well as concentrations of the total sarcoplasmic protein pool. This interrogation was prompted by literature suggesting that sarcoplasmic hypertrophy – or a disproportionate increase in the intracellular volume of the sarcoplasm with muscle hypertrophy – may occur following high-volume resistance training. To date, a handful of studies exist showing that sarcoplasmic hypertrophy may occur following months to years of resistance training [20, 21, 29, 30]. More recently, our laboratory has reported increases in sarcoplasmic protein concentrations with concomitant decreases in 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 men [19]. Critically, 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 paradigm [23]. When considering the findings from both studies, we hypothesized that HV training may facilitate sarcoplasmic hypertrophy, whereas HL training may facilitate what we have termed “conventional hypertrophy” – or, a proportional accretion of contractile and sarcoplasmic proteins with whole-muscle hypertrophy. In the current study, we

observed a decrease in sarcoplasmic protein concentrations for both conditions at POST with no changes in actin and myosin heavy chain protein concentrations. Although these data disagree with prior findings from Haun et al., it is important to note the key differences that exist between the high-volume components of each study. First, Haun et al. utilized a bilateral exercise paradigm using the barbell back squat and barbell stiff-legged deadlifts as the primary exercises, whereas the current study was a unilateral study design using a combination of unilateral leg press and leg extension. To this end, it could be posited that training stress on the legs was much greater in the Haun et al. study versus the current study. Second, Haun et al. used a 6-week intervention starting at 10 sets of 10 repetitions per week (for each exercise) and finishing with 32 sets of 10 repetitions per week where loads were standardized at 60% 1RM [31]. The current study started the HV leg at 10 sets of 10 repetitions per week (split between two exercises) at week 1 and finished with 20 sets of 10 repetitions per week at week 6 where loads were standardized at 60% 1RM. Thus, even though the HV leg was exposed to more training volume compared to the HL leg herein, the HV leg did not experience nearly the amount of volume as both legs incurred in participants by Haun et al. Moreover, the total training volume data in Figure 1 indicates that the HV leg was only exposed to 11% more volume compared to the HL leg. Thus, we speculate that the lack of protein concentration changes between legs over time may have been due to either: i) the lack of appreciable training volume differences between legs throughout the duration of the study, and/or ii) training volume in the HV leg not exceeding the participants' adaptive threshold. Alternatively stated, if sarcoplasmic hypertrophy is indeed a training phenomenon, we postulate that this physiological event may be prompted when training volumes are at or surpass the upper threshold of an individual's adaptive capacity.

Experimental Limitations

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 limited tissue yield. Due to this limitation, we lacked sufficient tissue to perform histology and assess fCSA values in each leg over time which would have been beneficial for this analysis. 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 men [31-33]. 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.

Conclusion

In conclusion, the data herein suggest that training loads between 60%-90% 1RM have minimal influence on the adaptive response to resistance training over a 6-week period. Additionally, the current data challenge the sarcoplasmic hypertrophy model due to: 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. We still maintain that extremely high-volume training above what an individual is accustomed to may stimulate features of sarcoplasmic hypertrophy. However, training within an individual's adaptive threshold, whether it is through HV or HL training, seemingly elicits conventional hypertrophy. Future research is still needed to determine if sarcoplasmic hypertrophy is a physiological occurrence.

MATERIALS AND METHODS

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).

Young 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 ≥1.5× bodyweight (estimated from a 3 repetition maximum [3RM] test) in accordance to standards designated by the National Strength and Conditioning Association [34]. 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.

Study design

A schematic of the study design is provided in Figure 5. 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 (POSTDL). The battery of tests performed are detailed further below, following a description of the training intervention and tracer methodologies.

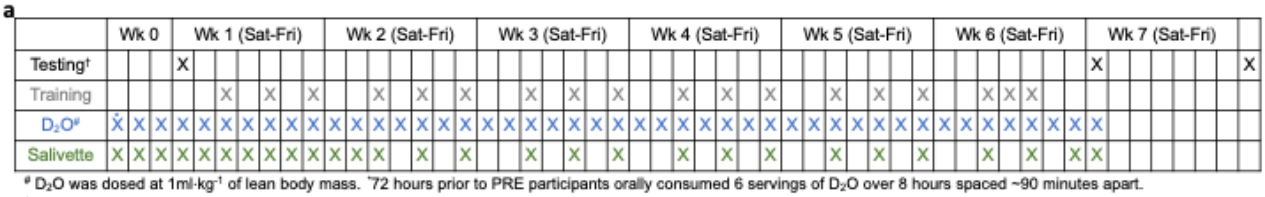
Resistance Training

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 5.

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, Nümbrect, 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.

Figure 4.5. ***Study Design***



b

Training Protocol*

		High-volume leg					High-load leg				
Wk	Leg Extensor Exercise	M	W	F	Total/wk	% 1RM	M	W	F	Total/wk	% 1RM
1	Unilateral Leg Press & Unilateral Leg Extension	2x10	1x10	2x10	5x10	60%	3x5	3x5	3x5	9x5	82.5%
2	Unilateral Leg Press & Unilateral Leg Extension	2x10	2x10	2x10	6x10	60%	3x5	3x5	3x5	9x5	85.0%
3	Unilateral Leg Press & Unilateral Leg Extension	2x10	3x10	2x10	7X10	60%	3x5	3x5	3x5	9x5	87.5%
4	Unilateral Leg Press & Unilateral Leg Extension	3x10	2x10	3x10	8x10	60%	3x5	3x5	3x5	9x5	90.0%
5	Unilateral Leg Press & Unilateral Leg Extension	3x10	3x10	3x10	9X10	60%	3x5	3x5	3x5	9x5	92.5%
6	Unilateral Leg Press & Unilateral Leg Extension	3x10	4x10	3x10	10X10	60%	3x5	3x5	3x5	9x5	95.0%
7	Cessation of training										

*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.

Abbreviations: Wk, week; D_2O, deuterium oxide. Panel a provides an overview of testing, training, D_2O administration, and saliva collection times. Panel b provides a schematic of training by day and total training for each week.

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 state [35] 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 laboratory [36] 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. 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. Importantly, all measurements were taken by the same investigator (S.C.O.) in order to minimize variability in measures as suggested in previous studies [37, 38]. Critically, this investigator was not privy to which leg from each participant was assigned to the HV or HL condition. Moreover, the location of measurements were marked 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 1 mm × 1 mm and slice thickness = 2.2 mm. An axial 3D 35.2mm thick slab (16 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 analyzed 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). Again, neither investigator was privy to which leg from each participant was assigned to 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 [39, 40]. 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 -80ºC for other molecular analyses described below.

Strength Testing. Following muscle skeletal muscle biopsies, participants underwent isokinetic dynamometry (Biodex System 4; Biodex Medical Systems, Inc., Shirly, 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 POSTDL.

Biochemical assays

Sarcoplasmic and myofibrillar protein isolation. Isolation of protein fractions was performed using the proteomic validated "MIST" or "myofibrillar isolation and solubilization technique" [41]. 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 -80ºC, 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 coefficients of variation for sarcoplasmic protein concentrations 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, 40, 42, 43]. 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 content [40]. 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.

Statistical Analyses

Statistical analyses were performed using SPSS (Version 26; IBM SPSS Statistics Software, Chicago, IL, USA), and open source softwares JASP (Version 0.11.0; JASP Team; 2019) and RStudio (Version 1.1.463, R Foundation for Statistical Computing, Vienna, AT). Notably, the same investigator performed all statistical analyses (P.W.M.), and this person was not involved with any aspect of data collection or training. 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 ± standard deviation (SD).

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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.

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