Differences between linear volume resistance training versus variable resistance training in skeletal muscle molecular adaptations

by

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ABSTRACT

Although several studies have examined the strength and hypertrophy adaptations in response to lower versus higher volume resistance training, examining the metabolic and morphological adaptations that occur to both forms of training is still in its infancy. Herein, healthy resistance-trained men (n = 20; 26 ± 3 years old, 25.6 ± 2.1 kg/m^2, 2.5 ± 1.1 years of self-reported training) performed eight weeks of unilateral leg resistance training (twice-weekly). In each participant, one leg was randomly assigned to perform a lower volume protocol (PC, Controlled linear volume), which consisted of 4 sets of unilateral leg press followed by 4 sets of unilateral leg extension exercise per workout (9–12 repetitions per exercise to concentric failure). The contralateral leg was subjected to a higher volume training protocol (PV, variable volume) with the same exercises which, over the 8-week period, led to 12.6% more total training volume ($p<0.05$). A pre-training vastus lateralis biopsy was obtained from a randomized single leg, and biopsies were obtained from both legs 48 h following the last training bout. Tissue was processed to examine metabolic and mitochondrial markers in the sarcoplasmic protein pool, and the myofibril pool was analyzed for the relative abundances of total myosin heavy chain (MHC) and actin proteins. Additionally, sections were stained using a fluorogenic-conjugated phalloidin stain to obtain contractile protein and non-contractile spacing attributes within myofibers. While neither form of training affected most of the assayed sarcoplasmic proteins related to metabolic processes (i.e., CKM, LDHA, PFK, PYGM, GLUT4, LAT1, IDH2. CPT1, or mitochondrial complexes I-IV), both forms of training increased hexokinase 2 protein levels while decreasing a mitochondrial beta-oxidation marker (VLCAD protein content). Interestingly, PV training decreased mitochondrial complex V protein content as well as the relative abundances of MHC and actin ($p<0.05$). Additionally, PV training decreased myofibril spacing within muscle fibers

($p<0.05$), and participants that began training with more myofibril packing seemingly experienced a larger increase in non-contractile protein spacing with PV training (association $r = -0.714$, $p=0.006$). In conclusion, these data are the second observation from our laboratory suggesting that higher volume training in previously-trained individuals significantly decreases myofibril volume in muscle fibers. However, unlike our previous report, this morphological adaptation did not coincide with metabolic adaptation. Further studies are needed to determine whether this morphological adaptation is transient and/or meaningful.

Keywords: myofibrils, myosin, actin, sarcoplasm

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LIST OF ABBREVIATIONS

1RM	one repetition maximum
ADP	adenosine diphosphate
ATP	adenosine triphosphate
CK	creatine kinase
fCSA	muscle fiber cross-sectional area
HL	higher load resistance training
HV	higher volume resistance training
HK	hexokinase
LC-MS/MS	liquid chromatography-mass spec/mass spectrometry
PCr	phosphocreatine
RT	resistance training
SERCA	SarcoEndoplasmic reticulum Calcium ATPase
TCA	citric acid cycle

CHAPTER 1: INTRODUCTION

Researchers have sought to determine whether modulating resistance training volume-load differentially affects training adaptations. Higher load (HL) resistance training involves participants lifting heavier weight for fewer repetitions (e.g. performing 3 to 5 sets of 85% or more of one repetition maximum (1RM) for 3-5 repetitions) [1]. Alternatively, higher-volume (HV) resistance training involves participants lifting lighter weight while performing more repetitions per set relative to HL training (e.g. sets containing >20 repetitions per set with ~30% of 1RM) [2]. A majority of research suggests HL training increases strength more so than HV training [3]. However, recent evidence suggests HL and HV training to failure results in similar hypertrophic adaptations [4, 5].

Although recent studies have compared strength and hypertrophy adaptations between HV and HL training [4-6], little evidence to date exists regarding the molecular, and specifically the metabolic adaptations, that occur with each form of training. Certain studies have reported that resistance training decreases the activities and/or concentrations of enzymes that generate intracellular ATP (e.g., hexokinase, creatine kinase, phosphofructokinase, and TCA cycle enzymes) [7, 8], whereas others have reported that resistance training increases or does not alter some of these variables [9, 10]. Although it is difficult to reconcile these disparate findings, these different outcomes may be related to the volume-load implemented in these studies. Our laboratory has reported that six weeks of HV training in previously trained males upregulates skeletal muscle proteins suggestive of increased creatine kinase and glycolytic activity in skeletal

muscle [11]. In a separate cohort of previously trained participants, however, we reported that 10 weeks of HL training did not affect these biomarkers [12]. Thus, it is unclear as to how volume-load modulates markers of skeletal muscle metabolism.

Our laboratory and others have also taken an interest as to how resistance training affects markers of mitochondrial function. We recently examined how six weeks of high volume versus high intensity resistance training affected markers of mitochondrial dynamics and biogenesis in previously trained college-aged males (Fox et al. *unpublished observations*). We reported that HV training increased certain protein markers indicative of mitochondrial biogenesis, fission and fusion, compared to HL training. Only one other study to date has extensively compared skeletal muscle mitochondrial biomarker adaptations between HL versus HV training. Specifically, Lim et al. [13] recruited college-aged males to perform a 10-week resistance training study. Participants performed either 80FAIL training (80% one-repetition max (1RM) to volitional fatigue), 30WM training (30% 1RM volume matched to 80FAIL), or 30FAIL training (30% 1RM to volitional fatigue). The authors reported 30WM, and 30FAIL training increased markers of mitochondrial biogenesis and function over 80FAIL training. Hence, it seems apparent that HV training likely facilities mitochondrial adaptations compared to HL training, although more research is needed in this area.

Aside from interrogating how resistance training affects metabolic adaptations at the tissue level, there has been a growing interest in determining how resistance training affects molecular attributes such as contractile versus non-contractile protein concentrations and/or mitochondrial volume density. We have recently postulated that higher volume training may facilitate fiber growth through a disproportionate increase in the volume of the sarcoplasm relative to myofibril protein accretion and the expansion of the mitochondrial reticulum [14].

This phenomenon has been termed sarcoplasmic hypertrophy. Interestingly, the aforementioned study by Haun et al. observed features of sarcoplasmic hypertrophy, which coincided with significant metabolic adaptations. From a spatial and bioenergetics perspective, we posited this mechanism allows the myofiber to prepare for the eventual increase in myofibril protein accretion. However, others have countered this hypothesis given that a large number of longitudinal studies have shown that specific tension is preserved in myofibers that have experienced radial growth as a result of increased mechanical loading [15]. Moreover, this mechanism is speculative due to the lack of overall research in the area, and more investigations are needed to validate the paradigm.

While many resistance training studies have focused on strength and hypertrophy adaptations for decades, studying on how resistance training affects molecular adaptations is still largely unexplored. Therefore, the purpose of the present study was to determine whether 8 weeks of unilateral leg lower-volume resistance training (LV) versus variable and, consequently, higher-volume resistance training (HV) differentially affects contractile protein content as well as select markers reflective of metabolic processes and mitochondrial function. Notably, the muscle specimens used for this study were from a previously published report [16]. Given the literature presented above, we hypothesized that HV training would promote a decrease in contractile protein content, and this would reflect potential sarcoplasmic hypertrophy. Additionally, we hypothesized that HV training would dynamically alter biomarkers related to metabolism and mitochondrial physiology compared to LV training.

CHAPTER II: LITERATURE REVIEW

This literature review is divided into various sections. First, it will provide a comprehensive overview of skeletal muscle physiology. Next, an overview of skeletal muscle ATP generation and utilization will be discussed. Metabolic biomarker adaptations to resistance training will then be outlined, and a discussion of sarcoplasmic hypertrophy and how this may coincide with metabolic adaptations will follow. Finally, I will propose knowledge gaps in the literature as well as the purpose of my dissertation project.

Properties of skeletal muscle

Skeletal muscle comprises roughly 50% of body mass in most humans, and provides important functions including force production for general movement and breathing, force generation for postural support, and heat production during cold stress. Moreover, skeletal muscle is an important tissue for bioenergetic homeostasis during rest and exercise. Skeletal muscle contains a hierarchical architecture, and is organized into several levels including: i) whole muscle surrounded by fascia, ii) bundles of muscles fibers called fascicles, iii) myofibrils within each single fiber, iv) sarcomeres within myofibrils, and v) proteins within sarcomeres including thick filaments (predominantly myosin) and thin filaments (predominantly actin). There are varying estimates regarding the space or volume contribution of myofibril versus non-myofibril components within muscle fibers. Experiments allocating transmission electron microscopy have indicated that myofibrils contribute to ~85% of the intracellular space, and the

remainder of the intracellular space is made up of the sarcoplasmic reticulum (~3-5%), the mitochondrial reticulum (~3-5%), and sarcoplasmic space (~5-10%) [17, 18]. It should also be noted that these characteristics can be influenced by muscle fiber type and training [17], and this will be discussed later on. Given that muscles contain mostly myofibrils from a spatial perspective, it would be expected that the number of myofibril proteins (e.g. actin, myosin, troponin, tropomyosin, titin, nebulin) outnumber other types of proteins (e.g., mitochondrial proteins or enzymes in the sarcoplasm). Indeed, one estimate in humans suggests ~60-70% of skeletal muscle mixed protein pool is myofibrillar, ~20-30% is sarcoplasmic, and ~5-10% is mitochondrial [19]. Recent molecular advances have continued to unveil the proportion of proteins that make up the skeletal muscle proteome. For instance, our laboratory has used mass spectrometry (LC-MS/MS) to characterize the muscle proteome in younger adults that were untrained versus resistance-trained, and we compared both of these groups to older untrained adults [20]. While interesting between-group differences existed, main themes of the proteomic data included the following:

i. Myofibril proteins constituted ~11-13% of muscle tissue weight, sarcoplasmic proteins constituted ~5-6% of muscle tissue weight, and fluid as well as other constituents (e.g., collagen, glycogen, IMTGs, mitochondrial reticulum) contributed to the remainder of the weight (~80-83%).

ii. The sum of all myosin isoforms made up ~60% of the myofibrillar protein pool, the sum of all actin isoforms made up roughly 5-10% of this pool, troponin isoforms made up roughly 4-6% of this pool, titin made up roughly 3% of this pool, and other sarcomeric proteins (e.g., tropomyosin, alpha-actinin, and nebulin) made of ~6% of this pool.

iii. The most enriched protein in the sarcoplasmic pool was the creatine kinase enzyme, which accounted for ~11% of all sarcoplasmic proteins. However, the sum of all glycolysis enzymes made up the largest percentage of sarcoplasmic proteins (roughly 25%), and mitochondrial proteins (i.e., beta-oxidation enzymes, Krebs cycle enzymes, and electron transport chain proteins) accounted for roughly 10% of all non-contractile proteins.

Gonzalez-Freire et al. [21] also used mass-spectrometry-based proteomics to show that most of the proteins in skeletal muscle (by percentage, not absolute amount) are involved in metabolic processes rather than muscle contraction. Specifically, the authors categorized around 40% of the total number of proteins in skeletal muscle as enzymes, and under 10% as contractile. Furthermore, ~20% of proteins were characterized as mitochondrial proteins that served roles in oxidative metabolism. However, as mentioned above, most of the intracellular mass of myofibers is not attributed to protein and, instead, is mainly attributed to fluid. On multiple occasions our laboratory has demonstrated that muscle tissue is ~75% fluid by mass when assessing wet versus dry tissue weights through lyophilization [11, 12, 20], and this agrees with other estimates [18]. Collectively, these data point to central themes regarding the intracellular environment of myofibers. First, myofibers contain mostly fluid when considering constituents by weight (again, ~75%). Second, myofibers spatially contain mostly myofibrils (~85%), and myosin isoforms make up ~60% of the proteins in the myofibrillar protein pool. Finally, although non-myofibril spacing within muscle fibers is sparse, these areas contain sarcoplasm, the mitochondrial and sarcoplasmic reticula, and stored energy substrates (e.g., glycogen and IMTGs). Moreover, a vast array of proteins exist in these non-myofibril spaces, and most of

these proteins by abundance are involved in ATP generation (e.g., creatine kinase, glycolysis enzymes, and mitochondrial enzymes and proteins).

Beyond these intracellular attributes, myofibers possess other unique properties worthy of discussion. Myofibers are morphologically unique relative to other cell types given that they are long, cylindrical cells that can measure from 3 cm up to 30 cm [22]. Additionally, myofibers can contain several hundred nuclei [23], and this is remarkable given that other cell types contain a single nucleus. Myofiber multinucleation occurs during development when multiple mononucleated myoblasts fuse to form mature myofibers [24]. However, biologists have deemed multinucleation essential for myofibers given the extreme length, and therefore, size of these cells. In this regard, the myonuclear domain theory has been posited, and this construct suggests muscle fiber growth requires the addition of myonuclei from satellite cells in order to support the accretion and maintenance of newly added intracellular material [25]. Muscle fibers are also unique in their ability to stretch and relax at various forms of either activity or inactivity; specifically, fibers can stretch up 10-15% of their resting length. To accomplish this, the plasma membrane (also termed the sarcolemma) contain indentions (termed caveolae) that provide the additional space needed to stretch.

Skeletal muscle can also be viewed in intracellular (i.e., beneath the muscle fiber membrane) and extracellular (i.e., outside the muscle fiber membrane) terms. While the extracellular compartment (or extracellular matrix) is primarily composed of connective tissue, there is also the presence of capillaries and non-muscle cells (e.g., satellite cells, pericytes, fibroblasts, and resident immune cells). Based on histological examinations, connective tissue occupies between ~1-9% of human skeletal muscle [26]. Satellite cells (i.e., resident stem cells) are mononucleated cells that exist in the extracellular space between the sarcolemma and the

basement membrane of muscle fibers [27]. Although beyond the scope of this review, it is notable that certain studies have deemed satellite cells as essential mediators of skeletal muscle hypertrophy due to their role in myonuclear addition [28]. Pericytes are mesenchymal cells residing in close proximity to capillaries. While the roles of these cells are complex, recent evidence in humans suggests pericytes may participant in exercise-induced angiogenesis [29]. Additionally, others have suggested pericytes play a supporting role in myofiber growth through the production and secretion of growth factors into the extracellular matrix that stimulate satellite proliferation [30]. Fibroblasts are spindle-shaped mononuclear cells that exist in the extracellular matrix, and are chiefly responsible for producing extracellular matrix proteins (e.g., the collogen I/II/IV isoforms) [31]. Finally, resident immune cells exist in the extracellular matrix and can include macrophages, neutrophils, and lymphocytes [30]. As with pericytes, the roles these cells have in the broader context of muscle physiology are complex. It is notable, however, that resident immune cells have been shown to secrete a majority of cytokines produced in muscle tissue in the post-exercise period, and this is thought to attract circulating immune and satellite cells to damaged areas in order to facilitate muscle repair [30].

An overview of ATP generation and utilization in skeletal muscle

Adenosine triphosphate (ATP), deemed the universal cellular energy substrate, provides energy for many cellular processes. The ATP molecule consists of three parts including: i) an adenine molecule, ii) a ribose sugar molecule and iii) three linked phosphates. The splitting (or hydrolysis) of the phosphoanhydride bond between the second and third phosphate via proteins with ATPase domains creates an exergonic reaction that cells can harness to facilitate enzymatic reactions. In skeletal muscle cells, there are variety of ATPase enzymes [32]. For instance,

Na^+/K^+ ATPase pump utilizes the energy derived from ATP hydrolysis to sustain intra- and extracellular sodium and potassium ion homeostasis. The SarcoEndoplasmic reticulum Calcium ATPase (SERCA) pump utilizes the energy liberated from ATP hydrolysis to promote myofiber relaxation by pumping calcium ions from the sarcoplasm into the lumen of the sarcoplasmic reticulum [33]. Myosin filaments are structurally organized into a head, neck and tail, and the head contains an actin-binding site as well as a myosin ATPase domain. The myosin ATPase domain utilizes the energy liberated from ATP hydrolysis to "walk" or "slide" along actin filaments, and this is the molecular basis of skeletal muscle contraction [34].

Several metabolic pathways are responsible for generating ATP in myofibers as well as other cell types. The ATP/ phosphocreatine (PCr) pathway consists of one enzymatic reaction (creatine kinase), and involves the interaction of PCr and ADP, which is catalyzed via creatine kinase. The byproduct ADP is phosphorylated by cytoplasmic creatine kinase, while PCr serves as a phosphate donor [35]. The byproduct, cytoplasmic creatine, is then rephosphorylated by mitochondrial CK. Phosphocreatine is a "high-energy phosphate" that cells can use to sustain ATP concentrations during periods of rapid energy consumption [36]. There are three different cytosolic oligomeric CK isoforms. These isoforms include the brain-specific BB-CK, as well as the sarcomeric muscle-type MM-CK and MB-CK forms. Creatine kinase also has two mitochondrial specific isoforms, a ubiquitous Mi_a-CK and a sarcomeric Mi_b-CK isoform. In fully differentiated skeletal muscle, MM-CK is the predominant isoform, although mitochondrial Mi_b-C is also present [37]. For the purpose of this study we used the MM-CK isoform to examine how exercise affects skeletal muscle creatine kinase protein levels.

Glycolysis is a metabolic pathway that occurs in the sarcoplasm of muscle cells and produces two net ATP and two pyruvate molecules [38]. Although glycolysis does not require

oxygen, it is critical for aerobic ATP production given that the pyruvate produced from glycolytic reactions are shuttled into the mitochondria for further oxidation. Mitochondria are cellular organelles that produce most of the ATP, and they require oxygen to carry out this process [39]. There are two prominent ATP-generating/substrate utilization mechanisms that occur in the mitochondria, and these include: i) the citric acid cycle (or TCA cycle), and ii) oxidative phosphorylation via the electron transport chain (ETC). The TCA cycle takes place in the mitochondrial matrix, and involves a series of eight enzymatic steps that oxidizes citrate to produce electron donors (NADH and $FADH_2$). As a result of citrate oxidation, carbon dioxide is produced, and the resultant NADH and $FADH_2$ ultimately transfer electrons to the ETC to begin the process of oxidative phosphorylation. Oxidative phosphorylation involves a series of delicate reactions where electrons received from NADH and $FADH_2$ traverse various complexes (complexes I-IV) embedded in the inner mitochondrial membrane. During this electron transfer process, an electrochemical gradient is established through the transport of hydrogen ions (i.e., protons) from the mitochondrial matrix into the inner membrane space. ATP syntheses occurs at the end of the chain where ATP synthase (referred to as complex V) facilitates proton flux back into the mitochondrial matrix, and this is coupled with the formation of ATP from ADP and inorganic phosphate [39]. At the end of the reaction, electrons are donated to oxygen, hydrogens are subsequently paired to oxygen as well, and this forms water. Interestingly, it has been estimated that ATP consumption can increase 1000-fold during periods of high intensity exercise [40], and this is mainly attributed to a robust increase in the activities of all the aforementioned ATPases.

Studies examining metabolic adaptations during resistance training

The first study to examine metabolic adaptations to exercise training in skeletal muscle was conducted by Dr. John Holloszy in 1967 who demonstrated that the activities of various mitochondrial enzymes in the hind limb muscles of endurance-trained rats were two-fold higher compared to age-matched sedentary controls [36]. Since then, several investigations in humans have shown that endurance training increases the following variables in a volume-dependent fashion: i) mitochondrial volume and function [36, 41, 42], ii) glycolytic enzyme content and activity levels for individual enzymes [36, 43, 44], iii) the intracellular storage of energy substrate (i.e., muscle glycogen and IMTGs) [45-47].

It widely accepted that resistance training leads to increases in strength and muscle hypertrophy. In untrained subjects, chronic resistance training generally leads to an increase in strength followed by an increase in muscle size [48]. This is predominantly due to neural adaptations including increased motor unit recruitment and synchronization, decreased co-contraction of antagonist muscles, increased neural drive from the central nervous system, and increased motor unit hypertrophy [49]. Strength adaptations are associated with lifting intensities in a manner where higher training loads (i.e., repetitions at 85-90% of 1RM) have been shown to result in greater strength adaptations compared to training at lower loads (i.e., repetitions at 55-65% of 1RM) [48, 50]. However, unlike the studies cited above that have delineated the metabolic adaptations to endurance training, literature examining skeletal muscle metabolic adaptations in response to resistance training is limited and equivocal. Tesch and colleagues conducted a six-month resistance training study where participants were divided into either heavy-resistance (HR) training or explosive strength training (EX) [51]. Hexokinase enzyme activity levels were reported to decrease in the HR and EX groups, and a decrease in creatine kinase (CK) enzyme activity levels occurred in the HR group. Additionally, a decrease

in all other muscle enzyme markers occurred with training regardless of group excluding lactate dehydrogenase (LDH), which was reported to be unaffected regardless of training. Tang et al. [9] reported a significant increase in hexokinase activity following 12 weeks of higher volume resistance training, while phosphofructokinase activity was not altered. Wang and colleagues observed an increase in the activities of certain glycolytic enzymes after 18 weeks of HL resistance training [10]. Chilibeck and colleagues conducted a linear variable high-load training three times per week for 12 weeks [8]. There were two groups including a strength-trained group and a control group that did not train. The authors reported a decrease in succinate dehydrogenase enzyme activity in the strength-trained group. Our laboratory has reported that six weeks of HV training in previously trained males upregulates skeletal muscle proteins suggestive of increased creatine kinase and glycolytic activity in skeletal muscle [11]. In a separate cohort of previously trained participants, however, we reported that 10 weeks of HL training did not affect the relative concentrations of these muscle enzymes [12]. The effects of resistance training on mitochondrial markers is also sparse and equivocal. Several studies indicate that markers representative of mitochondrial volume decrease during periods of resistance training (reviewed in Groenneback and Vissing) [52]. While perplexing, this consistent finding may indicate that a dilution of mitochondria occurs during periods of myofiber growth. Alternatively stated, myofibers do not experience decreased mitochondrial volume, but instead experience cell growth that outpaces the expansion of the mitochondrial reticulum. However, there is evidence to suggest resistance training enhances certain aspects of mitochondrial function in skeletal muscle. For instance, a review by Parry et al. [49] cites multiple studies suggesting that the protein and activity levels of mitochondrial complexes increase during periods of resistance training. It is also notable that the mitochondrial responses

to resistance training may be contingent on volume-load. For instance, we recently examined how six weeks of higher volume (HV) versus higher load (HL) resistance training affected markers of mitochondrial dynamics and biogenesis in previously trained college-aged males (Fox et al. *unpublished observations*). HV training increased select protein markers of mitochondrial biogenesis, fission and fusion, compared to HL training. To the best of our knowledge, only one other study has extensively examined skeletal muscle mitochondrial biomarker adaptations between HL versus HV training [13]. In this study, the authors recruited college-aged males to perform a 10-week resistance training study. Participants performed either 80FAIL training (80% one-repetition max (1RM) to volitional fatigue), 30WM training (30% 1RM volume matched to 80FAIL), or 30FAIL training (30% 1RM to volitional fatigue). Similar to our data, the authors reported that higher volume (30WM and 30FAIL) training increased markers of mitochondrial biogenesis and remodeling compared to higher load (80FAIL) training. Collectively, these data suggest that various metabolic adaptations can occur in skeletal muscle in response to resistance training, and the type of metabolic adaptations likely depend on the volume-load scheme implemented.

Studies examining sarcoplasmic hypertrophy during resistance training

As mentioned above, general resistance training adaptations include increases in muscle mass and strength. In this regard, it has been shown that weeks to months of resistance training increases skeletal muscle fiber cross-sectional area (fCSA) [53-57]. However, the intracellular/morphological adaptations that occur in muscle fibers during resistance training interventions remain largely unexplored. Our laboratory has recently hypothesized that higher volume resistance training may facilitate fiber growth through a disproportionate increase

sarcoplasmic volume relative to myofibril expansion [14]. This phenomenon has been termed *sarcoplasmic hypertrophy*, and there are a few studies discussed below that support this mechanism. Contrary to this model, the scientific community has generally maintained that conventional hypertrophy occurs in response to resistance training. Under this pretense, muscle fibers undergo training-induced radial growth through a proportional expansion of myofibrils and sarcoplasmic space. While there is little supporting mechanistic evidence for this paradigm, there are studies that do lend credence to this model. In this regard, a rodent study performed in 1964 by Goldspink is a landmark investigation in this area. Briefly, the author examined four groups of female mice where two groups were trained to obtain food through a resistance-loaded pulley apparatus, and the only variable differentiating these groups was the amount of food administered per day (i.e., 3.5 g/d and 5.0 g/d). The other two groups were not housed with pulley devices and served as feed-matched controls. Following the 25-day experiment, mice were sacrificed and biceps muscles histologically prepared and examined using light microscopy at 1000× magnification. Regardless of food amount administered, mice housed with the pulley apparatus possessed 30% larger myofibers compared to control animals. A strong linear relationship between mean fCSA and myofibril number in exercise-trained mice was also evident. Finally, larger/hypertrophied fibers in exercise-trained mice contained less sarcoplasmic space relative to smaller fibers. While limited to rodents, these data suggest: i) myofibril protein accretion accompanies resistance load-induced muscle fiber hypertrophy, ii) a marginal amount of myofibril packing – or a disproportionate increase in myofibril protein accretion relative to fiber growth – may occur during myofiber hypertrophy, and iii) an increase in myofibril number may accompany myofiber hypertrophy. Following this study, Goldspink authored a book chapter highlighting mechanisms of myofibril addition during exercise-induced muscle fiber

growth [14]. Goldspink cited numerous animal studies in his book suggesting: i) the generation of new myofibrils (i.e., *de novo* myofibrillogenesis) only occurs during embryonic development, ii) myofibril protein accretion in sexually mature animals likely involves newly synthesized proteins being added to the periphery of currently existing myofibrils, and iii) myofibrils can increase in number during periods of exercise training, although this is likely due to myofibril splitting. Human studies providing this level of morphological detail are currently lacking. However, long-term resistance-trained individuals have been shown to possess larger myofibers compared to untrained controls, and myofibril spacing was not reported to be different between groups [17]. Additionally, Luthi et al. [48] used TEM to examine morphological adaptations in vastus lateralis muscle fibers from eight untrained college-aged men that resistance trained for six weeks. Although the area of the entire vastus lateralis muscle increased by 8.4% with training, the density of myofibrils remained unchanged.

MacDougall et al. [58, 59] published the first studies in humans to suggest sarcoplasmic hypertrophy may coincide with muscle fiber hypertrophy. In these studies, previously trained and untrained individuals underwent six months of high load resistance training. TEM analysis showed a reduced mitochondrial and myofibrillar volume density following heavy resistance training, while a significant increase in cytoplasmic volume density was observed. Toth et al. [60] conducted an 18-week higher-volume resistance training study in older participants that were either apparently healthy or were heart failure patients. Similar to the aforementioned study, these authors used TEM to analyze space occupied by the myofibrils within myofibers. In short, regardless of disease state, the space occupied myofibrils decreased by approximately 15%. A study by Haun et al. [11] from our laboratory implemented high-volume resistance training in previously trained males over a six-week period. Participants lifted loads

corresponding to 60% 1RM, and sets of 10 repetitions were programmed for each exercise throughout the entirety of the study. The 3-day per week lifting protocol incorporated a progressive increase from 10 sets per week to 32 sets per week for each exercise, and by the last week of training, participants were performing 32 sets of 10 repetitions per exercise. Relative myosin and actin concentrations (per mg dry tissue) were assessed via SDS-PAGE, actin density per fiber was assessed via phalloidin staining, mitochondrial volume changes was assessed via citrate synthase activity assays, and relative sarcoplasmic protein concentrations were interrogated via proteomics. Actin and myosin concentrations decreased in lieu of mean fiber fCSA increases during training, and phalloidin staining confirmed that actin density per fiber followed a similar downtrend. Interestingly, of the 156 sarcoplasmic proteins identified via liquid chromatography-mass spec/mass spectrometry, 40 proteins were significantly upregulated from PRE to POST training. Performing KEGG pathway analysis on these 40 proteins indicated that the glycolysis/gluconeogenesis metabolic pathway was the only protein network in the sarcoplasmic protein pool that was significantly upregulated from PRE to POST training. Additionally, a significant upregulation in the CK enzyme occurred and, although mitochondrial dilution was observed through decreases in CS activity, it is notable that certain mitochondrial proteins involved with ATP generation were also found to be upregulated.

Do metabolic adaptations coincide with sarcoplasmic hypertrophy?

In a recent review, our laboratory posited sarcoplasmic hypertrophy may coincide with metabolic adaptations during higher volume resistance training in order to spatially and bioenergetically prepare myofibers for longer-term hypertrophy [14]. In this regard, it is common for myofibers to increase in radial size by ~15-30% in response to months of resistance training. Therefore,

myofibers need to generate an appreciable amount of intracellular space to accumulate more myofibril protein, and a potential mechanism to accomplish such growth could involve sarcoplasmic hypertrophy followed by "backfilling" of newly-generated sarcoplasmic space with myofibrils. Likewise, the synthesis of large myofibril proteins is an energetically costly proposition, with estimates suggesting 4 ATP are required per peptide bond [61]. Thus, upregulating ribosomes (via ribosome biogenesis) and ATP-generating enzymes in skeletal muscle during sarcoplasmic hypertrophy would furnish muscle cells with the machinery needed to synthesize and accumulate myofibril protein. While there is virtually no evidence to support this paradigm, it is notable that the study by Haun et al. demonstrated that features of sarcoplasmic hypertrophy coincided with ribosome biogenesis as well as an upregulation in ATP-producing enzymes during the 6-week high volume resistance training program. Notwithstanding, others have countered this hypothesis given that a large number of longitudinal studies have shown that specific tension is preserved in myofibers that have experienced radial growth as a result of increased mechanical loading [15]. Moreover, this mechanism is speculative due to the lack of overall research in the area, and more investigations are needed to validate the paradigm.

Purpose of the current study

The collective evidence presented above suggests certain phenomena may occur during periods of resistance training where volume-load differences exist. First, HV training may stimulate metabolic adaptations that coincide with sarcoplasmic hypertrophy, whereas these events are not as evident during paradigms that implement higher load and/or lower volume training. Additionally, while mitochondrial dilution has been reported to occur with resistance training,

higher volume resistance training may facilitate an improvement in mitochondrial function by: i) increasing the expression of mitochondrial proteins involved with ATP synthesis, and ii) "provoking" a reorganization of the mitochondrial reticulum through an alteration in dynamics markers. Therefore, the purpose of this study was to determine whether 8 weeks of unilateral leg lower-volume resistance training (LV) versus variable and, consequently, higher-volume resistance training (HV) differentially affects contractile protein content as well as select markers reflective of metabolic processes and mitochondrial function.

CHAPTER III: METHODS

Participants and ethical approval

The Human Research Ethics Committee of the Federal University of Sao Carlos approved the study (no. 2.226.596). Experimental procedures and associated risks were explained to each participant, who provided written and informed consent before participation. All procedures performed herein were in accordance with the ethical standards of the institutional and national research committee, and with the 1964 Helsinki Declaration and its later amendments. Healthy resistance-trained young men (n = 20) were recruited for the study; mean ± SD age: 26 ± 3 yr, body mass index: 25.6 ± 2.1 kg/m^2, and previous resistance training experience: 2.5 ± 1.1 yr. As inclusionary criteria, the participants had to be free from musculoskeletal disorders that would prevent adequate performance of the resistance training protocols, and participants did not use anabolic steroids.

General study design

All training and tissue procurement occurred at the Federal University of Sao Carlos and all wet lab analysis herein occurred at Auburn University. A within-subjects unilateral study design was utilized herein. All participants completed a familiarization session with all training protocols occurring before the onset of resistance training. Afterwards, participants performed twice weekly unilateral leg resistance training over 8 weeks. One leg was randomly assigned to a standard progressive resistance training paradigm (which was lower-volume, and abbreviated as

LV herein). The contralateral leg allocated to a variable resistance training volume protocol (which was higher-volume, and abbreviated as HV herein). The HV protocol systematically and sequentially modified exercise-related variables at each RT session (see full descriptions of RT protocols below). Participants completed all four VAR conditions in a counterbalanced randomized manner every two weeks of the 8-week training period. Thus, each participant performed each VAR condition four times during the 8-wk training program. Leg dominance was counterbalanced between protocols (i.e., 10 dominant and 10 nondominant legs in CON and VAR). Before and after the 8-week training period (3 days after the last RT session), bilateral vastus lateralis cross-sectional areas were measured using an ultrasound. The accumulated total training volume (TTV; sets x repetitions x load) performed by LV and HV legs over the training period was calculated. Finally, biopsy specimens prior to and 48 hours following the last training bout were obtained and assayed herein.

Resistance training protocol

The LV protocol consisted of 8 sets (4 sets of unilateral leg press followed by 4 sets of unilateral leg extension exercise) of 9–12 repetitions of resistance exercise to concentric failure with two-minute rest intervals between sets. Repetition range was achieved by increasing or decreasing the load between sets as previously described [16]. Each session of the HV protocol involved one of the following RT manipulations: i) variable-load, 8 sets (4 of leg press and 4 of leg extensions) of 25–30 reps to concentric failure/2 minutes between set rest interval; ii) variable-sets, 12 sets (6 of leg press and 6 of leg extension) of 9–12 reps to concentric failure/2 minutes between set rest intervals; iii) variation in contraction type, 8 sets (4 of leg press and 4 of leg extension) of 10 eccentric contractions at 110% of the load used in the LV leg/2 minutes between set rest

intervals; and iv) variations in rest periods, 8 sets (4 of leg press and 4 of leg extension) of 9–12 reps to concentric failure/4 minutes between set rest intervals.

Muscle biopsies

Biopsies of the vastus lateralis were performed using the percutaneous muscle biopsy technique with suction under local anesthesia 2-3 mL of 1% Xylocaine. Approximately 50 mg muscle tissue was dissected free from blood and connective tissue, and muscle tissue samples were immediately placed in cryogenic tubes, frozen in liquid nitrogen, and stored at -80°C until analyses described below. Separate pieces of muscle were preserved slow-frozen in OCT media, sectioned at a thickness of 10 µm using a cryostat, mounted on positively charged microscope slides, and stored at -80°C. Frozen samples and mounted sections were subsequently shipped to Auburn University on dry ice, and Auburn investigators received samples and stored them at -80°C until analyses described below.

Sarcoplasmic and myofibrillar protein isolations

Isolation of the sarcoplasmic and myofibrillar protein fractions from wet muscle tissue was performed using our recently published "MIST" or "myofibrillar isolation and solubilization technique" [62]. Briefly, 1.7 mL polypropylene tubes were pre-filled with ice-cold buffer (300 µL; Buffer 1: 25 mM Tris, pH 7.2, 0.5% Triton X-100, protease inhibitors) and placed on ice. Skeletal muscle foils were removed from -80 C, placed on a liquid nitrogen-cooled ceramic mortar and pestle, and tissue was pulverized into 2-4 mm^3 pieces. Approximately ~20 mg of crushed tissue was placed in tubes prefilled with buffer (described above), weighed using a scale with a sensitivity of 0.0001 g (Mettler-Toledo; Columbus, OH, USA), 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 (described below), and stored at -80°C until analysis occurred.

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 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 were 9.1%.

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[11, 63]. Briefly, SDS-PAGE sample preps were made using 10 µL resuspended myofibrils, 65 µL distilled water (diH_2O), and 25 µL 4x Laemmli

buffer. Samples (5 µL) were then loaded on precast gradients (4-15%) SDS-polyacrylamide gels(Bio-Rad Laboratories; Hercules, CA, USA) and subjected to electrophoresis at 180 V for 40 minutes using pre-made 1x SDS-PAGE running buffer (VWR International; Randor, PA, USA). Following electrophoresis, gels were rinsed in diH$_2$O 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 diH$_2$O for 60 minutes, and band densitometry was performed using a gel documentation system and associated software (ChemiDoc; Bio-Rad Laboratories). 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 relative 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 [11].

Western blotting of sarcoplasmic protein fraction

Sarcoplasmic protein resuspensions obtained above were prepared for Western blotting at 1 µg/µL using 4x Laemmli buffer. Following sample preparation, 15 µL samples were loaded onto pre-casted gradient (4–15%) SDS-polyacrylamide gels (Bio-Rad Laboratories) and subjected to electrophoresis (180 V for 45–60 min) using pre-made 1x SDS-PAGE running buffer (VWR International). Proteins were subsequently transferred (200 mA for 2 h) to polyvinylidene difluoride membranes (PVDF) (Bio-Rad Laboratories), Ponceau stained, and imaged to ensure equal protein loading between lanes. Membranes were then blocked for 1 h at room temperature with 5% nonfat milk powder in Tris-buffered saline with 0.1% Tween-20 (VWR International). Membranes were then incubated with the following antibody cocktails

overnight at 4 °C in TBST with 5% bovine serum albumin (BSA): 1) goat anti-human creatine kinase (CKM, 1:1000; Abcam Cat# ab174672, RRID:AB_2747709), 2) rabbit anti-human L-type amino acid transporter (LAT1, 1:1000, Cell Signaling Technology Cat# 5347, RRID:AB_10695104), 3) rabbit anti-human glucose transporter 4 (GLUT4, 1:1000, Cell Signaling Technology Cat# 2213, RRID:AB_823508), 4) rabbit anti-human lactate dehydrogenase (LDHA, 1:1000, Cell Signaling Technology Cat# 2012, RRID:AB_2137173), 5) rabbit anti-human phosphofructokinase (PFKM, 1:1000, catalog#: ab154804; Abcam), 6) rabbit anti-human total oxidative phosphorylation (OXPHOS) human cocktail (Abcam Cat# ab110411, RRID:AB_2756818), 7) rabbit anti-human COX IV (Cell Signaling Technology Cat# 4850, RRID:AB_2085424), 8) rabbit anti-human isocitrate dehydrogenase 2 (IDH2, 1:1000, Cell Signaling Technology Cat# 56439, RRID:AB_2799511), 9) rabbit anti-human acyl-CoA dehydrogenase very long chain (ACADVL, 1:1000, Abcam Cat# ab188872), 10) rabbit anti-human Carnitine O palmitoyltransferase I (CPT, 1:1000, Abcam Cat# ab134135, RRID:AB_2847833), and 11) rabbit anti-human hexokinase II (HK2, 1:1000, Cell Signaling Technology Cat# 2867, RRID:AB_2232946). The following day, membranes were incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (catalog #: 7074; Cell Signaling), or HRP-conjugated anti-goat IgG (catalog #: GTX628547-01; GeneTex) in TBST with 5% BSA at room temperature for 1 h (secondary antibodies diluted 1:2000). Membrane development was performed using an enhanced chemiluminescent reagent (Luminata Forte HRP substrate; EMD Millipore, Billerica, MA, USA), and band densitometry was performed using a gel documentation system and associated software (Bio-Rad Laboratories, Hercules, CA, USA). Raw densitometry values for each target were divided by whole-lane Ponceau densities. All

values were then divided by the mean of the PRE time point to depict relative protein concentrations.

Citrate synthesis activity assay

Sarcoplasmic protein resuspensions obtained above were prepared for citrate synthase activity assays as previously described by our laboratory [64]. This metric was used as a surrogate for mitochondrial content per the findings of Larsen et al. [65], suggesting citrate synthase activity correlates with transmission electron micrograph images of mitochondrial content ($r = 0.84$, $p < 0.001$). The assay principle is based upon the reduction of 5,50-dithiobis (2- nitrobenzoic acid) (DTNB) at 412 nm (extinction coefficient 13.6 mmol/L/cm) coupled to the reduction of acetyl-CoA by the citrate synthase reaction in the presence of oxaloacetate. Lysates from each biopsy (providing 12.5 µg protein) were assayed in duplicate where samples were added to a mixture composed of 0.125 mol/L Tris–HCl (pH 8.0), 0.03 mmol/L acetyl-CoA, and 0.1 mmol/L DTNB. The reaction was initiated by adding 5 µL of 50 mmol/L oxaloacetate and the absorbance change was recorded for 1 min. The coefficient of variation for all duplicates was 5.7%.

Phalloidin staining

For the determination of myofibril area per fiber, F-actin labelling using Alexa Fluor 488-conjugated (AF488) phalloidin was performed according to previous reports [11, 66, 67]. Briefly, serial sections were air-dried for 10 minutes followed by 10% formalin fixation for 10 minutes. Sections were then washed with PBS for 5 minutes, and blocked with 100% Pierce Super Blocker (Thermo Fisher Scientific) for 25 minutes. After blocking, a pre-diluted

commercially-available rabbit anti-dystrophin IgG1 antibody solution (catalog #: GTX15277; Genetex Inc.) and spiked in phalloidin-AF488 (1:35 dilution) was placed on sections in the dark for 30 minutes. Sections were subsequently washed in PBS for 5 minutes and incubated in the dark for 20 minutes with a secondary antibody solution containing Texas Red-conjugated anti-rabbit IgG (catalog #: TI-1000; Vector Laboratories; ~6.6 μL secondary antibody per 1 mL of blocking solution). Sections were washed in PBS for 5 minutes, and air-dried and mounted with fluorescent media containing DAPI (catalog #: GTX16206; Genetex Inc.). Following mounting, digital images were immediately captured with a fluorescent microscope (Nikon Instruments) using a 20x objective. Exposure times were 200 milliseconds for FITC, 800 milliseconds for TRITC imaging, and 100 milliseconds for DAPI imaging. This staining method allowed the identification of the sarcolemma (Texas Red filter), myofibrils (FITC filter), and myonuclei (DAPI filter). ImageJ (NIH) was used to quantify myofibril area per fiber. Briefly, images were split into RGB channels, and the green channel image was converted to grayscale. The threshold function in imageJ was then used to generate binary black/white images of stained versus unstained portions of fibers. Thereafter, fibers were manually traced using the polygon function, and myofibril areas were provided as a percentage per fiber area. A visual representation of this image analysis is provided in Figure 2 in the results section. Fibers that were quantified in this regard were manually matched to fibers on 10x images to derive myofibril areas for type I and type II fibers. Myonuclei from 20x images were also manually assigned to fibers to extrapolate myofibril-myonuclei relationships.

Statistical Analysis

Statistical analyses were performed in SPSS (Version 26; IBM SPSS Statistics Software, Chicago, IL, USA). Dependent variables were analyzed using dependent samples t-tests, comparing PRE to either LV or HV. Additionally, LV was compared to HV using dependent samples t-tests. Finally, select associations were performed using Pearson's correlations. Statistical significance for null hypothesis testing was set at $p<0.05$. Data are presented throughout as mean ± standard deviation values.

CHAPTER IV: PUBLICATION TO BE SUBMITTED TO PEER REVIEW

Molecular adaptations to different volume-loads of resistance training

Running title: Molecular adaptations to resistance training

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ABSTRACT

Although several studies have examined the training adaptations to lower versus higher volume resistance training, the examination of metabolic and morphological adaptations that occur to both forms of training is still in its infancy. Herein, healthy resistance-trained men (n = 20; 26 ± 3 years old, 25.6 ± 2.1 kg/m^2, 2.5 ± 1.1 years of self-reported training) performed eight weeks of unilateral leg resistance training (twice-weekly). For each participant, one leg was randomly assigned to perform a lower volume protocol (PC) consisting of 4 sets of unilateral leg press followed by 4 sets of unilateral leg extension exercise per workout (9–12 repetitions per exercise to concentric failure). The contralateral leg was subjected to a higher volume training protocol with the same exercises which, over the 8-week period, led to 12.6% more total training volume ($p<0.05$). A pre-training vastus lateralis biopsy was obtained from a randomized single leg, and biopsies were obtained from both legs 48 h following the last training bout. Tissue was processed to examine metabolic and mitochondrial markers in the sarcoplasmic protein pool, and the myofibril pool was analyzed for the relative abundances of total myosin heavy chain (MHC) and actin proteins. Additionally, sections analyzed using a fluorogenic-conjugated phalloidin stain to obtain contractile protein and non-contractile spacing attributes within myofibers. While neither form of training affected most of the assayed sarcoplasmic proteins related to metabolic processes (i.e., CKM, LDHA, PFK, PYGM, GLUT4, LAT1, IDH2. CPT1, or mitochondrial complexes I-IV), both forms of training increased hexokinase 2 protein levels while decreasing a mitochondrial beta-oxidation marker (VLCAD protein content). Interestingly, PV training decreased mitochondrial complex V protein content as well as the relative abundances of MHC and actin ($p<0.05$). Additionally, PV training decreased myofibril spacing within muscle fibers ($p<0.05$), and participants that began training with more myofibril packing seemingly

experienced a larger increase in non-contractile protein spacing with PV training (association r = -0.714, p=0.006). In conclusion, these data are the second observation from our laboratory suggesting higher volume training in previously-trained individuals decreases myofibril volume in muscle fibers. However, unlike our previous report, this morphological adaptation did not coincide with metabolic adaptation. Further studies are needed to determine whether this myofibril spacing alterations with higher volume training is transient and/or meaningful.

Keywords: myofibrils, myosin, actin, sarcoplasm

INTRODUCTION

Researchers have sought to determine whether modulating resistance training volume-load differentially affects training adaptations. Higher load resistance training involves executing lifts with heavier weight for fewer repetitions (e.g. performing 3 to 5 sets of 85% or more of one repetition maximum (1RM) for 3-5 repetitions) [1]. Conversely, higher-volume resistance training executing lifts with lighter weight while performing more repetitions per set relative to higher load training (e.g. sets containing >20 repetitions per set with ~30% of 1RM) [2]. A majority of research suggests high-load training increases strength more so than higher volume training [3]. However, recent evidence suggests both forms of training to failure result in similar hypertrophic adaptations [4, 5].

Although studies have compared strength and hypertrophy adaptations between higher volume and higher load training [4-6], examining the molecular adaptations, and specifically the metabolic adaptations, that occur with each form of training is still in its infancy. Certain studies have reported that resistance training decreases the activities and/or concentrations of enzymes that generate intracellular ATP (e.g., hexokinase, creatine kinase, phosphofructokinase, and TCA cycle enzymes) [7, 8], whereas others have reported that resistance training increases or does not alter some of these variables [9, 10]. Although it is difficult to reconcile, these different outcomes are likely related to the differences in volume-load implemented between studies and training status of subjects between studies. In this regard, we have reported that six weeks of higher volume training in previously-trained men upregulates skeletal muscle proteins suggestive of increased creatine kinase and glycolytic activity in skeletal muscle [11]. Conversely, 10 weeks of higher load training did not affect these biomarkers in a separate cohort of trained men

[12]. Thus, it remains possible that metabolic adaptation to resistance training may be enhanced through higher versus lower volume training.

Our laboratory and others have also taken an interest as to how resistance training affects mitochondrial markers. For instance, we recently examined how six weeks of higher volume versus higher load resistance training affected markers of mitochondrial dynamics and biogenesis in previously-trained men (*unpublished observations*). Interestingly, higher volume training increased certain protein markers indicative of mitochondrial biogenesis, fission and fusion, compared to higher load training. Only one other study to date has extensively compared skeletal muscle mitochondrial biomarker adaptations between higher volume and higher load training. Specifically, Lim et al. [13] recruited college-aged males to perform a 10-week resistance training study. Participants performed either 80FAIL training (80% one-repetition max (1RM) to volitional fatigue), 30WM training (30% 1RM volume matched to 80FAIL), or 30FAIL training (30% 1RM to volitional fatigue). The authors reported 30WM, and 30FAIL training increased markers of mitochondrial biogenesis and function over 80FAIL training. Hence, it seems apparent that higher volume training likely facilities mitochondrial adaptations compared to higher load training, although more research is needed in this area.

Aside from interrogating how resistance training affects metabolic adaptations at the tissue level, there has been a growing interest in determining how resistance training affects molecular attributes such as contractile versus non-contractile protein concentrations and/or mitochondrial volume density. We have recently postulated that higher volume training may facilitate fiber growth through a disproportionate increase in the volume of the sarcoplasm relative to myofibril protein accretion and the expansion of the mitochondrial reticulum (i.e., *sarcoplasmic hypertrophy*) [14]. Interestingly, the aforementioned study by Haun et al. observed

features of sarcoplasmic hypertrophy, which coincided with a significant upregulation in proteins involved with anaerobic and aerobic ATP production (i.e., metabolic adaptation). From a spatial and bioenergetics perspective, we posited this mechanism allows the myofiber to prepare for the eventual increase in myofibril protein accretion. However, others have countered this hypothesis given that a large number of longitudinal studies have shown that specific tension is preserved in myofibers that have experienced radial growth as a result of increased mechanical loading [15]. Moreover, this mechanism is speculative due to the lack of overall research in the area, and more investigations are needed to validate the paradigm.

The purpose of the present study was to determine whether 8 weeks of unilateral leg lower-volume resistance training (termed PC herein) versus variable and, consequently, higher-volume resistance training (termed PV herein) differentially affects contractile protein content as well as mitochondrial markers and select markers reflective of metabolic processes. Notably, the muscle specimens used for this study were from a previously-published report [16]. Given the literature presented above, we hypothesized PV training would increase the protein content of enzymes involved with ATP production as well as markers of mitochondrial content compared to PC training. Moreover, we hypothesized PV training would promote features of sarcoplasmic hypertrophy compared to PC training.

MATERIALS AND METHODS

Participants and ethical approval

The Human Research Ethics Committee of the Federal University of Sao Carlos approved this study prior to data collection (no. 2.226.596). Experimental procedures and associated risks were explained to each participant, who provided written and informed consent before participation.

All procedures performed herein were in accordance with the ethical standards of the institutional and national research committee, and with the 1964 Helsinki Declaration and its later amendments. Healthy resistance-trained young men (n = 20) were recruited for the study; mean ± SD age: 26 ± 3 yr, body mass index: 25.6 ± 2.1 kg/m^2, and previous resistance training experience: 2.5 ± 1.1 yr. As inclusionary criteria, the participants had to be free from musculoskeletal disorders that would prevent adequate performance of the resistance training protocols, and participants did not use anabolic steroids.

General study design

A summary figure of the study design is presented below (Fig. 1). All supervised training and tissue procurement occurred at the Federal University of Sao Carlos, and all wet lab analyses occurred at Auburn University. For resistance training, a within-subjects unilateral study design was utilized. All participants completed a familiarization session with all training protocols occurring before the onset of resistance training. Afterwards, participants performed twice-weekly unilateral leg resistance training over 8 weeks. One leg was randomly assigned to a standard progressive resistance training paradigm (PC). The contralateral leg allocated to a variable resistance training volume protocol (PV). The PV protocol systematically and sequentially modified exercise-related variables at each RT session (see full descriptions of RT protocols below). Before the 8-week training protocol, a single muscle biopsy was obtained from the vastus lateralis, where the leg used for collection was randomized. After the 8-week training period (2 days after the last RT session), bilateral vastus lateralis muscle biopsies were obtained. More details related to testing and training have been previously published [16].

INSERT FIGURE 1 HERE

Resistance training protocol

The PC protocol consisted of 8 sets (4 sets of unilateral leg press followed by 4 sets of unilateral leg extension exercise) of 9–12 repetitions of resistance exercise to concentric failure with two-minute rest intervals between sets. Repetition range was achieved by increasing or decreasing the load between sets as previously described [16]. Each session of the PV protocol involved one of the following RT manipulations: i) variable-load, 8 sets (4 of leg press and 4 of leg extensions) of 25–30 reps to concentric failure/2 minutes between set rest interval; ii) variable-sets, 12 sets (6 of leg press and 6 of leg extension) of 9–12 reps to concentric failure/2 minutes between set rest intervals; iii) variation in contraction type, 8 sets (4 of leg press and 4 of leg extension) of 10 eccentric contractions at 110% of the load used in the LV leg/2 minutes between set rest intervals; and iv) variations in rest periods, 8 sets (4 of leg press and 4 of leg extension) of 9–12 reps to concentric failure/4 minutes between set rest intervals. The accumulated total training volume (sets x repetitions x load) performed by PC and PV legs over the training period was calculated.

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Biopsies of the vastus lateralis were performed using the percutaneous muscle biopsy technique with suction under local anesthesia 2-3 mL of 1% Xylocaine. Approximately 50 mg muscle tissue was dissected free from blood and connective tissue, and muscle tissue samples were immediately placed in cryogenic tubes, frozen in liquid nitrogen, and stored at -80°C until analyses described below. Separate pieces of muscle were preserved slow-frozen in OCT media, sectioned at a thickness of 10 μm using a cryostat, mounted on positively charged microscope slides, and stored at -80°C. Frozen samples and mounted sections were subsequently shipped to

Auburn University on dry ice, and Auburn investigators received samples and stored them at -80°C until analyses described below. Notably, given that a pre-training biopsy was not obtained from both legs, this is an unresolved limitation to the study.

Sarcoplasmic and myofibrillar protein isolations

Isolation of the sarcoplasmic and myofibrillar protein fractions from wet muscle tissue was performed using our recently published "MIST" or "myofibrillar isolation and solubilization technique" [62]. Briefly, 1.7 mL polypropylene tubes were pre-filled with ice-cold buffer (300 µL; Buffer 1: 25 mM Tris, pH 7.2, 0.5% Triton X-100, protease inhibitors) and placed on ice. Skeletal muscle foils were removed from -80 C, placed on a liquid nitrogen-cooled ceramic mortar and pestle, and tissue was pulverized into 2-4 mm^3 pieces. Approximately ~20 mg of crushed tissue was placed in tubes prefilled with buffer (described above), weighed using a scale with a sensitivity of 0.0001 g (Mettler-Toledo; Columbus, OH, USA), 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 (described below), and stored at -80°C until analysis occurred.

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from each biopsy (providing 12.5 µg protein) were assayed in duplicate where samples were added to a mixture composed of 0.125 mol/L Tris–HCl (pH 8.0), 0.03 mmol/L acetyl-CoA, and 0.1 mmol/L DTNB. The reaction was initiated by adding 5 µL of 50 mmol/L oxaloacetate and the absorbance change was recorded for 1 min. The coefficient of variation for all duplicates was 5.7%.

Phalloidin staining

For the determination of myofibril area per fiber, F-actin labelling using Alexa Fluor 488-conjugated (AF488) phalloidin was performed according to previous reports [11, 66, 67]. Briefly, serial sections were air-dried for 10 minutes followed by 10% formalin fixation for 10 minutes. Sections were then washed with PBS for 5 minutes, and blocked with 100% Pierce Super Blocker (Thermo Fisher Scientific) for 25 minutes. After blocking, a pre-diluted commercially-available rabbit anti-dystrophin IgG1 antibody solution (catalog #: GTX15277; Genetex Inc.) and spiked in phalloidin-AF488 (1:35 dilution) was placed on sections in the dark for 30 minutes. Sections were subsequently washed in PBS for 5 minutes and incubated in the dark for 20 minutes with a secondary antibody solution containing Texas Red-conjugated anti-rabbit IgG (catalog #: TI-1000; Vector Laboratories; ~6.6 µL secondary antibody per 1 mL of blocking solution). Sections were washed in PBS for 5 minutes, and air-dried and mounted with fluorescent media containing DAPI (catalog #: GTX16206; Genetex Inc.). Following mounting, digital images were immediately captured with a fluorescent microscope (Nikon Instruments) using a 20x objective. Exposure times were 200 milliseconds for FITC, 800 milliseconds for TRITC imaging, and 100 milliseconds for DAPI imaging. This staining method allowed the identification of the sarcolemma (Texas Red filter), myofibrils (FITC filter), and myonuclei

(DAPI filter). ImageJ (NIH) was used to quantify myofibril area per fiber. Briefly, images were split into RGB channels, and the green channel image was converted to grayscale. The threshold function in imageJ was then used to generate binary black/white images of stained versus unstained portions of fibers. Thereafter, fibers were manually traced using the polygon function, and myofibril areas were provided as a percentage per fiber area. A visual representation of this image analysis is provided in the results section.

Statistical Analysis

Statistical analyses were performed in SPSS (Version 26; IBM SPSS Statistics Software, Chicago, IL, USA). Dependent variables were analyzed using dependent samples t-tests, comparing PRE to either PC or PV. Additionally, PC was compared to PV using dependent samples t-tests. Finally, select associations were performed using Pearson's correlations. Statistical significance for null hypothesis testing was set at $p<0.05$. Data are presented throughout as mean ± standard deviation values.

RESULTS

Total training volume per leg and mean fCSA changes with PC versus PV training

The total training volume for the PC leg was 193,259 ± 39,731 kg. The total training volume for the PV leg was 217,613 ± 43,834 kg. This equated to a 12.6% higher volume when comparing the PV to PC condition ($p<0.001$). Mean fCSA values prior to training was 5994 ±1000 μm^2, whereas mean fCSA following PC was 6770 ± 1000 μm^2 versus 6738 ±1070 μm^2 with PV training. When comparing fCSA values from PRE to PC, there was a significant difference ($p=0.003$). Additionally, when comparing fCSA values from PRE to PV, there was a

significant difference ($p=0.005$). However, when comparing fCSA values from PC to PV there was no difference (0.791).

Sarcoplasmic enzyme proteins

Changes in sarcoplasmic enzyme protein content are presented in Figure 2. Total sarcoplasmic protein content increased significantly from PRE (31.1±3.0 ug/mg of muscle weight) to PC (34.9±3.3 ug/mg of muscle weight, $p = 0.003$) (Fig. 2a). There was also a difference between PC and PV ($p = 0.009$). Additionally, we observed a significant change in HK2 protein levels from PRE to PC ($p < 0.001$) and PV ($p = 0.01$) (Fig. 2c). However, no significant difference was found between training conditions. CKM (Fig. 2b), LDHA (Fig. 2d), PFK (Fig. 2e), or PYGM (Fig. 2f), demonstrated no differences from PRE, or no differences between training conditions.

INSERT FIGURE 2 HERE

Select nutrient transporter protein levels

The nutrient transporter protein content changes are presented in Figure 3. No significant differences were found from PRE to PC or PV for LAT1 (Fig. 3a) and GLUT4 (Fig. 3b).

INSERT FIGURE 3 HERE

Mitochondrial enzyme markers

The protein content of select mitochondrial enzymes are presented in Figure 4. For VLCAD protein levels, PC and PV were lower compared to PRE values, albeit no difference existed between conditions (Fig. 4c). No significant differences were found from PRE to PC or PV for IDH2 (Fig. 4a) or CPT1 (Fig. 4b) protein levels.

INSERT FIGURE 4 HERE

Mitochondrial markers

A surrogate marker of mitochondrial volume (CS activity) and regulators of mitochondrial respiration (complexes I-V) are presented in Figure 5. Citrate synthase activity demonstrated no differences between PRE to PC or PV (Fig 5a). For Western targets only complex V showed any changes in protein value from PRE to PV ($p = 0.04$) (Fig. 5f), albeit no differences were observed between the PC or PV conditions. Complexes I/II/III/IV did not show any significant differences between PRE or post training values (Figs 5b-e).

INSERT FIGURE 5 HERE

Contractile protein markers

Changes in contractile protein markers are presented in Figure 6. MHC protein content (per mg tissue) decreased significantly in both groups from PRE to PC ($p = 0.004$) and PV ($p = 0.002$) (Fig. 6a). However, no differences observed between PC and PV groups for this variable. A significant decrease was also observed in actin protein content (per mg tissue) from PRE training post in the PV group ($P = 0.002$) (Fig. 6b). Interestingly, a decrease in percent area occupied by myofibrils was observed from PRE training values to post training values in the PV condition ($p = 0.039$), but not PC condition (Fig. 6d). Likewise, an increase in percent area occupied by non-contractile space was observed from PRE training values to post training values in the PV condition ($p = 0.039$), but not PC condition (Fig. 6e). Finally, when considering the ratio of space occupied by myofibrils versus the space occupied by non-myofibrils, PV was significantly lower than LC (Fig. 6f).

INSERT FIGURE 6 HERE

Contractile protein correlations with PV training

Given that we observed a decrease in myofibril area with PV training compared to PRE, we were interested in select associations with this variable. No association was observed between the

change in fCSA with PV training versus the PV-induced change in myofibril area (Fig. 7a). However, we observed a significant negative correlation between pre-training myofibril areas within muscle fibers versus the PV-induced change in myofibril area (r = -0.714, p = 0.006).

INSERT FIGURE 7 HERE

DISCUSSION

PC or PV training did not affect the protein content of several sarcoplasmic enzymes (CKM, LDHA, PFK, and PYGM), nutrient transporters (LAT1 and GLUT4), or mitochondrial proteins (COX I, II, III, IV). Additionally, a marker of mitochondrial volume (CS activity) did not significantly change with either training condition. However, we observed that the HK2 protein significantly increased in both PC and PV conditions from PRE. Additionally, the mitochondrial complex V protein decreased following PV training, and the CPT1 protein decreased following both forms of training. Perhaps most interesting is our finding that PV training decreased the intracellular area occupied by myofibrils. Moreover, individuals that began training with more myofibril packing (i.e., less non-contractile spacing) experienced a greater increase in non-myofibril space following PV training. These findings are discussed in greater detail below.

As mentioned above, no changes in CKM, PFK, PYGM, or LDHA protein levels occurred with either form of training, with the exception HK2 protein content, which increased with both forms of training. HK2 phosphorylates glucose-6-phosphate (G6P) in the first stage of glucose metabolism pathway. You could suggest that an increase in HK2 protein content regardless of stimulus, increases the muscles' ability to begin the breakdown of glucose. CKM is the only enzyme involved in the ATP-PCr pathway, PYGM is the rate-limiting enzyme for the breakdown of glycogen, PFK is the rate-limiting enzyme in the glycolytic pathway, and LDHA

catalyzes the interconversion of pyruvate to lactate. Hexokinase is responsible for the phosphorylation of glucose prior to glycolysis [68]. Collectively, these markers were chosen to reflect the ATP-PCr and glycolytic pathways. Recently our lab presented data showing that 6 weeks of high-volume resistance training in previously-trained individuals significantly increased many of these protein targets in the sarcoplasmic fraction [11], whereas 10 weeks of high-load training in a separate cohort of well-trained individuals did not alter these markers [12]. In lieu of these findings, we posited that higher volume resistance training may largely facilitate metabolic adaption, whereas higher load training does not. However, contrary to our hypothesis, this was not the case in the current study. While difficult to reconcile, not observing differences in most of the assayed sarcoplasmic proteins may be related to only a 12.6% difference in training volume being completed between legs. Stated alternatively, the PV leg may have needed to undergo much more training volume in order to observe elevations in the assayed sarcoplasmic proteins. Additionally, given that the subjects had prior resistance training experience, this may have influenced our ability to observe changes in these markers.

Determining how resistance training affects muscle nutrient transporter content is another understudied aspect of exercise physiology. LAT1 transports L-leucine and other essential amino acids into myofibers through a bi-transport system that simultaneously exports glutamine. This transporter is critical for muscle physiology for two reasons. First, leucine has been shown to stimulate the mechanistic target of rapamycin complex 1 (mTORC1) to increase muscle protein synthesis [69]. Second, skeletal muscle can catabolize BCAAs during higher-volume endurance exercise to produce ATP [70]. We hypothesized LAT1 protein levels might increase in the PV-trained leg versus the PC-trained leg in order to increase BCAA transport into muscle cells due to increased energy needs with higher-volume training. However, no differences

between legs were observed. Previous research has reported that the mRNA and protein levels of LAT1 increase in response to one to multiple training bouts [3, 71]. We have also reported that LAT1 protein levels increased in untrained subjects following 12 weeks of resistance training [72]. GLUT4 is the primary glucose transport protein expressed in skeletal muscle, and a plethora of animal and human data exist suggesting that endurance training increases the protein levels of this target [46]. Notwithstanding, the available data is limited regarding how resistance training affects this marker. Several studies dealing with special populations have examined the effects of resistance training and have observed large increases in GLUT4 protein expression [73, 74]. However, no changes were observed in this marker following PV and PC training. With the collective evidence in mind, it is possible that the modest differences between total volume-load and/or the prior training of the current participants status was likely responsible for our observations.

The effects that resistance training have on mitochondrial adaptations is still not well understood. We assayed three mitochondrial enzyme markers involved with substrate oxidation (IDH2, CPT1, and VLCAD) as well as the five protein complexes of the electron transport chain. Moreover, we examined citrate synthase activity levels, which is a surrogate marker of mitochondrial volume. Isocitrate dehydrogenase 2 (IDH2) is an enzyme that catabolizes the oxidative decarboxylation of isocitrate within the TCA cycle. Both legs experienced no changes in IDH2 protein expression. Carnitine palmitoyltransferase 1 (CPT1), which regulates the β-oxidation of fatty acids in mitochondria, is believed to play an important role in the fatty acid oxidation [75]. Again, neither form of training altered CPT1 protein expression. Very long chain Acyl CoA dehydrogenase (VLCAD) protein catalyzes the first step in beta-oxidation. Interestingly, both forms of training decreased VLCAD protein levels from PRE. These

decreases in VLCAD protein expression could be due in large to a shift from aerobic metabolism within the cell to glycolytic metabolism. To our knowledge, this marker has yet to be measured in skeletal muscle following periods of resistance training, and our findings indicate that resistance training (regardless of volume-load) may slightly affect the ability of skeletal muscle to undergo beta-oxidation. However, given that only one marker in this pathway was interrogated, more data are needed to validate this hypothesis. Interestingly, neither form of training affected 4 of the 5 complex proteins or citrate synthase activity levels. Indeed, there are disparate reports regarding how mitochondrial volume is affected with resistance training [49]. Various training studies have either shown that: i) mitochondrial volume increases in proportion with hypertrophy [76-78], ii) a dilution in mitochondrial volume occurs with training [7, 11, 79-81], or iii) increases in mitochondrial volume occurred more rapidly than increases in myofiber growth [9]. Given that mean fCSA values similarly increased in the PC and PV legs, our data agree with i) above. However, the general disagreement in findings between studies, while likely related to differences in study design/duration and participant characteristics, warrants the need for more research in this area using innovative techniques. To this end, analyzing muscle biopsies from resistance training studies using newer three-dimensional electron microscopy methods along with methods that monitor intricate substrate fluxes from mitochondrial isolates will undoubtedly yield much more insight as to how different resistance training protocols spatially and functionally affect the mitochondria [82].

A unique aim of this study was to compare how PC versus PV training affected the concentrations of major contractile proteins as well as myofibril spacing. This exploration 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

higher-volume resistance training [14]. Some studies have suggested that sarcoplasmic hypertrophy occurs with months to years of resistance training [59, 60, 83]. More recently, our laboratory has reported increases in sarcoplasmic protein concentrations with concomitant decreases in myosin heavy chain and actin protein abundances following 6 weeks of extremely high-volume resistance training in previously-trained college-aged men [11]. In the current study, we observed a decrease in the relative myosin heavy chain actin abundances following both forms of training, we also observed decreases in myofibril area following PV training. Suggesting that higher-volume training could potentially lead to an increase in the sarcoplasm surrounding the myofibril to spacetially and bioenergetically prime the cell for future myofibril growth. Finally, we observed that individuals who started training with a greater density of myofibrils typically experienced an increase in non-contractile protein space following PV training. All of these findings indicate that both forms of training, and in particular PV training given the histology data, may indeed lead to myofiber hypertrophy via a more rapid expansion of non-myofibril components. When considering the findings of Haun et al. [11], this is the second time we have observed this phenomenon. Mechanistically explaining these findings is difficult given that we did not obtain time-course data from each leg throughout the study. However, when considering studies that have observed features of sarcoplasmic hypertrophy, certain themes emerge. First, participants in the current study, participants from Haun et al. [11], and participants from MacDougall et al. [79] were all well-trained. Second, we are only confident in defending the occurrence of sarcoplasmic hypertrophy in the PV-trained leg given the cumulative SDS-PAGE and histology data, and again, the PV leg performed more volume compared to the PC leg. Hence, these findings suggest the possibility that, in well-trained individuals, unaccustomed training volume may elicit muscle fiber growth through the expansion

of non-contractile elements. While intriguing, more research still needs to be conducted in order to address the following questions: i) what is the purpose of cellular growth through the expansion of non-myofibril components? ii) is this form of hypertrophy transient and related to edema or fluid shifts from the extracellular environment, or rather, does this form of hypertrophy serve to spatially prime muscle cells for the eventual accretion of myofibrils and expansion of non-contractile components (e.g., the sarcoplasmic and mitochondrial reticula)? Finally, it has become recently apparent that myofibrils exist in a lattice-like network rather than parallel structures [84]. Thus, observations of increased non-myofibril spacing may simply be reflective of an enhanced rate of matrix remodeling rather than sarcoplasmic hypertrophy. Again, time-course studies that compare different volume loads between legs in trained versus untrained individuals will be critical in furthering our knowledge in this area. Additionally, the utilization of innovative techniques, such as three-dimensional electron microscopy, will be critical in this regard.

Conclusions

This study continues to expand upon the current knowledge regarding how the variation in resistance training volume-loads affect markers of metabolic and mitochondrial adaptations as well as contractile protein alterations. While the 12.6% difference in volume-load between legs is a limitation, it is notable that interesting and novel observations were made herein, especially in relation to myofibril spacing between training protocols. As posited in a recent review [85], performing similar resistance training studies while utilizing more advanced microscopy and biochemical techniques will continue to further our knowledge in relation to how different forms of training affect the molecular and metabolic milieu of skeletal muscle.

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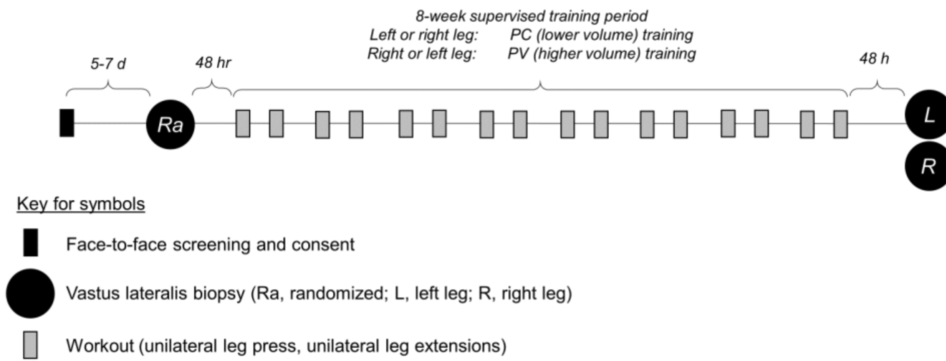
CONFLICTS OF INTEREST

M.D.R. and K.C.Y. receive laboratory funding from various industry sources in the form of fixed-priced contracts or laboratory gifts. M.D.R. has also been financially compensated from various industry entities for consultation work regarding scientific presentations and/or various scientific writing endeavors in accordance with Auburn University's Research Compliance and Ethics Guidelines. In relation to the current data, however, the authors declare that no conflicts of interest exist.

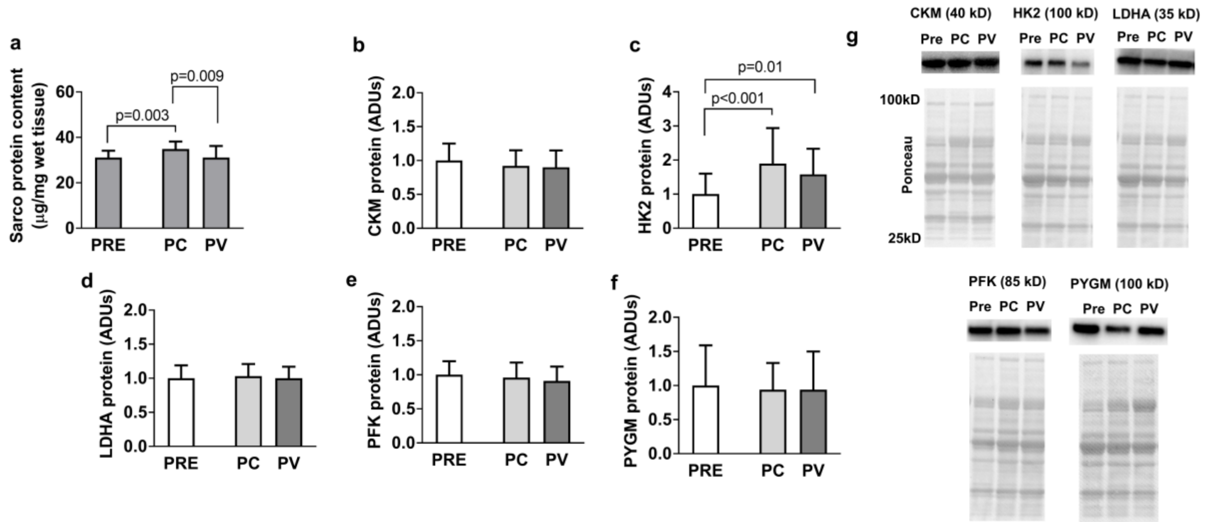
FIGURES AND LEGENDS

Figure 1. Study design



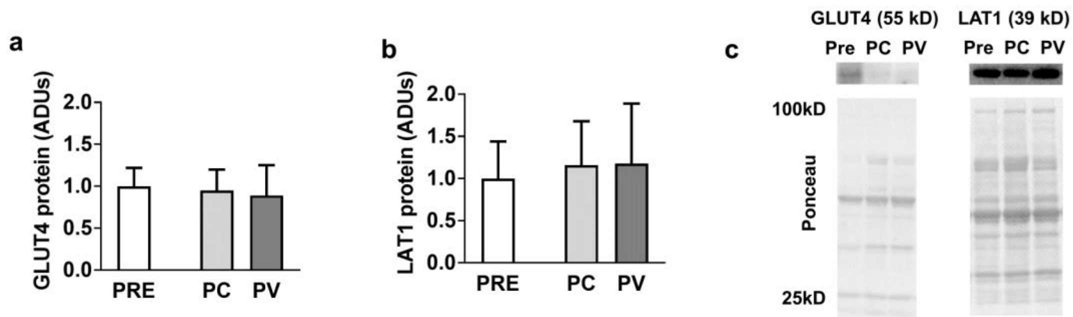
Legend: This shows the study design for the 8-week unilateral leg training paradigm

Figure 2. Alterations in select sarcoplasmic enzymes with PC versus PV training



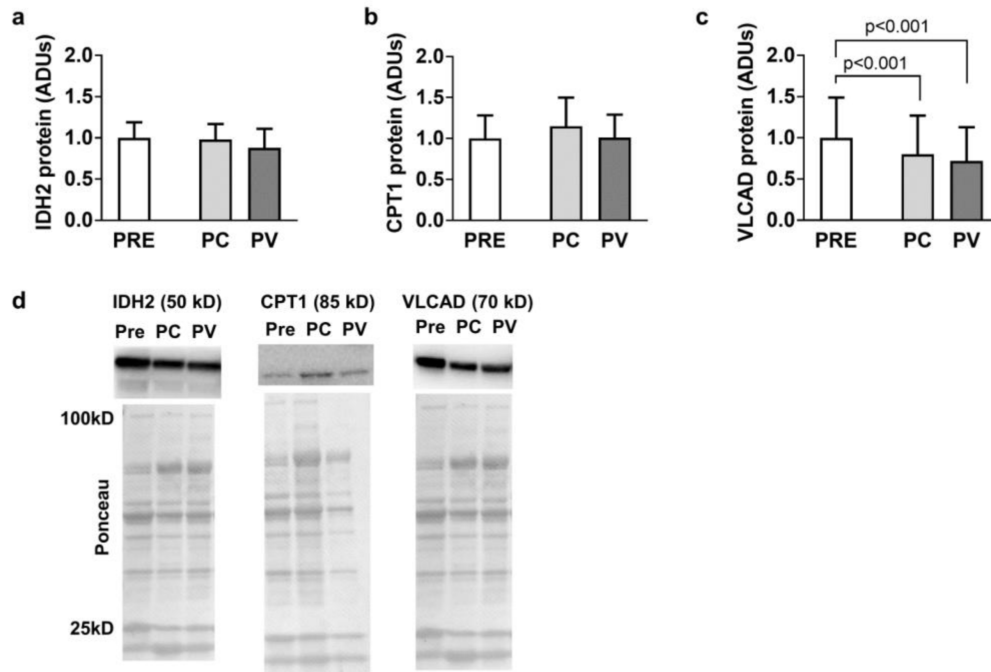
Legend: Data presented include sarcoplasmic protein concentrations from biopsied muscle (panel a), creatine kinase, M-type (CKM) protein levels from the sarcoplasmic protein pool (panel b), hexokinase 2 (HK2) protein levels from the sarcoplasmic protein pool (panel c), lactate dehydrogenase A (LDHA) protein levels from the sarcoplasmic protein pool (panel d), phosphofructokinase (PFK) protein levels from the sarcoplasmic protein pool (panel e), and glycogen phosphorylase (PYGM) protein levels from the sarcoplasmic protein pool (panel f). Data are presented as means with standard deviation bars. Panel g contains representative Western blot images for each target.

Figure 3. Alterations in select nutrient transporters with PC versus PV training



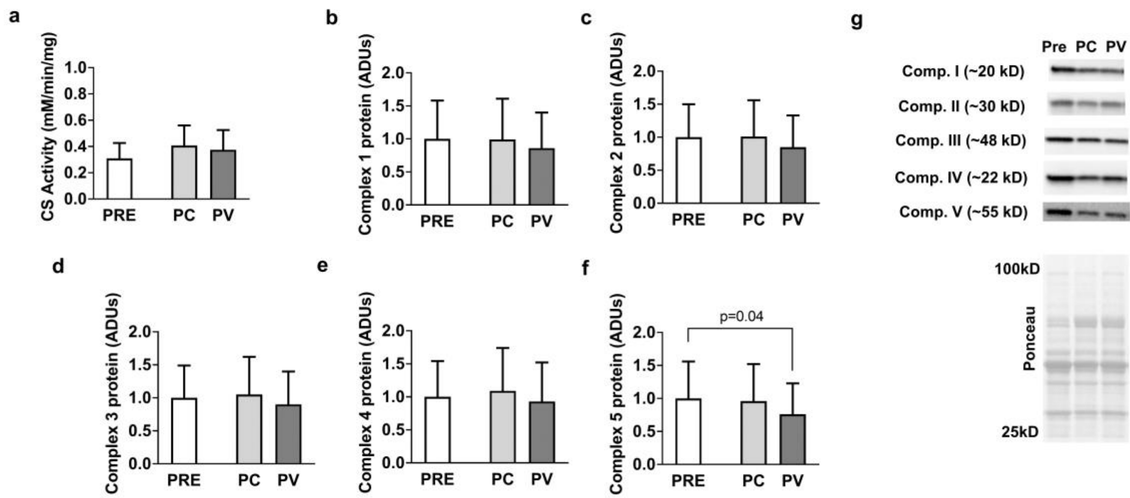
Legend: Data presented include glucose transporter 4 (GLUT4) protein levels from the sarcoplasmic protein pool (panel a), and L-Type Amino Acid Transporter 1 (LAT1) protein levels from the sarcoplasmic protein pool (panel b). Data are presented as means with standard deviation bars. Panel c contains representative Western blot images for each target.

Figure 4. Alterations in select mitochondrial enzymes with PC versus PV training



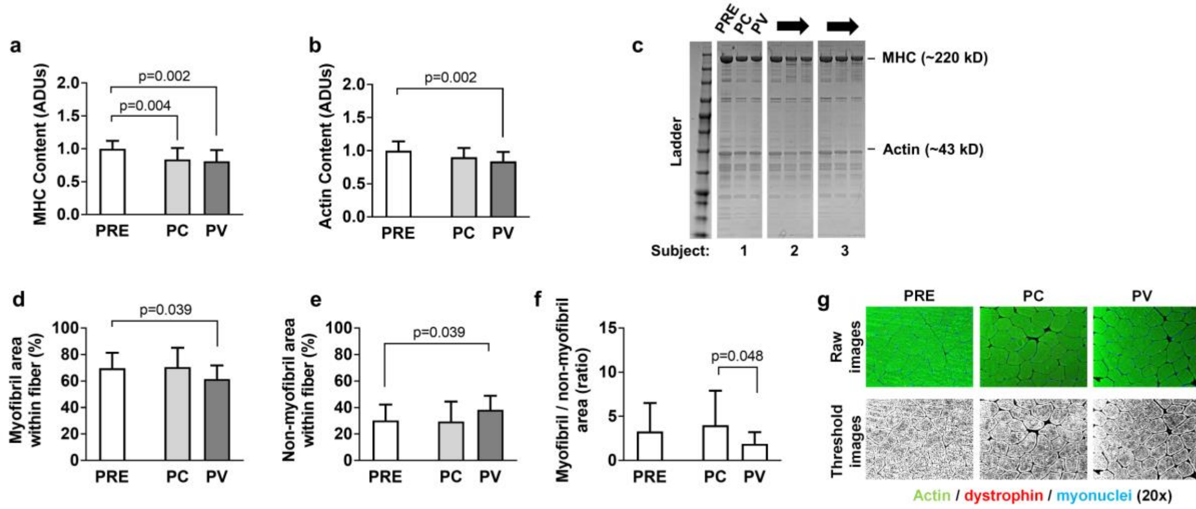
Legend: Data presented include isocitrate dehydrogenase 2 (IDH2) protein levels from the sarcoplasmic protein pool (panel a), carnitine palmitoyltransferase 1 (CPT1) protein levels from the sarcoplasmic protein pool (panel b), and very long-chain specific acyl-CoA dehydrogenase protein levels from the sarcoplasmic protein pool (panel c). Data are presented as means with standard deviation bars. Panel d contains representative Western blot images for each target.

Figure 5. Alterations in citrate synthase activity levels and mitochondrial electron transport chain proteins with PC versus PV training



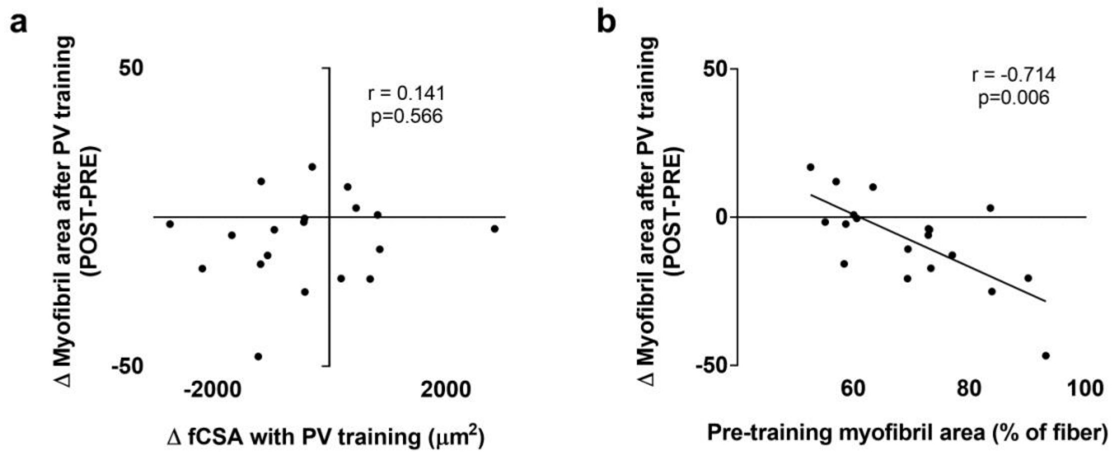
Legend: Data presented include citrate synthase (CS) activity levels from the sarcoplasmic protein pool (panel a), and the protein levels of complexes 1-5 from the sarcoplasmic protein pool (panels b-f). Data are presented as means with standard deviation bars. Panel g contains representative Western blot images for each target.

Figure 6. Alterations in contractile protein markers with PC versus PV training



Legend: Data presented include the relative abundances of myosin heavy (MHC) and actin protein levels from the myofibril protein pool (panels a and b) as determined by SDS-PAGE and Coomassie staining. Data in panels d-f were derived from phalloidin staining and image analysis. Data are presented as means with standard deviation bars. Panels c and g contain representative images of SDS-PAGE and phallodin staining, respectively.

Figure 7. Select contractile protein marker correlations with PV training



Legend: The first association is the pre-to-post training change in mean fCSA with PV training versus the change in myofibril area (panel a); no significant association was evident. The second is the pre-training myofibril area versus the change in myofibril area with PV training (panel b); a significant negative association was evident.

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