



RESEARCH ARTICLE

*Sex Differences in the Response to Exercise Training*

# Molecular predictors of resistance training outcomes in young untrained female adults

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## Abstract

We sought to determine 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, were associated with hypertrophic outcomes in females or differed in those who classified as higher (HR) or lower (LR) responders to resistance training (RT). Thirty-four untrained college-aged females ( $23.4 \pm 3.4$  kg/m<sup>2</sup>) completed a 10-wk RT protocol (twice weekly). Body composition and leg imaging assessments, a right leg vastus lateralis biopsy, and strength testing occurred before and following the intervention. A composite score, which included changes in whole body lean/soft tissue mass (LSTM), vastus lateralis (VL) muscle cross-sectional area (mCSA), midhigh mCSA, and deadlift strength, was used to delineate upper and lower HR ( $n = 8$ ) and LR ( $n = 8$ ) quartiles. In all participants, training significantly ( $P < 0.05$ ) increased LSTM, VL mCSA, midhigh mCSA, deadlift strength, mean muscle fiber cross-sectional area, satellite cell abundance, and myonuclear number. Increases in LSTM ( $P < 0.001$ ), VL mCSA ( $P < 0.001$ ), midhigh mCSA ( $P < 0.001$ ), and deadlift strength ( $P = 0.001$ ) were greater in HR vs. LR. The first-bout 24-hour MyoPS response was similar between HR and LR ( $P = 0.367$ ). While no significant responder  $\times$  time interaction existed for muscle total RNA concentrations (i.e., ribosome content) ( $P = 0.888$ ), satellite cell abundance increased in HR ( $P = 0.026$ ) but not LR ( $P = 0.628$ ). Pretraining LSTM ( $P = 0.010$ ), VL mCSA ( $P = 0.028$ ), and midhigh mCSA ( $P < 0.001$ ) were also greater in HR vs. LR. Female participants with an enhanced satellite cell response to RT, and more muscle mass before RT, exhibited favorable resistance training adaptations.

**NEW & NOTEWORTHY** This study continues to delineate muscle biology differences between lower and higher responders to resistance training and is unique in that a female population was interrogated. As has been reported in prior studies, increases in satellite cell numbers are related to positive responses to resistance training. Satellite cell responsivity, rather than changes in muscle ribosome content per milligrams of tissue, may be a more important factor in delineating resistance-training responses in women.

*myofibrillar protein synthesis; physiology; satellite cells; resistance training; ribosomes*

## INTRODUCTION

Resistance training (RT) adaptations have been studied for decades, mainly in young adult male participants (1, 2). A 2014 study examining three major sport science journals from 2011 to 2013 found 39% of study participants were female and 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). Therefore,

there is a heightened need to address the literature gap of female participant inclusion in sport science.

Two RT adaptations that have been well documented include strength increases (4–6) and muscle growth (or hypertrophy) (7, 8). Moreover, certain lines of evidence suggest resistance training can improve muscle quality (i.e., force generation per muscle cross section), especially in older females (9, 10). While it is generally presumed that all participants will exhibit improvements in these variables, there are various lines of evidence demonstrating that “lower” and “higher” responders exist regarding strength (11) and

hypertrophy outcomes (12–16), with most studies investigating hypertrophy responders. Importantly, these responder terms typically only apply to a few attributes and are not meant to imply a participant is a lower or nonresponder to exercise. A lower responder usually has a response to other attributes that might not be measured in that study. Specifically, 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 (17). Moreover, although lower hypertrophic responders can experience significant strength gains during training (13, 18, 19), there is some evidence suggesting lower hypertrophic responders to 12 wk of RT may also have impaired strength gains (11, 18). Thus deciphering variables that differentiate higher versus lower hypertrophic responders to RT is warranted, particularly in females.

Several studies from our laboratory (20–22) and others (16, 23, 24) have attempted to elucidate molecular variables that predict the hypertrophic response to RT. Collectively, these studies have demonstrated that three molecular variables, in particular, are associated with hypertrophy including 1) the ribosome biogenesis response to weeks of training, 2) the myofibrillar protein response days to weeks into training, and 3) the satellite cell response days to weeks into training. Nonetheless, there is debate pertaining to molecular variables being predictive of hypertrophic response to RT. Specifically, there is evidence in human, animal, and cell models to support (20, 25–32) and refute (7, 33) ribosome biogenesis and the myofibrillar protein synthetic responses being associated with muscle hypertrophy. Additionally, there are reports suggesting satellite cell proliferation and/or myonuclear accretion with training are (18, 34–37) and are not (38–40) associated with hypertrophic responses in animal models. Second, human studies have used both trained (5, 21, 41–45) and untrained participants (12, 16, 20, 31, 46–48). Training status influences adaptation and makes it difficult to compare findings between cohorts. Third, training interventions have ranged from 4 to 16 wk, raising the possibility of temporal variability among the reported molecular adaptations. Fourth, the criteria and statistics used to delineate responder clusters have vastly differed among studies, as well as what variable “responder” refers to (e.g., hypertrophy, strength, etc.). For example, some papers have used a single outcome variable to define the hypertrophic response, such as mean myofiber cross-sectional area (fCSA) (16), or ultrasound-determined muscle thickness (20, 49, 50). In contrast, others have adopted a composite score to delineate the hypertrophic response using multiple variables (16, 21, 22). Finally, most of these data are in males, and although women show a similar relative increase in muscle mass to RT (51), males and females adapt may show differential mechanistic responses to RT. For example, a prior study performed in younger and older males and females who underwent 16 wk of RT demonstrated that younger female participants generally did not exhibit hypertrophy assessed through mean fCSA (19). Likewise, it has been recently reported that men showed prolonged myofibrillar protein synthesis responses to a naïve resistance exercise bout compared to females (52).

To our knowledge, no one study has sought to determine if the acute myofibrillar protein synthetic response to a

naïve resistance exercise bout, or chronic changes in muscle ribosome content or satellite abundance to RT, are predictive of muscle hypertrophy in younger adult females. Thus the aims of this study were to examine the following in female participants: 1) if the 24-h myofibrillar protein synthesis response to a naïve bout of RT was associated with hypertrophic outcomes, 2) if chronic changes in muscle ribosome content (RNA per mg tissue) and satellite cell abundance after 10 wk of RT were associated with hypertrophic outcomes, and 3) determine whether these three variables differed between higher versus lower responders (via a composite score of strength and hypertrophy metrics). Based on the current literature we hypothesized all three outcome variables would be associated with hypertrophy in all participants. We also hypothesized that higher responders would exhibit more favorable changes in these molecular markers compared to lower responders.

## METHODS

### Ethical Approval and Prescreening

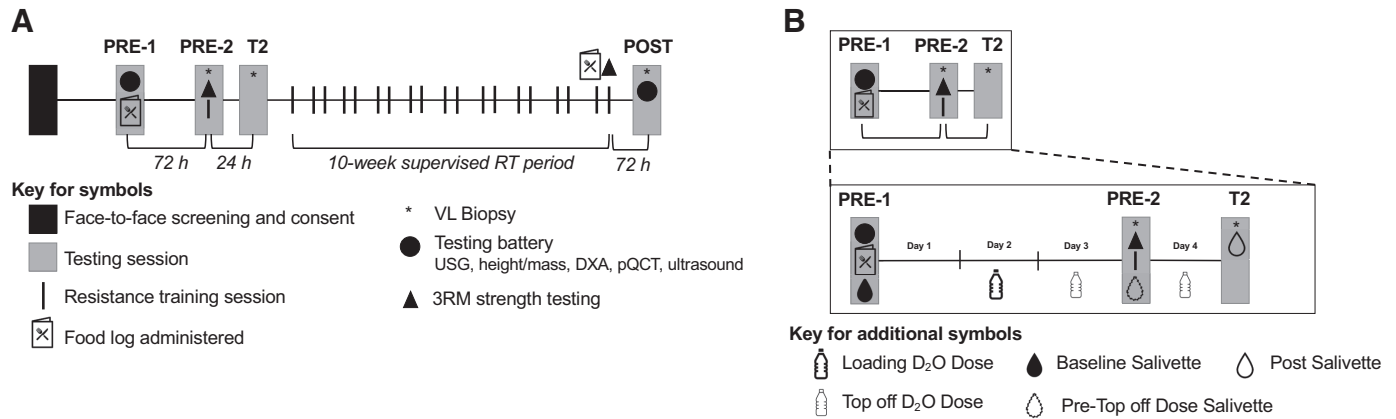
This study was approved by the Auburn University Institutional Review Board (IRB) (Protocol No. 19-249 MR 1907), conformed to the standards set by the latest revisions of the Declaration of Helsinki, and was registered as a clinical trial (NCT04707963). Thirty-four young, college-aged, untrained female participants 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 whether daily peanut protein (PP) supplementation, versus no supplementation, affects RT adaptations in college-aged males and females (53). In short, peanut protein 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 were defined as follows: between the ages of 18 and 30, body mass index <35 kg/m<sup>2</sup>; no recent participation in RT (less than one time per week for 6 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 6 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 who 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 pretesting (PRE).

### Study Design

The timeline and testing battery for each visit can be visualized in Fig. 1A and is described in greater detail below. Participants reported to the laboratory a total of 5 times for testing and 20 times for RT.

The PRE time sample was completed in two visits. During the first visit (PRE-1), participants reported to the laboratory



**Figure 1.** Study design. **A:** outline of the study design. More in-depth descriptions of procedures can be found in the text. **B:** outline of the protocol for D<sub>2</sub>O dosing and collection of salivette samples in relation to the points of data collection. DXA, dual energy X-ray absorptiometry; pQCT, peripheral quantitative computed tomography; 3RM, 3-repetition maximum; RT, resistance training; VL, vastus lateralis; PRE-1, first visit pretesting; PRE-2, second visit pretesting; T2, second skeletal muscle biopsy visit; USG, urine specific gravity.

after fasting for at least 4 hours 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: 1) height and weight measurements, 2) whole body dual X-ray absorptiometry (DXA), 3) right leg midthigh peripheral quantitative computed tomography (pQCT) scan, 4) VL ultrasound at the midthigh, and 5) isokinetic dynamometry of the right knee extensors. The participants then chewed a salivette (SARSTEDT, Nümbrecht, Germany) to provide saliva for the baseline assessment of whole body deuterium oxide (D<sub>2</sub>O). At the end of PRE-1, participants were given containers of D<sub>2</sub>O (70 atom percent; Sigma-Aldrich, St. Louis, MO) to take home and consume over the next 3 days and a 3-day food log. Participants consumed a total of 4.5 mL of D<sub>2</sub>O per kg of lean/soft tissue mass before the first muscle biopsy.

The second visit of PRE testing (PRE-2) occurred 3 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<sub>2</sub>O enrichment, consumed a top-off dose of D<sub>2</sub>O (0.5 mL/kg), and donated skeletal muscle from the right VL via biopsy before performing baseline strength testing on the bilateral 45° leg press, barbell bench press, and hex-bar deadlift. This strength testing, with the addition of 2 sets of 10 repetitions on the 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-h myofibrillar protein synthesis response).

Twenty-four hours after PRE-2, participants returned to the laboratory at least 4 hours fasted for their second skeletal muscle biopsy visit (T2) and final salivette sampling for D<sub>2</sub>O enrichment. After the T2 visit, participants completed their 10-wk RT program, wherein the last session included maximum strength assessments and isokinetic dynamometry. The last testing session (POST) was conducted ~72 h following the completion of the last RT session, and the following battery of tests was performed: 1) USG, 2) body mass, 3) DXA, 4) pQCT, 5) VL ultrasound, and 6) a muscle biopsy that was 1–2 cm proximal to the other two biopsy sites. The postintervention food log was given to participants in the week

leading up to POST. Each of these tests is described in greater detail below.

## Specific Testing Procedures

### Urine specific gravity.

At the beginning of PRE-1 and POST, participants provided a urine sample (~5 mL). The sample was immediately analyzed using a handheld refractometer (ATAGO; Bellevue, WA). All participants presented USG values  $\leq 1.020$  and were considered well hydrated.

### Body composition.

Following USG assessments, body mass and height were assessed using a laboratory scale (Seca 769; Hanover, MD). Participants were then subjected to a whole body DXA scan (Lunar Prodigy; GE Corporation, Fairfield, CT) to assess bone-free lean/soft tissue mass (LSTM) and fat mass (FM). This instrument was calibrated using a phantom device, and quality assurance was tested on each day scans were performed before 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-min rest period, 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<sub>3,1</sub>), standard error of the measurement (SE), and minimal difference to be considered real (MD) was previously determined for whole body LSTM on 10 participants scanned ~24 h apart. This resulted in an ICC<sub>3,1</sub> of 0.99, SE of 0.36 kg, and MD of 0.99 kg (54).

### Peripheral quantitative computed tomography.

After the DXA scan, a pQCT scan (Stratec XCT 3000, Stratec Medical, Pforzheim, Germany) of the right thigh was performed to measure midthigh muscle CSA (mCSA; cm<sup>2</sup>) and skeletal muscle density (mg/cm<sup>3</sup>) at 50% of the distance between the midinguinal crease and proximal patella. A permanent marker was used to indicate the precise transverse location of the scan, and a mark was made midbelly of the VL so that subsequent ultrasound images and muscle biopsy samples 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/s, 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 (55) freely available through ImageJ analysis software (NIH, Bethesda, MD). Importantly, all images were captured and analyzed by the same investigator who was blinded to group allocations. Test-retest reliability using ICC<sub>3,1</sub>, SE, and MD was previously determined for mCSA on 10 participants scanned ~24 h apart resulting in an ICC<sub>3,1</sub> of 0.99, SE of 0.84 cm<sup>2</sup>, and MD of 2.32 cm<sup>2</sup>.

### Ultrasound.

Real-time B-mode ultrasonography (NextGen LOGIQe R8, GE Healthcare) utilizing a multifrequency linear-array transducer (L4-12T, 4–12 MHz, GE Healthcare) was used to capture images of the VL in the transverse plane for measurement of VL CSA. Before image acquisition, subjects rested supine on an examination table for a minimum of 5 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 at which 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) 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; and dynamic range: 75), except for depth, were held constant across participants and sample times. One image per participant was obtained at each sample time. Images were analyzed using the freely available ImageJ software (National Institutes of Health, Bethesda, MD). VL CSA was calculated by 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 sample time. 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<sub>3,1</sub> of 0.99, SE of 0.60 cm<sup>2</sup>, and MD of 1.65 cm<sup>2</sup>.

### 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 the 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 with the initial load being an unloaded barbell/machine; the load was then incrementally increased based on 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 1–4 resulted in a weight increase of 25%, an RPE of 5–7 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 only 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 0.93 (56).

### Skeletal Muscle Biopsies

Skeletal muscle biopsies were collected from the right leg VL at PRE-2, T2, 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 T2 and POST the sample was taken 1–2 cm proximal of the initial biopsy site, using the previous scar as a reference. For biopsies, participants lay down on an athletic table and the upper thigh was shaved and cleansed with 70% isopropanol. A subcutaneous injection of 1% lidocaine (0.8 mL) was then administered. After 5 min, the area was cleansed with chlorhexidine solution (Hibiclens, Mölnlycke Health Care, Norcross, GA). Next, a pilot incision was made through the dermis with a single-use sterile No. 11 surgical blade (AD Surgical, Sunnyvale, CA). The 5-gauge biopsy needle was inserted into the pilot incision, through the muscle fascia, and ~2 cm into the muscle to collect a 50- to 100-mg sample while applying suction (57). Tissue was immediately removed from the needle, cleared of blood and connective tissue, and separated for RNA, histological, and tracer analysis. Tissue allocated for RNA and tracer analysis was placed in prelabeled foils and immediately frozen in liquid nitrogen and stored at –80°C. Tissue allocated to histological analysis was embedded in optimum cutting temperature (OCT) gel to prevent freeze damage, slow-frozen in liquid nitrogen-cooled isopentane, and stored at –80°C until sectioned and stained.

### Resistance Training Protocol

The RT protocol was modeled after a previous study from our laboratory (58), as well as American College of Sports Medicine Guidelines for Exercise Testing and Prescription (59). The protocol consisted of 10 wk in duration and consisted of 20 separate training sessions (2 days/wk; Table 1). The training session included the 45° bilateral leg press, barbell bench press (flat bench), seated leg extension, hex-bar deadlifts, and lat pull down. Each training session involved the following:

- 1) A general warm-up of 25 jumping jacks and 10 body weight squats,
- 2) A specific warm-up of 1 set of 10 reps at 50% of predetermined work-out weight, 1 set of 5 repetitions at 75% of work-out weight, and 1 set of 3 repetitions of 90% of work-out weight; and
- 3) Either *i*) 4 sets of 10 repetitions (higher volume day) or *ii*) 5 sets of 6 reps per exercise (higher load day), each done once per week at the prescribed weight.

To provide progressive overload, weekly loads increased by ~5% for the higher volume day and ~9% for the higher

**Table 1.** Resistance exercise training program

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

The training paradigm for the 10-wk progressive resistance training program is shown. Each training session included the 45° bilateral leg press, barbell bench press (flat bench), seated leg extension, hex-bar deadlifts, and lat pull down. 1-RM, 1-repetition maximum test; 3-RM, 3-repetition maximum test.

load day for the first 4 wk. 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 preprogrammed for all participants based on percentages of their 1-RM, participant RPE was used during each training session to ensure the appropriate load was implemented and the individual programs were adapted accordingly.

### Responder Composite Score

A composite pre- to postchange score consisting of the following variables was used to define “lower” versus “higher” responders: 1) whole body lean/soft tissue mass (LSTM) determined by dual X-ray absorptiometry (DXA), 2) vastus lateralis (VL) cross-sectional area (CSA) determined by ultrasound, 3) midthigh muscle CSA determined by peripheral quantitative computed tomography (pQCT), and 4) maximum deadlift strength determined by a three-repetition maximum test (3-RM). 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. All metrics carried the same weight within the composite score. 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 upper and lower

quartiles of  $n = 8$  higher and  $n = 8$  lower responders, respectively.

### Wet Laboratory Analyses

#### Immunohistochemistry.

PRE and POST biopsies preserved in OCT were batch processed for 1) cryostat sectioning, 2) antibody-based immunohistochemistry, and 3) imaging and analysis. Initially, all samples were sliced into 10  $\mu\text{m}$  thick sections and removed electrostatically from the cooled cryostat stage by a positively charged histology slide (Leica Biosystems; Buffalo Grove, IL). The slides were then stored at  $-80^{\circ}\text{C}$  until antibody staining.

Staining for muscle fCSA and myonuclear number per fiber was performed (60). Briefly, slides with sections were removed from  $-80^{\circ}\text{C}$  storage and dried for  $\sim 10$  min at room temperature. Triton-X (0.5%) in phosphate buffer solution (PBS) was then used to permeabilize the sections for 5 min. This was followed by a 5-min wash in PBS, and slides were subsequently incubated in a 100% concentration of blocking solution for 15 min (Pierce Super Blocker, Thermo Fisher Scientific). Slides were then incubated in primary antibody solution for 60 min. This solution contained PBS, 5% of Pierce Super Blocker Solution, and equal parts at 2% (1:50 dilution) of rabbit anti-dystrophin IgG1 (cat. no. GTX15277, Genetex Inc., Irvine, CA) and mouse anti-myosin I IgG1 (cat. no. A4.951 supernatant; Developmental Studies Hybridoma Bank, Iowa City, IA). Slides were then washed for 5 min in PBS and then incubated in a secondary antibody solution containing a 1× base of PBS and equal parts at 1% or (1:100 dilution) Texas Red-conjugated anti-rabbit IgG (cat. no. TI-1000; Vector Laboratories, Burlingame, CA) and Alexa Fluor 488-conjugated anti-mouse IgG1 (cat. no. A-11001; Thermo Fisher Scientific). This incubation occurred in the dark for 60 min. The slides were then washed for 5 min in PBS, dried, and mounted using a fluorescent media containing 4,6-diamidino-2-phenylindole (DAPI; cat. no. GTX16206; Genetex Inc.). Immediately following mounting, slides were imaged using a fluorescent microscope (Nikon Instruments, Melville, NY) with a  $\times 10$  objective lens. Exposure times were 200 ms for FITC, 600 ms for TRITC, and 100 ms for DAPI. Open-source software (MyoVision) was used to analyze all images for average fCSA, muscle fiber type, and myonuclear number per fiber (60). A conversion of  $0.964 \mu\text{m}/\text{pixel}$  was used to adjust the image for size and bit-depth, and a fiber size threshold was set at a minimum of  $500 \mu\text{m}^2$  and a maximum of  $15,000 \mu\text{m}^2$  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 MyoPS.

$\text{D}_2\text{O}$  was administered as a stable isotope tracer to determine MyoPS [as pioneered by Previs et al. (61) and the Fluckey laboratory (62)], and the timeline for  $\text{D}_2\text{O}$  loading and collection of salivette samples for the study is shown in Fig. 1B. Saliva samples obtained in the laboratory (or returned by participants) were stored at  $-20^{\circ}\text{C}$ . After study completion, salivette tubes were centrifuged for 2 min at  $1,000 g$  ( $2^{\circ}\text{C}$ ). Saliva obtained thereafter was frozen at  $-20^{\circ}\text{C}$ . Frozen samples were then shipped to Metabolic Solutions

(Nashua, NH) 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). 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. Intrarun precision using this method is generally <2 delta per mil (parts per thousand), and interrune precision is generally <3.5 delta per mil.

Muscle biopsy samples from PRE-2 and 24 h were batch processed using the myofibril isolation and solubilization technique as previously described by our laboratory (63). Isolated myofibrils were frozen at  $-80^{\circ}\text{C}$ , and frozen samples were shipped on dry ice to Metabolic Solutions for tracer analyses. This process first involved hydrolyzing myofibril pellets for 18 h with 3 mL of 6 N HCl ( $100^{\circ}\text{C}$ ). Dowex  $\text{H}^{+}$  resin (1 mL, 50WX8-100; Sigma-Aldrich, St. Louis, MO) was added to trap released alanine. Amino acid elution from the resin was performed using 2 mL of 3 N  $\text{NH}_4\text{OH}$ , and eluates were evaporated to dryness. Thereafter, the *N*-acetyl-*n*-propyl (NAP) derivative of alanine was prepared, and the propyl ester was formed by adding 200  $\mu\text{L}$  propyl acetate and 100  $\mu\text{L}$  BF<sub>3</sub>:propanol (14%). Samples were heated at  $110^{\circ}\text{C}$  for 30 min, and solutions evaporated to dryness under  $\text{N}_2$  gas at  $60^{\circ}\text{C}$ . The *N*-acetyl group was formed by adding 100  $\mu\text{L}$  of 0.1 M diethylamine (DEA) in hexane and 100  $\mu\text{L}$  of acetic anhydride. This reaction was incubated for 20 min at  $60^{\circ}\text{C}$  and was subsequently dried down with  $\text{N}_2$  gas and low heat. Samples were reconstituted in 100  $\mu\text{L}$  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 NAP ester of alanine was analyzed using a splitless injection with CTC Pal autosampler (1  $\mu\text{L}$ ). Injections used a Zebron ZB-5 column of 30 m  $\times$  0.25 mm  $\times$  0.50  $\mu\text{m}$  film thickness (Phenomenex, Torrance, CA), and the injection temperature was  $250^{\circ}\text{C}$ . The GC oven had an initial column temperature of  $80^{\circ}\text{C}$  with a 2-min hold and was followed by a ramp of  $30^{\circ}\text{C}$  per minute to  $330^{\circ}\text{C}$ . Compounds eluting off the column were directed into the pyrolysis reactor and heated to  $1,450^{\circ}\text{C}$  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. Intrarun precision for alanine measurements is generally <2 delta per mil, and interrune precision is generally <3 delta per mil.

Saliva and myofibril enrichments were used to calculate MyoPS rates over the 24-h period following the first naïve training bout. The equation follows the one published by Bell et al. (64) (see below; FSR, fractional synthesis rate).

$$\text{FSR } (\% \text{day}^{-1}) = \left[ \frac{(E_{\text{Ala}2} - E_{\text{Ala}1})}{E_{\text{BW}} \times t} \right] \times 3.7 \times 100$$

Briefly, the difference in deuterium ( $^2\text{H}$ ) enrichment from the first two biopsies ( $E_{\text{Ala}2}$  = 24-h muscle sample enrichment;

$E_{\text{Ala}2}$  = PRE-2 muscle sample enrichment) was divided by the product total body enrichment of  $^2\text{H}$  (in atom % excess) ( $E_{\text{BW}}$  =  $^2\text{H}$  from PRE-2 and 24-h saliva – baseline  $^2\text{H}$  from PRE-2 saliva) and the number of days that  $\text{D}_2\text{O}$  was consumed at the loading dose. This quotient was then multiplied by 3.7 to adjust for the number of  $^2\text{H}$  atoms typically bound to alanine. Finally, the resultant value was multiplied by 100 to achieve myofibrillar synthesis rate in percent per day.

#### Determination of satellite cells.

Satellite cells were quantified using Pax7 immunohistochemistry. For this analysis, separate slides with adjacent sections were removed from  $-80^{\circ}\text{C}$  storage and dried for  $\sim 10$  min at room temperature. Triton-X (0.5%) in PBS was used to permeabilize the sections for 5 min. This was followed by a 5-min wash in PBS, and slides were subsequently incubated in a 100% concentration of blocking solution for 15 min (Pierce Super Blocker). Slides were then incubated in primary antibody solution for 60 min. This solution contained PBS, 5% of Pierce Super Blocker Solution, and equal parts at 2% (1:50 dilution) of rabbit anti-dystrophin IgG1 (cat. no. GTX15277; Genetex Inc.; Irvine, CA) and mouse anti-Pax7 IgG (cat. no. PAX7 supernatant; Hybridoma Bank). Sections were then washed for 5 min in  $1\times$  PBS and incubated in the dark for 1 h 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  $\mu\text{L}$  of all secondary antibodies per 1 mL of blocking solution). Sections were then washed for 5 min 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  $\times 20$  objective lens. Exposure times were 200 ms for FITC, 600 ms for TRITC, and 100 ms 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 muscle ribosome content.

For total RNA analysis,  $\sim 15$ – $30$  mg of powdered tissue was weighed using an analytical scale with a sensitivity of 0.001 g (Mettler-Toledo, Columbus, OH). Tissue was then homogenized in 1.7-mL microcentrifuge tubes containing 500  $\mu\text{L}$  of Ribozol (Ameresco, Solon, OH) 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), and total RNA per wet muscle weight was used as a surrogate for muscle ribosome content as in past publications (65, 66).

#### Food Log Analysis

The 3-day food log packets were used to obtain nutritional intakes for 1 weekend day and 2 weekdays in the week leading up to PRE-2 and POST visits and ensure the lack of change in participant diet. Therefore, it could be assumed the change in the outcome metrics was due to the exercise intervention and not to other changes in participant habits. Each food log contained an example of 1 day, with three



additional blank sheets. Participants were instructed to write down as many details of their food as possible. If a food log was returned incomplete, we met with the participant to gather more information. Participants were asked to maintain their normal dietary habits throughout the duration of the study, except for 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/day, g/day, or g/kg/day) at each sample time.

### Additional Questionnaires

Self-reported sex, gender, race, and ethnicity were collected via questionnaires during the Informed Consent visit. Additionally, prior exercise history, menstrual cycle information, and contraceptive use were recorded during this visit. Day of the menstrual cycle was self-reported, with the first day of bleeding considered *day 1*. Finally, at POST, menstrual cycle information was collected again, as well as subjective sleep quality using the Pittsburgh Sleep Quality Index (67).

### Statistical Analysis

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

Dependent samples *t* tests were performed to examine changes in outcome variables for all participants. For menstrual cycle analysis, independent samples *t* tests were used to compare the 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. Pearson correlations were also performed between each individual variable in the composite score and VL, CSA, and mean fCSA, and these tests included all participants.

For the higher responder (HR) versus lower responder (LR) analysis, independent samples *t*-tests were used to compare the 24-h MyoPS rates, baseline differences in select phenotypes, as well as self-reported sleep duration between cohorts. Two-way (group  $\times$  time) repeated measure ANOVAs were used to determine changes in dependent variables before and following the training intervention in the HR versus LR cohorts. When a significant group  $\times$  time interaction was observed, least significant differences post hoc were performed to determine differences within each group from pre- to postintervention and between groups at each sample time. Statistical significance was established a priori at  $P < 0.05$ .

## RESULTS

### Participant Characteristics

Baseline participant characteristics can be found in Table 2. We assessed 81 females for eligibility (43 were excluded, with the majority waitlisted based on our maximum limit of participants), 38 female participants began the study, and 34 female participants ( $21 \pm 2.1$  yr,  $>90\%$  Caucasian, body mass index of  $23.4 \pm 3.4$  kg/m<sup>2</sup>) completed

the study. All participants completed above 90% 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 change significantly over time. However, LSTM significantly increased with training ( $43.16 \pm 4.27$  kg,  $P < 0.001$ ). 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:  $P = 0.001$ ; see Table 2 for values).

### Training Volume and Strength Metrics

The HR cohort engaged in a higher training volume compared to LR throughout the duration of the study (HR =  $151,101 \pm 18,478$  kg; LR =  $101,145 \pm 26,204$  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 =  $2203 \pm 348$ ; LR =  $1931 \pm 1076$ ;  $P = 0.506$ ).

All strength metrics are presented in Table 2. For all participants, 1-RM values increased by 102.4% for leg press, 24.6% for bench press, and 36.9% for deadlift from PRE to POST ( $P < 0.001$  for all). At PRE, the HR cohort had significantly greater 1-RM leg press and bench press (75.4% greater,  $P = 0.003$  and 22.9% greater,  $P = 0.026$ , respectively). All POST strength metrics were significantly higher in the HR than the LR cohort (leg press, 44% greater,  $P < 0.001$ ; bench press, 27.7% greater,  $P < 0.001$ ; and deadlift, 43.8% greater,  $P < 0.001$ ). Additionally, the change in strength between cohorts significantly differed for bench press (HR =  $9.8 \pm 1.7$  kg; LR =  $6.1 \pm 3.8$  kg;  $P = 0.025$ ) and deadlift (HR =  $36.5 \pm 14.7$  kg; LR =  $13.0 \pm 7.1$  kg;  $P = 0.001$ ) but not leg press (HR =  $73.0 \pm 14.5$  kg; LR =  $67.7 \pm 23.3$  kg;  $P = 0.588$ ).

### 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 1-RM deadlift. For all participants, the change values were as follows:  $1.07 \pm 1.11$  kg,  $2.67 \pm 2.70$  cm<sup>2</sup>,  $8.9 \pm 1.9$  cm<sup>2</sup>, and  $24 \pm 13$  kg, respectively (Fig. 2A,  $P < 0.001$ , for all variables from pre- to posttraining). The HR cohort showed increases in these variables from PRE to POST ( $P < 0.001$  for all variables). Mid-thigh mCSA (PRE =  $98.8 \pm 6.3$  cm<sup>2</sup>; POST =  $102.1 \pm 5.9$  cm<sup>2</sup>;  $P = 0.011$ ) and estimated 1-RM deadlift (PRE =  $51.7 \pm 12.6$  kg; POST =  $64.7 \pm 14.4$  kg;  $P = 0.001$ ) increased in the LR cohort. However, DXA LSTM (PRE =  $39.2 \pm 3.5$  kg; POST =  $39.5 \pm 3.7$  kg;  $P = 0.177$ ) and VL CSA (PRE =  $17.6 \pm 1.9$  cm<sup>2</sup>; POST =  $18.1 \pm 1.9$  cm<sup>2</sup>;  $P = 0.067$ ) did not. Finally, the HR cohort showed significantly greater increases than the LR cohort for all four variables (Fig. 2B,  $P < 0.001$ , for all variables).

### Mean fCSA Changes

Changes in mean fCSA and the responder analysis for this variable can be seen in Fig. 3, A and B. For these and other histology analyses, 31 participants were included due to 3 participant samples yielding low-quality sections at one of

**Table 2.** Participant characteristics and general training adaptations

Variable	All (n = 34)	HR (n = 8)	LR (n = 8)	Interaction P value	P Value HR vs. LR
Age, years	21 ± 2	21 ± 1	21 ± 1		0.693
Height, m	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1		0.909
PRE body mass, kg	68.2 ± 10.3	69.7 ± 11.1	62.5 ± 10.8	0.477	0.214
POST body mass, kg	68.2 ± 10.3	71.7 ± 10.9	63.5 ± 9.9		0.138
PRE DXA LSTM, kg	42.1 ± 4.1	44.2 ± 3.3	39.2 ± 3.5	<0.001	0.010
POST DXA LSTM, kg	43.2 ± 4.3*	46.5 ± 3.2†	39.5 ± 3.7		0.001
PRE DXA fat mass, kg	23.0 ± 8.0	22.4 ± 8.6	21.8 ± 8.2	0.490	0.876
POST DXA fat mass, kg	22.5 ± 7.5	22.6 ± 9.0	21.4 ± 7.1		0.763
PRE 1-RM leg press, kg	83 ± 39	111.8 ± 29	50.6 ± 39.4	0.580	0.003
POST 1-RM leg press, kg	168 ± 48 <sup>+</sup>	184.9 ± 28.8	118.3 ± 33.3		0.001
PRE 1-RM bench press, kg	32 ± 6	34.2 ± 5.4	27.2 ± 6.0	0.033	0.026
POST 1-RM bench press, kg	39 ± 7 <sup>+</sup>	44.0 ± 5.1	33.3 ± 4.6		0.001
PRE 1-RM deadlift, kg	60 ± 14	64.4 ± 11.6	51.7 ± 12.6	<0.001	0.055
POST 1-RM deadlift, kg	82 ± 23*	101.0 ± 11.1†	64.7 ± 14.4†		<0.001
PRE relative 1-RM deadlift, kg/kg body mass	0.91 ± 0.24	0.95 ± 0.25	0.84 ± 0.21	0.005	0.365
POST relative 1-RM deadlift, kg/kg body mass	1.22 ± 0.34*	1.43 ± 0.23†	1.03 ± 0.19†		0.002

DXA LSTM, lean/soft tissue mass according to the dual energy X-ray absorptiometry scans; 1-RM, 1-repetition maximum; HR, higher responder; LR, lower responder \*Significance between pretesting (PRE) and posttesting (POST) for all participants. †Increase within HR or LR from PRE to POST.

the sampling time points. Mean fCSA significantly increased from PRE to POST on average by 17.7% for all participants (PRE = 4,290 ± 989  $\mu\text{m}^2$ ; POST = 5,049 ± 1429  $\mu\text{m}^2$ ;  $P = 0.001$ ). However, there was no cluster  $\times$  time interaction observed for the responder analysis ( $P = 0.606$ ; Fig. 3B).

### Twenty-four-hour MyoPS Response to First Bout of Training

The 24-h integrated MyoPS response to the first naïve bout of resistance exercise and additional analyses related to this variable are presented in Fig. 4. For the initial analysis, 31 participants were included due to three participant samples lacking appropriate yield amounts from isolated myofibrillar proteins. MyoPS rates were 2.25 ± 1.05% per day during this 24-h period (Fig. 4A). There was no significant difference between HR and LR cohorts (HR = 1.88 ± 0.98% per day; LR = 2.43 ± 1.25% per day;  $P = 0.370$ ; Fig. 4B). There was no significant association between pre- to posttraining percentage change in VL CSA and the 24-h integrated MyoPS (Fig. 4C;  $r = 0.095$ ;  $P = 0.602$ ). Likewise, there was no significant association between the pre- to posttraining percentage change in mean fCSA and 24-h MyoPS response (Fig. 4D;  $r = 0.354$ ;  $P = 0.060$ ).

### Satellite Cell and Myonuclear Number

Satellite cell number changes from PRE to POST and correlations to hypertrophy can be seen in Fig. 5. Satellite cell number per muscle fiber increased in all participants by ~25% ( $P = 0.025$ ; Fig. 5A). A significant cluster  $\times$  time interaction was observed for the number of satellite cells per muscle fiber ( $P = 0.025$ ), but there was no main effect of time ( $P = 0.103$ ) or cluster ( $P = 0.563$ ; Fig. 5B). Post hoc testing indicated a significant increase occurred from PRE to POST in higher responders (PRE = 0.064 ± 0.020; POST = 0.113 ± 0.046;  $P = 0.026$ ), whereas no significant difference was evident from PRE to POST in lower responders (PRE = 0.083 ± 0.048; POST = 0.075 ± 0.043;  $P = 0.118$ ). Importantly, there was no significant difference between higher responders and lower responders at PRE ( $P = 0.305$ ). When considering all participants for correlations, there was no significant association between the

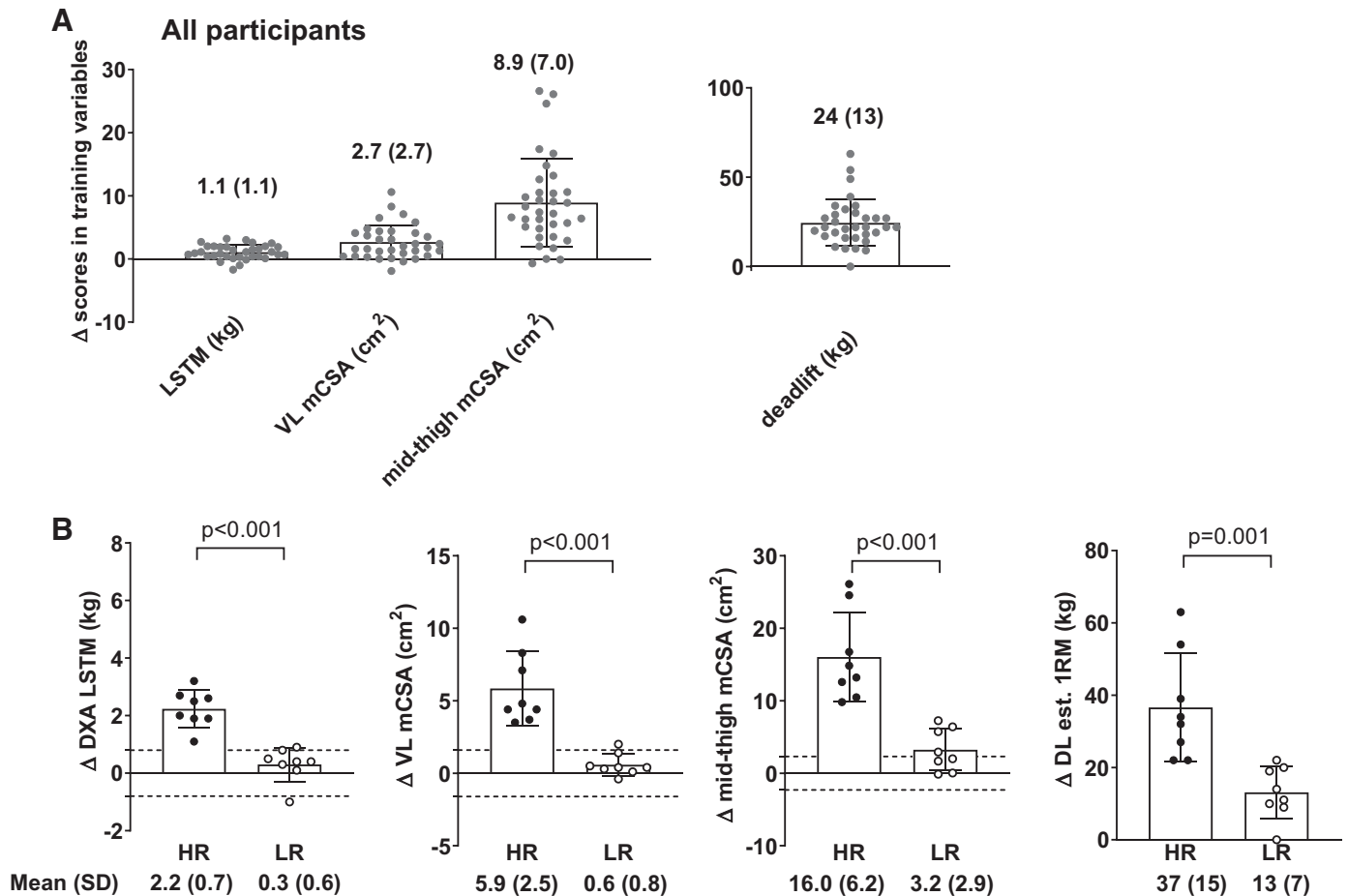
pre- to posttraining percentage changes in satellite cells per fiber and VL CSA ( $r = 0.095$ ;  $P = 0.609$ ; Fig. 5C). However, there was a positive correlation between the pre- to posttraining percentage changes in satellite cells per fiber and mean fCSA ( $r = 0.471$ ;  $P = 0.007$ ; Fig. 5D). Figure 5E provides a representative histology image for PRE. Figure 5F provides a representative histology image for POST of the same participant.

Change in myonuclear number per fiber from PRE to POST in all participants is presented in Fig. 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 (PRE = 1.16 ± 0.43 myonuclei/fiber; POST = 1.45 ± 0.64 myonuclei/fiber;  $P = 0.049$ ; Fig. 6B). However, the cluster effect and cluster  $\times$  time interaction was not significant ( $P = 0.163$  and  $P = 0.233$  respectively; Fig. 6B). There was a significant positive correlation between percent changes in myonuclei per fiber and mean fCSA from PRE to POST ( $r = 0.523$ ;  $P = 0.003$ ; Fig. 6D) but no significant correlation between percent change in VL CSA ( $P = 0.159$ ; Fig. 6C). Figure 6E provides a representative histology image for PRE. Figure 6F provides a representative histology image for POST of the same participant.

### Muscle Ribosome Content

Changes in a marker of muscle ribosome content (RNA per mg of wet muscle) and associations with hypertrophic outcomes can be found in Fig. 7. For this analysis, 33 participants were included due to 1 participant sample lacking adequate tissue amount to isolate RNA. There was no difference in RNA content per mg of wet muscle between PRE and POST for all participants (PRE = 1,221 ± 667 ng/mg; POST = 1,261 ± 729 ng/mg;  $P = 0.684$ ). However, because hypertrophy occurred in all participants (i.e., both fCSA and VL CSA), and the RNA content per mg per wet muscle did not change, this indicates the absolute amount of total RNA content increased. For responder analysis, the cluster  $\times$  time interaction and main effects of time and cluster were all not significant ( $P = 0.157$ ,  $P = 0.778$ , and  $P = 0.888$  respectively; Fig. 7B). The





**Figure 2.** Adaptations in variables used for classifying response groups. *A*: pre- to postintervention change scores for outcome variables used to generate response groups in all 34 female participants. *B*: pre- to postintervention change score differences between the higher ( $n = 8$ ) and lower ( $n = 8$ ) responders. For DXA LSTM, VL mCSA, and midthigh mCSA, dashed lines in *B* represent minimal difference scores based on test-retest statistics discussed in METHODS. All bar graphs are expressed as means  $\pm$  SD values and contain individual respondent values as well. DXA, dual energy X-ray absorptiometry; HR, higher responder; LR, lower responder; LSTM, lean/soft tissue mass; mCSA, muscle cross-sectional area; VL, vastus lateralis; 1RM, 1-repetition maximum; DL est., deadlift estimated.

correlations between percentage changes in muscle ribosome content and both hypertrophy measures were not significant (VL CSA:  $P = 0.060$ ; mean fCSA:  $P = 0.574$ ; Fig. 7, C and D). Furthermore, when removing on participant outlier from Fig. 7C, this yielded a lower association between changes in muscle ribosome content and VL mCSA ( $r = 0.014$ ;  $P = 0.937$ ).

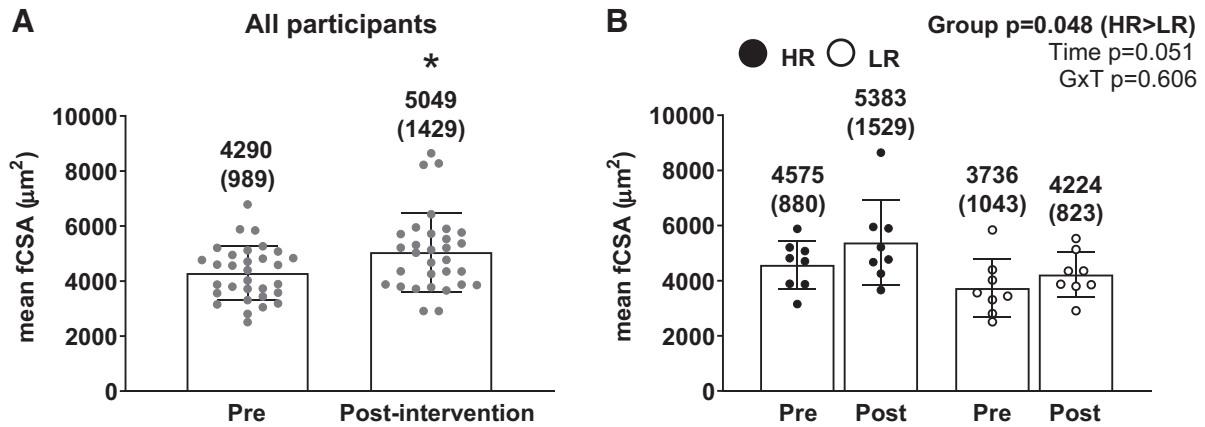
### Other Differences between Higher and Lower Responders

Table 3 compares other variables of interest between higher and lower responders. Along with Table 2 data indicating PRE DXA LSTM was greater in HR versus LR (HR PRE =  $44.2 \pm 3.3$  kg; LR PRE =  $39.2 \pm 3.5$  kg;  $P = 0.010$ ), PRE midthigh mCSA (HR =  $116.6 \pm 8.0$  cm<sup>2</sup>; LR PRE =  $98.8 \pm 6.3$  cm<sup>2</sup>), and PRE VL mCSA (HR =  $20.1 \pm 2.2$  cm<sup>2</sup>; LR =  $17.6 \pm 1.9$  cm<sup>2</sup>) were significantly greater in HR versus LR ( $P < 0.001$  and  $P = 0.028$ , respectively). PRE self-reported energy intake, POST self-reported energy intake, self-reported sleep, and self-reported day of menstrual cycle at PRE were not significantly different between response groups.

## DISCUSSION

The main findings from the present study include 1) 24-h integrated MyoPS following the first training bout did not predict hypertrophy outcomes, 2) muscle ribosome content (per mg tissue) did change with training, and 3) satellite cell proliferation only occurred in higher responders. Furthermore, there was a moderately strong positive correlation between the percent change in satellite cell number and mean fCSA in all participants ( $r = 0.471$ ;  $P = 0.007$ ). 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 ( $r = 0.523$ ;  $P = 0.003$ ). Finally, females that began training with more lean tissue mass and larger leg muscles (as assessed using DXA and ultrasound, respectively) were typically higher responders.

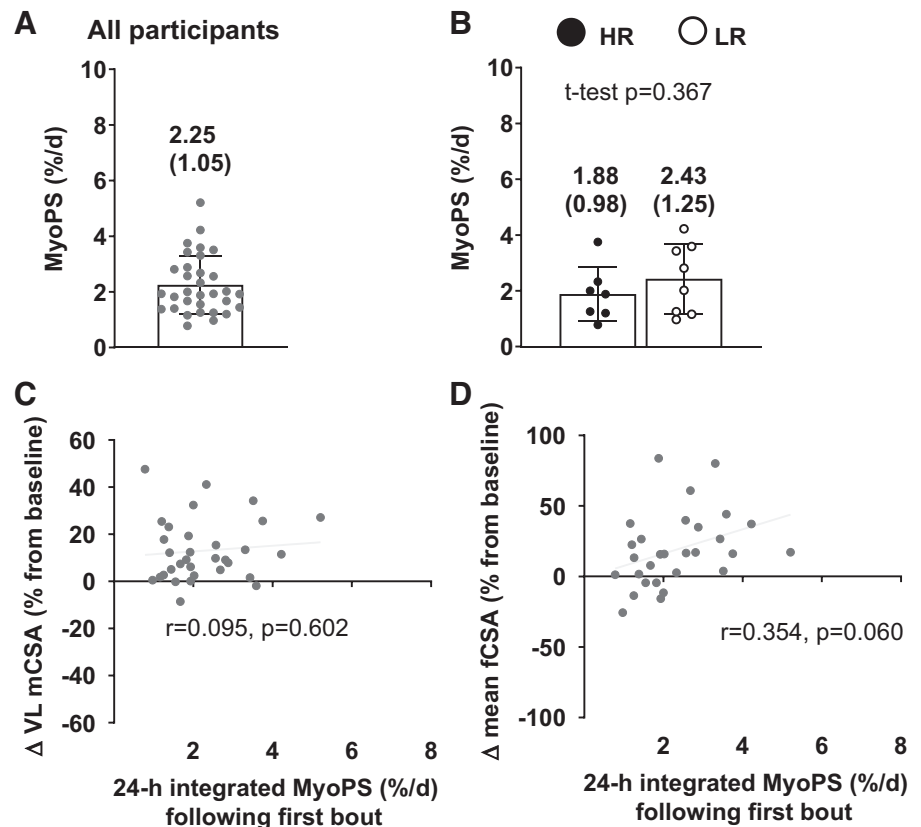
Satellite cells are distinctively defined by their proximity to skeletal muscle fibers and are positioned between the sarcolemma and basement membrane. Mechanical overload stimulates satellite cell proliferation, differentiation, and fusion to preexisting myofibers (68), and an increase in satellite cell-



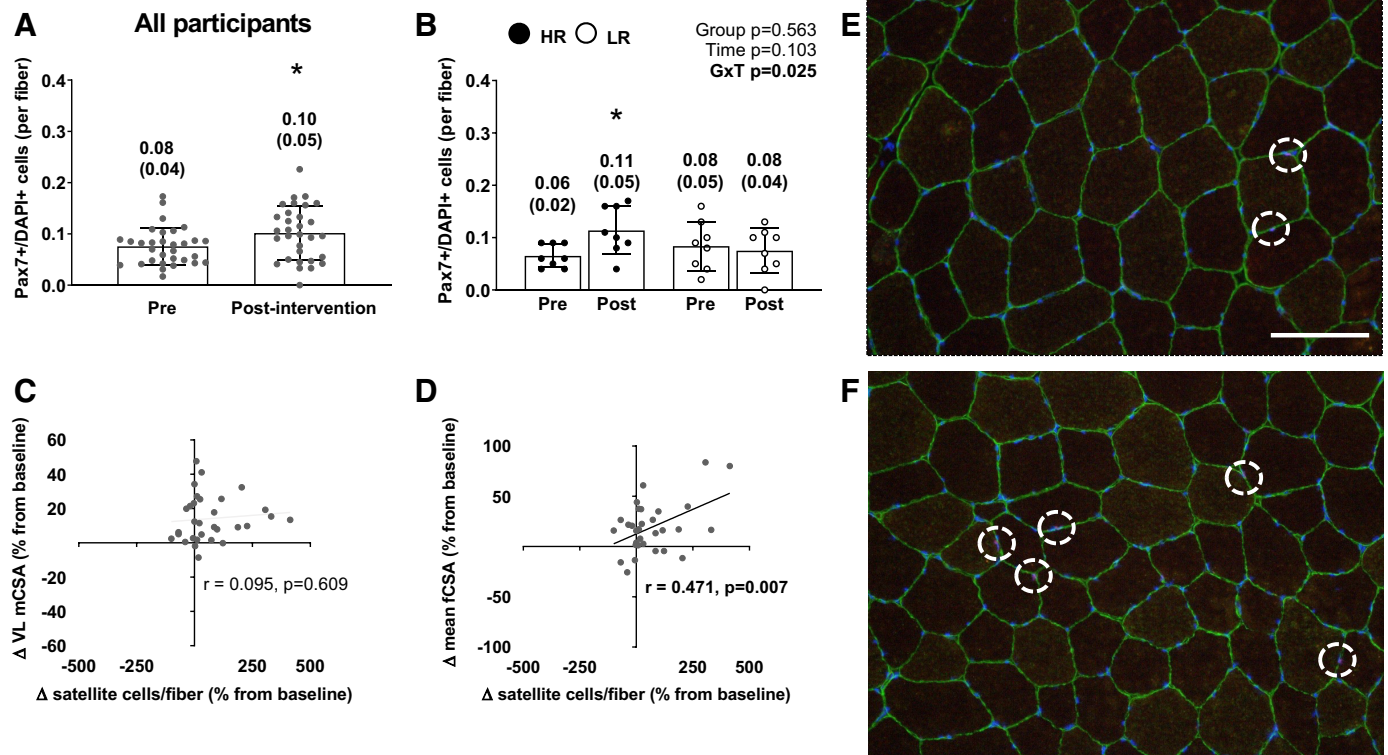
**Figure 3.** Mean fCSA changes in all participants and higher vs. lower responders. A: pre- and postintervention mean fCSA values in the 31 of 34 female participants that yielded enough tissue for analyses (\* $P < 0.05$  comparing pre to post). B: pre- and postintervention mean fCSA values between the higher ( $n = 8$ ) and lower ( $n = 8$ ) responders. All bar graphs are expressed as means  $\pm$  SD values and contain individual respondent values as well. fCSA, fiber cross-sectional area; GxT, group  $\times$  time interaction; HR, higher responder; LR, lower responder.

mediated myonuclear addition in growing myofibers likely provides cellular support for the increased number of cellular macromolecules (e.g., ribosomes and muscle proteins) (69). Importantly, several groups have reported that satellite cell proliferation increases in response to one bout of resistance exercise (70–75). Moreover, our findings align with those of several studies that have demonstrated a similar response to multiple weeks of RT in males (76–78) and females (78–80) and continue to support the body of literature suggesting an increase in satellite cell number to one or multiple bouts of resistance training differentiates higher and lower responders (71, 80).

Several studies have shown that increased muscle RNA content per mg tissue through ribosome biogenesis coincides with muscle hypertrophy following weeks to months of mechanical overload in both animal models (30, 81–83) and humans (25, 84, 85). Contrary to these data, the female participants herein did not demonstrate increases in muscle RNA content per milligram of tissue, and changes in this variable were not predictive of hypertrophy (i.e.,  $\Delta$ VL CSA or  $\Delta$ VL mean fCSA). This lack of predictability does not discount the role that ribosome biogenesis plays in muscle hypertrophy, and it is possible that more dynamic responses could have



**Figure 4.** Integrated MyoPS response to the first bout of training in all participants and higher vs. lower responders. A: 24-h integrated MyoPS response to the first bout of training in the 32 of 34 female participants that yielded enough tissue for analyses. B: same values as in A between the higher ( $n = 7$ ) and lower ( $n = 8$ ) responders. All bar graphs are expressed as means  $\pm$  SD values and contain individual respondent values as well. C: scatterplot showing the association between the MyoPS responses presented in A and the pre- to postchange in VL mCSA in all participants. D: scatterplot showing the association between the MyoPS responses presented in panel A and the pre- to postchange in mean fCSA in all participants. mCSA, muscle cross-sectional area; fCSA, fiber cross-sectional area; HR, higher responder; LR, lower responder; MyoPS, myofibrillar protein synthetic; VL, vastus lateralis.



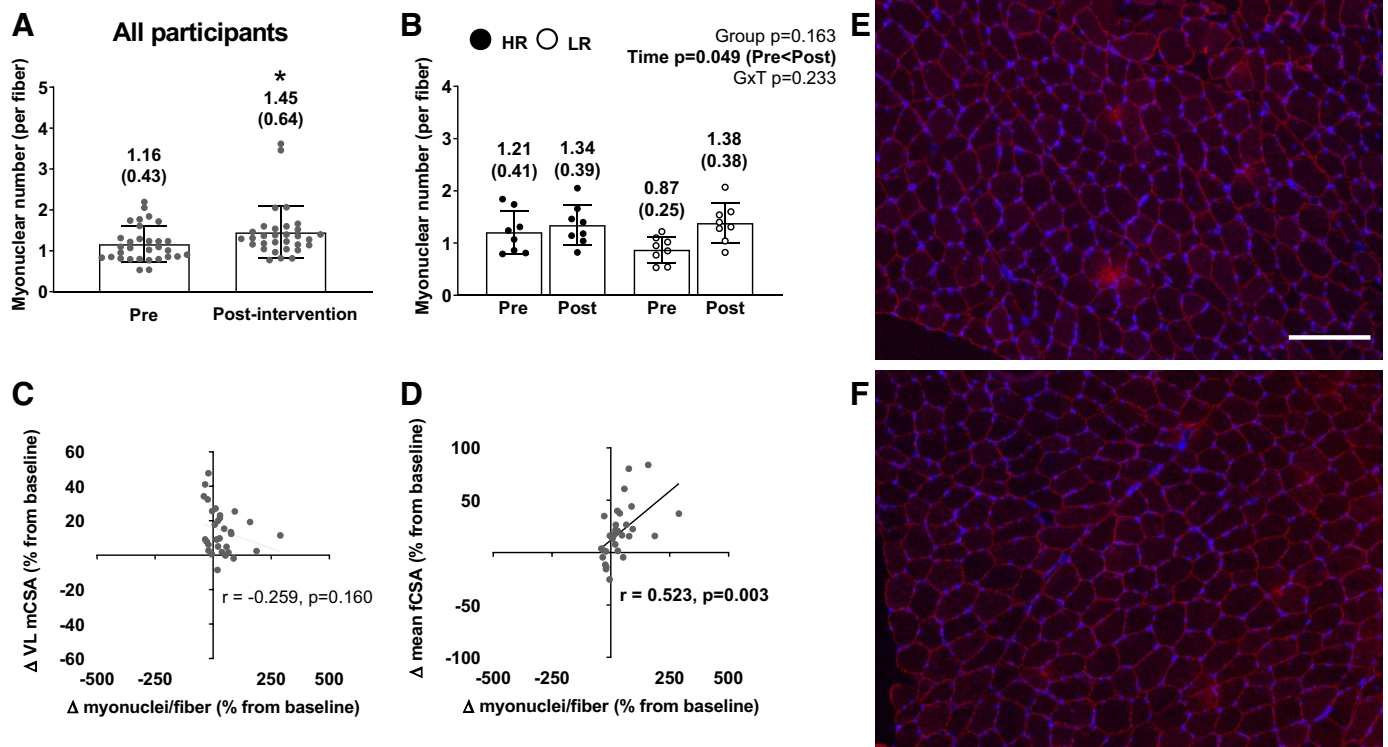
**Figure 5.** The satellite cell response to training in all participants and higher vs. lower responders. **A:** PAX7 + /DAPI + (or satellite cell) response to 10 wk of training in the 31 of 34 female participants that yielded enough tissue for analyses. **B:** same values as in **A** between the higher ( $n = 8$ ) and lower ( $n = 8$ ) responders. \*Increase in HR from pre to post. All bar graphs are expressed as means  $\pm$  SD values and contain individual respondent values as well. **C:** scatterplot showing the association between the pre- to postchange in satellite cells and the pre- to postchange in VL mCSA in all participants. **D:** scatterplot showing the association between pre- to postchange in satellite cells and the pre- to postintervention change in mean fCSA in all participants. **E:**  $\times 20$  representative image of PRE (scale bar = 100  $\mu$ m). **F:** representative image of POST of the same participant (white circles identify satellite cells; colors: green, laminin; blue, DAPI; red, Pax-7; purple, satellite cell). mCSA, muscle cross-sectional area; fCSA, fiber cross-sectional area; GxT, group  $\times$  time interaction; HR, higher responder; LR, lower responder; VL, vastus lateralis.

occurred earlier in training. However, this hypothesis is speculative given that we did not obtain time course biopsies throughout training. Moreover, and as stated in the previous section, while ribosome content (RNA per mg tissue) remained unaltered with chronic training, we contend that ribosome biogenesis did occur given that muscle hypertrophy occurred in lieu of ribosome content values remained stable. However, unlike past studies in previously untrained men indicating that an increased ribosome content per milligram of muscle tissue delineates higher and lower hypertrophic responders after shorter term (i.e., 4 wk) (31) and longer term (i.e., 12 wk) resistance training (20), we did not observe that phenomenon in previously untrained women following a 10-wk resistance training protocol.

As well, the 24-h integrated MyoPS response to the first bout of resistance exercise was not predictive of hypertrophy (i.e.,  $\Delta$ VL CSA or  $\Delta$ VL mean fCSA), agreeing with prior studies and a review reporting low associations exist between the muscle protein synthetic response to a naïve bout of resistance exercise and muscle mass changes weeks into training (7, 86). The measured level of MyoPS ( $2.29 \pm 1.05\%$ ) aligns with previous studies showing heightened MyoPS rates 24 h following RE in males (7, 87). 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 is likely increased due to the novelty of the stimulus and the initial muscle damage that ensues. Therefore, this level of breakdown activity will affect 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 become more refined as individuals become more trained (88, 89). Thus it is likely that we assessed the “damage-synthesis” MyoPS response, whereas ascertaining this variable later in training may have yielded more insightful findings. Notably, this phenomenon has been observed in previously untrained men by Damas et al. (86) who reported that the elevations in MyoPS to a naïve training bout, when muscle damage metrics were concomitantly elevated, were not significantly associated with muscle hypertrophy after 10 wk of resistance training. However, the MyoPS response to a week 10 training bout was strongly correlated ( $r \sim 0.90$ ) with the muscle hypertrophy induced by 10 wk of resistance training. Whether this finding holds true in females, however, remains to be determined. What finally should be noted is that our approach may not have been sensitive enough to detect between-cluster differences in MyoPS given that a large majority of  $D_2O$ -labeled alanine was likely integrated into myofibrils by the first biopsy based on our dosing schematic shown in Fig. 1.



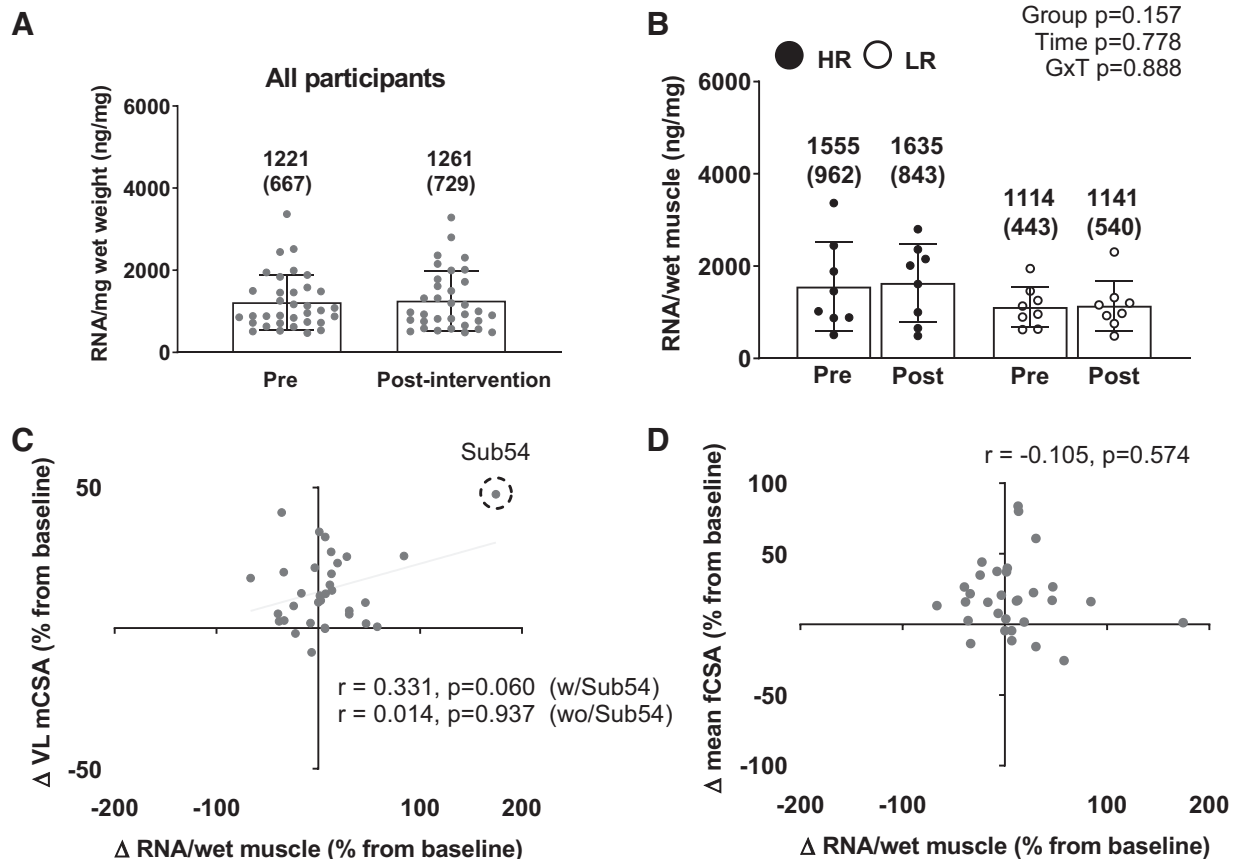


**Figure 6.** The myonuclear response to training in all participants and higher vs. lower responders. *A*: myonuclear number response to 10 wk of training in the 31 of 34 female participants that yielded enough tissue for analyses. *B*: same values as in *A* between the higher ( $n = 8$ ) and lower ( $n = 8$ ) responders. All bar graphs are expressed as means  $\pm$  SD values and contain individual respondent values as well. *C*: scatterplot showing the association between the pre- to postchange in myonuclear number and the pre- to postchange in VL mCSA in all participants. *D*: scatterplot showing the association between pre- to postchange in myonuclear number and the pre- to postchange in mean fCSA in all participants. *E* and *F*:  $\times 10$  representative image of PRE (*E*) and representative image of POST (*F*) of the same participant (colors: red, dystrophin; blue, DAPI; white scale bar = 200  $\mu$ m). mCSA, muscle cross-sectional area; fCSA, fiber cross-sectional area; GxT, group  $\times$  time interaction; HR, higher responder; LR, lower responder.

It has been implied that satellite cell-mediated myonuclear accretion coincides with hypertrophy (15, 77, 90). However, hypertrophy without myonuclear accretion has also been observed in humans and, more specifically, in previously untrained female participants. For instance, Herman-Montemayor et al. (91) 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. (71) reported similar findings after 16 wk of knee extensor training in untrained female participants. 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 demonstrated a main effect of training but not group  $\times$  time interaction. There are speculative explanations for this 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, rodent data indicate that satellite cells have nonfusion roles. For example, satellite cells secrete exosomes that communicate with fibroblasts to downregulate collagen production (92, 93). 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 notion that training-induced increases in satellite cell numbers coincide with skeletal muscle hypertrophy and may play a role in the responsiveness to RT.

Other findings herein 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 a growing interest in the field investigating the impact of the amount of sleep (94) and timing within the menstrual cycle (95–97) on training adaptations. While the present study collected these data, 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. This agrees in principle with a study by Van Etten et al. (13) who reported that individuals with a “solid body build” presented gains in lean body mass, whereas a separate cohort of those who possessed a “slender” build did not. In contrast to these reports, however, 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). While these data allude to the role that pretraining body composition plays in the response to RT, more research is needed to fully address this relationship.



**Figure 7.** The muscle ribosome content response to training in all participants and higher vs. lower responders. **A:** muscle total RNA concentration (per wet tissue mass, or ribosome density) response to 10 wk of training in the 33 of 34 female participants that yielded enough tissue for analyses. **B:** same values as in **A** between the higher ( $n = 8$ ) and lower ( $n = 8$ ) responders. All bar graphs are expressed as means  $\pm$  SD values and contain individual respondent values as well. **C:** scatterplot showing the association between the pre- to postchange in ribosome density and the pre- to postchange in VL mCSA in all participants. Notably, the removal of one subject (subject 54) changes the  $P$  value from 0.060 to 0.937. **D:** scatterplot showing the association between the pre- to postchange in ribosome density and the pre- to postchange score in mean fCSA in all participants. mCSA, muscle cross-sectional area; fCSA, fiber cross-sectional area; GxT, group  $\times$  time interaction; HR, higher responder; LR, lower responder; VL, vastus lateralis.

## Limitations

As with any study, there are limitations that must be considered, and the first is the length of training. Previous literature demonstrates that as little as 3–6 wk of RT increases hypertrophy measures in untrained male participants (45, 98, 99); however, less investigation has been done in

female participants. While the present study was 10 wk in duration, a longer intervention would have broadened the opportunity to observe training adaptations. Second, the analysis of “higher responder” versus “non/lower responder” is highly debated. Some researchers attribute this differentiation to a technical error in the measurements being used to assess skeletal muscle hypertrophy or plainly interindividual variation. Conversely, others (including our laboratory) believe there are characteristics that lead an individual to respond to RT in varying magnitudes. A key limitation to the responder analysis itself is contingent on the training regimen used; therefore, a higher or lower responder is referring to the magnitude of response for the specific training intervention used. For example, the training stimulus may have been too low for a lower responder to see similar increases in performance outcomes as a higher responder (i.e., if participants trained 4 or 5 times a week and/or completed more volume, we could see different results). Additionally, 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 are presented separately for both responder clusters as well as all participants with associations. It also should be noted that

**Table 3.** Other differences between higher and lower responders

Variable	HR ( $n = 8$ )	LR ( $n = 8$ )	$P$ Value
PRE midhigh mCSA, $\text{cm}^2$	117 $\pm$ 8	99 $\pm$ 6	<0.001
PRE VL mCSA, $\text{cm}^2$	20.1 $\pm$ 2.2	17.6 $\pm$ 1.9	0.028
PRE energy intake, kcal/day	1,268 $\pm$ 388	1,512 $\pm$ 474	0.279
POST energy intake, kcal/day	1,312 $\pm$ 448	1,627 $\pm$ 618	0.263
PRE protein intake, g/day	61 $\pm$ 32	65 $\pm$ 30	0.807
POST protein intake, g/day	68 $\pm$ 22	77 $\pm$ 31	0.521
PRE HEI-2015	48.1 $\pm$ 7.3	46.4 $\pm$ 12.7	0.691
POST HEI-2015	46.8 $\pm$ 8.1	44.9 $\pm$ 10.3	0.492
Self-reported sleep, h/night	9.3 $\pm$ 1.1	9.4 $\pm$ 2.1	0.870
Day of menstrual cycle (at PRE)	15 $\pm$ 8	18 $\pm$ 7	0.480

HR, higher responder; LR, lower responder; HEI-2015, healthy eating index-2015; mCSA, muscle cross-sectional area; VL, vastus lateralis; PRE, pretesting; POST, posttesting.

histology was performed on a small section of skeletal muscle, and analyses were performed in cross-section. As our laboratory has contended in the past, limitations in what information can be gleaned with skeletal muscle biopsy analyses whereby two-dimensional analysis is used to extrapolate three-dimensional attributes of multinucleated cells should be more recognized by readership (100). Finally, the methods to collect information on sleep were self-reported, and therefore, those data are limited to the discretion of the participant. Using wearables to collect this data would increase the accuracy of this data.

## Conclusions

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

## DATA AVAILABILITY

All raw data can be obtained upon reasonable request and establishment of a material transfer agreement by emailing the corresponding author (mdr0024@auburn.edu).

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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

M.A.S., A.D.F., L.B.G., and M.D.R. conceived and designed research; M.A.S., C.L.S., K.A.S., S.C.O., J.S.G., J.P.B., and B.A.R., K.C.Y., and M.D.R. performed experiments; M.A.S., C.L.S., J.S.G., and J.P.B. analyzed data; M.A.S. and J.S.G. interpreted results of experiments; M.A.S. prepared figures; M.A.S. drafted manuscript; M.A.S., C.L.S., K.A.S., S.C.O., J.S.G., J.P.B., B.A.R., M.D.G., J.L.E., A.D.F., A.T.R., L.B.G., K.C.Y., and M.D.R. edited and revised manuscript; M.A.S., C.L.S., K.A.S., S.C.O., J.S.G., J.P.B., B.A.R., M.D.G., J.L.E., A.D.F., A.T.R., L.B.G., K.C.Y., and M.D.R. approved final version of manuscript.

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