Molecular predictors of the hypertrophy response to resistance training in young untrained female adults

by

Morgan A. Smith

A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy in Kinesiology

Auburn, Alabama
August 6, 2022

Keywords: female, resistance training, ribosome biogenesis, muscle protein synthesis, satellite cells

Approved by

Michael Roberts, PhD, Chair, Associate Professor of Kinesiology
L. Bruce Gladden, PhD, Professor of Kinesiology
Austin Robinson, PhD, Assistant Professor of Kinesiology
Karyn Esser, PhD, Professor, Department of Physiology and Functional Genomics
Kaelin Young, PhD, Associate Professor of Cell Biology and Physiology (VCOM)

ABSTRACT

We determined if the myofibrillar protein synthetic (MyoPS) response to a naïve resistance exercise (RE) bout, or chronic changes in satellite cell number and muscle ribosome content, varied in females that classified as higher (HR) or lower (LR) responders following resistance training (RT). Thirty-four untrained college-aged females completed a 10-week full-body RT protocol (twice weekly). Body composition, right leg muscle imaging, a right leg vastus lateralis biopsy, and strength testing occurred prior to and following the intervention. A composite pre-to-post change score consisting of four variables was used to define HR (n=8) and LR (n=8): i) whole body lean/soft tissue mass (LSTM), ii) VL muscle cross-sectional area (CSA), iii) mid-thigh muscle cross-sectional area (mCSA), and iv) maximal deadlift strength. For all participants, training increased LSTM (+1.1±1.1 kg, $p<0.001$), VL CSA (+2.7±2.7 cm^2, $p<0.001$), mid-thigh mCSA (+8.9±7.0 cm^2, $p<0.001$), deadlift strength (24±13 kg, $p<0.001$), mean muscle fiber cross-sectional area (+759±1198 μm^2, $p=0.001$), satellite cell number (+0.026±0.061 cells/fiber, $p=0.025$), and myonuclear number (+0.29±0.37 nuclei/fiber, $p=0.013$). HR exhibited superior training responses to LR in LSTM (2.2±0.7 kg versus 0.3±0.6 kg, respectively, $p < 0.001$), VL CSA (5.9±2.5 cm^2 versus 0.6±0.8 cm^2, $p<0.001$), mid-thigh mCSA (16.0±6.2 cm^2 versus 3.2±2.9 cm^2, $p<0.001$), and deadlift strength (37±15 kg versus 13±7 kg, $p=0.001$). The 24-hour MyoPS response to the first RE bout was not different between HR and LR ($p=0.367$). A significant group*time interaction was found for satellite cell number after RT ($p=0.025$), but not muscle ribosome content ($p=0.888$). Specifically, satellite cell number increased in HR ($p=0.026$), but not LR ($p=0.628$). Pre-training values multiple variables were also greater in HR versus LR: i) LSTM (44.2±3.3 kg versus 39.2±3.5 kg, $p=0.010$), ii) VL CSA (20.1±2.2 cm^2 versus 17.6±1.9 cm^2, $p<0.028$), and iii) mid-thigh mCSA (117±8 cm^2 versus 99 ±

6 cm^2, $p<0.001$). Fat mass prior to RT, sleep duration, energy intake, body mass-normalized training volume, and menstrual cycle days at pre- and post-data collections were not significantly different between response groups. These data suggest that untrained female participants with an enhanced satellite cell response to RT, and more muscle mass prior to RT, exhibit more favorable RT adaptations.

Key words: females; resistance training; hypertrophy; satellite cells; myofibrillar protein synthesis; ribosomes

ACKNOWLEDGEMENTS

I am very grateful to be awarded the opportunity to receive my doctoral degree, and I am even more grateful for the army that was behind me in the process.

Dr. Roberts, you took a chance on me, the first student to want to go industry off the bat. You made sure I was caught up to speed on all the techniques I needed to learn, while also giving me the freedom to work independently. Your guidance and support have been crucial during my process. To my committee, thank you for challenging me and molding me into the scientist I am today.

Karyn, you essentially set me on my current path and have mentored and supported me the entire way. What once started as a summer job in your lab (because I was hurting for cash), turned into a mentor-mentee, trainer-trainee, friend to friend relationship and I am very appreciative for all that you have done for me thus far.

To Krissy, my co-coordinator turned great friend – thank you. Thank you for helping me in ensuring everything was going smoothly and always being available when I needed your support. Bradley, my lab bestie, thank you for always saying 'yes' when I needed help and assisting me in all my wet lab analyses. Dr. Vann – although you weren't present for my study, you had a huge impact on me as a scientist and as a coach.

Next, my team, my homies, my family. Thank you for keeping me sane, motivated, and recharged. In times of burnout or when my eyes were glossed over from tirelessly working, y'all made sure to check in on me, kept me fed, and made sure I had fun during this time.

And finally, to my greatest support system, Mo – we look back on the days in my first year of the program where I said, "this isn't that bad" and you said "yeah, just wait", and we laugh. Now I can say that you were right, and that I made it.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

1-RM	one-repetition maximum
^{2}H	deuterium
3-RM	three-repetition maximum
CSA	cross-sectional rea
D_2O	deuterium oxide
DEA	diethylamine
DNA	deoxyribonucleic acid
DXA	dual x-ray absorptiometry
fCSA	myofiber cross-sectional area
FM	fat mass
GLI3	GLI Family Zinc Finger 3
GWA	genome wide association
HR	high responder
HRT	hormone replacement therapy
ICC $_{3,1}$	intraclass correlation coefficients $_{3,1}$
LR	low responder
LSTM	lean/soft tissue mass
mCSA	muscle cross-sectional area
MD	minimal difference to be considered real
MHC	myosin heavy chain
MHC IIx	type IIx fibers

MHC IIa	type IIa fibers
MPB	muscle protein breakdown
MPS	muscle protein synthesis
mRNA	messenger RNA
MyoPS	myofibrillar protein synthesis/synthetic response
NADH	nicotinamide adenine dinucleotide
NAP	N-acetyl-n-propyl
NDSR	nutrition data system for research
Pax7	paired box7
PBS	phosphate buffer solution
Pol I	RNA polymerase I
Pol II	RNA polymerase II
Pol III	RNA polymerase III
pQCT	peripheral quantitative computed tomography
rDNA	ribosomal DNA
RE	resistance exercise
RNA	ribonucleic acid
RPE	rate of perceived exertion
rRNA	ribosomal RNA
RT	resistance training
SDH	succinate dehydrogenase
SEM	standard error of measurement
SNP	single nucleotide polymorphism

USG	urine specific gravity
VL	vastus lateralis

CHAPTER I

INTRODUCTION

Resistance training (RT) adaptations have been studied over decades, mainly in young adult males [1, 2]. Female participants are significantly under-represented in the sport science literature. In this regard, a 2014 study examined three major sport science journals (Medicine and Science in Sports and Exercise, British Journal of Sports Medicine, and American Journal of Sports Medicine) from 2011-2013, and found 39% of study participants were female, but only 4-13% of studies contained participant groups that were females only [3]. While advances have been made in the field, younger adult males do not appropriately represent how other populations will adapt to exercise (e.g., younger females, older individuals, older males).

Two RT adaptations that have been well-documented include strength [4-6] and muscle growth (or hypertrophy) [7, 8]. However, while it is generally presumed that all participants will show improvements in these variables, there are various lines of evidence to show that "lower" and "higher" responders exist regarding strength [9] and hypertrophy outcomes [10-14]. Being a lower hypertrophic responder could be a potential barrier to long-term training adherence, given that one of the chief motivations for a novice trainee is to gain skeletal muscle mass [15]. Moreover, although lower hypertrophic responders can experience significant strength gains during training [16, 17], there is some evidence suggesting lower hypertrophic responders to 12 weeks of RT may have impaired strength gains [16]. Thus, deciphering variables that differentiate higher versus lower hypertrophic responders to RT is warranted, particularly in females.

Several studies, some from our laboratory, have attempted to elucidate molecular variables that predict the hypertrophic response to RT. Collectively, these studies have shown

that three molecular variables are predictive of hypertrophy, and these variables include: i) the ribosome biogenesis response to weeks of training, ii) the myofibrillar protein response days to weeks into training, and iii) the satellite cell response days to weeks into training. While insightful, there are some nuances and limitations in the existing literature on molecular variables being predictive of hypertrophic response to RT. Specifically, there is evidence to support [18-25], and refute [7, 26] ribosome biogenesis and myofibrillar protein synthetic responses as being predictive of muscle hypertrophy. Additionally, although there are reports suggesting satellite cell proliferation and/or myonuclear accretion with training predicts hypertrophic responses [16, 27-31], there are conflicting data [32-34]. Second, the data generated in this area is varied in that some studies have used trained [5, 35-40] and untrained participants [10, 14, 24, 25, 41-43], which can have an impact on the level of adaptation seen and makes it difficult to compare findings. Third, training interventions have ranged from 4-16 weeks and there could be temporal variability pertaining to the molecular adaptations within this range of duration. Fourth, criterion variables and statistics used to delineate responders versus non-responders have vastly differed between studies, as well as what 'responder' refers to (e.g., hypertrophic, strength, etc.). For example, some papers have used a single outcome variable to define the hypertrophic response (e.g. mean myofiber cross-sectional area (fCSA) [14], or ultrasound-determined muscle thickness [25, 44, 45]), whereas others have adopted a composite score using a multifactorial approach to delineate the hypertrophic response using different variables [14, 36, 46]. Finally, most of these data are in males, and we cannot assume that males and females adapt in the same way or via similar mechanisms. A landmark study in this area by Bamman and colleagues was performed in younger and older males and females who underwent 16 weeks of RT [17]. However, the authors noted that younger females generally did not exhibit hypertrophy as

determined through mean fCSA. Thus, the current data on molecular variables that predict the hypertrophic response in females is limited.

Given the collective evidence presented above, it is currently unknown if ribosome biogenesis, myofibrillar protein synthetic responses, and satellite cell response to RT are predictive of muscle hypertrophy in adult females. Therefore, the primary aims of this study were threefold: i) examine chronic responses of ribosome biogenesis and satellite cell proliferation and possible predictive ability to hypertrophy, ii) examine predictive ability of 24-hour myofibrillar protein synthesis response post a single bout of RT to long-term hypertrophy, and iii) determine whether these three variables differentiated higher versus lower responders following 10 weeks of RT in previously untrained, younger adult females. Herein, 34 females completed a 10-week supervised RT protocol (twice weekly). A composite pre-to-post change score consisting of the following variables was used to define 'lower' versus 'higher' responders: i) whole body lean/soft tissue mass determined by dual x-ray absorptiometry (DXA), ii) vastus lateralis (VL) cross-sectional area (CSA) determined by ultrasound, iii) mid-thigh muscle CSA determined by peripheral quantitative computed tomography (pQCT), and iv) maximum deadlift strength determined by a three-repetition maximum test (3RM). Crucially, both strength and hypertrophy measures were included in the multidimensional composite score to be more inclusive in capturing various levels of response and not limiting the assessment to strength or hypertrophy exclusively. Therefore, 'responder' refers specifically to the sum of the composite score variables used in the present study. Based on the resultant composite scores, we identified quartiles of n=8 higher and n=8 lower responders.

Our hypotheses are as follows: i) responses in ribosome biogenesis and satellite cell proliferation will correlate with hypertrophy (the greater response correlated with greater

hypertrophy), ii) the 24-hour MyoPS response will be predictive of the hypertrophic response, and iii) response variation will occur, and higher and lower responders will be evident via the composite score. In addition to the responder analysis, we also performed correlations for all participants between outcomes representative of hypertrophy (e.g., change in VL CSA and mean fiber CSA (fCSA)), changes in ribosome biogenesis, myofibrillar protein synthetic, and satellite cell responses.

CHAPTER II

REVIEW OF LITERATURE

This literature review is divided into multiple sections. First, it will briefly explain general RT adaptations. Next, an overview of the three main variables of interest (ribosome biogenesis, muscle protein synthesis, and satellite cells) will be discussed in relation to each variable's contribution to muscle hypertrophy. Literature examining lower and higher responders to RT will then be outlined, and a discussion of the biological sex gap in exercise physiology research (i.e., lack of representation of females) will follow. While not an emphasis in the current study, the effects of contraceptives on RT adaptations will be briefly explained, as well as exercise adherence and its potential relationship to the responder analysis. Finally, I will propose the purpose of the present study, provide a brief overview of the study design, and propose my hypotheses.

To preface, it is important to note the difference between gender-based comparisons (e.g., man versus woman) and sex-based comparisons (e.g., male versus female). Dichotomizing both gender and sex is not completely inclusive. However, I will define all results in terms of sex because the adaptations being compared are referring to biology instead of gender. For the present study, we collected both gender and sex data from participants, and all female participants also identified as women.

General Resistance Training Adaptations

Skeletal muscle adaptations to RT have been well documented for over 50 years [47], and chief adaptations include increases in strength [4-6] and skeletal muscle hypertrophy [44, 48]. A review by the American College of Sports Medicine, which included more than 100 strength

program studies, documented that in response to months of training, on average, participant's muscular strength was improved in the untrained by 40%, moderately trained by 20%, trained by 16%, advanced by 10%, and elite participants by 2% [49]. Muscle hypertrophy has been measured in various ways, including assessments of: i) muscle cross-sectional area (mCSA) at the mid-thigh via magnetic resonance imaging (MRI) [7, 8], ii) mCSA at the mid-thigh via peripheral quantitative computed tomography (pQCT) [50], iii) dual-energy x-ray absorptiometry (DXA) for whole body or appendicular lean tissue mass assessments [14, 21, 51], and iv) measuring vastus lateralis (VL) thickness or CSA via ultrasound [25, 44, 52-54].

It is generally accepted that neural adaptations ensue rapidly within the first few weeks of RT, and these include enhanced motor unit recruitment and firing frequency of agonist muscles [55, 56]. These neural adaptations largely drive observed increases in strength. Although increases in muscle size, muscle strength, and neural adaptations are all adaptations to RT, they do not necessarily correlate well with one another. For instance, Ahtiainen et al. [9] demonstrated a low association between muscle size and strength, alluding to the important role of neural adaptions in mediating maximum strength.

Weeks to months into RT, there is generally a shift in muscle fiber type [57]. Although the nuanced classification of muscle fiber type is beyond the scope of this literature review, it should be noted that various histological methods have been used including nicotinamide adenine dinucleotide (NADH) staining, succinate dehydrogenase (SDH) staining, and staining for the predominant myosin heavy chain (MHC) isotype [57]. Herein, we will differentiate fiber type based on the latter of these techniques. With RT, we typically see a shift from MHC IIx (type IIx) to MHC IIa (type IIa) in both males and females [17, 58], meaning the fibers are transitioning from highly fatigable to fibers that are more resistant to fatigue.

Additionally, type IIx fCSA increases over time in response to chronic RT [17], whereas type I fCSA may or may not increase [57]. In this regard, significant hypertrophy of all major muscle fiber types have been seen in the VL in untrained females following six weeks of RT [43]. However, in males, more robust increases in type II fibers seemingly occur [35, 59].

Although sex differences do exist between males and females for certain phenotypes (e.g., lean body mass, percentage body fat, absolute strength), these differences do not seemingly contribute to sex-differences in RT adaptations [6, 58, 60, 61]. A recent meta-analysis by Roberts et al. [62], which included 63 training studies, suggests there are no differences in hypertrophy or strength gains between male and female response to RT. Moreover, these authors suggest that females may gain relative strength more rapidly compared to men [62].

Ribosome Biogenesis

Myofiber size is, in part, determined by of the ability of ribosomes to facilitate muscle protein synthesis. It has been well-documented that RT increases muscle protein synthesis (MPS) rates above homeostatic levels [22, 39, 63, 64]. Importantly, the number of ribosomes and their activity are critical in determining protein synthesis rates, as ribosomes are the sole macromolecules in muscle cells (also referred to as myofibers) that catalyze this process. An increase in ribosome number, or translational capacity (increased ability to catalyze proteins), increases the ability of myofibers to facilitate protein synthesis.

Several recent reviews have been published detailing the process of ribosome biogenesis in skeletal muscle, and the following description of the process has been adapted from these reviews [65-67]. Ribosome biogenesis occurs almost exclusively in the nucleolar portion of myonuclei and requires participation of all three RNA polymerases (Pol I, Pol II, Pol III). First,

Pol I transcribes ribosomal DNA (rDNA) to form the 47S pre-RNA transcript. This transcript is processed into the 18S, 5.8S, and 28S ribosomal RNAs (rRNAs) by cleavage enzymes. Pol III transcribes 5S rDNA, and the resultant transcript is the fourth rRNA needed to assemble a mature 80S ribosome. Aside from containing rRNA, the mature ribosome is comprised of nearly 80 ribosomal proteins encoded by messenger RNA (mRNA), and these are transcribed by Pol II.

Typically, translational capacity is assessed by assaying total RNA per milligram of wet tissue as a surrogate measure for muscle ribosome content. Considering 85% of total RNA is rRNA, and two thirds of a ribosome is rRNA, an increase in total RNA per milligram of wet tissue demonstrates an increase in rRNA concentrations and inherently, ribosome biogenesis.

Translational efficiency, or the amount of protein synthesis per unit RNA via existing ribosomes, has also been hypothesized to play a critical role in muscle hypertrophy. Although translational efficiency can be modulated through initiation, elongation, and termination, initiation is the main point of control in mammals [68] and is the rate limiting step of the regulation of translational efficiency [69]. However, a more recent emphasis has been placed on the role that enhanced translational capacity has on facilitating muscle hypertrophy in response to RT [65, 69].

Some of the first papers to demonstrate that ribosome biogenesis contributes to skeletal muscle hypertrophy in rodents were conducted by Hamosch et al. in 1967 [70] and Goldberg et al. [71] the same year. Since these landmark investigations, several studies have shown ribosome biogenesis coincides with muscle hypertrophy following chronic RT in both animal models [23, 72-74] and humans [18, 58, 75]. For example, Nakada et al. [23] demonstrated plantaris 18+28S rRNA content increased following synergist ablation in male rats, and rRNA content strongly correlated with muscle weight. Collectively, these data support the notion that the magnitude of

ribosome biogenesis may be a molecular adaptation that coincides with, and could potentially predict skeletal muscle hypertrophy during periods of RT. Moreover, in line with general training adaptations, there may not be biological sex differences in this response. In this regard, Figueiredo et al. [75] reported that the observed upregulation of ribosome biogenesis was not different between male and females 24 hours post an acute bout of RT.

Muscle Protein Synthesis

It is widely accepted that in fully mature mammals, a positive protein balance is a chief mechanism that facilitates muscle hypertrophy from chronic RT. This is accomplished through routine feeding and exercise, where both summed phenomena stimulate a muscle protein synthetic response that is appreciably greater than muscle protein breakdown (MPB) [20]. Though MPB has been examined in this relationship, it is broadly believed that muscle protein synthesis rates are much more adaptable and responsive to feeding and exercise. Thus, muscle protein synthesis likely has a greater impact on net protein balance and has therefore received the greatest attention [20, 76]. Overall, MPS rates under homeostatic conditions [77, 78] and following single RE bouts [79] appear to be similar between males and females.

Several laboratories have used delicate tissue separation and tracer techniques to interrogate mixed, myofibrillar, and sarcoplasmic protein synthesis rates to feeding and exercise paradigms [39, 63, 64, 80-88]. This review, however, focuses on myofibrillar protein synthesis (MyoPS), given that several studies have shown rates to increase following one or multiple exercise bouts [82, 87, 89-93]. MyoPS rates are assessed in humans using either infused or ingested stable isotope tracers that contain amino acids with carbon, nitrogen, or hydrogen stable isotopes. With infusion studies, the tracer is administered hours prior to, during, and following a single bout

of RE, and the amino acid tracer is integrated into muscle proteins during the protein synthesis process. Enrichment levels can then be measured by processing muscle tissue samples and monitoring the presence of the tracer using mass spectrometry-based methods [77]. However, a major drawback with infused tracers, is that they require intravenous cannulations, which restricts studies to a shorter time frame (e.g., 24 hours or less).

A relatively newer method, and one that our laboratory has utilized, is the quantification of MyoPS through orally ingested deuterium oxide (D_2O) [94, 95]. Repeated D_2O ingestion allows for the determination of protein synthesis over longer periods (i.e., days to months) [94, 96, 97]. Wilkinson et al. [94] published the first study to demonstrate the utility of this method over a period not feasible with infused tracers (8 days), illustrating a key advantage to using the D_2O method. Importantly, synthesis rates are termed "integrated rates" given the longer relative time frame of measurement.

Several RT studies have shown increases in the integrated MyoPS response 1-24 hours post a single bout of RE [22, 26, 80, 98], but few have investigated the relationship between that response and skeletal muscle hypertrophy after long term RT. While tracer studies are informative, low associations between the muscle protein synthetic response to a bout of RE and chronic muscle mass changes have been reported [7, 99]. For example, Mitchell et al. [7] demonstrated that the MPS response 6 hours after the first bout of exercise did not correlate with muscle hypertrophy after 16 weeks of RT in untrained males. Moreover, Damas et al. [99] demonstrated that the MyoPS response to a naïve bout of RE is not associated with hypertrophy. However, a recent review has highlighted these discrepancies and suggests that the relationship between these variables, while not perfect, are similar in directionality [100].

Satellite Cells

Within multinucleated skeletal muscle fibers, the cytoplasmic area regulated by one myonuclei is defined as the myonuclear domain. There is great debate whether the myonuclear domain is flexible or rigid. This theory was first put forth by Cheek et al. [101], with the idea that the number of myonuclei per fiber must increase to maintain a constant myonuclear domain during periods of muscle fiber hypertrophy. In short, the domain is a fixed volume that cannot change. However, if the myonuclear domain is flexible, then it can increase in volume during muscle fiber hypertrophy. Because mature mammalian myofibers are differentiated and cannot undergo mitosis, new myonuclei must come from an external source, which is achieved via satellite cell-mediated myonuclear accretion (increase in myonuclei).

Satellite cells are distinctively defined by their close proximity to skeletal muscle fibers, positioned between the plasma membrane and basement membrane. In addition to location, satellite cells are also identified by the expression of several genes, including paired box 7 (Pax7), a common target gene in experimental models to identify satellite cells [28]. Certain stressors, like exercise, stimulate satellite cell proliferation, differentiation, and fusion to pre-existing myofibers [102, 103]. The ultimate outcome of this process yields myofibers with more myonuclei, via satellite cell-mediated myonuclear accretion. Critically, a recent rodent study has shown that an increase in satellite cell–mediated myonuclear addition during a period of resisted wheel running provides cellular support for increased ribosome production [104], and this increases translational capacity.

Mauro published the first paper in muscle demonstrating the existence of satellite cells, specifically in the tibialis anticus muscle of the frog, and suggested they might be important for skeletal muscle regeneration [105]. Similar cells were discovered around that research era in the

sartorius and ileofibularis muscles of the frog and the sartorius and tongue muscle of laboratory rats [106]. Mauro suggested that because these cell types were found in two vertebrates, it is likely the case these cell types exist in all vertebrates [105].

Since these foundational studies, it has been widely advocated that the expansion of the myonuclear domain during periods of RT necessitates the addition of myonuclei via satellite cells, and that this mechanism increases the size potential of muscle fibers [29, 107]. Indeed, elegant rodent studies have supported this construct through various techniques. Historically, researchers have used γ-irridation or other chemical agents to knock out satellite cells, and such methods have been used to demonstrate satellite cell proliferation as a prerequisite for muscle hypertrophy [30, 108]. However, these methods are problematic due to the lack of cellular specificity of targeting satellite cells [33]. Alternatively stated, the radiation used likely affects other resident mitotic cells in muscle tissue and can lead to non-specific effects. The Pax7-DTA mouse has revolutionized this area of muscle biology. Upon administering tamoxifen, a robust depletion of satellite cells (~90%) can occur, and an abrogation can be seen with muscle regeneration, maturation, and overload-induced hypertrophy [109-112]. Moreover, there are presumably less unwanted side effects using the Pax7-DTA model. However, a common criticism of this model is the implied limitations of it being a rodent model.

Multiple studies point to the important relationship between myonuclear accretion and satellite cell number in animal models. Early experiments by Schiaffino and colleagues demonstrated myonuclear accretion accompanied load-induced hypertrophy in rodents, specifically the rat extensor digitorum longus, using injections of ^{3}H-thymidine at three time points post synergist ablation [27]. Importantly, on the other side of the debate, some researchers have also observed muscle hypertrophy in satellite-cell depleted muscle and an increase in

myonuclear domain size [32, 34, 113]. For example, McCarthy et al. [32] reported the depletion of satellite cells in Pax7-DTA mice administered with tamoxifen did not impair overload-induced muscle hypertrophy.

While such mechanistic studies are not possible in humans, there are studies showing that satellite cell proliferation increases in response to one bout of RE [114-119]. Additionally, several studies have shown a similar response to weeks of training in males [107, 120, 121] and females [121, 122]. For example, Kadi and Thornell analyzed muscle fiber area, myonuclear number, and satellite cell number of the trapezius in untrained females following 10 weeks of supervised upper body RT and documented a 36%, 70%, and 46% increase, respectively [122].

Some of these aforementioned studies imply that myonuclear accretion coincides with hypertrophy [13, 120, 123]. However, hypertrophy without myonuclear accretion has also been observed in humans, and more specifically, untrained females. Herman-Montemayor et al. [124] demonstrated a 30% increase in type II fiber hypertrophy, a 29% increase in the size of myonuclear domains, and no myonuclear accretion (i.e., no new myonuclei). Petrella et al. [115] reported similar findings after 16 weeks of knee extensor RT in untrained females. Specifically, mean fiber area and myonuclear domain size increased, but myonuclei number remained unchanged [115]. A conclusive stance on the need for satellite cells in facilitating RT-induced hypertrophy remains equivocal. Notwithstanding, the previously mentioned literature, particularly in human trials, suggests that markers of satellite cell activity (e.g., increase in mRNA expression reflective of proliferation and differentiation, increased satellite cell number, increased myonuclear accretion) coincide with and are likely needed for myofiber hypertrophy.

Lower and Higher Hypertrophic Responders

Interestingly, participants can perform the same RT program but exhibit highly individualized responses, with some observing no training adaptations in size or strength, decreases in muscle mass [9, 11, 13, 14, 24, 25, 125], or even decreases in strength [9]. Before expanding on the literature in this space, it is important to note two key points: i) research using 'high' and 'low' responders are referring specifically to the variables investigated in the study, and ii) the dose-response relationship is different between individuals, therefore a particular intervention may not be the appropriate exercise stress for a lower responder to adapt. When 'lower' and 'higher' responder language is used throughout this chapter, it will be referring to the variables used in the presented studies, or using the exact phrasing from the study, not stating that individuals are a 'low' or 'non' responder to exercise.

In 2007, Bamman [10] published a milestone paper demonstrating the presence of "extreme responders", "modest responders", and "non-responders" based on the magnitude of VL fCSA changes to 16 weeks of RT. In brief, many of the findings from this work suggested satellite cell proliferation and subsequent myonuclear accretion differentiated the hypertrophic response [10]. Other responder research in the RT arena has since been published. For instance, Ahtiainen et al. [9] reported that almost 7% of male and female participants (group n≅20, total n=287 total) did not demonstrate increases in max strength over 5 to 6 months of RT. Additionally, they classified ~3% of those participants as 'low' responders for both muscle size and strength [9]. However, most low responders for muscle size or strength were considered a responder for the other trait [9, 11]. This critical finding suggests that, although an individual may not express hypertrophy after RT over time, they may still increase strength, some as much as the 'higher' hypertrophic responders. Importantly, this indicates that all individuals can derive some benefit from RT [9]. These studies suggest multiple variables should be considered in a

responder analysis research due to the independent adaptations in outcome variables. Alternatively stated, 'low' responders likely show impairments in some indices reflective of hypertrophy and strength, but not all.

A 2018 review by Roberts and colleagues provided a "state of the union" on the molecular variables that may delineate 'higher' versus 'lower' hypertrophic responders to RT. What is seemingly evident are the findings by several laboratories suggesting ribosome biogenesis differentiates 'higher' and 'lower' hypertrophic responders following weeks to months of RT [18, 24, 25]. As discussed in the review, as well as introduced above, various research also implies that an increase in satellite cell number in response to one bout of RE [126], as well as following months of RT [115], predict the hypertrophic response to RT.

Beyond the three main molecular variables presented in this chapter, other variables thought to play a role in the magnitude of response to RT have been investigated. For example, Ogasawara et al. [127] reported that certain muscle microRNAs were evident in higher versus lower hypertrophic responders following 12 weeks of RT, and two studies in males have suggested that increases in muscle androgen receptor protein levels predicted the hypertrophic response to weeks of training [7, 128]. A more recent study published from our laboratory investigated the potential genetic contributors to the muscle heterogenic hypertrophic response to RT by utilizing a genome-wide association (GWA) approach [129]. In short, 109 younger adult males were included in the analysis, and over 315,000 genetic polymorphisms were interrogated. Interestingly, no polymorphisms were associated with the change in whole-body lean mass assessed via DXA. However, two polymorphisms, one being annotated as a single nucleotide polymorphism (SNP) of the GLI Family Zinc Finger 3 (GLI3), and the other being unannotated, were shown to be associated with the change in mean fCSA [129]. The GLI3 polymorphism and

myofiber hypertrophy association is interesting given that the encoded protein acts as transcription factor that drives the expression of cyclin D1 as well as myogenic regulatory factors, which are involved in regulating cell cycle progression [130]. Notably, although the 'high' responder versus 'low' responder literature has provided excellent data that has informed the scientific community as to how muscle cells adapt to RT, most of these studies have been performed in males. Nonetheless, this research begins to potentially explain how a person's genetics could influence RT-induced alterations in ribosome biogenesis and satellite cell proliferation.

The Biological Sex Gap

As previously noted, studies examining skeletal muscle adaptations from RT are considerably less common in females versus males. Virginia Miller noted that human physiology has historically been defined in terms of adaptations of the 'typical 70-kg man', including animal models mainly focusing on the male sex [2]. Sociological, cultural and personal factors all have contributed to the sex bias in general physiology, exercise physiology, and sport science research [2]. In a review of various physiological responses based on sex, Sheel describes there are noticeable differences between sexes that are likely caused by hormones [1]. However, the author underscored the need for additional research in this area. There are data in females, albeit limited, showing that the aforementioned molecular adaptations can and do occur in response to RT.

In terms of strength metrics, several studies have indicated that there is no difference between relative responses in these variables between males and females, and that females possibly gain more relative isometric and dynamic strength with both lower body [9, 131] and

upper body training [48, 132, 133]. Ahtiainen et al. [9] demonstrated that sex did not affect muscle size or strength adaptations in healthy, untrained males and females over 20-24 weeks of RT interventions. Similarly, Kell [132] observed that females were more responsive than men regarding strength gains to a 12-week, periodized strength training program (women ≥ 30% and men ≥ 25%). Like strength, hypertrophic adaptations also appear to occur independent of sex [9, 44]. While informative, none of these studies have determined if the aforementioned molecular variables predict hypertrophy, and if they differentiated 'higher' versus 'lower' hypertrophic responders.

Female Sex Hormones, Menstrual Cycle, and Resistance Training Adaptations

Research on females has, in part, been limited given the challenges in accounting for hormonal fluctuations, which have been considered major barriers to conducting exercise physiology and sport science research in females [44]. Several studies have reported similar observations of females being perceptually affected by their menstrual cycle on various performance outcomes [134, 135]. One study reported 55.4% of exercising females and elite female athletes perceived their training and/or performance is impacted by their menstrual cycle impacts [136]. A recent review by Carmichael et al. [137] reported that a large percentage of female athletes feel their training and competition performance is impacted due to their menstrual cycle (50-71% and 49-65% respectively). Objectively, however, literature has shown that strength [138, 139], hypertrophy [138], skeletal muscle protein metabolism [140], or volume load lifted is different between phases of the menstrual cycle [141]. However, there are studies that have reported an effect on both strength [142, 143] and hypertrophy [138]. More details on these variables are provided in subsequent paragraphs.

The menstrual cycle consists of three phases including menstruation, follicular phase, and luteal phase. Briefly, menstruation is characterized by bleeding, the follicular phase is characterized by low estrogen, and the luteal phase is characterized by relatively high estrogen. Additionally, females can further modify their hormone levels with exogenous hormones (e.g., oral contraceptives, IUD, implant, patch, or shot). However, there have been equivocal reports suggesting either muscular strength and endurance do not differ significantly during the menstrual cycle [138, 144-147], or they do, but only to a marginal degree [148-150]. The same has been seen with menstrual cycle phase and hypertrophy [138]. Interestingly, when comparing MPS rates between young males and females, West et al. [79] disregarded the phase of the female menstrual cycle during testing because of previous findings that skeletal muscle protein metabolism is not influenced by menstrual cycle phase. Additionally, these authors reported no sex-based difference in the MPS response at rest [151], to a single bout of RE [79, 152], and in a fasted state [153]. Miller et al. [140] also did not observe any differences in MyoPS rates between the follicular or luteal phases in young, untrained females after an acute bout of exercise. These findings are perhaps anticipated, because when normalized to lean mass, males and female express similar basal muscle protein synthesis and degradation rates [151]. Collectively, these data suggest different phases of the menstrual cycle may minimally impact certain molecular outcome variables in female RT studies.

However, there is aging literature that suggests female hormones (namely progesterone and estrogen) can impact certain physiological outcomes, though the mechanisms are not as well characterized as testosterone [154]. The production of female sex hormones falls at menopause and can be correlated with skeletal muscle mass loss [155, 156]. Conversely, females who combat this decrease in hormone production tend to have better performance outcomes (e.g.,

strength and hypertrophy). For instance, older females on hormone replacement therapy (HRT) have slightly greater muscle strength compared to those not on HRT [157-159]. Phillips et al. [60] reported that HRT had a protective effect on the natural decline in specific force production in post-menopausal women. Additionally, similar effects have been observed with skeletal muscle hypertrophy [158, 160], albeit there is conflicting literature in this area [161, 162]. Ronkainen et al. [163] demonstrated protective effects in muscle power, relative muscle size, and maximum walking speed in female monozygotic twin pairs where one was using long-term HRT and the other not. Thus, HRT appears to have protective effects in older females in attenuating muscle mass and strength decrements. While older females are not the target population of the present study, this literature provides important insight to the function of female sex hormones and performance outcomes (strength and hypertrophy).

Aging also decreases the satellite cell pool and, again, this may be due to hormone levels. In males, aging diminishes the regenerative capacity of skeletal muscle [164]. The same also holds true in females. Larson et al. [165] demonstrated that satellite cell number was 41-43% lower in placebo mice than in estradiol-treated ovariectomized mice, which indicates age-associated decreases in female sex hormones may contribute to a loss in satellite cells. This is an important discovery, alongside work that has suggested exercise-induced activation of satellite cells is decreased in the absence of estradiol [166]. Collins et al. [167] reported similar findings in mice, where the tibialis anterior in ovariectomized mice had lower satellite cell numbers by roughly 50%, with the decline being dependent on the duration of hormone deficiency. They also determined estradiol as the hormone responsible by treating a subset of ovariectomized mice with 17β-estradiol, and this subset rescued their satellite cell number [167]. This research has important indications for the influence of female sex hormones on satellite cell number, and

possibly function. Additionally, estrogen treatment has been shown to positively regulate rRNA synthesis, and therefore ribosome biogenesis, although this was examined in breast cancer cells, not skeletal muscle [168]. Outside of that study, the role of estrogens in ribosome biogenesis research is very limited.

While not a focus of the present study, the literature is mixed regarding how contraceptives affect RT adaptations. If taking contraceptives, the concentrations of circulating sex hormones are contingent upon the type, dose, and brand, leaving room for a large amount of variation. However, there have been studies demonstrating no difference in maximal strength in oral-contraceptive users versus non-users acutely [169, 170] and chronically [171]. Similar findings have been shown with contraceptive use and hypertrophy [171, 172].

Exercise Adherence

As stated, 'low' responders may have adherence barriers to exercise programs if the desired results are not seen or felt. If a person does not experience visible changes (e.g., increased muscle definition or a reduction in hip or waist circumference), this can persuade them to decrease their frequency or stop exercising all together due to not seeing adaptations on the surface. Gjestvang and colleagues reported that in new fitness club members, increased physical fitness was associated with adherence to exercise [15, 173]. While subjective, these perceptions play an important role in an individual continuing to exercise or participate in RT.

Conversely, Dishman and Buckworth's meta-analysis on 127 studies observed a moderately large effect in increasing participant physical activity after the completion of an exercise intervention [174]. This is a crucial finding, considering mere participation in a RT research study can increase adherence to exercise without adherence being a primary study aim.

Around 50% of previously inactive adults relapse to physical inactivity or a less active lifestyle within months after initiation of exercise [175]. Understanding a person's responsiveness to a certain type and dose of RT may facilitate individualized training programs to optimize muscle health and performance. There is a wide array of optimal nutrition and exercise practices available in the literature and in the fitness industry. Therefore, obtaining a greater understanding how individuals respond to RT can assist fitness practitioners in better catering to the general population.

Purpose of This Study

The purpose of this study was multifaceted. First, we sought to elucidate molecular adaptations that occur in previously untrained, young adult females that resistance train. Second, we sought to determine whether the chronic responses in ribosome biogenesis, satellite cell proliferation, and/or myonuclear addition predicted hypertrophic outcomes. Moreover, we wanted to determine if the myofibrillar protein synthetic response to a naïve training bout predicted the hypertrophic response. Lastly, we sought to investigate if other variables obtained during the study differed between response clusters. Herein, 34 females completed a 10-week RT protocol, and a composite pre-to-post change score consisting of the following variables were used to define 'lower' versus 'higher' responders (LR and HR, respectively): i) whole body LSTM determined by DXA, ii) VL CSA determined by ultrasound, iii) mid-thigh mCSA determined by pQCT, and iv) deadlift strength. Based on these criteria, we identified upper and lower quartiles of n=8 higher and n=8 lower responders, respectively.

We chose to adopt this multifactorial approach in determining responders to RT given two papers from our laboratory that analyzed molecular predictors of muscle hypertrophy in

male participants [16, 25]. In our first paper, we implemented a K-means cluster analysis based solely on changes in VL thickness to generate 'lower', 'moderate', and 'higher' hypertrophic responders [25]. This prior approach resembled several reports that have used one metric (e.g., RT-induced changes in mean or type II fCSA changes) as a clustering variable [10, 13, 17, 24]. However, more recent data from Davidsen et al. [14] used a combination of metrics to define 'higher' versus 'lower' responders; specifically, changes in mean fCSA, changes in whole-body lean tissue mass, and changes in strength gains for three leg exercises. Thus, a second paper from our laboratory [16] adopted a similar approach by generating hypertrophic response clusters based on percent changes in the following variables after 12 weeks of RT: i) DXA total body muscle mass, ii) VL thickness, and iii) mean fCSA. Critically, we did not observe a between-cluster interaction for 3RM squat strength in our first publication. Alternatively stated, both 'lower' and 'higher' responders experienced similar increases in lower body strength. However, in our second paper using the multifactoral approach in classifying 'higher' versus 'lower' responders, we observed that higher responders gained more lower-body strength. Therefore, given that our prior multifactorial approach of defining 'lower' versus 'higher' hypertrophic responders yielded differences in a functional strength outcome, we feel using multiple metrics to define responders is a more robust approach relative to using a single metric in the current project.

Based on the current literature my hypotheses are as follows: i) the 24-hour MyoPS response to the first bout of RT will be greater in higher versus lower responders, and ii) muscle ribosome content and satellite cell number will increase more in higher versus lower responders. In addition to the responder analysis, I also performed associations in all participants between outcomes representative of hypertrophy (e.g., change in VL CSA and mean fCSA) and changes

in satellite cell, ribosome biogenesis, and myofibrillar protein synthetic responses. Again, I hypothesize significant correlations will be evident.

CHAPTER III

METHODS

Ethical Approval and Pre-screening

This study was approved by the Auburn University Institutional Review Board (IRB) (Protocol # 19-249 MR 1907), conformed the standards set by the latest revisions of the Declaration of Helsinki, and was registered as a clinical trial (NCT04707963). Young, college-aged, untrained females were recruited from Auburn University's campus and the surrounding area via email, flyers, and word of mouth. The intent of the originally approved trial is to determine if daily peanut protein (PP) supplementation, versus no supplementation, affected RT adaptations [176]. In short, PP supplementation did not affect hypertrophic or strength outcomes, so this study is a secondary analysis of the female participants from the trial.

The eligibility criteria for those interested was defined as follows: between the ages of 18-30, BMI <35 kg/m^2; no participation in RT more than one time per week for six months prior, no known peanut allergy; free of metal implants that may interfere with x-ray procedures, no medically necessary radiation exposure (excluding dental x-rays) for six months prior; free of obvious cardiovascular or metabolic disease; blood pressure below 140/90 mmHg (with or without medication); free of conditions contraindicating participation in exercise programs or donating muscle biopsies (i.e. taking blood thinners or blood clotting disorders); and not pregnant or trying to become pregnant. Those that were deemed eligible and agreed to participate received an informed consent packet and were verbally informed of all study procedures. Following verbal and written consent, participants completed a health history questionnaire and scheduled a time for pre-testing (PRE).

Study design

The timeline and testing battery for each visit can be visualized in Figure 1 and is described in greater detail below. Participants reported to the laboratory a total of five times for testing, and 20 times for RT.

INSERT FIGURE 1

The PRE time point was completed in two visits. During the first pre-testing visit (PRE-1), participants reported to the laboratory at least four hours fasted and underwent an array of testing beginning with urine specific gravity (USG) to ensure hydration and a rapid pregnancy test. The following examinations were completed thereafter: i) height and weight measurements, ii) whole-body DXA, iii) right leg mid-thigh pQCT scan, iv) VL ultrasound at the mid-thigh, and v) isokinetic dynamometry of the knee extensors. The participants then chewed a salivette (SARSTEDT AG &Co, Nümbrect, Germany) to provide saliva for the baseline assessment of whole-body deuterium oxide (D_2O). At the end of PRE-1, participants were given containers of D_2O (70 atom percent; Sigma-Aldrich, St. Louis, MO, USA) to take home and consume over the next 3 days, and a three-day food log. Regarding the self-administration of D_2O, participants consumed a total of 4.5 mL per kg of lean/soft tissue mass prior to the first muscle biopsy.

The first biopsy/strength assessment visit for baseline metrics (PRE-2) took place three days after PRE-1. During PRE-2, participants chewed a salivette to monitor D_2O enrichment, consumed a top-off dose of D_2O (0.5 mL/kg), and donated a skeletal muscle via biopsy prior to subsequently performing baseline strength testing on the bilateral 45° leg press, barbell bench press, and hex-bar deadlift. This strength testing, with the addition of two sets of 10 repetitions on leg press, bench press, and deadlifts at 50% of their estimated 1-repetition maximum strength (1-RM), was considered the first/naïve training session.

Twenty-four hours after PRE-2, participants returned to the laboratory at least four hours fasted for their second skeletal muscle biopsy visit (24-Hr) and final salivette sampling for D_2O enrichment. After the 24-Hr visit, participants completed their 10-week RT program, where the last session included maximum strength testing and isokinetic dynamometry. The last testing session (POST) was conducted approximately 72 hours following the last RT session was completed, and the following battery of tests were performed: i) USG, body mass, ii) DXA, iii) pQCT, iv) VL ultrasound, and v) a muscle biopsy which was 1-2 cm proximal to the other two biopsy sites. Each of these tests are described in greater detail below.

Specific testing procedures

Urine specific gravity. At the beginning of PRE-1 and POST, participants donated a urine sample (~5 mL). The sample was immediately analyzed using a handheld refractometer (ATAGO; Bellevue, WA, USA) USG levels. Participants with a USG value $\leq$ 1.020 were considered well hydrated. Those who exceeded a USG of 1.020 were excluded from the analysis.

Body composition. Following USG assessments, body mass and height were assessed using a laboratory scale (Seca 769; Hanover, MD, USA). Participants were then subjected to a whole-body DXA scan (Lunar Prodigy; GE Corporation, Fairfield, CT, USA) to assess bone-free LSTM and fat mass (FM). This instrument was calibrated using a phantom device, and quality-assurance was tested on each day that scans were performed. Prior to the test, participants were asked to remove any metallic objects and to lie supine on the DXA scanner table underneath the scanner arm. Following a 5-minute period of allowing the participant to lay on the table, the scan was performed. Importantly, all scans were performed by the same investigator at both the

PRE and POST. Test-retest reliability from our laboratory using intraclass correlation coefficient $_{3,1}$ (ICC $_{3,1}$), standard error of the measurement (SEM) and minimal difference to be considered real (MD) was previously determined for whole-body LTM on ten participants scanned approximately 24 hours apart. This resulted in an ICC $_{3,1}$ of 0.99, SEM of 0.36 kg, and MD of 0.99 kg, respectively [177].

Peripheral quantitative computed tomography. After undergoing the DXA scan, participants had a pQCT scan (Stratec XCT 3000, Stratec Medical, Pforzheim, Germany) of the right thigh to measure mid-thigh mCSA (cm^2) and skeletal muscle density (mg/cm^3) at 50% of the distance between the mid-inguinal crease and proximal patella. A permanent marker was used to indicate the precise transverse location of the scan and a mark was made mid-belly of the VL so that subsequent ultrasound images and a muscle biopsy sample could be acquired from the exact location being imaged. Moreover, the biopsy scar was used as a reference point to ensure that POST images were captured at the same location as PRE. Each pQCT scan was captured using a scan speed of 20 mm/sec, a 2.4 mm slice thickness and a voxel size of 0.4 mm. Images were analyzed for mCSA and muscle density using the pQCT BoneJ plugin [178] freely available through ImageJ analysis software (NIH, Bethesda, MD, USA). Importantly, all images were captured and analyzed by the same investigator who was blinded to group allocations. Test-retest reliability using ICC $_{3,1}$, SEM and MD was previously determined for mCSA on ten participants scanned approximately 24 hours apart resulting in an ICC $_{3,1}$ of 0.99, SEM of 0.84 cm^2, and MD of 2.32 cm^2, respectively.

Ultrasound. Real-time B-mode ultrasonography (NextGen LOGIQe R8, GE Healthcare, USA) utilizing a multi-frequency linear-array transducer (L4-12T, 4-12 MHz, GE Healthcare, USA) was used to capture images of the VL in the transverse plane for measurement of VL CSA.

Prior to image acquisition, subjects rested supine on an examination table for a minimum of five minutes with the hip and knee fully extended. Images were captured at the same anatomical location as the pQCT scan as previously described above. For VL thickness, images were collected at a depth where the edge of the femur was visible, and this depth was held constant for POST image collection. For VL CSA measurements, a high-density cork pad was placed around the circumference of the thigh and secured using an adjustable strap. The pad was used as a guide for the consistent placement and movement of the probe in the transverse plane. All VL CSA images were captured using a panoramic function (LogicView, GE Healthcare, USA) with probe placement starting at the lateral aspect of the thigh and moving medially until the rectus femoris muscle was visible within the image. For all ultrasound images, a generous amount of water-soluble transmission gel was applied to both the skin and probe and care was taken to apply a consistent probe pressure to maximize image quality without compressing the underlying tissue. All ultrasound settings (frequency: 10 MHz, gain: 50 dB, dynamic range: 75), except for depth, were held constant across participants and time points. One image per participant was obtained at each time point. Images were analyzed using the freely available ImageJ software (National Institutes of Health, Bethesda, MD, USA). VL CSA was calculated manually tracing the border of the VL using the polygon function, with care taken to exclude any connective tissue within the region of interest. Again, these measurements were performed one time for each participant at each time point. All ultrasound images were captured and analyzed by the same investigator with a previously determined test-retest reliability in 10 participants resulting in an ICC $_{3,1}$, of 0.99, SEM of 0.60 cm^2, and MD of 1.65 cm^2.

Strength testing. All participants began the testing session with a general warm-up consisting of 25 jumping jacks and 10 bodyweight squats, after which they completed a battery

of strength testing in order of 45° bilateral leg press, barbell bench press (flat bench), and hex-bar deadlifts. Each of the exercises began with 3-5 warm-up sets where the load was incrementally increased based upon the participants' perceived difficulty. As each set was completed, the participant was asked to rate the difficulty on a rating of perceived exertion (RPE) scale of 1-10 ("really easy" to "really hard"). An RPE of 1 resulted in a weight increase of 25%, an RPE of 5 with a 10% weight increase, and an RPE of 8-9 with a 2-3% weight increase. A load easily performed for 10 repetitions was first used and was followed by a load that could be completed for five repetitions. Testing finished with 1-3 sets of three repetitions with each of those sets increasing in intensity. When a three-repetition maximum (3-RM) was achieved, that number was used to calculate an estimated 1-RM by dividing the 3-RM by .93.

Skeletal muscle biopsies

Skeletal muscle biopsies were collected from the right leg VL at PRE-2, 24-Hr, and POST visits. At PRE-2, the biopsy was collected from the VL at the same location as the ultrasound and pQCT scans, and at 24-Hr and POST the sample was taken 1-2 cm proximal of the initial biopsy sight, using the scar as a reference. During biopsies, participants laid down on an athletic table where the upper thigh was shaved and cleansed with 70% isopropanol. A subcutaneous injection of 1% lidocaine (0.8 mL) was then administered. After 5 minutes, the area was cleansed with chlorhexidine solution (Hibiclens; Mölnlycke Health Care, Norcross, GA, USA). Thereafter, a pilot incision was made through the dermis with a single-use sterile No. 11 surgical blade (AD Surgical; Sunnyvale, CA, USA). The 5-gauge biopsy needle was inserted into the pilot incision, through the muscle fascia, and ~2 cm into the muscle where a 50-100 mg sample was collected while applying suction [179]. Tissue was immediately removed

from the needle, teased of blood and connective tissue, and separated for RNA, histological, and tracer analysis. Tissue allocated for RNA and tracer analysis was placed in pre-labeled foils and immediately frozen via liquid nitrogen and stored at -80°C. Tissue allocated to histological analysis were embedded in optimum cutting temperature (OCT) gel to prevent freeze damage, slow-frozen in liquid nitrogen-cooled isopentane, and transported to -80°C for storage until sectioned and stained.

Resistance training protocol

The training protocol was 10 weeks in duration and consisted of 20 separate training sessions (2 days/week; Table 1). Each training session involved:

i) A general warm-up of 25 jumping jacks and 10 body weight squats,

ii) A specific warm-up of 1 set of 10 reps at 50% of pre-determined working weight, 1 set of 5 repetitions at 75% of working weight, and 1 set of 3 repetitions of 90% of working weight,

iii) Either a) 4 sets of 10 repetitions (higher volume day) or b) 5 sets of 6 reps per exercise (higher load day), each done once per week

Regarding progressive overload, weekly loads increased by ~5% for the higher volume day and ~9% for the higher load day for the first 4 weeks. Participants were then given a week of reduced load training at week 5 (deload), consisting of 50% intensity for both training sessions. During week 6, progressive loading ensued from week 4 values. Although loads were pre-programmed for all participants based on percentages of their 1-RM, participant RPE was used during each training session to ensure the appropriate load is implemented and their program was adapted accordingly.

INSERT TABLE 1

Wet laboratory analyses

Immunohistochemistry. PRE-1 and POST biopsies preserved in OCT were batch processed for: i) cryostat sectioning, ii) antibody-based immunohistochemistry, and iii) imaging and analysis. Initially, all samples were sliced into 10 μm thick sections where they were electrostatically removed from the cooled cryostat stage by a positively charged histology slide (Leica Biosystems; Buffalo Grove, IL, USA). The slides were then stored at -80°C until all samples were ready to undergo antibody staining.

Staining for fCSA and myonuclear number per fiber was performed [180], as well as staining for muscle fiber type (either type I or II) as was performed as previously described by our laboratory, but this was not included in the analysis [181]. Briefly, slides with sections were removed from -80°C storage and dried ~10 minutes at room temperature. Triton-X (0.5%) in phosphate buffer solution (PBS) was then used to permeabilize the sections for 5 minutes. This was followed by a 5-minute wash in PBS, and slides were subsequently incubated in an 100% concentration of blocking solution for 15 minutes (Pierce Super Blocker, Thermo Fisher Scientific). Slides were then incubated in primary antibody solution for 60 minutes. This solution contained a 1x base of PBS, 5% of Pierce Super Blocker Solution, equal parts at 2% (1:50 dilution) of rabbit anti-dystrophin IgG1 (catalog #: GTX15277; Genetex Inc.; Irvine, CA, USA) and mouse anti-myosin I IgG1 (catalog #: A4.951 supernatant; Developmental Studies Hybridoma Bank, Iowa City, IA, USA). Slides were then washed for 5 minutes in PBS and then incubated in a secondary antibody solution containing a 1x base of PBS, equal parts at 1% or (1:100 dilution) Texas Red-conjugated anti-rabbit IgG (catalog #: TI-1000; Vector Laboratories, Burlingame, CA, USA) and Alexa Fluor 488-conjugated anti-mouse IgG1 (catalog #: A-11001;

Thermo Fisher Scientific). This incubation occurred in the dark for 60 minutes. The slides were then washed for 5 minutes in PBS, dried, and mounted using a 4,6-diamidino-2-phenylindole containing florescent media (DAPI; catalog #: GTX16206; Genetex Inc.). Immediately following mounting, slides were imaged using a fluorescent microscope (Nikon Instruments, Melville, NY, USA) with a 10x objective lens. Exposure times were 200 milliseconds for FITC, 600 milliseconds for TRITC, and 100 milliseconds for DAPI. Open-sourced software (MyoVision) was used to analyze all images for average fiber cross-sectional area (fCSA), muscle fiber type, and myonuclear number per fiber [180]. A conversion of 0.964 μm/pixel was used to adjust the image for size and bit-depth, and a fiber size threshold was set at a minimum of 500 μm^2 and a maximum of 15,000 μm^2 to ensure the exclusion of spaces between fibers or fibers in an oblong orientation. Resultant images were visually inspected for erroneous detection of fibers.

Determination of satellite cells. Satellite cells were quantified using Pax7 immunohistochemistry. For this analysis, separate slides with adjacent sections were removed from -80°C storage and dried ~10 minutes at room temperature. Triton-X (0.5%) in PBS was used to permeabilize the sections for 5 minutes. This was followed by a 5-minute wash in PBS, and slides were subsequently incubated in a 100% concentration of blocking solution for 15 minutes (Pierce Super Blocker). Slides were then incubated in primary antibody solution for 60 minutes. This solution contained a 1x base of PBS, 5% of Pierce Super Blocker Solution, equal parts at 2% (1:50 dilution) of rabbit anti-dystrophin IgG1 (catalog #: GTX15277; Genetex Inc.; Irvine, CA, USA) and mouse anti-Pax7 IgG (catalog #: PAX7 supernatant; Hybridoma Bank). Sections were then washed for 5 minutes in 1x PBS and incubated in the dark for 1 hour with a secondary antibody solution containing Alexa Fluor 488-conjugated anti-rabbit IgG (Vector

Laboratories), and Texas Red-conjugated anti-mouse IgG (Thermo Fisher Scientific) (10 µL of all secondary antibodies per 1 mL of blocking solution). Sections were then washed for 5 minutes in PBS thereafter, air-dried, and mounted with fluorescent media containing DAPI (Genetex). Following mounting, slides were imaged using a fluorescent microscope (Nikon Instruments) with a 20x objective lens. Exposure times were 200 milliseconds for FITC, 600 milliseconds for TRITC, and 100 milliseconds for DAPI. Satellite cells were manually counted by an investigator blinded to the PRE and POST time points using a grid function in ImageJ and a handheld tally counter. Due to imaging constraints (i.e., 3 fluorescent detection filters), satellite cells were not specified as type I or type II fiber-specific.

Determination of MyoPS. Saliva samples obtained in the laboratory (or returned by participants) were stored at -20°C. Following the conclusion of the study, salivette tubes were centrifuged for 2 minutes at 1000 *g* (2°C). Saliva obtained thereafter was frozen at -20°C. Frozen samples were then shipped to Metabolic Solutions (Nashua, NH, USA) on dry ice for analysis. Saliva analysis for deuterium enrichment occurred using cavity ring-down spectroscopy. Instrumentation included Liquid Water Isotope Analyzer with an automated injection system and a version 2 upgrade (Los Gatos Research, Mountain View, CA, USA). Samples were vortexed and spun at 8,000 rpm to remove any particulates. The aqueous phase of saliva was injected 6 times, and the last three measurements were averaged for data analysis. Standard curves were generated before and after sample runs for the determination of deuterium enrichment. Intra-run precision using this method is generally < 2 delta per mil (parts per thousand) and inter-run precision is generally < 3.5 delta per mil.

Muscle biopsy samples from PRE-1 and PRE-2 were batch-processed using our laboratory's MIST method [182]. Isolated myofibrils were frozen at -80°C, and frozen samples

were shipped on dry ice to Metabolic Solutions for tracer analyses. This process first involved hydrolyzing myofibril pellets for 18 hours with 3 mL of 6 N HCl (100°C). Dowex H^+ resin (1 mL, 50Wx8-100; Sigma-Aldrich, Saint Louis, MO, USA) was added to trap released alanine. Amino acid elution from the resin was performed using 2 mL of 3N NH_4OH, and eluates evaporate to dryness. Thereafter, the N-acetyl-n-propyl (NAP) derivative of alanine was prepared, and the propyl ester was formed by adding 200 uL propyl acetate and 100 uL BF3:Propanol (14%). Samples were heated at 110°C for 30 minutes, and solutions evaporated to dryness under N_2 gas at 60°C. The N-acetyl group was formed by adding 100 uL of 0.1M diethylamine (DEA) in hexane and 100 uL of acetic anhydride. This reaction incubated for 20 minutes at 60°C and was subsequently dried down with N_2 gas and low heat. Samples were reconstituted in 100 uL ethyl acetate and pipetted into autosampler vials. Deuterated alanine from myofibrillar preparations was detected using a Thermo Finnigan Delta V IRMS coupled to a Thermo Trace GC Ultra with a GC combustion interface III and Conflow IV. The N-acetyl-n-Propyl ester of alanine was analyzed using a splitless injection with CTC Pal autosampler (1 µL). Injections used a Zebron ZB-5 column of 30 m x 0.25 mm x 0.50 µm film thickness (Phenomenex, Torrance, CA, USA), and the injection temperature was 250°C. The GC oven had an initial column temperature of 80°C with a 2-minute hold and was followed by a ramp of 30°C per minute to 330°C. Compounds eluting off the column were directed into the pyrolysis reactor and heated to 1450°C hydrogen gas conversion. Deuterium enrichment was first expressed in delta values compared to a calibrated hydrogen gas. These values were then converted to atom % D by standard equations. Methylpalmitate was used as the calibration standard for the reference hydrogen gas. Intra-run precision for alanine measurements is generally < 2 delta per mil, and inter-run precision is generally < 3 delta per mil.

Saliva and myofibril enrichments were used to calculate MyoPS rates over the 24-hour period following the first naïve training bout. The equation follows the one published by Bell et al. [183] (see below).

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

Briefly, the difference in deuterium (^2H) enrichment from the first two biopsies (E_{Ala2} = 24-Hr muscle sample enrichment, E_{Ala2} = PRE-2 muscle sample enrichment) was divided by the product total body enrichment of ^2H (in atom % excess) (E_{BW} = ^2H from PRE-2 and 24-Hr saliva – Baseline ^2H from PRE-1 saliva) and number of days that D_2O was consumed at the loading dose. This quotient was then multiplied by 3.7 to adjust for the number of ^2H atoms typically bound to alanine. Finally, the resultant value was multiplied by 100 to achieve myofibrillar synthesis rate in percent per day.

Determination of muscle ribosome content. For total RNA analysis, ~15–30 mg of powdered tissue was weighed using an analytical scale with a sensitivity of 0.001 g (Mettler-Toledo; Columbus, OH, United States). Tissue was then homogenized in 1.7 mL microcentrifuge tubes containing 500 µL of Ribozol (Ameresco; Solon, OH, United States) via micropestle manipulation and RNA isolation was performed per manufacturer recommendations. Total RNA concentrations were determined in duplicate using a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific; Waltham, MA, United States), and total RNA per wet muscle weight was used as a surrogate for muscle ribosome content as in past publications [184, 185].

Food log analysis

The three-day food log packets were used to obtain nutritional intakes for one weekend day and two weekdays in the week leading up to PRE-2 and POST visits. Participants were

asked to maintain their normal dietary habits through the duration of the study, except daily consumption of the protein supplement in the PP group. Data from the food logs was entered into the Nutrition Data System for Research (NDSR) (NDSR 2014: University of Minnesota). Calories and macronutrients were averaged from the three days of food logs for a mean intake (kcal/d, g/d or g/kg/d) at each time point.

Additional questionnaires

Self-reported sex, gender, race, and ethnicity was collected via questionnaires during the Informed Consent visit. Additionally, prior exercise history, menstrual cycle information, and contraceptive use was gathered during this visit. Menstrual cycle phases were self-estimated, where the first day of bleeding was considered day 1. Finally, at POST, menstrual cycle information was collected again, as well as subjective sleep quality was obtained using the Pittsburgh Sleep Quality Index [186].

Statistical analysis

Statistical analysis was performed in SPSS v26.0 (IBM Corp, Armonk, NY, USA). Prior to statistical analysis, normality testing was performed on all dependent variables using Shapiro-Wilk tests at PRE and POST time points.

For the HR versus LR responder analysis, independent samples t-tests were used to compare the 24-hour MyoPS rates between responder cohorts. Independent samples t-tests were used to examine baseline differences in select phenotypes as well as self-reported sleep quality between HR versus LR cohorts. Two-way (group*time) repeated measure ANOVAs were used to determine changes in dependent variables prior to and following the training intervention in

the HR versus LR cohorts. When a significant group*time interaction was observed, LSD post hocs were performed to determine differences within each group from pre- to post-intervention and between groups at each time point. Pearson correlations were also performed between select variables, and these tests included all participants. For analysis on all participants, dependent samples t-tests were performed. For menstrual cycle analysis, independent t-tests were used to compare average day of cycle during PRE and POST testing. Statistical significance was established at $p<0.05$.

CHAPTER IV

COMPLETED MANUSCRIPT (to be submitted to J Appl Physiol in Summer 2022)

Molecular predictors of the hypertrophy response to resistance training in young untrained female adults

Morgan A. Smith[1], Casey L. Sexton[1], Kristen A. Smith[2], Shelby C. Osburn[1], Joshua L. Godwin[1], Jonathan P. Beausejour[1], Bradley A. Ruple[1], Michael D. Goodlett[3,4], Joseph L. Edison[3,4], Andrew D. Fruge[2], Kaelin C. Young[1,4], Michael D. Roberts[1,4,*]

Affiliations: [1]School of Kinesiology, Auburn University, Auburn, AL, USA; [2]Department of Nutrition, Dietetics and Hospitality Management, Auburn University, Auburn, AL, USA; [3]Athletics Department, Auburn University, Auburn, AL, USA; [4]Edward Via College of Osteopathic Medicine, Auburn, AL, USA

Short title: Molecular predictors of resistance training adaptations in females

Conflicts of interest: None of the authors has financial or other conflicts of interest to report in relation to the current data.

[*]Address correspondence to:
Michael D. Roberts, PhD
Associate Professor, School of Kinesiology
Director, Molecular and Applied Sciences Laboratory
Director, Applied Physiology Laboratory
Auburn University
301 Wire Road, Office 260
Auburn, AL 36849
Phone: 334-844-1925
Fax: 334-944-1467
E-mail: mdr0024@auburn.edu

ABSTRACT

We determined if the myofibrillar protein synthetic (MyoPS) response to a naïve resistance exercise (RE) bout, or chronic changes in satellite cell number and muscle ribosome content, varied in females that classified as higher (HR) or lower (LR) responders following resistance training (RT). Thirty-four untrained college-aged females completed a 10-week full-body RT protocol (twice weekly). Body composition, right leg muscle imaging, right leg vastus lateralis biopsy, and strength testing occurred prior to and following the intervention. A composite pre-to-post change score consisting of four variables was used to define HR (n=8) and LR (n=8): i) whole body lean/soft tissue mass (LSTM), ii) VL cross-sectional area (CSA), iii) mid-thigh muscle cross-sectional area (mCSA), and iv) maximal deadlift strength. For all participants, training increased LSTM (+1.1±1.1 kg, $p<0.001$), VL CSA (+2.7±2.7 cm^2, $p<0.001$), mid-thigh mCSA (+8.9±7.0 cm^2, $p<0.001$), deadlift strength (24±13 kg, $p<0.001$), mean muscle fiber cross-sectional area (+759±1198 μm^2, $p=0.001$), satellite cell number (+0.026±0.061 cells/fiber, $p=0.025$), and myonuclear number (+0.29±0.37 nuclei/fiber, $p=0.013$). HR exhibited superior training responses to LR concerning changes in LSTM (2.2±0.7 kg versus 0.3±0.6 kg, respectively, $p<0.001$), VL CSA (5.9±2.5 cm^2 versus 0.6±0.8 cm^2, $p<0.001$), mid-thigh mCSA (16.0±6.2 cm^2 versus 3.2±2.9 cm^2, $p<0.001$), and deadlift strength (37±15 kg versus 13±7 kg, $p=0.001$). The 24-hour MyoPS response to the first RE bout was not different between HR and LR ($p=0.367$). A significant group*time interaction was found for satellite cell number after RT ($p=0.025$), but not muscle ribosome content ($p=0.888$). Specifically, satellite cell number increased in HR ($p=0.026$), but not LR ($p=0.628$). Pre-training values for the following variables were also greater in HR versus LR: i) LSTM (44.2±3.3 kg versus 39.2±3.5 kg, $p=0.010$), ii) VL CSA (20.1±2.2 cm^2 versus 17.6±1.9 cm^2, $p<0.028$), and iii) mid-thigh mCSA (117±8 cm^2 versus

99 ± 6 cm^2, p<0.001). Fat mass prior to RT, sleep duration, energy intake, RT volume normalized to body mass, and menstrual cycle day during the pre- and post-RT data collection days were not significantly different between response groups. These data suggest that untrained female participants with an enhanced satellite cell response to RT, and more muscle mass prior to RT, exhibit more favorable adaptations.

Key words: females; resistance training; hypertrophy; satellite cells; myofibrillar protein synthesis; ribosomes

INTRODUCTION

Resistance training (RT) adaptations have been studied over decades, mainly in young adult males [1, 2]. Female participants are significantly under-represented in the sport science literature. In this regard, a 2014 study examined three major sport science journals (Medicine and Science in Sports and Exercise, British Journal of Sports Medicine, and American Journal of Sports Medicine) from 2011-2013, and found 39% of study participants were female, but only 4-13% of studies contained participant groups that were females only [3]. While advances have been made in the field, younger adult males do not appropriately represent how other populations will adapt to exercise (e.g., younger females, older individuals, older males).

Two RT adaptations that have been well-documented include strength [4-6] and muscle growth (or hypertrophy) [7, 8]. However, while it is generally presumed that all participants will show improvements in these variables, there are various lines of evidence to show that "lower" and "higher" responders exist regarding strength [9] and hypertrophy outcomes [10-14]. Being a lower hypertrophic responder could be a potential barrier to long-term training adherence, given that one of the chief motivations for a novice trainee is to gain skeletal muscle mass [15]. Moreover, although lower hypertrophic responders can experience significant strength gains during training [16, 17], there is some evidence suggesting lower hypertrophic responders to 12 weeks of RT may have impaired strength gains [16]. Thus, deciphering variables that differentiate higher versus lower hypertrophic responders to RT is warranted, particularly in females.

Several studies, some from our laboratory, have attempted to elucidate molecular variables that predict the hypertrophic response to RT. Collectively, these studies have shown that three molecular variables are predictive of hypertrophy, and these variables include: i) the

ribosome biogenesis response to weeks of training, ii) the myofibrillar protein response days to weeks into training, and iii) the satellite cell response days to weeks into training. While insightful, there are some nuances and limitations in the existing literature on molecular variables being predictive of hypertrophic response to RT. Specifically, there is evidence to support [18-25], and refute [7, 26] ribosome biogenesis and myofibrillar protein synthetic responses as being predictive of muscle hypertrophy. Additionally, although there are reports suggesting satellite cell proliferation and/or myonuclear accretion with training predicts hypertrophic responses [16, 27-31], there are conflicting data [32-34]. Second, the data generated in this area is varied in that some studies have used trained [5, 35-40] and untrained participants [10, 14, 24, 25, 41-43], which can have an impact on the level of adaptation seen and makes it difficult to compare findings. Third, training interventions have ranged from 4-16 weeks and there could be temporal variability pertaining to the molecular adaptations within this range of duration. Fourth, criterion variables and statistics used to delineate responders versus non-responders have vastly differed between studies, as well as what 'responder' refers to (e.g., hypertrophic, strength, etc.). For example, some papers have used a single outcome variable to define the hypertrophic response (e.g. mean myofiber cross-sectional area (fCSA) [14], or ultrasound-determined muscle thickness [25, 44, 45]), whereas others have adopted a composite score using a multifactorial approach to delineate the hypertrophic response using different variables [14, 36, 46]. Finally, most of these data are in males, and we cannot assume that males and females adapt in the same way via similar mechanisms. A landmark study in this area by Bamman and colleagues was performed in younger and older males and females who underwent 16 weeks of RT [17]. However, the authors noted that younger females generally did not exhibit hypertrophy assessed through mean fCSA. Importantly, this study did not assess the present molecular predictive

variables. Thus, the current data on molecular variables that predict the hypertrophic response in females is limited.

Given the collective evidence presented above, it is currently unknown if ribosome biogenesis, myofibrillar protein synthetic responses, and satellite cell response to RT are predictive of muscle hypertrophy in adult females. Therefore, the primary aims of this study were threefold: i) examine chronic responses of ribosome biogenesis and satellite cell proliferation and possible predictive ability to hypertrophy, ii) examine predictive ability of 24-hour myofibrillar protein synthesis response post a single bout of RT to long-term hypertrophy, and iii) determine whether these three variables differentiated higher versus lower responders following 10 weeks of RT in previously untrained, younger adult females. Herein, 34 females completed a 10-week supervised RT protocol (twice weekly). A composite pre-to-post change score consisting of the following variables was used to define 'lower' versus 'higher' responders: i) whole body lean/soft tissue mass (LSTM) determined by dual x-ray absorptiometry (DXA), ii) vastus lateralis (VL) cross-sectional area (CSA) determined by ultrasound, iii) mid-thigh muscle CSA determined by peripheral quantitative computed tomography (pQCT), and iv) maximum deadlift strength determined by a three-repetition maximum test (3RM). Crucially, both strength and hypertrophy measures were included in the multidimensional composite score to be more inclusive in capturing various levels of response and not limiting the assessment to strength or hypertrophy exclusively. Therefore, 'responder' refers specifically to the sum of the composite score variables used in the present study. Based on the resultant composite scores, we identified quartiles of n=8 higher and n=8 lower responders.

Based on the current literature our hypotheses are as follows: i) responses in ribosome biogenesis and satellite cell proliferation will correlate with hypertrophy (the greater response

correlated with greater hypertrophy), ii) the 24-hour MyoPS response will be predictive of the hypertrophic response, and iii) response variation will occur, and higher and lower responders will be evident via the composite score. In addition to the responder analysis, we also performed correlations for all participants between outcomes representative of hypertrophy (e.g., change in VL CSA and mean fiber CSA (fCSA)), changes in ribosome biogenesis, myofibrillar protein synthetic, and satellite cell responses.

MATERIALS AND METHODS

Ethical Approval and Pre-screening

This study was approved by the Auburn University Institutional Review Board (IRB) (Protocol # 19-249 MR 1907), conformed to the standards set by the latest revisions of the Declaration of Helsinki, and was registered as a clinical trial (NCT04707963). Young, college-aged, untrained females were recruited from Auburn University's campus and the surrounding area via email, flyers, and word of mouth. The intent of the originally approved trial is to determine if daily peanut protein (PP) supplementation, versus no supplementation, affected RT adaptations in college-aged males and females [47]. In short, PP supplementation did not affect hypertrophic or strength outcomes, so this study is a secondary analysis of the female participants from the trial.

The eligibility criteria for participation was defined as follows: between the ages of 18-30, BMI <35 kg/m^2; no participation in RT (less than one time per week for six months prior); no known peanut allergy; free of metal implants that may interfere with x-ray procedures, no medically necessary radiation exposure (excluding dental x-rays) for six months prior; free of obvious cardiovascular or metabolic disease; blood pressure below 140/90 mmHg (with or

without medication); free of conditions contraindicating participation in exercise programs or donating muscle biopsies (i.e. taking blood thinners or blood clotting disorders); and not pregnant or trying to become pregnant. Those that were deemed eligible and agreed to participate received an informed consent packet and were verbally informed of all study procedures. Following verbal and written consent, participants completed a health history questionnaire and scheduled a time for pre-testing (PRE).

Study design

The timeline and testing battery for each visit can be visualized in Figure 1 and is described in greater detail below. Participants reported to the laboratory a total of five times for testing, and 20 times for RT.

INSERT FIGURE 1

The PRE time point was completed in two visits. During the first visit (PRE-1), participants reported to the laboratory at least four hours fasted and underwent an array of testing, beginning with urine specific gravity (USG) to ensure hydration and a rapid pregnancy test. The following examinations were completed thereafter: i) height and weight measurements, ii) whole-body DXA, iii) right leg mid-thigh pQCT scan, iv) VL ultrasound at the mid-thigh, and v) isokinetic dynamometry of the right knee extensors. The participants then chewed a salivette (SARSTEDT AG &Co, Nümbrect, Germany) to provide saliva for the baseline assessment of whole-body deuterium oxide (D_2O). At the end of PRE-1, participants were given containers of D_2O (70 atom percent; Sigma-Aldrich, St. Louis, MO, USA) to take home and consume over the next three days, and a three-day food log. Regarding the self-administration of D_2O, participants consumed a total of 4.5 mL per kg of lean/soft tissue mass prior to the first muscle biopsy.

The second visit of PRE testing (PRE-2) occurred three days following PRE-1, and consisted of the baseline biopsy, strength assessment, and initial/naïve training bout. During PRE-2, participants chewed a salivette to monitor D_2O enrichment, consumed a top-off dose of D_2O (0.5 mL/kg), and donated skeletal muscle from the right VL via biopsy prior to performing baseline strength testing on the bilateral 45° leg press, barbell bench press, and hex-bar deadlift. This strength testing, with the addition of two sets of 10 repetitions on leg press, bench press, and deadlifts at 50% of their estimated 1-repetition maximum strength (1-RM), was considered the first/naïve training session (training bout used for the 24-hr myofibrillar protein synthesis response).

Twenty-four hours after PRE-2, participants returned to the laboratory at least four hours fasted for their second skeletal muscle biopsy visit (24-Hr) and final salivette sampling for D_2O enrichment. After the 24-Hr visit, participants completed their 10-week RT program, where the last session included maximum strength assessments and isokinetic dynamometry. The last testing session (POST) was conducted approximately 72 hours following the completion of the last RT session, and the following battery of tests were performed: i) USG, ii) body mass, iiI) DXA, iv) pQCT, v) VL ultrasound, and vi) a muscle biopsy which was 1-2 cm proximal to the other two biopsy sites. Each of these tests are described in greater detail below.

Specific testing procedures

Urine specific gravity. At the beginning of PRE-1 and POST, participants donated a urine sample (~5 mL). The sample was immediately analyzed using a handheld refractometer (ATAGO; Bellevue, WA, USA). Participants with a USG value $\leq$ 1.020 were considered well hydrated. Those who exceeded a USG of 1.020 were excluded from the analysis.

Body composition. Following USG assessments, body mass and height were assessed using a laboratory scale (Seca 769; Hanover, MD, USA). Participants were then subjected to a whole-body DXA scan (Lunar Prodigy; GE Corporation, Fairfield, CT, USA) to assess bone-free LSTM and fat mass (FM). This instrument was calibrated using a phantom device, and quality-assurance was tested on each day scans were performed. Prior to the test, participants were asked to remove any metal objects and to lie supine on the DXA scanner table underneath the scanner arm. Following a 5-minute period allowing the participant to lay on the table, the scan was performed. Importantly, all scans were performed by the same investigator at both PRE and POST. Test-retest reliability from our laboratory using intraclass correlation coefficient $_{3,1}$ (ICC $_{3,1}$), standard error of the measurement (SEM), and minimal difference to be considered real (MD) was previously determined for whole-body LSTM on ten participants scanned approximately 24 hours apart. This resulted in an ICC $_{3,1}$ of 0.99, SEM of 0.36 kg, and MD of 0.99 kg [48].

Peripheral quantitative computed tomography. After undergoing the DXA scan, participants had a pQCT scan (Stratec XCT 3000, Stratec Medical, Pforzheim, Germany) of the right thigh to measure mid-thigh muscle CSA (mCSA, cm^2) and skeletal muscle density (mg/cm^3) at 50% of the distance between the mid-inguinal crease and proximal patella. A permanent marker was used to indicate the precise transverse location of the scan and a mark was made mid-belly of the VL so that subsequent ultrasound images and muscle biopsy sample could be acquired from the exact location being imaged. Moreover, the biopsy scar was used as a reference point to ensure that POST images were captured at the same location as PRE. Each pQCT scan was captured using a scan speed of 20 mm/sec, a 2.4 mm slice thickness, and a voxel size of 0.4 mm. Images were analyzed for mCSA and muscle density using the pQCT BoneJ

plugin [49] freely available through ImageJ analysis software (NIH, Bethesda, MD, USA). Importantly, all images were captured and analyzed by the same investigator who was blinded to group allocations. Test-retest reliability using ICC $_{3,1}$, SEM, and MD was previously determined for mCSA on ten participants scanned approximately 24 hours apart resulting in an ICC $_{3,1}$ of 0.99, SEM of 0.84 cm^2, and MD of 2.32 cm^2.

Ultrasound. Real-time B-mode ultrasonography (NextGen LOGIQe R8, GE Healthcare, USA) utilizing a multi-frequency linear-array transducer (L4-12T, 4-12 MHz, GE Healthcare, USA) was used to capture images of the VL in the transverse plane for measurement of VL CSA. Prior to image acquisition, subjects rested supine on an examination table for a minimum of five minutes with the hip and knee fully extended. Images were captured at the same anatomical location as the pQCT scan as previously described above. For VL thickness, images were collected at a depth where the edge of the femur was visible, and this depth was held constant for POST image collection. For VL CSA measurements, a high-density cork pad was placed around the circumference of the thigh and secured using an adjustable strap. The pad was used as a guide for the consistent placement and movement of the probe in the transverse plane. All VL CSA images were captured using a panoramic function (LogicView, GE Healthcare, USA) with probe placement starting at the lateral aspect of the thigh and moving medially until the rectus femoris muscle was visible within the image. For all ultrasound images, a generous amount of water-soluble transmission gel was applied to both the skin and probe, and care was taken to apply a consistent probe pressure to maximize image quality without compressing the underlying tissue. All ultrasound settings (frequency: 10 MHz, gain: 50 dB, dynamic range: 75), except for depth, were held constant across participants and time points. One image per participant was obtained at each time point. Images were analyzed using the freely available ImageJ software

(National Institutes of Health, Bethesda, MD, USA). VL CSA was calculated manually tracing the border of the VL using the polygon function, with care taken to exclude any connective tissue within the region of interest. Again, these measurements were performed one time for each participant at each time point. All ultrasound images were captured and analyzed by the same investigator with a previously determined test-retest reliability in 10 participants resulting in an ICC $_{3,1}$, of 0.99, SEM of 0.60 cm^2, and MD of 1.65 cm^2.

Strength assessments. All participants began the testing session with a general warm-up consisting of 25 jumping jacks and 10 bodyweight squats, after which they completed a battery of strength testing in order of 45° bilateral leg press, barbell bench press (flat bench), and hex-bar deadlifts. Each of the exercises began with three to five warm-up sets where the load began with an unloaded barbell/machine and was incrementally increased based upon the participants' perceived difficulty. As each set was completed, the participant was asked to rate the difficulty on a rating of perceived exertion (RPE) scale of 1-10 ("really easy" to "really hard"). An RPE of 1 resulted in a weight increase of 25%, an RPE of 5 with a 10% weight increase, and an RPE of 8-9 with a 2-3% weight increase. These guidelines were followed until the participant reached a load that could be completed for five repetitions. Testing on each exercise finished with 1-3 sets of three repetitions with each of those sets increasing in intensity if the participant was able to complete all three repetitions. When a three-repetition maximum (3-RM) was achieved, that number was used to calculate an estimated 1-RM by dividing the 3-RM by .93.

Skeletal muscle biopsies

Skeletal muscle biopsies were collected from the right leg VL at PRE-2, 24-Hr, and POST visits. At PRE-2, the biopsy was collected from the VL at the same location as the

ultrasound and pQCT scans, and at 24-Hr and POST the sample was taken 1-2 cm proximal of the initial biopsy sight, using the previous scar as a reference. During biopsies, participants laid down on an athletic table where the upper thigh was shaved and cleansed with 70% isopropanol. A subcutaneous injection of 1% lidocaine (0.8 mL) was then administered. After 5 minutes, the area was cleansed with chlorhexidine solution (Hibiclens; Mölnlycke Health Care, Norcross, GA, USA). Thereafter, a pilot incision was made through the dermis with a single-use sterile No. 11 surgical blade (AD Surgical; Sunnyvale, CA, USA). The 5-gauge biopsy needle was inserted into the pilot incision, through the muscle fascia, and ~2 cm into the muscle where a 50-100 mg sample was collected while applying suction [50]. Tissue was immediately removed from the needle, teased of blood and connective tissue, and separated for RNA, histological, and tracer analysis. Tissue allocated for RNA and tracer analysis was placed in pre-labeled foils and immediately frozen via liquid nitrogen and stored at -80°C. Tissue allocated to histological analysis were embedded in optimum cutting temperature (OCT) gel to prevent freeze damage, slow-frozen in liquid nitrogen-cooled isopentane, and transported to -80°C for storage until sectioned and stained.

Resistance training protocol

The RT protocol was 10 weeks in duration and consisted of 20 separate training sessions (2 days/week; Table 1). Each training session involved:

i) A general warm-up of 25 jumping jacks and 10 body weight squats,

ii) A specific warm-up of 1 set of 10 reps at 50% of pre-determined working weight, 1 set of 5 repetitions at 75% of working weight, and 1 set of 3 repetitions of 90% of working weight,

iii) Either a) 4 sets of 10 repetitions (higher volume day) or b) 5 sets of 6 reps per exercise (higher load day), each done once per week

Regarding progressive overload, weekly loads increased by ~5% for the higher volume day and ~9% for the higher load day for the first 4 weeks. Participants were then given a week of reduced load training at week 5 (deload), consisting of 50% intensity for both training sessions. During week 6, progressive loading ensued from week 4 values. Although loads were pre-programmed for all participants based on percentages of their 1-RM, participant RPE was used during each training session to ensure the appropriate load is implemented and their program was adapted accordingly.

INSERT TABLE 1

Wet laboratory analyses

Immunohistochemistry. PRE and POST biopsies preserved in OCT were batch processed for: i) cryostat sectioning, ii) antibody-based immunohistochemistry, and iii) imaging and analysis. Initially, all samples were sliced into 10 μm thick sections where they were electrostatically removed from the cooled cryostat stage by a positively charged histology slide (Leica Biosystems; Buffalo Grove, IL, USA). The slides were then stored at -80°C until all samples were ready to undergo antibody staining.

Staining for muscle fiber cross-sectional (fCSA) and myonuclear number per fiber was performed [51], as well as staining for muscle fiber type (either type I or II) was performed as previously described by our laboratory, but this was not included in the analysis [52]. Briefly, slides with sections were removed from -80°C storage and dried ~10 minutes at room temperature. Triton-X (0.5%) in phosphate buffer solution (PBS) was then used to permeabilize the sections for 5 minutes. This was followed by a 5-minute wash in PBS, and slides were

subsequently incubated in an 100% concentration of blocking solution for 15 minutes (Pierce Super Blocker, Thermo Fisher Scientific). Slides were then incubated in primary antibody solution for 60 minutes. This solution contained a 1x base of PBS, 5% of Pierce Super Blocker Solution, equal parts at 2% (1:50 dilution) of rabbit anti-dystrophin IgG1 (catalog #: GTX15277; Genetex Inc.; Irvine, CA, USA) and mouse anti-myosin I IgG1 (catalog #: A4.951 supernatant; Developmental Studies Hybridoma Bank, Iowa City, IA, USA). Slides were then washed for 5 minutes in PBS and then incubated in a secondary antibody solution containing a 1x base of PBS, equal parts at 1% or (1:100 dilution) Texas Red-conjugated anti-rabbit IgG (catalog #: TI-1000; Vector Laboratories, Burlingame, CA, USA) and Alexa Fluor 488-conjugated anti-mouse IgG1 (catalog #: A-11001; Thermo Fisher Scientific). This incubation occurred in the dark for 60 minutes. The slides were then washed for 5 minutes in PBS, dried, and mounted using a 4,6-diamidino-2-phenylindole containing florescent media (DAPI; catalog #: GTX16206; Genetex Inc.). Immediately following mounting, slides were imaged using a fluorescent microscope (Nikon Instruments, Melville, NY, USA) with a 10x objective lens. Exposure times were 200 milliseconds for FITC, 600 milliseconds for TRITC, and 100 milliseconds for DAPI. Open-sourced software (MyoVision) was used to analyze all images for average fCSA, muscle fiber type, and myonuclear number per fiber [51]. A conversion of 0.964 μm/pixel was used to adjust the image for size and bit-depth, and a fiber size threshold was set at a minimum of 500 μm^2 and a maximum of 15,000 μm^2 to ensure the exclusion of spaces between fibers or fibers in an oblong orientation. Resultant images were visually inspected for erroneous detection of fibers.

Determination of satellite cells. Satellite cells were quantified using Pax7 immunohistochemistry. For this analysis, separate slides with adjacent sections were removed from -80°C storage and dried ~10 minutes at room temperature. Triton-X (0.5%) in PBS was

used to permeabilize the sections for 5 minutes. This was followed by a 5-minute wash in PBS, and slides were subsequently incubated in a 100% concentration of blocking solution for 15 minutes (Pierce Super Blocker). Slides were then incubated in primary antibody solution for 60 minutes. This solution contained a 1x base of PBS, 5% of Pierce Super Blocker Solution, equal parts at 2% (1:50 dilution) of rabbit anti-dystrophin IgG1 (catalog #: GTX15277; Genetex Inc.; Irvine, CA, USA) and mouse anti-Pax7 IgG (catalog #: PAX7 supernatant; Hybridoma Bank). Sections were then washed for 5 minutes in 1x PBS and incubated in the dark for 1 hour with a secondary antibody solution containing Alexa Fluor 488-conjugated anti-rabbit IgG (Vector Laboratories), and Texas Red-conjugated anti-mouse IgG (Thermo Fisher Scientific) (10 µL of all secondary antibodies per 1 mL of blocking solution). Sections were then washed for 5 minutes in PBS thereafter, air-dried, and mounted with fluorescent media containing DAPI (Genetex). Following mounting, slides were imaged using a fluorescent microscope (Nikon Instruments) with a 20x objective lens. Exposure times were 200 milliseconds for FITC, 600 milliseconds for TRITC, and 100 milliseconds for DAPI. Satellite cells were manually counted by an investigator blinded to the PRE and POST time points using a grid function in ImageJ and a handheld tally counter. Due to imaging constraints (i.e., three fluorescent detection filters), satellite cells were not specified as type I or type II fiber-specific.

Determination of MyoPS. Saliva samples obtained in the laboratory (or returned by participants) were stored at -20°C. Following the conclusion of the study, salivette tubes were centrifuged for 2 minutes at 1000 *g* (2°C). Saliva obtained thereafter was frozen at -20°C. Frozen samples were then shipped to Metabolic Solutions (Nashua, NH, USA) on dry ice for analysis. Saliva analysis for deuterium enrichment occurred using cavity ring-down spectroscopy. Instrumentation included Liquid Water Isotope Analyzer with an automated

injection system and a version 2 upgrade (Los Gatos Research, Mountain View, CA, USA). Samples were vortexed and spun at 8,000 rpm to remove any particulates. The aqueous phase of saliva was injected six times, and the last three measurements were averaged for data analysis. Standard curves were generated before and after sample runs for the determination of deuterium enrichment. Intra-run precision using this method is generally < 2 delta per mil (parts per thousand) and inter-run precision is generally < 3.5 delta per mil.

Muscle biopsy samples from PRE-2 and 24-hr were batch-processed using our laboratory's MIST method [53]. Isolated myofibrils were frozen at -80°C, and frozen samples were shipped on dry ice to Metabolic Solutions for tracer analyses. This process first involved hydrolyzing myofibril pellets for 18 hours with 3 mL of 6 N HCl (100°C). Dowex H^+ resin (1 mL, 50Wx8-100; Sigma-Aldrich, Saint Louis, MO, USA) was added to trap released alanine. Amino acid elution from the resin was performed using 2 mL of 3N NH_4OH, and eluates evaporate to dryness. Thereafter, the N-acetyl-n-propyl (NAP) derivative of alanine was prepared, and the propyl ester was formed by adding 200 uL propyl acetate and 100 uL BF3:Propanol (14%). Samples were heated at 110°C for 30 minutes, and solutions evaporated to dryness under N_2 gas at 60°C. The N-acetyl group was formed by adding 100 uL of 0.1M diethylamine (DEA) in hexane and 100 uL of acetic anhydride. This reaction incubated for 20 minutes at 60°C and was subsequently dried down with N_2 gas and low heat. Samples were reconstituted in 100 uL ethyl acetate and pipetted into autosampler vials. Deuterated-alanine from myofibrillar preparations was detected using a Thermo Finnigan Delta V IRMS coupled to a Thermo Trace GC Ultra with a GC combustion interface III and Conflow IV. The N-acetyl-n-Propyl ester of alanine was analyzed using a splitless injection with CTC Pal autosampler (1 µL). Injections used a Zebron ZB-5 column of 30 m x 0.25 mm x 0.50 µm film thickness

(Phenomenex, Torrance, CA, USA), and the injection temperature was 250°C. The GC oven had an initial column temperature of 80°C with a 2-minute hold and was followed by a ramp of 30°C per minute to 330°C. Compounds eluting off the column were directed into the pyrolysis reactor and heated to 1450°C hydrogen gas conversion. Deuterium enrichment was first expressed in delta values compared to a calibrated hydrogen gas. These values were then converted to atom % D by standard equations. Methylpalmitate was used as the calibration standard for the reference hydrogen gas. Intra-run precision for alanine measurements is generally < 2 delta per mil, and inter-run precision is generally < 3 delta per mil.

Saliva and myofibril enrichments were used to calculate myofibrillar protein synthesis (MyoPS) rates over the 24-hour period following the first naïve training bout. The equation follows the one published by Bell et al. [54] (see below).

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

Briefly, the difference in deuterium (^2H) enrichment from the first two biopsies (E_{Ala2} = 24-Hr muscle sample enrichment, E_{Ala2} = PRE-2 muscle sample enrichment) was divided by the product total body enrichment of ^2H (in atom % excess) (E_{BW} = ^2H from PRE-2 and 24-Hr saliva – Baseline ^2H from PRE-2 saliva) and number of days that D_2O was consumed at the loading dose. This quotient was then multiplied by 3.7 to adjust for the number of ^2H atoms typically bound to alanine. Finally, the resultant value was multiplied by 100 to achieve myofibrillar synthesis rate in percent per day.

Determination of muscle ribosome content. For total RNA analysis, ~15–30 mg of powdered tissue was weighed using an analytical scale with a sensitivity of 0.001 g (Mettler-Toledo; Columbus, OH, United States). Tissue was then homogenized in 1.7 mL microcentrifuge tubes containing 500 µL of Ribozol (Ameresco; Solon, OH, United States) via micropestle

manipulation and RNA isolation was performed per manufacturer recommendations. Total RNA concentrations were determined in duplicate using a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific; Waltham, MA, United States), and total RNA per wet muscle weight was used as a surrogate for muscle ribosome content as in past publications [55, 56].

Food log analysis

The three-day food log packets were used to obtain nutritional intakes for one weekend day and two weekdays in the week leading up to PRE-2 and POST visits. Participants were asked to maintain their normal dietary habits through the duration of the study, except daily consumption of the protein supplement in the PP group. Data from the food logs was entered into the Nutrition Data System for Research (NDSR) (NDSR 2014: University of Minnesota). Calories and macronutrients were averaged from the three days of food logs for a mean intake (kcal/d, g/d or g/kg/d) at each time point.

Additional questionnaires

Self-reported sex, gender, race, and ethnicity was collected via questionnaires during the Informed Consent visit. Additionally, prior exercise history, menstrual cycle information, and contraceptive use was gathered during this visit. Day of the menstrual cycle was self-reported, where the first day of bleeding was considered day 1. Finally, at POST, menstrual cycle information was collected again, as well as subjective sleep quality obtained using the Pittsburgh Sleep Quality Index [57].

Statistical analysis

Statistical analysis was performed in SPSS v26.0 (IBM Corp, Armonk, NY, USA). Prior to statistical analysis, normality testing was performed on all dependent variables using Shapiro-Wilk tests at PRE and POST time points.

For analysis on all participants, dependent samples t-tests were performed. For menstrual cycle analysis, independent samples t-tests were used to compare average day of cycle during PRE and POST testing. Additionally, dependent samples t-tests were used to examine differences in menstrual cycle day within each cohort.

For the higher responder (HR) versus lower responder (LR) responder analysis, independent samples t-tests were used to compare the 24-hour MyoPS rates between responder cohorts. Independent samples t-tests were used to examine baseline differences in select phenotypes as well as self-reported sleep quality between HR versus LR cohorts. Two-way (group*time) repeated measure ANOVAs were used to determine changes in dependent variables prior to and following the training intervention in the HR versus LR cohorts. When a significant group*time interaction was observed, LSD post hocs were performed to determine differences within each group from pre- to post-intervention and between groups at each time point. Pearson correlations were also performed between each individual variable in the composite score and VL CSA and fCSA, and these tests included all participants. Statistical significance was established *a priori* at $p<0.05$.

RESULTS

Participant characteristics

Baseline participant characteristics can be found in Table 2. Briefly, 34 untrained college-aged females (21 $\pm$ 2.1 years, >90% Caucasian, body mass index of 23.4±3.4 kg/m^2) completed

the study. All participants completed 90-100% of the workouts (i.e., at least 18 training sessions).

At PRE, participants weighed 68.2 $\pm$ 10.3 kg, with 42.1 $\pm$ 4.1 kg being LSTM and 23.0 $\pm$ 8.0 kg being FM. Body weight and FM did not significantly change over time. However, LSTM significantly increased with training (p<0.001). There were no baseline differences between the HR and LR cohorts regarding age, height, or FM. Interestingly, at both PRE and POST, the HR cohort had significantly higher LSTM (PRE p = 0.010; POST p = 0.001).

INSERT TABLE 2

Training Volume and Strength Metrics

The HR cohort engaged in a higher training volume compared to LR throughout the duration of the study (HR = 151101 $\pm$ 18478 kg, LR = 101145 $\pm$ 26204 kg, p < 0.001). However, this may have been due to HR participants weighing more. When considering relative training volume throughout the study (i.e., total training volume divided by PRE body mass), no difference was evident between the HR and LR cohorts (HR: 2203 ± 348, LR: 1931 ± 1076; p = 0.506).

All strength metrics are presented in Table 2. For all participants, 1RM values increased for leg press, bench press, and deadlift from PRE to POST (p < 0.001 for all). At PRE, the HR cohort had significantly greater 1RM leg press and bench press (p = 0.003 and p = 0.026, respectively). All POST strength metrics were significantly higher in the HR than the LR cohort (leg press p < 0.001, bench press p < 0.001, deadlift p < 0.001). Additionally, the change in strength between cohorts significantly differed for bench press (p = 0.025) and deadlift (p = 0.001), but not leg press (p = 0.588).

Responder Cohorts and Clustering Variables

Figure 2 displays the training adaptations from PRE to POST for the four variables used in the composite score for the responder analysis including DXA LSTM, VL CSA, mid-thigh mCSA, and estimated 1RM deadlift. For all participants, the change values were as follows: 1.07 $\pm$ 1.11 kg, 2.67 $\pm$ 2.70 cm^2, 8.9 $\pm$ cm^2, 24 $\pm$ 13 kg, respectively (Figure 2a, $p < 0.001$ for all variables from pre-to-post training). The HR cohort showed increases in these variables from PRE to POST ($p < 0.001$ for all variables). Although mid-thigh mCSA and estimated 1-RM deadlift increased in the LR cohort ($p = 0.011$ and $p = 0.001$, respectively), DXA LSTM ($p = 0.177$) and VL CSA ($p = 0.067$) did not. Finally, the HR cohort showed significantly greater increases than the LR cohort for all four variables (Figure 2b-e, $p < 0.001$ for all variables).

INSERT FIGURE 2

Hypertrophy Adaptations

Changes in mean fCSA and the responder analysis for this variable can be seen in Figure 3a-b. Mean fCSA significantly increased from PRE to POST by on average by 17.7% for all participants (PRE = 4290 $\pm$ 989 μm^2, POST = 5049 $\pm$ 1429 μm^2, $p = 0.001$). However, there was no cluster×time interaction observed for the responder analysis ($p = 0.606$; Figure 3b).

VL CSA significantly increased in all participants on average by 13.46% (PRE = 19.4 $\pm$ 3.0 cm^2, POST = 22.1 $\pm$ 4.5 cm^2, $p < 0.001$; Figure 3c). For the responder analysis, a significant cluster×time interaction ($p < 0.001$), a main effect of time ($p < 0.001$), and a main effect of cluster were also observed ($p = 0.001$) (Figure 3d).

Mid-thigh mCSA significantly increased in all participants on average by 8.3% (PRE: 107.1 ± 10.9 cm^2, POST: 116.0 ± 14.2 cm^2, $p < 0.001$; Figure 3e). For the responder analysis, a significant cluster×time interaction ($p < 0.001$), a main effect of time ($p < 0.001$), and main effect of cluster were also observed ($p = 0.001$) (Figure 3f).

INSERT FIGURE 3

24-Hour MyoPS Response to First Bout of Training

The 24-hr integrated MyoPS response to the first naïve bout of resistance exercise (RE) and additional analyses related to this variable are presented in Figure 4. For the initial analysis, 32 participants were included due to two participant samples lacking appropriate yield amounts from isolated myofibrillar proteins. MyoPS rates were 2.25 ± 1.05% per day during this 24-hour period (Figure 4a). There was no significant difference between HR and LR cohorts ($p = 0.370$, Figure 4b). There was no significant association between the pre-to-post training percentage change in mean fCSA and 24-hr MyoPS response (Figure 4c, $r = 0.389$, $p = 0.059$). Likewise, there was no significant association between pre-to-post training percentage change in VL CSA and the 24-hr integrated MyoPS (Figure 4d, $r = 0.096$, $p = 0.600$, $n = 31$).

INSERT FIGURE 4

Satellite Cell and Myonuclear Number

Satellite cell number changes from PRE to POST and correlations to hypertrophy can be seen in Figure 5. Satellite cell number per muscle fiber increased in all participants by 34.1% ($p = 0.025$, Figure 5a). A significant cluster×time interaction was observed for the number of satellite cells per muscle fiber ($p = 0.029$), but there was no main effect of time or cluster ($p =$

0.098 and p = 0.615 respectively, Figure 5b). Post hoc testing indicated a significant increase occurred from PRE to POST in higher responders (p = 0.026), whereas no significant difference was evident from PRE to POST in lower responders (p = 0.118). When considering all participants for correlations, there was no significant association between the pre-to-post training percentage changes in satellite cells per fiber and VL CSA (r = .095, p = 0.610, Figure 5c). However, there was a positive correlation between the pre-to-post training percentage changes in satellite cells per fiber and mean fCSA (r = 0.471, p = 0.008, Figure 5d).

INSERT FIGURE 5

Change in myonuclear number per fiber from PRE to POST in all participants is presented in Figure 6a. There was a significant increase in myonuclei per fiber from PRE to POST in all participants (p = 0.013). With the responder cluster analysis, there was a significant main effect of time from PRE to POST (p = 0.049; Figure 6b). However, the cluster effect and cluster×time interaction were not significant (p = 0.160 and p = 0.236 respectively; Figure 6b). There was a significant positive correlation between percent changes in myonuclei per fiber and mean fCSA from PRE to POST (r = 0.523. p = 0.003; Figure 6d), but no significant correlation between percent change in VL CSA (p = 0.159; Figure 6c).

INSERT FIGURE 6

Muscle Ribosome Content

Changes in muscle ribosome content and associations with hypertrophic outcomes can be found in Figure 7. There was no difference in muscle ribosome content between PRE and POST for all participants (p = 0.684). The cluster×time interaction and main effects of time and cluster

were all not significant ($p = 0.157$, $p = 0.778$, $p = 0.888$ respectively, Figure 7b). The correlations between percentage changes in muscle ribosome content and both hypertrophy measures were not significant (VL CSA $p = 0.060$, mean fCSA $p = 0.574$; Figure 7c/d).

INSERT FIGURE 7

Menstrual Cycle

Menstrual cycle data is presented in Table 3. At PRE, women who had a consistent menstrual cycle, on average, were on day 18 of their cycle (Table 3). At POST testing, the average was day 16 of the menstrual cycle (Table 3). There was no significant difference between average day of the menstrual cycle between PRE and POST ($p = 0.512$). Importantly there was no difference in date of cycle during PRE or POST between response clusters (see Table 3).

INSERT TABLE 3

Self-Reported Nutritional Intakes

Total energy, carbohydrate, and fat intake did not significantly differ from PRE to POST for all participants (see Table 4). However, protein intake for all participants was significantly higher at POST ($p = 0.022$). There was no difference in nutritional intake between responder clusters (see Table 4 for values).

INSERT TABLE 4

Sleep

On average, participants reported obtaining 3703 ± 419 minutes (8.82 hours) of sleep a night (Table 5). There was no significant difference between the average hours of sleep between HR and LR cohorts. These values are portrayed in Table 5.

INSERT TABLE 5

DISCUSSION

Main findings from the present study include: (i) 24-hr integrated MyoPS following the first training bout did not predict hypertrophy outcomes, (ii) muscle ribosome content did change with training and/or predict hypertrophy outcomes, and (iii) satellite cell proliferation occurred in higher responders. As discussed in later paragraphs, these main findings will be compared to what has been observed in the literature. Notably, most of the literature is in males (both human and animal), so this is taken into consideration. From the data presented prior, although males and females show similar changes in hypertrophic outcomes with RT, mechanisms may differ in response to training. Therefore, these biological sex comparisons can be used as a basis to guide future research.

Changes in muscle ribosome content (i.e., total muscle RNA concentrations) was not predictive of hypertrophy (i.e., ΔVL CSA or ΔVL mean fCSA), and in fact did not significantly change over the 10 weeks of training for all participants, regardless of responder group. These findings contrast multiple studies that have reported an increase in muscle ribosome content with 6 – 12 weeks of RT [26, 36, 58]. However, this study is unique given that females were examined herein. This finding does not discount the role that ribosome biogenesis plays in muscle hypertrophy, and it is possible that more dynamic responses could have occurred earlier in training. However, given that we only captured muscle ribosome content at select time points,

more research is needed to further the investigation of sex differences in ribosome biogenesis mechanisms in response to RT.

As previously stated, the 24-hour integrated MyoPS response to the first bout of resistance exercise was not predictive of hypertrophy (i.e., ΔVL CSA or ΔVL mean fCSA). The measured level of MyoPS (2.29 $\pm$ 1.05%) aligns with previous literature in studies showing heightened MyoPS rates 24 hours following RE in males [7, 58]. We hypothesize that this could be due to other factors affecting net muscle protein balance early into training. Specifically, muscle protein breakdown in an untrained individual during the initial sessions of RT are likely increased due to the naivety of the stimulus on the body. Therefore, this level of breakdown activity will impact the net protein balance, and therefore hypertrophy. Additionally, the MyoPS response over the entirety of the training intervention was not measured, and the magnitude and duration of MyoPS increases can change with training within the same individual [59, 60]. Clearly, this study continues to demonstrate that acute measures of MyoPS are not proxy measures for hypertrophy, and this has been articulated by Witard and colleagues in a recent review article as well [61].

The key finding from the present study is that satellite cell number increased with training in the HR cluster versus the LR cluster, and there was a moderately strong positive correlation between the percentage change in satellite cell number and mean fCSA in all participants ($r = 0.471$, $p=0.007$). Additionally, myonuclear number per fiber increased significantly following training, regardless of response cluster, and a moderate positive correlation was found between percentage changes in this variable and mean fCSA in all participants ($r = 0.523$, $p=0.003$). Satellite cells are distinctively defined by their proximity to skeletal muscle fibers and are positioned between the sarcolemma and basement membrane. In

addition to location, satellite cells are also identified by the expression of several genes, including paired box 7 (PAX7), a common target gene in experimental models [28]. Mechanical overload stimulates satellite cell proliferation, differentiation, and fusion to pre-existing myofibers [62], and an increase in satellite cell–mediated myonuclear addition in growing myofibers provides cellular support for the increased of cellular macromolecules (e.g., ribosomes and muscle proteins) [63]. Importantly, there are studies showing that satellite cell proliferation increases in response to one bout of resistance exercise [64-69]. Moreover, our findings agree with several studies that have shown a similar response to weeks of RT in males [70-72] and females [72-74].

It has been implied that satellite cell-mediated myonuclear accretion coincides with hypertrophy [13, 71, 75]. However, hypertrophy without myonuclear accretion has also been observed in humans, and more specifically, untrained females. For instance, Herman-Montemayor et al. [76] demonstrated a 30% increase in type II fiber hypertrophy, a 29% increase in the size of myonuclear domains, and no myonuclear accretion. Petrella et al. [65] reported similar findings after 16 weeks of knee extensor training in untrained females. Specifically, mean fCSA and myonuclear domain size increased while myonuclear number remained unchanged.

Interestingly, we observed a significant increase in satellite cell number in the HR cluster, whereas the LR cluster did not exhibit this phenotype. However, greater increases in satellite cell number did not translate to enhancements in myonuclear number in HR, as the latter metric showed a main effect of training but not responder group-by-time interaction. There are speculative explanations for this latter observation. First, an enhancement in myonuclear accretion may have eventually become evident in HR if the training program was longer in duration. Second, beyond contributing to the myonuclear pool satellite cells have multiple roles

that not only impact adaptation to RT, but also cell health and structure. For example, satellite cells have been shown to secrete exosomes that communicate with fibroblasts to down-regulate collagen production [77, 78]. Given the pervasiveness of collagen in the extracellular matrix, an increase in satellite cell number in HR may have acted to reduce collagen synthesis to better facilitate myofiber and tissue growth. However, changes in muscle tissue collagen characteristics, satellite cell exosome cargo, and other molecular variables to test this hypothesis were not performed. Notwithstanding, these data support the contention that training-induced increases in satellite cell number coincides with skeletal muscle hypertrophy and may play a role in the responsiveness to RT.

Other secondary findings are also notable. First, the nutritional intake, average hours of sleep, and day of menstrual cycle at PRE and POST were not statistically different between responder clusters. However, there is growing interest in the field investigating the impact of the amount of sleep [79] and timing within the menstrual cycle [80-82] on training adaptations. While the present study collected data on these metrics, it did not thoroughly investigate these variables. An interesting finding in the present study is the HR cluster had higher lean mass values at PRE. Conversely, our laboratory has observed different findings in males, seeing that there was no difference in lean tissue mass, and, tangentially, LR had higher type II fCSA values at PRE than HR [36]. These data allude to the role body composition plays in the response to RT. However, more research is needed to fully address this relationship.

Limitations

As with any study, there are limitations that must be considered, and the first is the length of training. Previous literature has shown that as little as 3-6 weeks of RT increases hypertrophy

measures in untrained males [40, 83, 84], however less investigation has been done in females. While the present study was 10 weeks in duration, a longer intervention would have broadened the opportunity to observe training adaptations. Secondly, the analysis of 'higher responder' versus 'non/lower responder' is highly debated. Some researchers attribute this differentiation to technical error in the measurements being used to assess skeletal muscle hypertrophy or plainly inter-individual variation. Conversely, others (including our laboratory) believe there are characteristics that lead an individual to respond to RT in varying magnitudes. In the present study, we did see delineation into clusters based on the composite score. There is a lack of standardization in how response clusters are defined in the field, making this analysis seemingly subjective. However, to address this concern, data is both presented in the responder clusters as well as all participants with associations.

CONCLUSIONS

These data support prior research suggesting satellite cells are involved with skeletal muscle hypertrophy. As discussed throughout, more research is needed in determining other molecular factors that delineate lower versus higher responders in females to RT. Moreover, designing lifestyle and training interventions to optimize exercise adaptations in lower responders is warranted.

Availability of data and material

All raw data can be obtained by emailing the corresponding author (mdr0024@auburn.edu).

Competing interests

None of the authors has financial or other conflicts of interest to report regarding these data.

Funding

This study was made possible by a grant provided by the Peanut Institute Foundation (TPIF, Albany, GA, USA) to A.D.F., K.C.Y., and M.D.R. TPIF did not have a role in study design or analysis.

Authors' contributions

This experiment was performed at Auburn University's School of Kinesiology in the Molecular and Applied Sciences Laboratory. M.A.S., K.C.Y., and M.D.R. were responsible for the conception and design of the experiment. M.A.S. and M.D.R. primarily drafted the manuscript. All authors were involved in different aspects of data collection, medical oversight, or critical intellectual insight. All authors read and approved the final manuscript.

Acknowledgements

We thank the participants for partaking in the study. We also thank Dr. David Wagner (Metabolic Solutions) for his assistance regarding the mass spectrometry work. Finally, we thank Dr. Karyn Esser (University of Florida) for her insight.

REFERENCES FOR CHAPTER IV

1. Sheel, A.W., *Sex differences in the physiology of exercise: an integrative perspective.* Exp Physiol, 2016. **101**(2): p. 211-2.
2. Miller, V.M., *Sex-based physiology prior to political correctness.* Am J Physiol Endocrinol Metab, 2005. **289**(3): p. E359-60.
3. Costello, J.T., F. Bieuzen, and C.M. Bleakley, *Where are all the female participants in Sports and Exercise Medicine research?* Eur J Sport Sci, 2014. **14**(8): p. 847-51.
4. Kraemer, W.J., N.A. Ratamess, and D.N. French, *Resistance training for health and performance.* Curr Sports Med Rep, 2002. **1**(3): p. 165-71.
5. Stone, M., et al., *Comparison of the Effects of Three Different Weight-Training Programs on the One Repetition Maximum Squat.* The Journal of Strength & Conditioning Research, 2000. **14**.
6. Staron, R.S., et al., *Skeletal muscle adaptations during early phase of heavy-resistance training in men and women.* J Appl Physiol (1985), 1994. **76**(3): p. 1247-55.
7. Mitchell, C.J., et al., *Acute post-exercise myofibrillar protein synthesis is not correlated with resistance training-induced muscle hypertrophy in young men.* PLoS One, 2014. **9**(2): p. e89431.
8. Nordez, A., et al., *Comparison of methods to assess quadriceps muscle volume using magnetic resonance imaging.* J Magn Reson Imaging, 2009. **30**(5): p. 1116-23.
9. Ahtiainen, J.P., et al., *Heterogeneity in resistance training-induced muscle strength and mass responses in men and women of different ages.* Age (Dordr), 2016. **38**(1): p. 10.
10. Bamman, M.M., et al., *Cluster analysis tests the importance of myogenic gene expression during myofiber hypertrophy in humans.* J Appl Physiol (1985), 2007. **102**(6): p. 2232-9.
11. Van Etten, L.M., F.T. Verstappen, and K.R. Westerterp, *Effect of body build on weight-training-induced adaptations in body composition and muscular strength.* Med Sci Sports Exerc, 1994. **26**(4): p. 515-21.
12. Yannoulopoulos, B. and J.B. De Meeus, *[Arterial hypertension and pregnancy: detection and monitoring].* Soins Gynecol Obstet Pueric Pediatr, 1991(127-128): p. 22-6.
13. Petrella, J.K., et al., *Potent myofiber hypertrophy during resistance training in humans is associated with satellite cell-mediated myonuclear addition: a cluster analysis.* J Appl Physiol (1985), 2008. **104**(6): p. 1736-42.
14. Davidsen, P.K., et al., *High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression.* J Appl Physiol (1985), 2011. **110**(2): p. 309-17.
15. Gjestvang, C., et al., *Motives and barriers to initiation and sustained exercise adherence in a fitness club setting-A one-year follow-up study.* Scand J Med Sci Sports, 2020. **30**(9): p. 1796-1805.
16. Roberts, M.D., et al., *Physiological Differences Between Low Versus High Skeletal Muscle Hypertrophic Responders to Resistance Exercise Training: Current Perspectives and Future Research Directions.* Front Physiol, 2018. **9**: p. 834.
17. Kosek, D.J., et al., *Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults.* J Appl Physiol (1985), 2006. **101**(2): p. 531-44.

18. Figueiredo, V.C., et al., *Ribosome biogenesis adaptation in resistance training-induced human skeletal muscle hypertrophy.* Am J Physiol Endocrinol Metab, 2015. **309**(1): p. E72-83.
19. Terzis, G., et al., *Resistance exercise-induced increase in muscle mass correlates with p70S6 kinase phosphorylation in human subjects.* Eur J Appl Physiol, 2008. **102**(2): p. 145-52.
20. Phillips, S.M., *A brief review of critical processes in exercise-induced muscular hypertrophy.* Sports Med, 2014. **44 Suppl 1**: p. S71-7.
21. Hartman, J.W., et al., *Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters.* Am J Clin Nutr, 2007. **86**(2): p. 373-81.
22. Burd, N.A., et al., *Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men.* PLoS One, 2010. **5**(8): p. e12033.
23. Nakada, S., et al., *Correlation between Ribosome Biogenesis and the Magnitude of Hypertrophy in Overloaded Skeletal Muscle.* PLoS One, 2016. **11**(1): p. e0147284.
24. Stec, M.J., et al., *Ribosome biogenesis may augment resistance training-induced myofiber hypertrophy and is required for myotube growth in vitro.* Am J Physiol Endocrinol Metab, 2016. **310**(8): p. E652-E661.
25. Mobley, C.B., et al., *Biomarkers associated with low, moderate, and high vastus lateralis muscle hypertrophy following 12 weeks of resistance training.* PLoS One, 2018. **13**(4): p. e0195203.
26. Mitchell, C.J., et al., *Resistance exercise load does not determine training-mediated hypertrophic gains in young men.* J Appl Physiol (1985), 2012. **113**(1): p. 71-7.
27. Schiaffino, S., S.P. Bormioli, and M. Aloisi, *The fate of newly formed satellite cells during compensatory muscle hypertrophy.* Virchows Arch B Cell Pathol, 1976. **21**(2): p. 113-8.
28. Seale, P., et al., *Pax7 is required for the specification of myogenic satellite cells.* Cell, 2000. **102**(6): p. 777-86.
29. Murach, K.A., et al., *Fusion-Independent Satellite Cell Communication to Muscle Fibers During Load-Induced Hypertrophy.* Function (Oxf), 2020. **1**(1): p. zqaa009.
30. Rosenblatt, J.D. and D.J. Parry, *Gamma irradiation prevents compensatory hypertrophy of overloaded mouse extensor digitorum longus muscle.* J Appl Physiol (1985), 1992. **73**(6): p. 2538-43.
31. O'Connor, R.S. and G.K. Pavlath, *Point:Counterpoint: Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy.* J Appl Physiol (1985), 2007. **103**(3): p. 1099-100.
32. McCarthy, J.J., et al., *Effective fiber hypertrophy in satellite cell-depleted skeletal muscle.* Development, 2011. **138**(17): p. 3657-66.
33. McCarthy, J.J. and K.A. Esser, *Counterpoint: Satellite cell addition is not obligatory for skeletal muscle hypertrophy.* J Appl Physiol (1985), 2007. **103**(3): p. 1100-2; discussion 1102-3.
34. Jackson, J.R., et al., *Satellite cell depletion does not inhibit adult skeletal muscle regrowth following unloading-induced atrophy.* Am J Physiol Cell Physiol, 2012. **303**(8): p. C854-61.

35. Vann, C.G., et al., *Effects of High-Volume versus High-Load Resistance Training on Skeletal Muscle Growth and Molecular Adaptations.* bioRxiv, 2021: p. 2021.07.01.450728.
36. Haun, C.T., et al., *Pre-training Skeletal Muscle Fiber Size and Predominant Fiber Type Best Predict Hypertrophic Responses to 6 Weeks of Resistance Training in Previously Trained Young Men.* Front Physiol, 2019. **10**: p. 297.
37. Morton, R.W., et al., *Muscle Androgen Receptor Content but Not Systemic Hormones Is Associated With Resistance Training-Induced Skeletal Muscle Hypertrophy in Healthy, Young Men.* Front Physiol, 2018. **9**: p. 1373.
38. Phillips, S.M., et al., *Resistance training reduces the acute exercise-induced increase in muscle protein turnover.* Am J Physiol, 1999. **276**(1): p. E118-24.
39. Damas, F., et al., *A review of resistance training-induced changes in skeletal muscle protein synthesis and their contribution to hypertrophy.* Sports Med, 2015. **45**(6): p. 801-7.
40. Haun, C.T., et al., *Muscle fiber hypertrophy in response to 6 weeks of high-volume resistance training in trained young men is largely attributed to sarcoplasmic hypertrophy.* PLoS One, 2019. **14**(6): p. e0215267.
41. Walker, D.K., et al., *Exercise, amino acids, and aging in the control of human muscle protein synthesis.* Med Sci Sports Exerc, 2011. **43**(12): p. 2249-58.
42. Haun, C.T., et al., *A Critical Evaluation of the Biological Construct Skeletal Muscle Hypertrophy: Size Matters but So Does the Measurement.* Front Physiol, 2019. **10**: p. 247.
43. Staron, R.S., et al., *Strength and skeletal muscle adaptations in heavy-resistance-trained women after detraining and retraining.* J Appl Physiol (1985), 1991. **70**(2): p. 631-40.
44. Abe, T., et al., *Time course for strength and muscle thickness changes following upper and lower body resistance training in men and women.* Eur J Appl Physiol, 2000. **81**(3): p. 174-80.
45. Räntilä, A., et al., *High Responders to Hypertrophic Strength Training Also Tend to Lose More Muscle Mass and Strength During Detraining Than Low Responders.* J Strength Cond Res, 2021. **35**(6): p. 1500-1511.
46. Roberts, M.D., et al., *Skeletal muscle mitochondrial volume and myozenin-1 protein differences exist between high versus low anabolic responders to resistance training.* PeerJ, 2018. **6**: p. e5338.
47. Sexton, C.L., et al., *Effects of Peanut Protein Supplementation on Resistance Training Adaptations in Younger Adults.* Nutrients, 2021. **13**(11).
48. Kephart, W.C., et al., *Ten weeks of branched-chain amino acid supplementation improves select performance and immunological variables in trained cyclists.* Amino Acids, 2016. **48**(3): p. 779-789.
49. Rantalainen, T., et al., *An open source approach for regional cortical bone mineral density analysis.* J Musculoskelet Neuronal Interact, 2011. **11**(3): p. 243-8.
50. Evans, W.J., S.D. Phinney, and V.R. Young, *Suction applied to a muscle biopsy maximizes sample size.* Med Sci Sports Exerc, 1982. **14**(1): p. 101-2.
51. Wen, Y., et al., *MyoVision: software for automated high-content analysis of skeletal muscle immunohistochemistry.* J Appl Physiol (1985), 2018. **124**(1): p. 40-51.

52. Vann, C.G., et al., *Molecular Differences in Skeletal Muscle After 1 Week of Active vs. Passive Recovery From High-Volume Resistance Training.* J Strength Cond Res, 2021. **35**(8): p. 2102-2113.
53. Roberts, M.D., et al., *An optimized procedure for isolation of rodent and human skeletal muscle sarcoplasmic and myofibrillar proteins.* J Biol Methods, 2020. **7**(1): p. e127.
54. Bell, K.E., et al., *Integrated Myofibrillar Protein Synthesis in Recovery From Unaccustomed and Accustomed Resistance Exercise With and Without Multi-ingredient Supplementation in Overweight Older Men.* Front Nutr, 2019. **6**: p. 40.
55. Nader, G.A., T.J. McLoughlin, and K.A. Esser, *mTOR function in skeletal muscle hypertrophy: increased ribosomal RNA via cell cycle regulators.* Am J Physiol Cell Physiol, 2005. **289**(6): p. C1457-65.
56. Mobley, C.B., et al., *Comparative effects of whey protein versus L-leucine on skeletal muscle protein synthesis and markers of ribosome biogenesis following resistance exercise.* Amino Acids, 2016. **48**(3): p. 733-750.
57. Buysse, D.J., et al., *The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research.* Psychiatry Res, 1989. **28**(2): p. 193-213.
58. Brook, M.S., et al., *Skeletal muscle hypertrophy adaptations predominate in the early stages of resistance exercise training, matching deuterium oxide-derived measures of muscle protein synthesis and mechanistic target of rapamycin complex 1 signaling.* FASEB J, 2015. **29**(11): p. 4485-96.
59. Tang, J.E., et al., *Resistance training alters the response of fed state mixed muscle protein synthesis in young men.* Am J Physiol Regul Integr Comp Physiol, 2008. **294**(1): p. R172-8.
60. Burd, N.A., et al., *Resistance exercise volume affects myofibrillar protein synthesis and anabolic signalling molecule phosphorylation in young men.* J Physiol, 2010. **588**(Pt 16): p. 3119-30.
61. Witard, O.C., L. Bannock, and K.D. Tipton, *Making Sense of Muscle Protein Synthesis: A Focus on Muscle Growth During Resistance Training.* Int J Sport Nutr Exerc Metab, 2021: p. 1-13.
62. Fry, C.S., et al., *Regulation of the muscle fiber microenvironment by activated satellite cells during hypertrophy.* FASEB J, 2014. **28**(4): p. 1654-65.
63. Kirby, T.J., et al., *Myonuclear transcription is responsive to mechanical load and DNA content but uncoupled from cell size during hypertrophy.* Mol Biol Cell, 2016. **27**(5): p. 788-98.
64. Crameri, R.M., et al., *Changes in satellite cells in human skeletal muscle after a single bout of high intensity exercise.* J Physiol, 2004. **558**(Pt 1): p. 333-40.
65. Petrella, J.K., et al., *Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women.* Am J Physiol Endocrinol Metab, 2006. **291**(5): p. E937-46.
66. Kim, J.S., et al., *Resting and load-induced levels of myogenic gene transcripts differ between older adults with demonstrable sarcopenia and young men and women.* J Appl Physiol (1985), 2005. **99**(6): p. 2149-58.
67. Kim, J.S., J.M. Cross, and M.M. Bamman, *Impact of resistance loading on myostatin expression and cell cycle regulation in young and older men and women.* Am J Physiol Endocrinol Metab, 2005. **288**(6): p. E1110-9.

68. Walker, D.K., et al., *PAX7+ satellite cells in young and older adults following resistance exercise.* Muscle Nerve, 2012. **46**(1): p. 51-9.
69. O'Reilly, C., et al., *Hepatocyte growth factor (HGF) and the satellite cell response following muscle lengthening contractions in humans.* Muscle Nerve, 2008. **38**(5): p. 1434-1442.
70. Conceicao, M.S., et al., *Muscle Fiber Hypertrophy and Myonuclei Addition: A Systematic Review and Meta-analysis.* Med Sci Sports Exerc, 2018. **50**(7): p. 1385-1393.
71. Kadi, F., et al., *The effects of heavy resistance training and detraining on satellite cells in human skeletal muscles.* J Physiol, 2004. **558**(Pt 3): p. 1005-12.
72. Roth, S.M., et al., *Skeletal muscle satellite cell characteristics in young and older men and women after heavy resistance strength training.* J Gerontol A Biol Sci Med Sci, 2001. **56**(6): p. B240-7.
73. Kadi, F. and L.E. Thornell, *Concomitant increases in myonuclear and satellite cell content in female trapezius muscle following strength training.* Histochem Cell Biol, 2000. **113**(2): p. 99-103.
74. Abou Sawan, S., et al., *Satellite cell and myonuclear accretion is related to training-induced skeletal muscle fiber hypertrophy in young males and females.* J Appl Physiol (1985), 2021. **131**(3): p. 871-880.
75. Bruusgaard, J.C., et al., *Myonuclei acquired by overload exercise precede hypertrophy and are not lost on detraining.* Proc Natl Acad Sci U S A, 2010. **107**(34): p. 15111-6.
76. Herman-Montemayor, J.R., R.S. Hikida, and R.S. Staron, *Early-Phase Satellite Cell and Myonuclear Domain Adaptations to Slow-Speed vs. Traditional Resistance Training Programs.* J Strength Cond Res, 2015. **29**(11): p. 3105-14.
77. Fry, C.S., et al., *Myogenic Progenitor Cells Control Extracellular Matrix Production by Fibroblasts during Skeletal Muscle Hypertrophy.* Cell Stem Cell, 2017. **20**(1): p. 56-69.
78. Murach, K.A., et al., *Starring or Supporting Role? Satellite Cells and Skeletal Muscle Fiber Size Regulation.* Physiology (Bethesda), 2018. **33**(1): p. 26-38.
79. Knowles, O.E., et al., *Inadequate sleep and muscle strength: Implications for resistance training.* J Sci Med Sport, 2018. **21**(9): p. 959-968.
80. Bruinvels, G., et al., *Sport, exercise and the menstrual cycle: where is the research?* Br J Sports Med, 2017. **51**(6): p. 487-488.
81. Carmichael, M.A., et al., *The Impact of Menstrual Cycle Phase on Athletes' Performance: A Narrative Review.* Int J Environ Res Public Health, 2021. **18**(4).
82. Blagrove, R.C., G. Bruinvels, and C.R. Pedlar, *Variations in strength-related measures during the menstrual cycle in eumenorrheic women: A systematic review and meta-analysis.* J Sci Med Sport, 2020. **23**(12): p. 1220-1227.
83. Seynnes, O.R., M. de Boer, and M.V. Narici, *Early skeletal muscle hypertrophy and architectural changes in response to high-intensity resistance training.* J Appl Physiol (1985), 2007. **102**(1): p. 368-73.
84. DeFreitas, J.M., et al., *An examination of the time course of training-induced skeletal muscle hypertrophy.* Eur J Appl Physiol, 2011. **111**(11): p. 2785-90.

TABLES, FIGURES, AND LEGENDS

Table 1. Resistance training program.

Week	Day	Sets x Repetitions	%1RM
1	1	3-RM Testing (+ 2 x 10)	50%
	2	5 x 6	56%
2	3	4 x 10	55%
	4	5 x 6	65%
3	5	4 x 10	60%
	6	5 x 6	74%
4	7	4 x 10	65%
	8	5 x 6	84%
5	9	4 x 10	50%
	10	5 x 6	50%
6	11	4 x 10	65%
	12	5 x 6	84%
7	13	4 x 10	70%
	14	5 x 6	90%
8	15	4 x 10	75%
	16	5 x 6	96%
9	17	4 x 10	80%
	18	5 x 6	98%
10	19	5 x 6	102%
	20	3-RM Testing	---

Legend: This table represents the training paradigm for the 10-week progressive resistance training program. Abbreviations: 3-RM, three-repetition maximum test.

Table 2. Participant Characteristics

Variable	**All (n = 34)**	**HR (n = 8)**	**LR (n = 8)**	***p* value HR vs. LR**
Age (years)	21 ± 2.1	21 ± 1.2	21.3 ± 1.3	0.693
Height (m)	1.7 ± 0.1	1.7 ± .1	1.7 ± .1	0.909
PRE body mass (kg)	68.2 ± 10.3	69.7 ± 11.1	62.5 ± 10.8	0.214
POST body mass (kg)	68.2 ± 10.3	71.7 ± 10.9	63.5 ± 9.9	0.138
PRE LSTM (kg)	42.09 ± 4.07	44.2 ± 3.3	39.2 ± 3.5	**0.010**
POST LSTM (kg)	43.16 ± 4.27^{+}	46.5 ± 3.2	39.5 ± 3.7	**0.001**
PRE FM (kg)	22.98 ± 7.99	22.4 ± 8.6	21.8 ± 8.2	0.876
POST FM (kg)	22.52 ± 7.53	22.6 ± 9.0	21.4 ± 7.1	0.763
PRE 1RM LEG PRESS (KG)	83 ± 39	111.8 ± 29	50.6 ± 39.4	**0.003**
POST 1RM LEG PRESS (KG)	168 ± 48^{+}	184.9 ± 28.8	118.3 ± 33.3	**0.001**
PRE 1RM BENCH PRESS (KG)	32 ± 6	34.2 ± 5.4	27.2 ± 6.0	**0.026**
POST 1RM BENCH PRESS (KG)	39 ± 7^{+}	44.0 ± 5.1	33.3 ± 4.6	**0.001**
PRE 1RM DEADLIFT (KG)	60 ± 14	64.4 ± 11.6	51.7 ± 12.6	0.055
POST 1RM DEADLIFT (KG)	82 ± 23^{+}	101.0 ± 11.1	64.7 ± 14.4	**<0.001**

Key: LSTM, lean/soft tissue mass; FM, fat mass; 1RM, one-repetition maximum; $^{+}$ denotes significance between PRE and POST for all participants. Significance is noted by bolded *p* values.

Table 3. Menstrual Cycle Comparison Between Responder Clusters

	All	**HR**	**LR**	***p-value of t-test between HR and LR***
Date of Cycle Pre-testing	18	15	18	0.480
Date of Cycle Post-testing	16	12	10	0.801

Key: HR = higher responder; LR = lower responder. Legend: PRE n for All = 26, POST n for All = 28, HR n PRE = 4, HR n POST = 5, LR n PRE = 7, LR n PRE = 7

Table 4. Nutritional Intake

Variable	**All (n = 34)**	**HR (n = 8)**	**LR (n = 8)**	***p* value HR vs. LR**
PRE Total Energy (kcal)	1459 ± 438	1268 ± 388	1512 ± 474	0.279
POST Total Energy (kcal)	1490 ± 447	1312 ± 448	1627 ± 618	0263
p value PRE VS. POST	0.597	0.683	0.443	--
PRE Protein (g)	65 ± 27	61 ± 32	65 ± 30	0.807
POST Protein (g)	76 ± 26	68 ± 22	77 ± 31	0.521
p value PRE VS. POST	**0.022**	0.658	0.149	--
PRE Carbohydrate (g)	157.21 ± 70.91	117 ± 51	189 ± 84	0.058
POST Carbohydrate (g)	159.29 ± 56.45	132 ± 42	195 ± 80	0.071
p value PRE VS. POST	0.793	0.095	0.822	--
PRE Fat (g)	63.61 ± 18.71	62 ± 19	58 ± 18	0.629
PRE Fat (g)	62.81 ± 21.20	58 ± 26	63 ± 29	0.728
p value PRE VS. POST	0.819	0.607	0.728	--

Key: HR = higher responder, LR = lower responder. Legend: All n = 34; HR n = 8; LR n = 8.

Bold face illustrates significance within the paired t-tests from PRE to POST.

Table 5. Sleep Characteristics

	All	**HR**	**LR**	***p-value HR vs. LR***
Average hours of sleep	8.82 ± 1.00	8.74 ± 1.05	8.54 ± 0.75	0.664

Key: HR = higher responder; LR = lower responder. Legend: All n = 34; HR n = 8; LR n = 8.

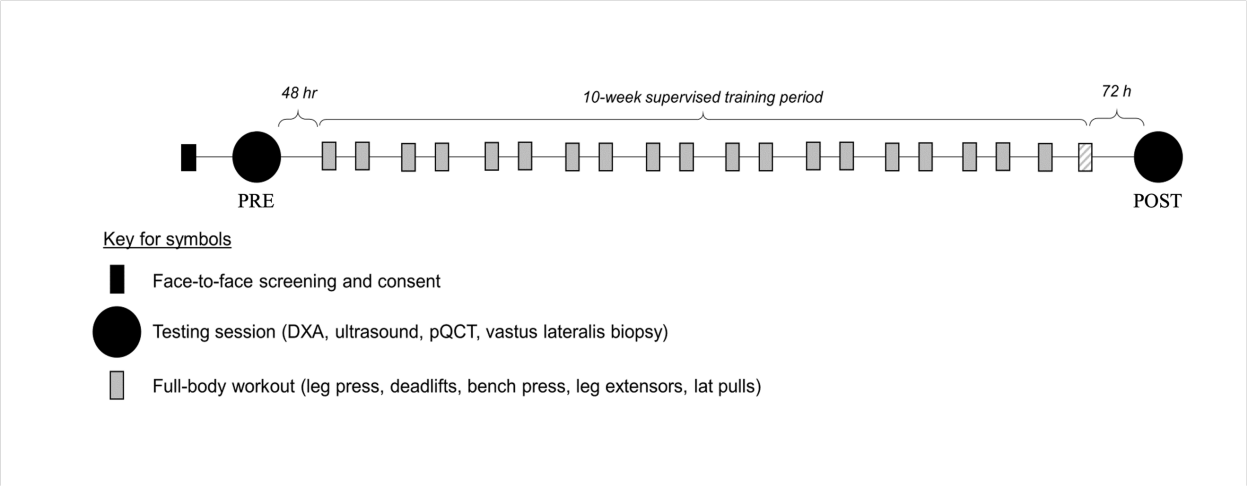

Figure 1. Study Design. Legend: The figure above outlines the study design. More in-depth descriptions of procedures can be found in-text.

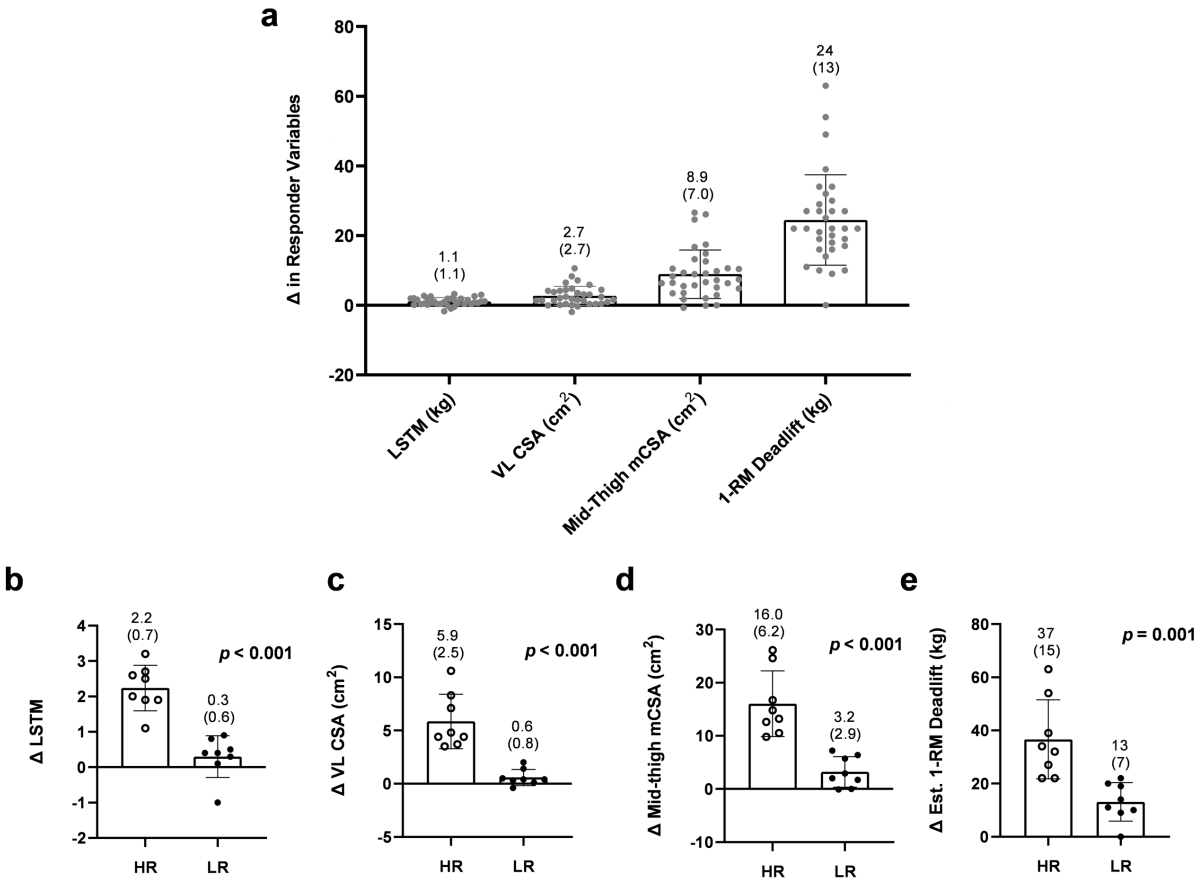

Figure 2. Responder Analysis. The four variables used for the responder analysis for all participants (panel a). Change in LSTM (panel b), VL CSA (panel c), mid-thigh mCSA (panel d), and estimated 1-RM deadlift (panel e) compared between HR and LR clusters. All data are presented as mean (standard deviation), and each circle is one participant. The grey circles (panel a) are all participants, the white circles are the HR cluster (panel b-e), and the black circles are the LR cluster (panel b-e). Significance is noted by bolded *p* values Key: HR = higher responders; LR = lower responders.

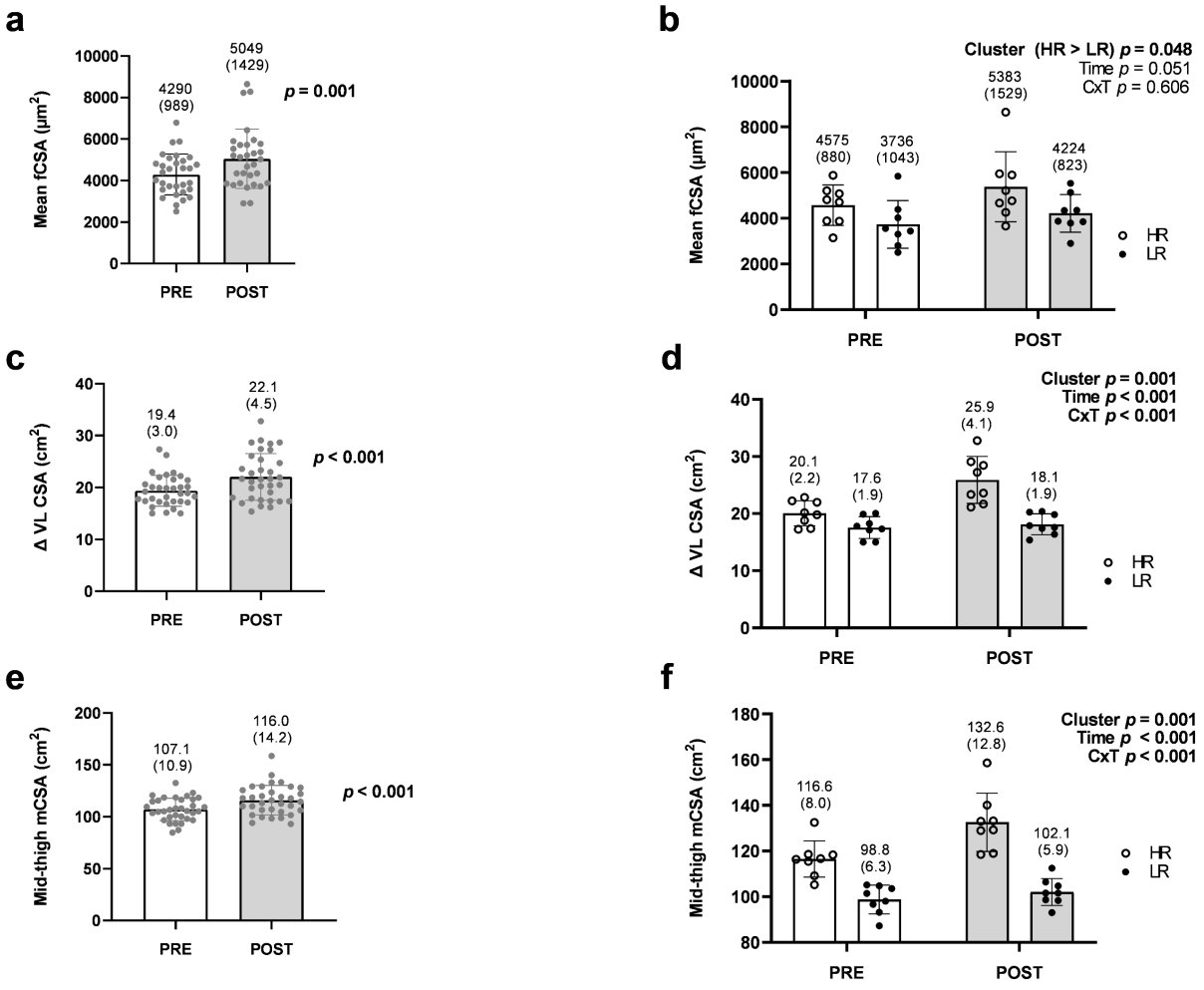

Figure 3. Hypertrophy measures. Mean fCSA data comparing PRE to POST is presented in panel a. Mean fCSA changes in HR and LR clusters over time is presented in panel b. Change in VL CSA over time is presented in panel c, as well as change over time broken into responder groups (panel d). Mid-thigh mCSA data is presented in panels e and f. All data are presented as mean (standard deviation), and each circle is one participant. The grey circles (panel a) are all participants, the white circles are the HR cluster (panel b-e), and the black circles are the LR cluster (panel b-e). Panel a, c, and e n = 34. Significance is noted by bolded *p* values. Key: HR = higher responders; LR = lower responders.

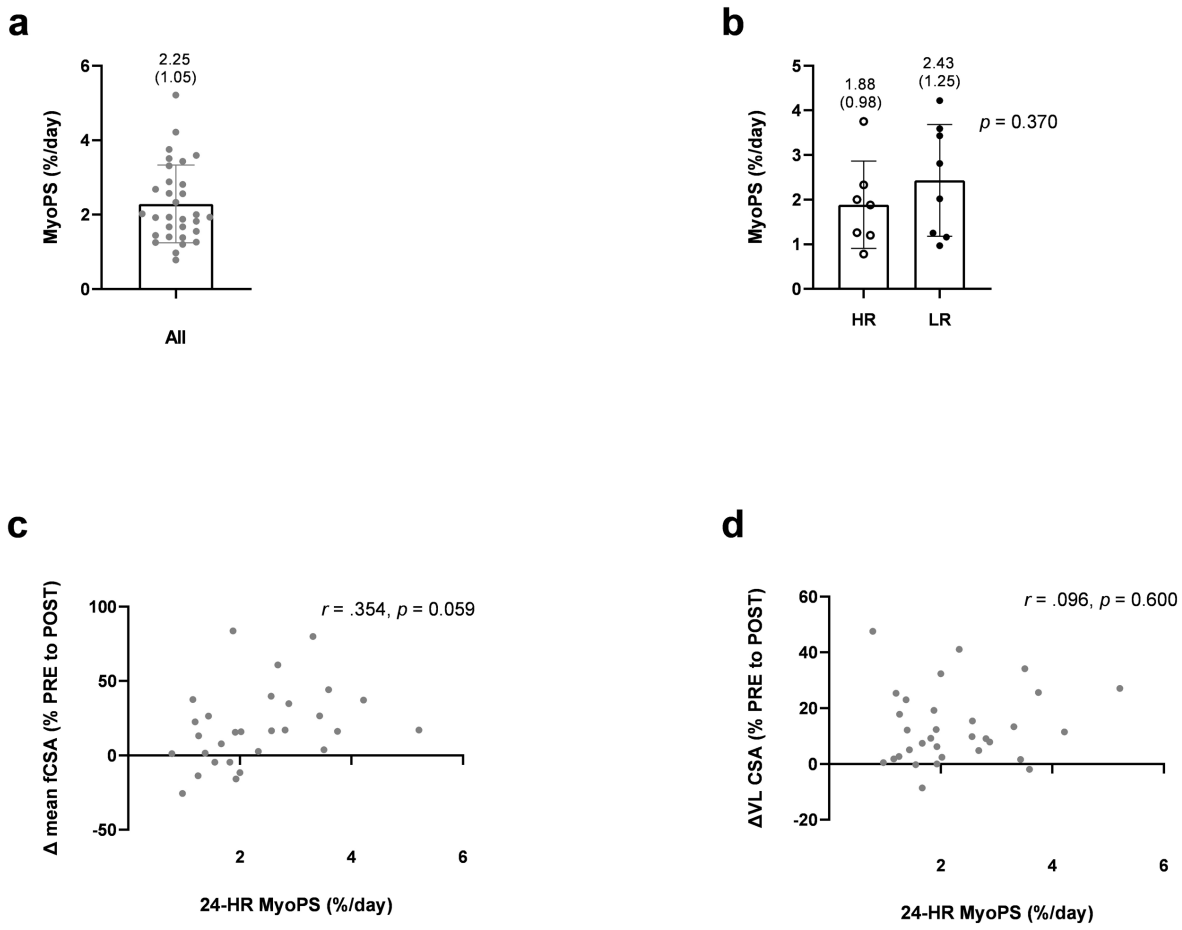

Figure 4. Myofibrillar protein synthesis rates 24 hours following the first bout of training. All data are presented as mean (standard deviation), and each circle is one participant. The grey circles (panel a) are all participants, the white circles are the HR cluster (panel b-d), and the black circles are the LR cluster (panel b-d). Significance is noted by bolded *p* values. Panel a n = 31; panel c n = 29; panel d n = 32. Key: HR = higher responders; LR = lower responders.

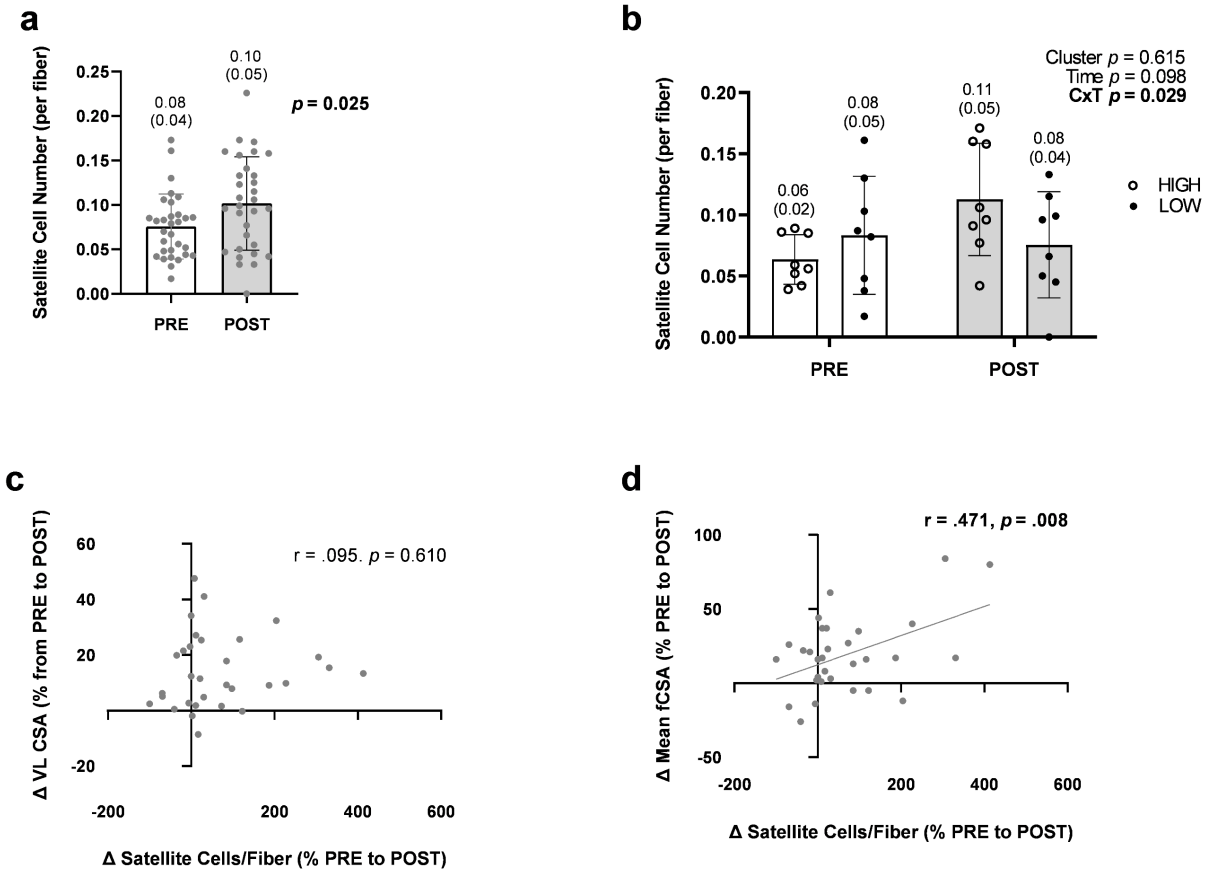

Figure 5. Satellite cell number from PRE to POST and correlations with hypertrophy. All data are presented as mean (standard deviation), and each circle is one participant. The grey circles (panel a) are all participants, the white circles are the HR cluster (panel b-d), and the black circles are the LR cluster (panel b-d). Significance is noted by bolded *p* values. n=31 for all panels Key: HR = higher responders; LR = lower responders.

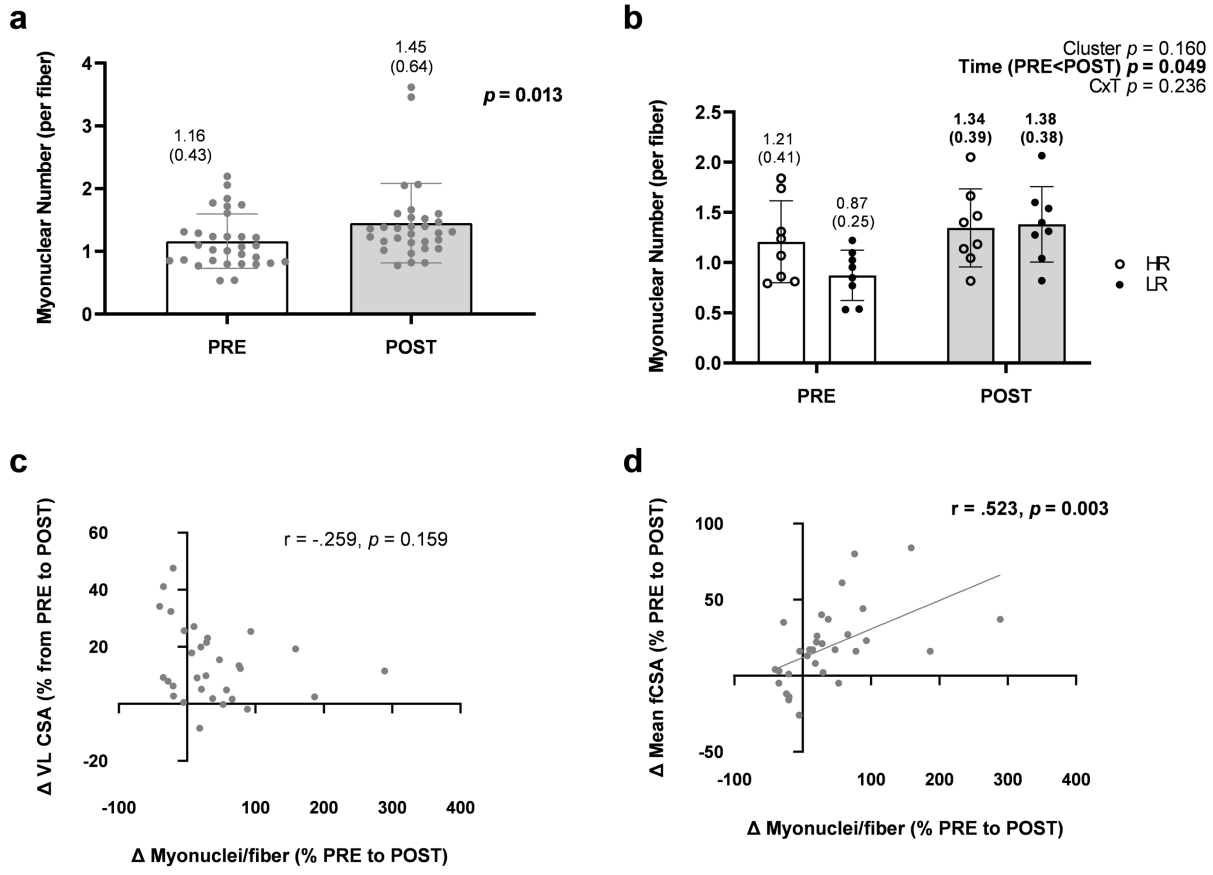

Figure 6. Myonuclear number changes over time and correlation to hypertrophy. All data are presented as mean (standard deviation), and each circle is one participant. The grey circles (panel a) are all participants, the white circles are the HR cluster (panel b-d), and the black circles are the LR cluster (panel b-d). Significance is noted by bolded *p* values. n = 31. Key: HR = higher responders; LR = lower responders.

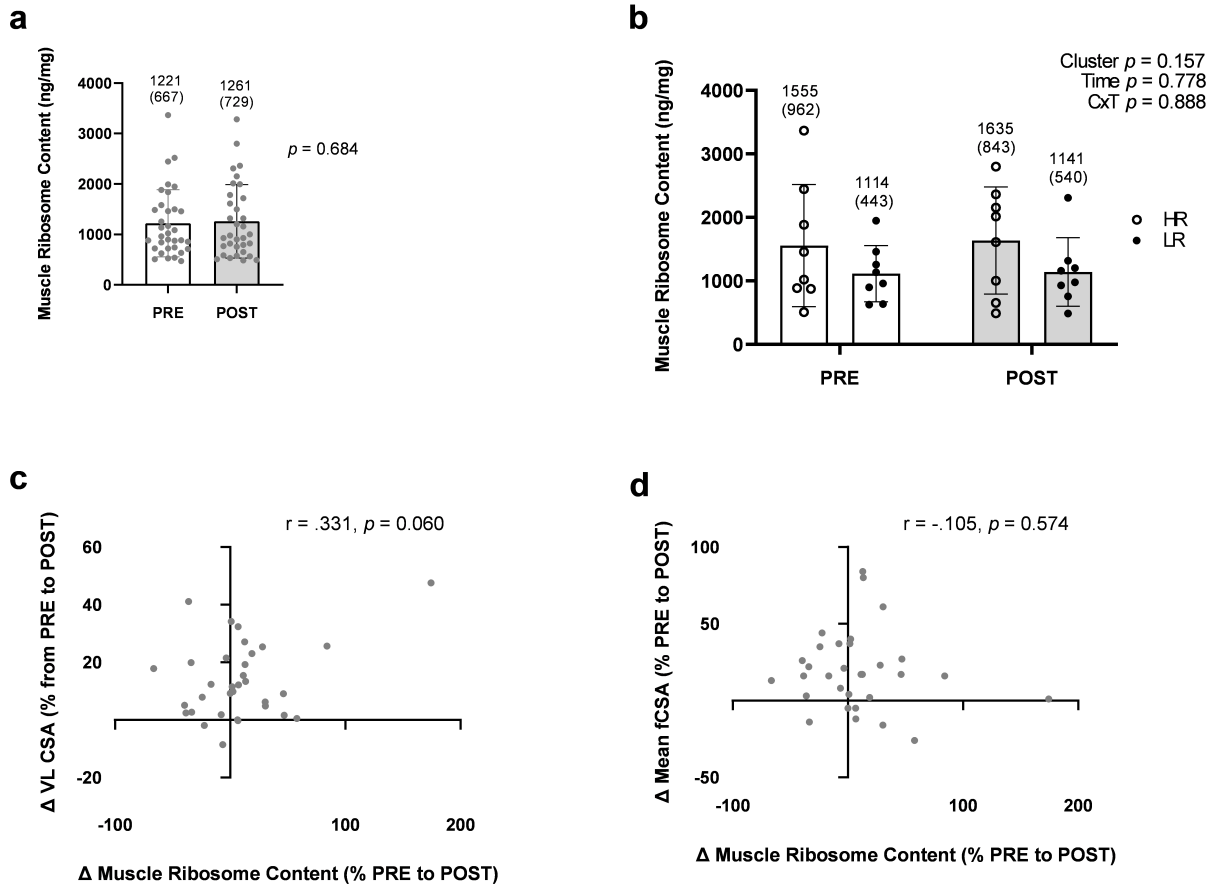

Figure 7. Muscle ribosome content changes over time, between responder clusters, and correlations to hypertrophy. All data are presented as mean (standard deviation), and each circle is one participant. The grey circles (panel a) are all participants, the white circles are the HR cluster (panel b-d), and the black circles are the LR cluster (panel b-d). Significance is noted by bolded *p* values. n = 33. Key: HR = higher responders; LR = lower responders.

REFERENCES FOR CHAPTERS I-III

1. Sheel, A.W., *Sex differences in the physiology of exercise: an integrative perspective.* Exp Physiol, 2016. **101**(2): p. 211-2.
2. Miller, V.M., *Sex-based physiology prior to political correctness.* Am J Physiol Endocrinol Metab, 2005. **289**(3): p. E359-60.
3. Costello, J.T., F. Bieuzen, and C.M. Bleakley, *Where are all the female participants in Sports and Exercise Medicine research?* Eur J Sport Sci, 2014. **14**(8): p. 847-51.
4. Kraemer, W.J., N.A. Ratamess, and D.N. French, *Resistance training for health and performance.* Curr Sports Med Rep, 2002. **1**(3): p. 165-71.
5. Stone, M., et al., *Comparison of the Effects of Three Different Weight-Training Programs on the One Repetition Maximum Squat.* The Journal of Strength & Conditioning Research, 2000. **14**.
6. Staron, R.S., et al., *Skeletal muscle adaptations during early phase of heavy-resistance training in men and women.* J Appl Physiol (1985), 1994. **76**(3): p. 1247-55.
7. Mitchell, C.J., et al., *Acute post-exercise myofibrillar protein synthesis is not correlated with resistance training-induced muscle hypertrophy in young men.* PLoS One, 2014. **9**(2): p. e89431.
8. Nordez, A., et al., *Comparison of methods to assess quadriceps muscle volume using magnetic resonance imaging.* J Magn Reson Imaging, 2009. **30**(5): p. 1116-23.
9. Ahtiainen, J.P., et al., *Heterogeneity in resistance training-induced muscle strength and mass responses in men and women of different ages.* Age (Dordr), 2016. **38**(1): p. 10.
10. Bamman, M.M., et al., *Cluster analysis tests the importance of myogenic gene expression during myofiber hypertrophy in humans.* J Appl Physiol (1985), 2007. **102**(6): p. 2232-9.
11. Van Etten, L.M., F.T. Verstappen, and K.R. Westerterp, *Effect of body build on weight-training-induced adaptations in body composition and muscular strength.* Med Sci Sports Exerc, 1994. **26**(4): p. 515-21.
12. Yannoulopoulos, B. and J.B. De Meeus, *[Arterial hypertension and pregnancy: detection and monitoring].* Soins Gynecol Obstet Pueric Pediatr, 1991(127-128): p. 22-6.
13. Petrella, J.K., et al., *Potent myofiber hypertrophy during resistance training in humans is associated with satellite cell-mediated myonuclear addition: a cluster analysis.* J Appl Physiol (1985), 2008. **104**(6): p. 1736-42.
14. Davidsen, P.K., et al., *High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression.* J Appl Physiol (1985), 2011. **110**(2): p. 309-17.
15. Gjestvang, C., et al., *Motives and barriers to initiation and sustained exercise adherence in a fitness club setting-A one-year follow-up study.* Scand J Med Sci Sports, 2020. **30**(9): p. 1796-1805.
16. Roberts, M.D., et al., *Physiological Differences Between Low Versus High Skeletal Muscle Hypertrophic Responders to Resistance Exercise Training: Current Perspectives and Future Research Directions.* Front Physiol, 2018. **9**: p. 834.
17. Kosek, D.J., et al., *Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults.* J Appl Physiol (1985), 2006. **101**(2): p. 531-44.

18. Figueiredo, V.C., et al., *Ribosome biogenesis adaptation in resistance training-induced human skeletal muscle hypertrophy.* Am J Physiol Endocrinol Metab, 2015. **309**(1): p. E72-83.
19. Terzis, G., et al., *Resistance exercise-induced increase in muscle mass correlates with p70S6 kinase phosphorylation in human subjects.* Eur J Appl Physiol, 2008. **102**(2): p. 145-52.
20. Phillips, S.M., *A brief review of critical processes in exercise-induced muscular hypertrophy.* Sports Med, 2014. **44 Suppl 1**: p. S71-7.
21. Hartman, J.W., et al., *Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters.* Am J Clin Nutr, 2007. **86**(2): p. 373-81.
22. Burd, N.A., et al., *Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men.* PLoS One, 2010. **5**(8): p. e12033.
23. Nakada, S., et al., *Correlation between Ribosome Biogenesis and the Magnitude of Hypertrophy in Overloaded Skeletal Muscle.* PLoS One, 2016. **11**(1): p. e0147284.
24. Stec, M.J., et al., *Ribosome biogenesis may augment resistance training-induced myofiber hypertrophy and is required for myotube growth in vitro.* Am J Physiol Endocrinol Metab, 2016. **310**(8): p. E652-E661.
25. Mobley, C.B., et al., *Biomarkers associated with low, moderate, and high vastus lateralis muscle hypertrophy following 12 weeks of resistance training.* PLoS One, 2018. **13**(4): p. e0195203.
26. Mitchell, C.J., et al., *Resistance exercise load does not determine training-mediated hypertrophic gains in young men.* J Appl Physiol (1985), 2012. **113**(1): p. 71-7.
27. Schiaffino, S., S.P. Bormioli, and M. Aloisi, *The fate of newly formed satellite cells during compensatory muscle hypertrophy.* Virchows Arch B Cell Pathol, 1976. **21**(2): p. 113-8.
28. Seale, P., et al., *Pax7 is required for the specification of myogenic satellite cells.* Cell, 2000. **102**(6): p. 777-86.
29. Murach, K.A., et al., *Fusion-Independent Satellite Cell Communication to Muscle Fibers During Load-Induced Hypertrophy.* Function (Oxf), 2020. **1**(1): p. zqaa009.
30. Rosenblatt, J.D. and D.J. Parry, *Gamma irradiation prevents compensatory hypertrophy of overloaded mouse extensor digitorum longus muscle.* J Appl Physiol (1985), 1992. **73**(6): p. 2538-43.
31. O'Connor, R.S. and G.K. Pavlath, *Point:Counterpoint: Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy.* J Appl Physiol (1985), 2007. **103**(3): p. 1099-100.
32. McCarthy, J.J., et al., *Effective fiber hypertrophy in satellite cell-depleted skeletal muscle.* Development, 2011. **138**(17): p. 3657-66.
33. McCarthy, J.J. and K.A. Esser, *Counterpoint: Satellite cell addition is not obligatory for skeletal muscle hypertrophy.* J Appl Physiol (1985), 2007. **103**(3): p. 1100-2; discussion 1102-3.
34. Jackson, J.R., et al., *Satellite cell depletion does not inhibit adult skeletal muscle regrowth following unloading-induced atrophy.* Am J Physiol Cell Physiol, 2012. **303**(8): p. C854-61.

35. Vann, C.G., et al., *Effects of High-Volume versus High-Load Resistance Training on Skeletal Muscle Growth and Molecular Adaptations.* bioRxiv, 2021: p. 2021.07.01.450728.
36. Haun, C.T., et al., *Pre-training Skeletal Muscle Fiber Size and Predominant Fiber Type Best Predict Hypertrophic Responses to 6 Weeks of Resistance Training in Previously Trained Young Men.* Front Physiol, 2019. **10**: p. 297.
37. Morton, R.W., et al., *Muscle Androgen Receptor Content but Not Systemic Hormones Is Associated With Resistance Training-Induced Skeletal Muscle Hypertrophy in Healthy, Young Men.* Front Physiol, 2018. **9**: p. 1373.
38. Phillips, S.M., et al., *Resistance training reduces the acute exercise-induced increase in muscle protein turnover.* Am J Physiol, 1999. **276**(1): p. E118-24.
39. Damas, F., et al., *A review of resistance training-induced changes in skeletal muscle protein synthesis and their contribution to hypertrophy.* Sports Med, 2015. **45**(6): p. 801-7.
40. Haun, C.T., et al., *Muscle fiber hypertrophy in response to 6 weeks of high-volume resistance training in trained young men is largely attributed to sarcoplasmic hypertrophy.* PLoS One, 2019. **14**(6): p. e0215267.
41. Walker, D.K., et al., *Exercise, amino acids, and aging in the control of human muscle protein synthesis.* Med Sci Sports Exerc, 2011. **43**(12): p. 2249-58.
42. Haun, C.T., et al., *A Critical Evaluation of the Biological Construct Skeletal Muscle Hypertrophy: Size Matters but So Does the Measurement.* Front Physiol, 2019. **10**: p. 247.
43. Staron, R.S., et al., *Strength and skeletal muscle adaptations in heavy-resistance-trained women after detraining and retraining.* J Appl Physiol (1985), 1991. **70**(2): p. 631-40.
44. Abe, T., et al., *Time course for strength and muscle thickness changes following upper and lower body resistance training in men and women.* Eur J Appl Physiol, 2000. **81**(3): p. 174-80.
45. Räntilä, A., et al., *High Responders to Hypertrophic Strength Training Also Tend to Lose More Muscle Mass and Strength During Detraining Than Low Responders.* J Strength Cond Res, 2021. **35**(6): p. 1500-1511.
46. Roberts, M.D., et al., *Skeletal muscle mitochondrial volume and myozenin-1 protein differences exist between high versus low anabolic responders to resistance training.* PeerJ, 2018. **6**: p. e5338.
47. Goldberg, A.L., *Work-induced growth of skeletal muscle in normal and hypophysectomized rats.* Am J Physiol, 1967. **213**(5): p. 1193-8.
48. Cureton, K.J., et al., *Muscle hypertrophy in men and women.* Med Sci Sports Exerc, 1988. **20**(4): p. 338-44.
49. Medicine, A.C.o.S., *American College of Sports Medicine position stand. Progression models in resistance training for healthy adults.* Med Sci Sports Exerc, 2009. **41**(3): p. 687-708.
50. Lamb, D.A., et al., *The effects of resistance training with or without peanut protein supplementation on skeletal muscle and strength adaptations in older individuals.* J Int Soc Sports Nutr, 2020. **17**(1): p. 66.
51. DeFreitas, J.M., et al., *An examination of the time course of training-induced skeletal muscle hypertrophy.* Eur J Appl Physiol, 2011. **111**(11): p. 2785-90.

52. Bemben, M.G., *Use of diagnostic ultrasound for assessing muscle size.* J Strength Cond Res, 2002. **16**(1): p. 103-8.
53. Moquin, P.A., et al., *Lean Body Mass and Muscle Cross-Sectional Area Adaptations Among College Age Males with Different Strength Levels across 11 Weeks of Block Periodized Programmed Resistance Training.* Int J Environ Res Public Health, 2021. **18**(9).
54. Häkkinen, K., et al., *Changes in agonist-antagonist EMG, muscle CSA, and force during strength training in middle-aged and older people.* J Appl Physiol (1985), 1998. **84**(4): p. 1341-9.
55. Moritani, T. and H.A. deVries, *Neural factors versus hypertrophy in the time course of muscle strength gain.* Am J Phys Med, 1979. **58**(3): p. 115-30.
56. Häkkinen, K., et al., *Changes in muscle morphology, electromyographic activity, and force production characteristics during progressive strength training in young and older men.* J Gerontol A Biol Sci Med Sci, 1998. **53**(6): p. B415-23.
57. Plotkin, D.L., et al., *Muscle Fiber Type Transitions with Exercise Training: Shifting Perspectives.* Sports (Basel), 2021. **9**(9).
58. Hammarström, D., et al., *Benefits of higher resistance-training volume are related to ribosome biogenesis.* J Physiol, 2020. **598**(3): p. 543-565.
59. Ruple, B.A., et al., *Myofibril and Mitochondrial Area Changes in Type I and II Fibers Following 10 Weeks of Resistance Training in Previously Untrained Men.* Front Physiol, 2021. **12**: p. 728683.
60. Phillips, S.K., et al., *Muscle weakness in women occurs at an earlier age than in men, but strength is preserved by hormone replacement therapy.* Clin Sci (Lond), 1993. **84**(1): p. 95-8.
61. Taipale, R.S., et al., *A 10-Week Block of Combined High-Intensity Endurance and Strength Training Produced Similar Changes in Dynamic Strength, Body Composition, and Serum Hormones in Women and Men.* Front Sports Act Living, 2020. **2**: p. 581305.
62. Roberts, B.M., G. Nuckols, and J.W. Krieger, *Sex Differences in Resistance Training: A Systematic Review and Meta-Analysis.* J Strength Cond Res, 2020. **34**(5): p. 1448-1460.
63. Atherton, P.J. and K. Smith, *Muscle protein synthesis in response to nutrition and exercise.* J Physiol, 2012. **590**(5): p. 1049-57.
64. MacDougall, J.D., et al., *The time course for elevated muscle protein synthesis following heavy resistance exercise.* Can J Appl Physiol, 1995. **20**(4): p. 480-6.
65. Figueiredo, V.C. and J.J. McCarthy, *Regulation of Ribosome Biogenesis in Skeletal Muscle Hypertrophy.* Physiology (Bethesda), 2019. **34**(1): p. 30-42.
66. Chaillou, T., T.J. Kirby, and J.J. McCarthy, *Ribosome biogenesis: emerging evidence for a central role in the regulation of skeletal muscle mass.* J Cell Physiol, 2014. **229**(11): p. 1584-94.
67. von Walden, F., *Ribosome biogenesis in skeletal muscle: coordination of transcription and translation.* J Appl Physiol (1985), 2019. **127**(2): p. 591-598.
68. Pestova, T.V., et al., *Molecular mechanisms of translation initiation in eukaryotes.* Proc Natl Acad Sci U S A, 2001. **98**(13): p. 7029-36.
69. Hannan, R.D., et al., *Cardiac hypertrophy: a matter of translation.* Clin Exp Pharmacol Physiol, 2003. **30**(8): p. 517-27.
70. Hamosch, M., et al., *Enhanced protein synthesis in a cell-free system from hypertrophied skeletal muscle.* Science, 1967. **157**(3791): p. 935-7.

71. Goldberg, A.L., et al., *Mechanism of work-induced hypertrophy of skeletal muscle.* Med Sci Sports, 1975. **7**(3): p. 185-98.
72. Kirby, T.J., et al., *Blunted hypertrophic response in aged skeletal muscle is associated with decreased ribosome biogenesis.* J Appl Physiol (1985), 2015. **119**(4): p. 321-7.
73. Chaillou, T., et al., *Time course of gene expression during mouse skeletal muscle hypertrophy.* J Appl Physiol (1985), 2013. **115**(7): p. 1065-74.
74. Laurent, G.J. and M.P. Sparrow, *Changes in RNA, DNA and protein content and the rates of protein synthesis and degradation during hypertrophy of the anterior latissimus dorsi muscle of the adult fowl (Gallus domesticus).* Growth, 1977. **41**(4): p. 249-62.
75. Figueiredo, V.C., et al., *Genetic and epigenetic regulation of skeletal muscle ribosome biogenesis with exercise.* J Physiol, 2021. **599**(13): p. 3363-3384.
76. Glynn, E.L., et al., *Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise.* Am J Physiol Regul Integr Comp Physiol, 2010. **299**(2): p. R533-40.
77. Parise, G., et al., *Effects of acute creatine monohydrate supplementation on leucine kinetics and mixed-muscle protein synthesis.* J Appl Physiol (1985), 2001. **91**(3): p. 1041-7.
78. Jahn, L.A., et al., *Tissue composition affects measures of postabsorptive human skeletal muscle metabolism: comparison across genders.* J Clin Endocrinol Metab, 1999. **84**(3): p. 1007-10.
79. West, D.W., et al., *Sex-based comparisons of myofibrillar protein synthesis after resistance exercise in the fed state.* J Appl Physiol (1985), 2012. **112**(11): p. 1805-13.
80. Wilkinson, S.B., et al., *Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage.* Am J Clin Nutr, 2007. **85**(4): p. 1031-40.
81. Phillips, S.M., et al., *Mixed muscle protein synthesis and breakdown after resistance exercise in humans.* Am J Physiol, 1997. **273**(1 Pt 1): p. E99-107.
82. Churchward-Venne, T.A., et al., *Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: a double-blind, randomized trial.* Am J Clin Nutr, 2014. **99**(2): p. 276-86.
83. Bennet, W.M., et al., *Increase in anterior tibialis muscle protein synthesis in healthy man during mixed amino acid infusion: studies of incorporation of [1-13C]leucine.* Clin Sci (Lond), 1989. **76**(4): p. 447-54.
84. Moore, D.R., et al., *Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men.* Am J Clin Nutr, 2009. **89**(1): p. 161-8.
85. Roberts, M.D., et al., *Sarcoplasmic Hypertrophy in Skeletal Muscle: A Scientific "Unicorn" or Resistance Training Adaptation?* Front Physiol, 2020. **11**: p. 816.
86. Moore, D.R., et al., *Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions.* Am J Physiol Endocrinol Metab, 2005. **288**(6): p. E1153-9.
87. Moore, D.R., et al., *Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise.* J Physiol, 2009. **587**(Pt 4): p. 897-904.
88. Biolo, G., et al., *Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans.* Am J Physiol, 1995. **268**(3 Pt 1): p. E514-20.

89. Burd, N.A., et al., *Resistance exercise volume affects myofibrillar protein synthesis and anabolic signalling molecule phosphorylation in young men.* J Physiol, 2010. **588**(Pt 16): p. 3119-30.
90. Kumar, V., et al., *Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men.* J Physiol, 2009. **587**(1): p. 211-7.
91. Wilkinson, S.B., et al., *Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle.* J Physiol, 2008. **586**(15): p. 3701-17.
92. Burd, N.A., et al., *Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men.* J Nutr, 2011. **141**(4): p. 568-73.
93. Kim, P.L., R.S. Staron, and S.M. Phillips, *Fasted-state skeletal muscle protein synthesis after resistance exercise is altered with training.* J Physiol, 2005. **568**(Pt 1): p. 283-90.
94. Wilkinson, D.J., et al., *A validation of the application of D(2)O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans.* Am J Physiol Endocrinol Metab, 2014. **306**(5): p. E571-9.
95. Brook, M.S., et al., *A novel D.* Am J Physiol Endocrinol Metab, 2017. **313**(6): p. E681-E689.
96. Busch, R., et al., *Measurement of protein turnover rates by heavy water labeling of nonessential amino acids.* Biochim Biophys Acta, 2006. **1760**(5): p. 730-44.
97. Brook, M.S., et al., *Skeletal muscle hypertrophy adaptations predominate in the early stages of resistance exercise training, matching deuterium oxide-derived measures of muscle protein synthesis and mechanistic target of rapamycin complex 1 signaling.* FASEB J, 2015. **29**(11): p. 4485-96.
98. Mayhew, D.L., et al., *Translational signaling responses preceding resistance training-mediated myofiber hypertrophy in young and old humans.* J Appl Physiol (1985), 2009. **107**(5): p. 1655-62.
99. Damas, F., et al., *Resistance training-induced changes in integrated myofibrillar protein synthesis are related to hypertrophy only after attenuation of muscle damage.* J Physiol, 2016. **594**(18): p. 5209-22.
100. Witard, O.C., L. Bannock, and K.D. Tipton, *Making Sense of Muscle Protein Synthesis: A Focus on Muscle Growth During Resistance Training.* Int J Sport Nutr Exerc Metab, 2021: p. 1-13.
101. CHEEK, D.B., G.K. POWELL, and R.E. SCOTT, *GROWTH OF MUSCLE MASS AND SKELETAL COLLAGEN IN THE RAT. I. NORMAL GROWTH.* Bull Johns Hopkins Hosp, 1965. **116**: p. 378-87.
102. McCormick, K.M. and D.P. Thomas, *Exercise-induced satellite cell activation in senescent soleus muscle.* J Appl Physiol (1985), 1992. **72**(3): p. 888-93.
103. Darr, K.C. and E. Schultz, *Exercise-induced satellite cell activation in growing and mature skeletal muscle.* J Appl Physiol (1985), 1987. **63**(5): p. 1816-21.
104. Englund, D.A., et al., *Satellite Cell Depletion Disrupts Transcriptional Coordination and Muscle Adaptation to Exercise.* Function (Oxf), 2021. **2**(1): p. zqaa033.
105. MAURO, A., *Satellite cell of skeletal muscle fibers.* J Biophys Biochem Cytol, 1961. **9**: p. 493-5.

106. Gross, P., et al., *Hemodynamic effects of isradipine and nifedipine in chronic sustained hypertension.* J Cardiovasc Pharmacol, 1992. **19 Suppl 3**: p. S84-6.
107. Conceição, M.S., et al., *Muscle Fiber Hypertrophy and Myonuclei Addition: A Systematic Review and Meta-analysis.* Med Sci Sports Exerc, 2018. **50**(7): p. 1385-1393.
108. Rosenblatt, J.D., D. Yong, and D.J. Parry, *Satellite cell activity is required for hypertrophy of overloaded adult rat muscle.* Muscle Nerve, 1994. **17**(6): p. 608-13.
109. Oustanina, S., G. Hause, and T. Braun, *Pax7 directs postnatal renewal and propagation of myogenic satellite cells but not their specification.* EMBO J, 2004. **23**(16): p. 3430-9.
110. Fry, C.S., et al., *Regulation of the muscle fiber microenvironment by activated satellite cells during hypertrophy.* FASEB J, 2014. **28**(4): p. 1654-65.
111. Murach, K.A., et al., *Differential requirement for satellite cells during overload-induced muscle hypertrophy in growing versus mature mice.* Skelet Muscle, 2017. **7**(1): p. 14.
112. Egner, I.M., J.C. Bruusgaard, and K. Gundersen, *Satellite cell depletion prevents fiber hypertrophy in skeletal muscle.* Development, 2016. **143**(16): p. 2898-906.
113. Murach, K.A., et al., *Starring or Supporting Role? Satellite Cells and Skeletal Muscle Fiber Size Regulation.* Physiology (Bethesda), 2018. **33**(1): p. 26-38.
114. Crameri, R.M., et al., *Changes in satellite cells in human skeletal muscle after a single bout of high intensity exercise.* J Physiol, 2004. **558**(Pt 1): p. 333-40.
115. Petrella, J.K., et al., *Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women.* Am J Physiol Endocrinol Metab, 2006. **291**(5): p. E937-46.
116. Kim, J.S., et al., *Resting and load-induced levels of myogenic gene transcripts differ between older adults with demonstrable sarcopenia and young men and women.* J Appl Physiol (1985), 2005. **99**(6): p. 2149-58.
117. Kim, J.S., J.M. Cross, and M.M. Bamman, *Impact of resistance loading on myostatin expression and cell cycle regulation in young and older men and women.* Am J Physiol Endocrinol Metab, 2005. **288**(6): p. E1110-9.
118. Walker, D.K., et al., *PAX7+ satellite cells in young and older adults following resistance exercise.* Muscle Nerve, 2012. **46**(1): p. 51-9.
119. O'Reilly, C., et al., *Hepatocyte growth factor (HGF) and the satellite cell response following muscle lengthening contractions in humans.* Muscle Nerve, 2008. **38**(5): p. 1434-1442.
120. Kadi, F., et al., *The effects of heavy resistance training and detraining on satellite cells in human skeletal muscles.* J Physiol, 2004. **558**(Pt 3): p. 1005-12.
121. Roth, S.M., et al., *Skeletal muscle satellite cell characteristics in young and older men and women after heavy resistance strength training.* J Gerontol A Biol Sci Med Sci, 2001. **56**(6): p. B240-7.
122. Kadi, F. and L.E. Thornell, *Concomitant increases in myonuclear and satellite cell content in female trapezius muscle following strength training.* Histochem Cell Biol, 2000. **113**(2): p. 99-103.
123. Bruusgaard, J.C., et al., *Myonuclei acquired by overload exercise precede hypertrophy and are not lost on detraining.* Proc Natl Acad Sci U S A, 2010. **107**(34): p. 15111-6.
124. Herman-Montemayor, J.R., R.S. Hikida, and R.S. Staron, *Early-Phase Satellite Cell and Myonuclear Domain Adaptations to Slow-Speed vs. Traditional Resistance Training Programs.* J Strength Cond Res, 2015. **29**(11): p. 3105-14.

125. Kim, J.S., et al., *Load-mediated downregulation of myostatin mRNA is not sufficient to promote myofiber hypertrophy in humans: a cluster analysis.* J Appl Physiol (1985), 2007. **103**(5): p. 1488-95.
126. Bellamy, L.M., et al., *The acute satellite cell response and skeletal muscle hypertrophy following resistance training.* PLoS One, 2014. **9**(10): p. e109739.
127. Ogasawara, R., et al., *MicroRNA expression profiling in skeletal muscle reveals different regulatory patterns in high and low responders to resistance training.* Physiol Genomics, 2016. **48**(4): p. 320-4.
128. Ahtiainen, J.P., et al., *Heavy resistance exercise training and skeletal muscle androgen receptor expression in younger and older men.* Steroids, 2011. **76**(1-2): p. 183-92.
129. Vann, C.G., et al., *An intron variant of the GLI family zinc finger 3 (GLI3) gene differentiates resistance training-induced muscle fiber hypertrophy in younger men.* FASEB J, 2021. **35**(5): p. e21587.
130. Renault, M.A., et al., *Gli3 regulation of myogenesis is necessary for ischemia-induced angiogenesis.* Circ Res, 2013. **113**(10): p. 1148-58.
131. Hubal, M.J., et al., *Variability in muscle size and strength gain after unilateral resistance training.* Med Sci Sports Exerc, 2005. **37**(6): p. 964-72.
132. Kell, R.T., *The influence of periodized resistance training on strength changes in men and women.* J Strength Cond Res, 2011. **25**(3): p. 735-44.
133. Gentil, P., et al., *Comparison of upper body strength gains between men and women after 10 weeks of resistance training.* PeerJ, 2016. **4**: p. e1627.
134. Jacobson, B.H. and W. Lentz, *Perception of physical variables during four phases of the menstrual cycle.* Percept Mot Skills, 1998. **87**(2): p. 565-6.
135. Solli, G.S., et al., *Changes in Self-Reported Physical Fitness, Performance, and Side Effects Across the Phases of the Menstrual Cycle Among Competitive Endurance Athletes.* Int J Sports Physiol Perform, 2020: p. 1-10.
136. Bruinvels, G., et al., *The Prevalence and Impact of Heavy Menstrual Bleeding (Menorrhagia) in Elite and Non-Elite Athletes.* PLoS One, 2016. **11**(2): p. e0149881.
137. Carmichael, M.A., et al., *The Impact of Menstrual Cycle Phase on Athletes' Performance: A Narrative Review.* Int J Environ Res Public Health, 2021. **18**(4).
138. Sakamaki-Sunaga, M., et al., *Effects of Menstrual Phase-Dependent Resistance Training Frequency on Muscular Hypertrophy and Strength.* J Strength Cond Res, 2016. **30**(6): p. 1727-34.
139. Lebrun, C.M., et al., *Effects of menstrual cycle phase on athletic performance.* Med Sci Sports Exerc, 1995. **27**(3): p. 437-44.
140. Miller, B.F., et al., *No effect of menstrual cycle on myofibrillar and connective tissue protein synthesis in contracting skeletal muscle.* Am J Physiol Endocrinol Metab, 2006. **290**(1): p. E163-E168.
141. Kraemer, R.R., et al., *Follicular and luteal phase hormonal responses to low-volume resistive exercise.* Medicine & Science in Sports & Exercise, 1995. **27**(6).
142. Rodrigues, P., M. de Azevedo Correia, and L. Wharton, *Effect of menstrual cycle on muscle strength.* Journal of Exercise Physiology Online, 2019. **22**(5): p. 89-96.
143. Graja, A., et al., *Physical, Biochemical, and Neuromuscular Responses to Repeated Sprint Exercise in Eumenorrheic Female Handball Players: Effect of Menstrual Cycle Phases.* J Strength Cond Res, 2020.

144. Fridén, C., A.L. Hirschberg, and T. Saartok, *Muscle strength and endurance do not significantly vary across 3 phases of the menstrual cycle in moderately active premenopausal women.* Clin J Sport Med, 2003. **13**(4): p. 238-41.
145. Janse de Jonge , X.A., et al., *The influence of menstrual cycle phase on skeletal muscle contractile characteristics in humans.* J Physiol, 2001. **530**(Pt 1): p. 161-6.
146. DiBrezzo, R., I.L. Fort, and B. Brown, *Relationships among strength, endurance, weight and body fat during three phases of the menstrual cycle.* J Sports Med Phys Fitness, 1991. **31**(1): p. 89-94.
147. Lebrun, C.M., *Effect of the different phases of the menstrual cycle and oral contraceptives on athletic performance.* Sports Med, 1993. **16**(6): p. 400-30.
148. Thompson, B., et al., *The Effect of the Menstrual Cycle and Oral Contraceptives on Acute Responses and Chronic Adaptations to Resistance Training: A Systematic Review of the Literature.* Sports Med, 2020. **50**(1): p. 171-185.
149. McNulty, K.L., et al., *The Effects of Menstrual Cycle Phase on Exercise Performance in Eumenorrheic Women: A Systematic Review and Meta-Analysis.* Sports Med, 2020. **50**(10): p. 1813-1827.
150. Blagrove, R.C., G. Bruinvels, and C.R. Pedlar, *Variations in strength-related measures during the menstrual cycle in eumenorrheic women: A systematic review and meta-analysis.* J Sci Med Sport, 2020. **23**(12): p. 1220-1227.
151. Fujita, S., et al., *Basal muscle intracellular amino acid kinetics in women and men.* Am J Physiol Endocrinol Metab, 2007. **292**(1): p. E77-83.
152. Dreyer, H.C., et al., *Resistance exercise increases leg muscle protein synthesis and mTOR signalling independent of sex.* Acta Physiol (Oxf), 2010. **199**(1): p. 71-81.
153. Smith, G.I., et al., *No major sex differences in muscle protein synthesis rates in the postabsorptive state and during hyperinsulinemia-hyperaminoacidemia in middle-aged adults.* J Appl Physiol (1985), 2009. **107**(4): p. 1308-15.
154. Anderson, L.J., H. Liu, and J.M. Garcia, *Sex Differences in Muscle Wasting.* Adv Exp Med Biol, 2017. **1043**: p. 153-197.
155. Thomas, F., et al., *International variability of ages at menarche and menopause: patterns and main determinants.* Hum Biol, 2001. **73**(2): p. 271-90.
156. Iannuzzi-Sucich, M., K.M. Prestwood, and A.M. Kenny, *Prevalence of sarcopenia and predictors of skeletal muscle mass in healthy, older men and women.* J Gerontol A Biol Sci Med Sci, 2002. **57**(12): p. M772-7.
157. Collins, B.C., E.K. Laakkonen, and D.A. Lowe, *Aging of the musculoskeletal system: How the loss of estrogen impacts muscle strength.* Bone, 2019. **123**: p. 137-144.
158. Taaffe, D.R., et al., *Estrogen replacement, muscle composition, and physical function: The Health ABC Study.* Med Sci Sports Exerc, 2005. **37**(10): p. 1741-7.
159. Greising, S.M., et al., *Hormone therapy and skeletal muscle strength: a meta-analysis.* J Gerontol A Biol Sci Med Sci, 2009. **64**(10): p. 1071-81.
160. Sipilä, S., et al., *Effects of hormone replacement therapy and high-impact physical exercise on skeletal muscle in post-menopausal women: a randomized placebo-controlled study.* Clin Sci (Lond), 2001. **101**(2): p. 147-57.
161. Figueroa, A., et al., *Effects of exercise training and hormone replacement therapy on lean and fat mass in postmenopausal women.* J Gerontol A Biol Sci Med Sci, 2003. **58**(3): p. 266-70.

162. Bemben, D.A. and D.B. Langdon, *Relationship between estrogen use and musculoskeletal function in postmenopausal women.* Maturitas, 2002. **42**(2): p. 119-27.
163. Ronkainen, P.H., et al., *Postmenopausal hormone replacement therapy modifies skeletal muscle composition and function: a study with monozygotic twin pairs.* J Appl Physiol (1985), 2009. **107**(1): p. 25-33.
164. Shefer, G., et al., *Satellite-cell pool size does matter: defining the myogenic potency of aging skeletal muscle.* Dev Biol, 2006. **294**(1): p. 50-66.
165. Larson, A.A., et al., *Oestradiol affects skeletal muscle mass, strength and satellite cells following repeated injuries.* Exp Physiol, 2020. **105**(10): p. 1700-1707.
166. Enns, D.L. and P.M. Tiidus, *Estrogen influences satellite cell activation and proliferation following downhill running in rats.* J Appl Physiol (1985), 2008. **104**(2): p. 347-53.
167. Collins, B.C., et al., *Estrogen Regulates the Satellite Cell Compartment in Females.* Cell Rep, 2019. **28**(2): p. 368-381.e6.
168. Ray, S., et al., *Androgens and estrogens stimulate ribosome biogenesis in prostate and breast cancer cells in receptor dependent manner.* Gene, 2013. **526**(1): p. 46-53.
169. Elliott, K.J., N.T. Cable, and T. Reilly, *Does oral contraceptive use affect maximum force production in women?* Br J Sports Med, 2005. **39**(1): p. 15-9.
170. Peters, C. and M. Burrows, *Androgenicity of the progestin in oral contraceptives does not affect maximal leg strength.* Contraception, 2006. **74**(6): p. 487-91.
171. Dalgaard, L.B., et al., *Influence of Oral Contraceptive Use on Adaptations to Resistance Training.* Front Physiol, 2019. **10**: p. 824.
172. Oxfeldt, M., et al., *Molecular markers of skeletal muscle hypertrophy following 10 wk of resistance training in oral contraceptive users and nonusers.* J Appl Physiol (1985), 2020. **129**(6): p. 1355-1364.
173. Gjestvang, C., T. Stensrud, and L.A.H. Haakstad, *Are changes in physical fitness, body composition and weight associated with exercise attendance and dropout among fitness club members? Longitudinal prospective study.* BMJ Open, 2019. **9**(4): p. e027987.
174. Dishman, R.K. and J. Buckworth, *Increasing physical activity: a quantitative synthesis.* Med Sci Sports Exerc, 1996. **28**(6): p. 706-19.
175. Marcus, B.H., et al., *Physical activity behavior change: issues in adoption and maintenance.* Health Psychol, 2000. **19**(1S): p. 32-41.
176. Sexton, C.L., et al., *Effects of Peanut Protein Supplementation on Resistance Training Adaptations in Younger Adults.* Nutrients, 2021. **13**(11).
177. Kephart, W.C., et al., *Ten weeks of branched-chain amino acid supplementation improves select performance and immunological variables in trained cyclists.* Amino Acids, 2016. **48**(3): p. 779-789.
178. Rantalainen, T., et al., *An open source approach for regional cortical bone mineral density analysis.* J Musculoskelet Neuronal Interact, 2011. **11**(3): p. 243-8.
179. Evans, W.J., S.D. Phinney, and V.R. Young, *Suction applied to a muscle biopsy maximizes sample size.* Med Sci Sports Exerc, 1982. **14**(1): p. 101-2.
180. Wen, Y., et al., *MyoVision: software for automated high-content analysis of skeletal muscle immunohistochemistry.* J Appl Physiol (1985), 2018. **124**(1): p. 40-51.
181. Vann, C.G., et al., *Molecular Differences in Skeletal Muscle After 1 Week of Active vs. Passive Recovery From High-Volume Resistance Training.* J Strength Cond Res, 2021. **35**(8): p. 2102-2113.

182. Roberts, M.D., et al., *An optimized procedure for isolation of rodent and human skeletal muscle sarcoplasmic and myofibrillar proteins.* J Biol Methods, 2020. **7**(1): p. e127.
183. Bell, K.E., et al., *Integrated Myofibrillar Protein Synthesis in Recovery From Unaccustomed and Accustomed Resistance Exercise With and Without Multi-ingredient Supplementation in Overweight Older Men.* Front Nutr, 2019. **6**: p. 40.
184. Nader, G.A., T.J. McLoughlin, and K.A. Esser, *mTOR function in skeletal muscle hypertrophy: increased ribosomal RNA via cell cycle regulators.* Am J Physiol Cell Physiol, 2005. **289**(6): p. C1457-65.
185. Mobley, C.B., et al., *Comparative effects of whey protein versus L-leucine on skeletal muscle protein synthesis and markers of ribosome biogenesis following resistance exercise.* Amino Acids, 2016. **48**(3): p. 733-750.
186. Buysse, D.J., et al., *The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research.* Psychiatry Res, 1989. **28**(2): p. 193-213.