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Short-term aerobic conditioning prior to resistance training augments muscle hypertrophy and satellite cell content in healthy young men and women

Aaron C. Q. Thomas, Alex Brown, Aidan A. Hatt, Katherine Manta, Anamaria Costa-Parke, Michael Kamal, Sophie Joanisse, Chris McGlory, Stuart M. Phillips, Dinesh Kumbhare, Gianni Parise

Abstract

Factors influencing inter-individual variability of responses to resistance training (RT) remain to be fully elucidated. We have proposed the importance of capillarization in skeletal muscle for the satellite cell (SC) response to RT-induced muscle hypertrophy, and hypothesized that aerobic conditioning (AC) would augment RT-induced adaptations. Fourteen healthy young (22 ± 2 years) men and women underwent AC via 6 weeks of unilateral cycling followed by 10 weeks of bilateral RT to investigate how AC alters SC content, activity, and muscle hypertrophy following RT. Muscle biopsies were taken at baseline (unilateral), post AC (bilateral), and post RT (bilateral) in the aerobically conditioned (AC + RT) and unconditioned (RT) legs. Immunofluorescence was used to determine muscle capillarization, fiber size, SC content, and activity. Type I and type II fiber cross-sectional area (CSA) increased following RT, and when legs were analyzed independently, AC + RT increased type I, type II, and mixed-fiber CSA, where the RT leg tended to increase type II ($p = .05$), but not type I or mixed-fiber CSA. SC content, activation, and differentiation increased with RT, where type I total and quiescent SC content was greater in AC + RT compared to the RT leg. Those with the greatest capillary-to-fiber perimeter exchange index before RT had the greatest change in CSA following RT and a significant relationship was observed between type II fiber capillarization and the change in type II-fiber CSA with RT ($r = 0.35$). This study demonstrates that AC prior to RT can augment RT-induced muscle adaptions and that these differences are associated with increases in capillarization.

Abbreviations: AC, aerobic conditioning; ANOVA, analysis of variance; AT, aerobic training; CSA, cross-sectional area; C/Fi, capillary to fibre ratio; CFPE, capillary to fibre perimeter exchange ratio; DXA, dual-energy x-ray absorptiometry; FFM, fat-free mass; MICT, moderate-intensity continuous training; MyoD, myoblast determination protein 1; MHCI/MHCII, myosin heavy chain I/II; OCT, optimal cutting temperature; PBS, phosphate-buffered saline; PBST, phosphate-buffered saline with Tween-20®; PFA, paraformaldehyde; Pax7, paired-box 7; RT, resistance training; rpm, revolutions per minute; SC, satellite cells; VEGF, vascular endothelial growth factor; W, watts; WGA, wheat germ agglutinin; 1RM, 1-repetition maximum.

1 | INTRODUCTION

Resistance exercise training (RT) can increase skeletal muscle mass (hypertrophy) and strength in humans. However, there is a high degree of heterogeneity in the adaptive response to RT, where up to 20% of people are deemed as low- or non-responders from a hypertrophy or strength perspective.[1] While RT typically leads to improved insulin sensitivity, reduced blood pressure, and increased muscle mass, "low-responders" appear to exhibit an impaired capacity to adapt throughout an RT protocol. Previously, the variability of the hypertrophic response to RT has been attributed to cellular and molecular regulation of mechanisms involving the muscle transcriptome,[2,3] microRNA expression,[4] and activation of signaling proteins involved in muscle protein synthesis.[5] Recently, it has been demonstrated that older adults with higher relative skeletal muscle capillarization before RT showed greater myofiber hypertrophy following 24 weeks of RT than those with lower, indicating the potential impact of muscle capillarization on supporting RT outcomes; however, this association has yet to be characterized in younger individuals.[6]

Satellite cells (SC) are skeletal muscle stem cells that play an essential role in skeletal muscle maintenance, repair, and growth.[7-9] Typically residing in a quiescent state, SC activates in response to a stimulus or for basal muscle maintenance.[10] Upon activation, SC proliferate, and either return to quiescence to replenish the SC pool or differentiate and fuse to existing myofibres.[11,12] The SC pool increases in both number and activity following RT, where myonuclei are donated to support transcriptional activity within muscle fibers.[13,14] In fact, SC pool expansion has been directly linked to the degree of muscle hypertrophy following RT and are therefore thought to be significant contributors to the muscle hypertrophic response.[15] We recently showed that muscle capillary content might be a significant regulator of SC activation and proliferation in support of muscle hypertrophy following RT in young men.[16] Individuals with greater skeletal muscle capillarization had an augmented SC response to myofibrillar damage and activated SC that was closer to capillaries than quiescent SC.[17,18] It is hypothesized that SC situated closer to capillaries are exposed to higher concentrations of blood-borne signaling factors (such as hormones, growth factors, and cytokines) that regulate the SC response to stimuli.[17,19,20]

Aerobic training (AT) has been shown to augment skeletal muscle capillary density in humans.[21,22] In addition to increasing capillarization, AT influences SC function. Both moderate-intensity interval training and sprint interval training increase activated and differentiating SC in remodeling fibers following a non-hypertrophic stimulus, indicating SC contribution to adaptation and remodeling with AT.[21,22] Although findings are inconsistent as to whether AT increases SC pool content, AT alters SC function, and may potentially prime SC to respond better when presented with future adaptive stimuli.

To increase skeletal muscle capillarization prior to RT, we utilized a single-leg AT model to aerobically condition (AC) one leg with the intent to increase capillarization and followed that with a bilateral RT program. This model allows for a robust within-subject comparison of two separate conditions, eliminates between-subject variability in confounding variables, such as diet, and potentiates a greater physiological adaptative response by training one limb instead of two.[23] Histological, biochemical, and functional outcomes are similar between legs at baseline when randomized based on dominance.[24,25] Although there exists a potential systemic cross-over effect of the exercised limb to the sedentary control, it is unlikely that myokines and cytokines released from the skeletal muscle of a single exercising limb would be of sufficient concentration in systemic circulation to cause a meaningful difference in the non-trained limbs.[26–28]

The purpose of this study was to examine the impact of AC on muscle hypertrophy and SC content and activation following RT. We hypothesized that AC (to increase capillarization) prior to RT would augment muscle hypertrophy and SC content and activation following RT compared to RT alone.

2 | METHODS

2.1 | Participants

Fourteen healthy young male ($n = 8$) and female ($n = 6$) participants were recruited to participate in this study (baseline characteristics highlighted in Table 1). All participants were recreationally active with no formal weight training experience in the previous 6 months. Exclusion criteria included smoking, diabetes, the use of non-steroidal anti-inflammatory drugs or statins, and a history of respiratory disease and/or any major orthopedic disability. Participants were informed about the nature and risks of the experimental procedures before their written consent was obtained. The study was approved by the Hamilton Health Sciences Integrated Research Ethics Board (HiREB Number; 3885) and conformed to the guidelines outlined

TABLE 1 Overview of baseline participant characteristics

Characteristic	Overall ($n = 14$)	Males ($n = 8$)	Females ($n = 6$)
Age (years)	21.1 ± 1.6	21.0 ± 1.7	21.0 ± 1.5
Height (cm)	169.8 ± 8.6	175.4 ± 4.3*	162.4 ± 7.2
Body mass (kg)	74.1 ± 17.6	84.2 ± 15.5*	60.6 ± 9.4
BMI ($kg \cdot m^{-2}$)	25.4 ± 4.5	27.3 ± 4.8	22.9 ± 2.2
VO_2 work peak (W)	271.6 ± 66.5	318.8 ± 40.3*	208.8 ± 35.6
VO_2 relative ($ml \cdot min^{-1} \cdot kg^{-1}$)	39.1 ± 7.1	42.3 ± 6.9	34.8 ± 4.6

Note: Values are means ± SD.

*$p < .05$ significant effect between group (M vs. F).

in the Declaration of Helsinki. Participants gave their informed written consent before their inclusion in the study.

2.2 | Bilateral VO_2 peak

To assess eligibility to participate in the study, participants performed a standard (bilateral) incremental ramp test to volitional exhaustion on an electronically braked cycle ergometer (Excalibur Sport, version 2.0; Lode, Groningen, The Netherlands) to determine whole-body peak oxygen uptake (VO_2 peak) and peak power output (Watts). Following a 1-min warm-up at 50 watts (W), workload was increased by 1 W every 2 s until the participant reached volitional exhaustion, defined by cycling cadence dropping below 60 revolutions per minute (rpm). Expired gases were analyzed using an online gas collection system (Moxus modular oxygen uptake system; AEI Technologies, Pittsburgh, PA, USA) and VO_2 peak was determined from the greatest 30 s average of VO_2 peak. Participants were deemed as "recreationally active" if their VO_2 peak scores were less than 45 $ml \cdot min^{-1} \cdot kg^{-1}$ in men and 40 $ml \cdot min^{-1} \cdot kg^{-1}$ in women.

2.3 | Single-leg VO_2 Peak

At least 48 h following the double-leg ramp test, participants were familiarized with a single-leg cycling technique modeled after previous work.[23,25,29] One crank on an electronically braked cycle ergometer (Velotron; RacerMate, Seattle, WA, USA) was fitted with a custom-machined pedal that held an 11.4 kg counterweight. Participants pedaled using one leg, with the non-exercising leg resting on a stationary platform. The counterweight assisted with the upstroke phase of the revolution, eliminating the need to pull up on the pedal and participants performed an incremental exercise test to volitional exhaustion with each leg. Methods for the single-leg tests were similar to the double-legged tests, except the rate at which the workload increased was reduced by half (1 W every 4 s). Legs were tested in a randomized order, followed 10 min later by the contralateral leg as previous data shows that fatigue does not transfer to the non-exercised leg.[30] Participants repeated single-leg VO_2 peak testing for each leg following aerobic conditioning (post AC) and following resistance training (post RT).

2.4 | Muscle biopsy sampling

A total of five percutaneous needle biopsies were taken from the mid-portion of the *vastus lateralis* under local anesthetic (1% lidocaine [lignocaine]) using a 5-mm Bergström needle custom-adapted for manual suction.[31] One muscle biopsy was obtained prior to AC (baseline) at rest from a randomized leg, this leg was then assigned to the AC protocol. After 6 weeks of single-leg AC (post AC), biopsies were obtained from the aerobically conditioned (AC + RT) and unconditioned (RT) legs. Following 10 weeks of RT (post RT), a final biopsy was obtained from both legs. Approximately 150 mg of muscle tissue was collected from each biopsy. Following collection of the sample, the muscle was dissected free of adipose and connective tissue and flash-frozen in liquid nitrogen, then stored at −80°C for later analysis. For immunofluorescence, a fresh piece of muscle (approximately 50 mg) was dissected from the biopsies, orientated in cross-section, mounted in OCT compound (Tissue-Tek, Sakura Finetek, USA) and frozen in isopentane cooled with liquid nitrogen. The mounted samples were stored at −80°C and then sectioned (7 μm) at −20°C using a cryostat (Thermo Scientific, Microm HM 550, Walldorf, Germany). The cross-sections were mounted on slides and stored at −80°C for later immunofluorescent analysis.

2.5 | Body composition

Whole-body and regional lean soft tissue mass (i.e. fat-free and bone-free mass), fat mass, and bone mineral content were measured with the use of dual-energy X-ray absorptiometry (DXA) (GE-Lunar iDXA; Aymes Medical) after a 10–12 h overnight fast. Body composition was measured at baseline, post AC, and post RT.

2.6 | Aerobic conditioning

All AC was performed on the same cycle-ergometer adapted for single-leg cycling as that used for baseline single-leg VO_2 peak testing (Excalibur Sport, version 2.0; Lode, Groningen, The Netherlands). Random assignment (based on leg dominance) determined which leg would complete the AC protocol while the other would remain untrained. Participants completed 45-min sessions of progressive, moderate-intensity continuous training (MICT) 3 times per week for 6 weeks, as this form of AT is most effective to induce angiogenesis in skeletal muscle.[32–34] Six weeks of AC was chosen as this has been demonstrated to be a sufficient period for increasing capillary content in sedentary individuals.[32] Initial workload was determined by 50% of the average work peak (W) achieved in the participant's single-leg VO_2 peak test. Participants progressed in workload by 2–4% every 4 sessions. All training sessions included a 3-min warm-up at 25 W, followed by 40-min of MICT during which participants were instructed to maintain a cadence of approximate 80–90 rpm and concluded with a 2-min cool-down at 25 W. Heart rate and rating of perceived exertion were recorded during each session at the 2, 7, 40, and 44-min time points.

2.7 | Resistance exercise training

Starting approximately 2 weeks following the AC period, participants performed progressive bilateral lower body RT three times per week for 10 weeks, primarily targeting the thigh (quadriceps) muscles. On each visit, participants completed five lower body exercises: squats, leg press, leg extension, hamstring curls, and calf raises. Squats, leg press, and leg extension were performed at 70%–80% of the participant's 1-repetition max (1-RM) for three sets of 10–12 repetitions, with the last set completed to failure for each exercise. Hamstring curls and calf raises were also performed for three sets of 10–12 repetitions with the last set performed until failure at 10–12 repetitions.

2.8 | Muscle strength

Bilateral 1-RM testing was performed according to American College of Sports Medicine guidelines for exercise testing and prescription on squat, leg extension, and leg press exercises pre RT (post AC), and post RT.[35] Prior to 1-RM testing, participants warmed up with a set of submaximal repetitions. An initial load of ~75% of participant's predicted 1RM was set, and was progressively increased as necessary until they were no longer able to complete a repetition with the full range of motion. If repetition failed, the participant's were given one opportunity to repeat the repetition for the selected load. The final load that the participant was able to complete was recorded as their 1RM.

2.9 | Supplementation

Immediately following each RT session, 25 g of whey protein isolate (including 2.7 g leucine) (Ascent, Vanilla Bean) was ingested by each participant to support optimal adaptation to RT.

2.10 | Immunofluorescence

Muscle tissue cross-sections (7 μm) were prepared from unfixed OCT embedded samples, allowed to air dry for 30 min, and stored at −80°C. Samples were stained for subsequent analyses for immunofluorescent detection, and appropriate secondary antibodies were used (detailed antibody information can be found in Table 2).

TABLE 2 Details for antibodies used for immunofluorescent analyses

Antibody	Species	Source	Details	Primary	Secondary
Anti-Pax7	Mouse	DHSB	Pax7	Neat	Alexa Fluor 594 goat anti-mouse (1:500)
Anti-MyoD	Mouse	DAKO	5.8A	1:100	Biotin goat anti-mouse (1:200), streptavidin 594 (1:200)
Anti-laminin	Rabbit	Abcam	Ab11575	1:500	Alexa Fluor 488, 647 goat anti-rabbit (1:500)
Wheat-germ Agglutinin	N/A	Invitrogen	W11262	1:200	Conjugated to 594
Anti-MHCI	Mouse	DHSB	A4.951 Slow Isoform	Neat	Alexa Fluor 488 goat anti-mouse (1:500)
Anti-MHCII	Rabbit	Abcam	Ab51263	1:1000	Alexa Fluor 647 goat anti-rabbit (1:500)
Anti-CD31	Rabbit	Abcam	Ab28364	1:50	Alexa Fluor 594 goat anti-rabbit (1:500)

Abbreviations: *CD31*; Endothelial cell adhesion molecule 31; *MHCI*, Myosin heavy chain I; *MHCII*, Myosin heavy chain II; *MyoD*, Myoblast determination protein 1; *Pax7*, Paired box protein 7.

2.10.1 | Satellite cell content and muscle fibers

SC content and fiber cross-sectional area (CSA) were assessed through staining for Pax7, MHCI, and MHCII, respectively, using laminin to reference SC location. After fixing the muscle cross-sections with 4% paraformaldehyde (PFA) for 10 min, slides were washed for 5 min each in 1X phosphate-buffered saline (PBS) and 1X PBS with Tween-20® (Fischer Scientific) (PBST). Samples were blocked for 90 min at room temperature and were incubated overnight at 4°C with primary antibodies for laminin and Pax7. The next day, slides were washed three times for 5 min in PBST and were subsequently incubated with respective secondary antibodies in 1% bovine serum albumin (BSA) for 2 h at room temperature. Slides were then washed (3 × 5 min in PBST), re-fixed with 4% PFA for 10 min, washed again (two times for 5 min in PBST) and incubated overnight with MHCI and MHCII (diluted in MHCI) at 4°C. For the final day, slides were washed (three times for 5 min) in PBST, incubated with secondary antibodies in 1% BSA for 2 h at room temperature, washed again (three times for 5 min in PBST) and nuclei were labeled with 4′,6-diamidino-2-phenylindole (DAPI; 1:20000, Sigma-Aldrich, Oakville, ON, Canada) for 10 min prior to a final wash (5 min PBST, 5 min PBS), drying, and cover slipping with fluorescent mounting media (DAKO, Burlington, ON, Canada).

2.10.2 | Capillaries

Muscle capillarization was visualized through a CD31 stain to assess capillary to fiber ratio (C/Fi; capillary content) and capillary to fiber perimeter exchange ratio (CFPE). Slides were fixed, blocked, and incubated overnight at 4°C with primary antibodies for MHCI and laminin. The next day, slides were washed and subsequently incubated with respective secondary antibodies. Slides were then washed, re-fixed, washed again, and incubated overnight with CD31 diluted in 1% BSA at 4°C. For the final day, slides were washed, incubated with secondary antibodies, washed again, and nuclei were labeled with DAPI (1:20,000) prior to a final wash, drying, and cover slipping.

2.10.3 | Satellite cell activation

SC activation status was assessed by co-staining for MyoD and Pax7, with MHCII used to determine fiber-type and wheat germ agglutinin (WGA; lectin glycoprotein predominantly located on the sarcolemma)[36] to reference SC location. Quiescent SC was defined as $Pax7^{+}/MyoD^{-}$, activated as $Pax7^{+}/MyoD^{+}$, and differentiating as $Pax7^{-}/MyoD^{+}$ cells colocalized to DAPI. After fixing the muscle cross-sections, samples were blocked and then incubated with the primary antibody for MyoD overnight at 4°C. The next day, slides were washed and subsequently incubated with biotin in 1% BSA for 60 min at room temperature, washed again, and incubated with respective streptavidin antibodies for 60 min at room temperature. Slides were once again washed, re-fixed, washed, and incubated overnight with Pax7 and MHCII (diluted in Pax7) at 4°C. For the final day, slides were washed incubated with respective secondary, washed, re-fixed, washed again, and incubated with wheat germ agglutinin for 25 min. Slides were washed, labeled with DAPI (1:20 000) for 10 min, washed, dried, and cover slipped.

All staining procedures were verified using negative controls for primary (primary, no secondary), and secondary (no primary, secondary only) antibodies to ensure appropriate staining specificity. Slides were viewed with the Nikon Eclipse Ti Microscope (Nikon Instruments, Inc., USA), equipped with a high-resolution Photometrics CoolSNAP HQ2 fluorescent camera (Nikon Instruments, Melville, NY, USA). Images were captured and analyzed using the Nikon NIS Elements AR 3.2 software (Nikon Instruments, Inc., USA). All images were obtained with the 20X objective. The muscle fiber SC content was quantified by identifying the number of SC (Pax7+ colocalized with DAPI, between sarcolemma and basal lamina) per 100 myofibers (type I or type II) within the muscle sample. Total SC content was determined by identifying total Pax7+/DAPI+ cells on the perimeter of the myofibre. Quiescent SC content was determined by identifying Pax7+/MyoD-/DAPI+ cells, activated SC content determined by Pax7+/MyoD+/DAPI+ cells, and differentiating SC content determined by Pax7-/MyoD+/DAPI+ cells. Sixty muscle fibers/participant/time points were used to quantify capillary content. Based on the work from Hepple et al.,[37] we determined the capillary-to-fiber ratio (C/Fi) and the capillary-to-fiber perimeter exchange index (CFPE) as an estimate of the capillary-to-fiber surface area as a proxy measure of perfusion.[34] Myonuclear content was calculated via automated counting using Fiji ImageJ2 (Version 2.3.0), where each image was split into its respective channels and the DAPI channel was thresholded to determine the number of DAPI+ cells in a defined area of the muscle cross-section.

All immunofluorescent analyses were completed in a blinded fashion.

2.11 | Responder analyses

Individual legs (AC + RT or RT) were ranked from high to low based on the change (Δ) in SC content from post AC

to post RT, ΔCSA from post AC to post RT and CFPE at post AC (14 participants × 2 legs). High and low responders were determined using the top 10 and bottom 10 values from each measure, respectively.

2.12 | Statistical testing

Unpaired T-Tests were used to compare baseline participant characteristics between males and females. One-way repeated measures analysis of variance (ANOVA) with an effect of time was used to compare VO_2 peak, C/Fi, CFPE, and fiber-type distribution for type I and type II fibers. Upon detection of significance, pre-planned post hoc testing, involving Holm–Sidak's multiple comparison tests, were performed. Two-way repeated measures ANOVA for time (post AC and post RT) and condition (AC + RT and RT) were used to analyze fiber CSA, myonuclear content, SC content, and activation status. Similarly, this test was used to assess fat-free mass with the addition of baseline in the AC + RT and RT legs. A two-way repeated measures ANOVA with factors of fiber-type (type I or type II) and responder status (high or low) was used to assess differences between high and low responders. Prior to analysis, a Shapiro–Wilk test was performed on the data to test normality. Wilcoxon signed-rank tests were used to assess indices of muscle strength, 1-RM leg press, and squat. Lastly, Pearson's r correlation was utilized to observe the relationship between fiber-specific CFPE and ΔCSA from post AC to post RT. Analysis was conducted using GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA, USA). Statistical significance was set at $p < .05$. All data are represented as mean ± standard deviation (SD). Figures for capillary, hypertrophy, myonuclei, and SC data are presented as box and whisker plots, where the box denotes the median, 25th, and 75th percentiles, the cross represents the mean value and whiskers represent the maximum and minimum values.

3 | RESULTS

3.1 | Participant characteristics

Complete participant characteristics are reported in Table 1. Significant differences in height (175 ± 4 cm, 162 ± 7 cm), body mass (84 ± 16 kg, 61 ± 9 kg), and Work Peak (319 ± 40 W, 209 ± 36 W) were observed between males and females, respectively ($p < .05$).

3.2 | VO_2 relative to leg fat-free mass ($ml \cdot min^{-1} \cdot kgFFMLeg^{-1}$)

The RT leg did not change VO_2 peak ($ml \cdot min^{-1} \cdot kgFFMLeg^{-1}$) relative to leg fat-free mass from baseline (261.4 ± 37.8) to post AC (265.6 ± 42.1; $p > .05$) or post RT (246.5 ± 47.2; $p > .05$) (Figure 1A). The AC + RT leg increased VO_2 peak relative to leg fat-free mass from baseline (256.5 ± 37.6) to post AC (285.4 ± 47.2; $p < .05$) and returned to baseline post RT (260.2 ± 45.4; $p > .05$). VO_2 peak relative to leg fat-free mass was greater in the AC + RT leg compared to the RT leg at both post AC and post RT ($p < .05$).

3.3 | VO_2 work peak (W_{peak})

The RT leg did not change VO_2 work peak (W) from baseline (144.7 ± 36.0) to post AC (148.3 ± 34.4; $p > .05$) or post RT (150.4 ± 31.4; $p > .05$) (Figure 1B). The AC + RT leg increased work peak from baseline (148.1 ± 40.0) to post AC (166.9 ± 35.1; $p < .01$) and returned to baseline at post RT (161.1 ± 31.6; $p > .05$). Work peak was greater in

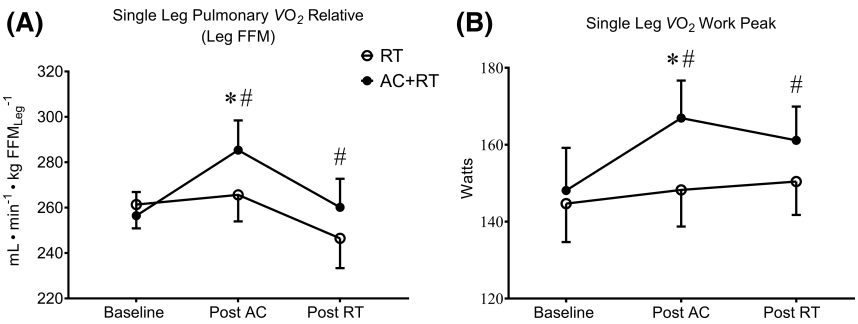

FIGURE 1 Single leg VO_2 peak test outcomes before aerobic conditioning (baseline), after aerobic conditioning (post AC) and after resistance training (post RT) in the unconditioned (RT; white circles) and aerobically conditioned (AC + RT; black circles) legs. (A) Single leg pulmonary VO_2 relative to respective leg fat-free mass. (B) Single leg VO_2 work peak. Data are expressed as means ± SD. *$p < .05$, difference from baseline. #$p < .05$, group difference (RT vs. AC + RT).

the AC + RT leg compared to the RT leg at both post AC ($p < .001$) and post RT ($p < .05$).

3.4 | C/Fi

Type I fiber C/Fi increased from baseline (1.3 ± 0.2) to post AC in both the AC + RT (1.8 ± 0.4; $p < .001$) and RT (1.6 ± 0.4; $p < .05$) legs (Figure 2A). No differences in type I fiber C/Fi was observed between the AC + RT and RT legs at post AC ($p > .05$). Type II fiber C/Fi also increased from baseline (1.2 ± 0.3) to post AC in the AC + RT (1.7 ± 0.2; $p < .001$) and RT (1.6 ± 0.5; $p < .05$) legs (Figure 2B). No differences in type II fiber C/Fi were observed between the AC + RT and RT legs at post AC ($p > .05$).

3.5 | CFPE

Type I fiber CFPE (capillaries · 1000 μm^{-1}) increased from baseline (4.9 ± 0.8) to post AC in the AC + RT leg (7.0 ± 1.2; $p < .0001$) but not the RT leg (5.1 ± 1.1; $p > .05$) (Figure 2C). In addition, type I fiber CFPE of the AC + RT leg was greater relative to the RT leg at post AC ($p < .01$). Type II fiber CFPE also increased from baseline (4.6 ± 1.0) to post AC in the AC + RT leg (5.6 ± 1.2; $p < .05$) but not the RT leg (4.8 ± 1.1; $p > .05$) (Figure 2D).

3.6 | CSA

Muscle fiber CSA (μm^2) increased from post AC to post RT (effect of time) in type I fibers (5149.9 ± 1265.5 vs. 5694.6 ± 1349.6; $p < .05$) (Figure 3A) and type II fibers (5312.6 ± 1677.2 vs. 6723.7 ± 2486.7; $p < .01$) (Figure 3B). When the AC + RT and RT legs were analyzed separately, the AC + RT leg increased type I (5150.3 ± 1429.3 vs. 6147.7 ± 1471.0; $p < .05$), type II (5049.8 ± 1619.4 vs. 7091.6 ± 2910.9; $p < .05$) and mixed fiber (5100.0 ± 1425.5 vs. 6619.7 ± 2100.1; $p < .05$) CSA from post AC to post RT, where the RT leg tended to increase type II fiber CSA (5575.4 ± 169 2.8 ± 6355.8 ± 1872.6; $p = .05$) only (Supplementary 1).

3.7 | Fiber-type Proportion

No differences in type I (Figure 3E) or type II (Figure 3F) fiber-type proportion (%) were observed at any timepoint in the RT or the AC + RT legs ($p > .05$). The RT leg had similar fiber-type proportions at post AC (*type I* 37 ± 11, *type II* 63 ± 11) and post RT (*type I* 41 ± 10, *type II* 59 ± 10),

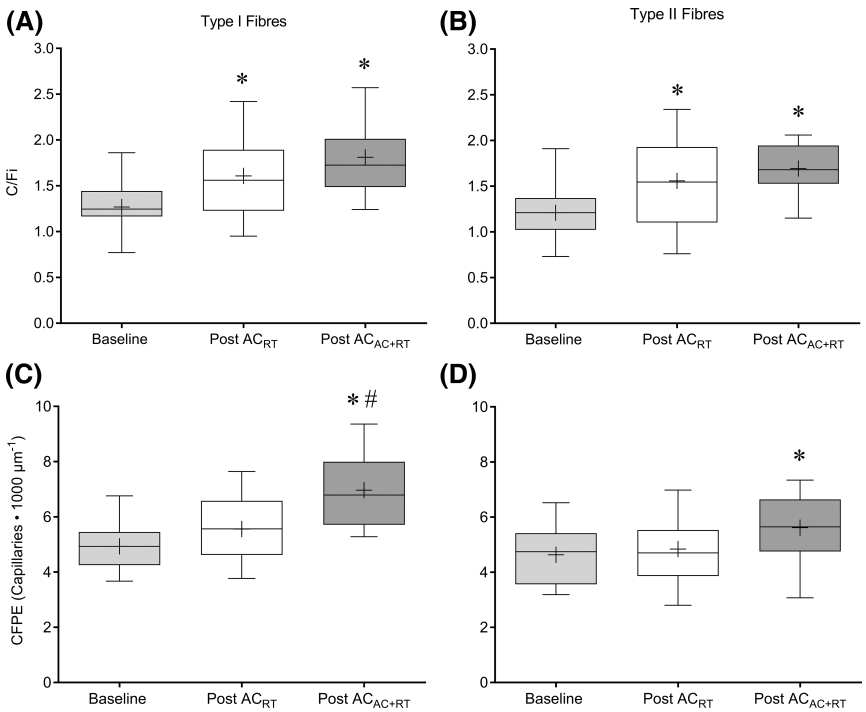

FIGURE 2 Fiber type-specific capillary measures before (baseline) and after aerobic conditioning (post AC) in the unconditioned leg (RT) and the aerobically conditioned leg (AC + RT). (A and B) C/Fi specific to type I (A) and type II (B) muscle fibers. (C and D) CFPE specific to type I (C) and type II (D) muscle fibers. Data are expressed as box and whisker plots, where + represents the mean, horizontal lines represent the first quartile (bottom), median (middle), and third quartile (top), and error bars represent the minimum (bottom) and maximum (top) values. *$p < .05$, difference from baseline. #$p < .05$, group difference (RT vs. AC + RT).

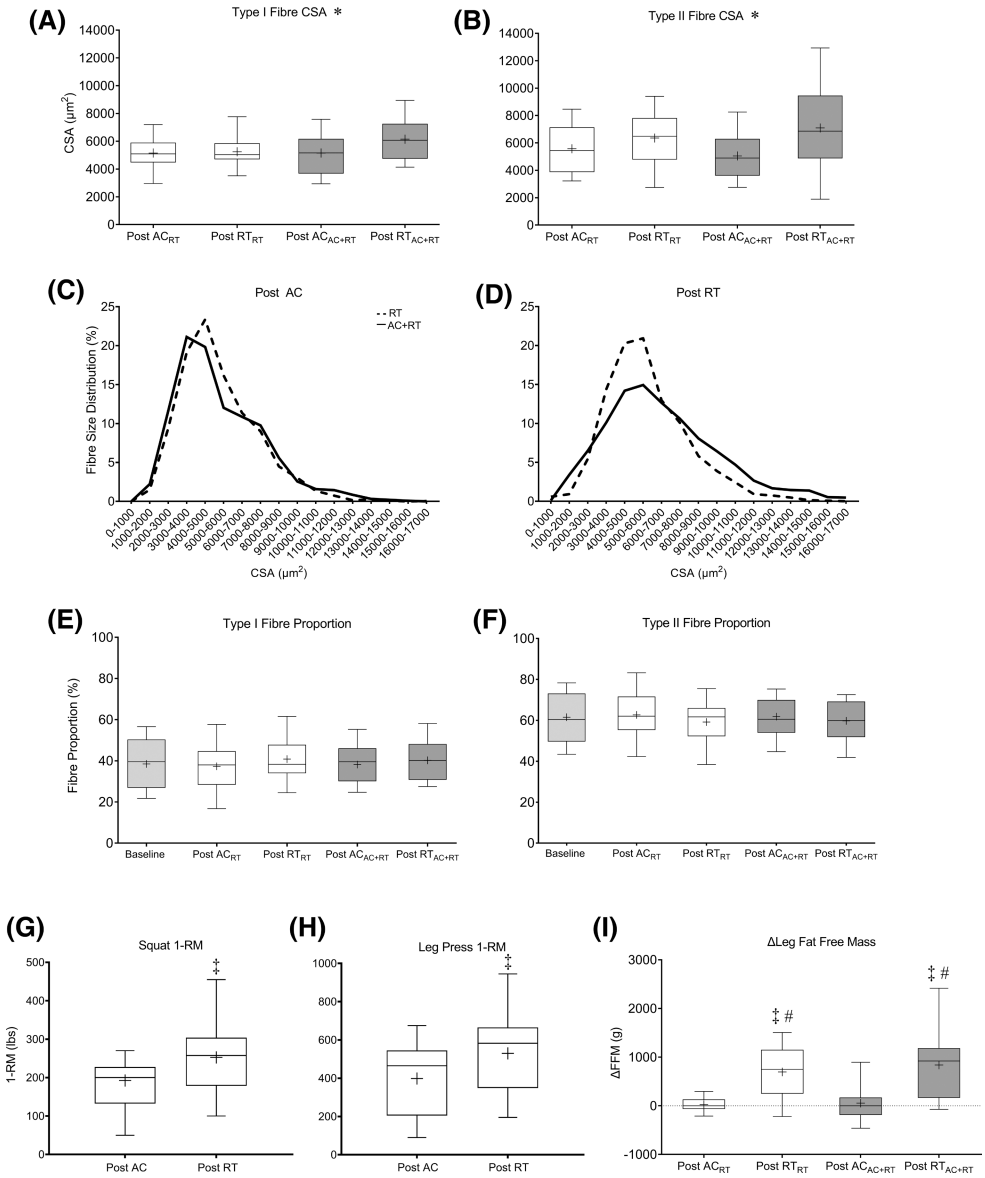

FIGURE 3 Muscle fiber type-specific cross-sectional area, fiber size distribution, fiber-type proportion, strength measurements, and leg fat-free mass. (A and B) Cross-sectional area of type I (A) and type II (B) muscle fibers before (post AC) and after resistance training (post RT) in the unconditioned (RT) and aerobically conditioned (AC + RT) legs. (C and D) Muscle fiber size distribution at post AC (C), and post RT (D) in the RT and AC + RT legs. (E and F) Fiber type proportion of type I (F) and type II (G) muscle fibers post AC and post RT in RT leg, baseline, post AC, and post RT in AC + RT leg. (G and H) Double leg, 1-repetition max strength measurements for squat (G) and leg-press (H) post AC and post RT. (I) Leg-specific fat-free mass at baseline, post AC, and post RT in RT and AC + RT legs. Data are expressed as box and whisker plots, where + represents the mean, horizontal lines represent the first quartile (bottom), median (middle), and third quartile (top), and error bars represent the minimum (bottom) and maximum (top) values. *$p < .05$, effect of time (post AC vs. post RT). ‡$p < .05$, difference from post AC. #$p < .05$, difference from baseline.

as did the AC + RT leg at baseline (*type I* 38 ± 11, *type II* 62 ± 11), post AC (*type I* 38 ± 9, *type II* 62 ± 9) and post RT (*type I* 40 ± 9, *type II* 60 ± 9) ($p > .05$).

3.8 | Strength

Bilateral 1-RM (lbs) strength measures for squat (192 ± 84 vs. 252 ± 85; $p < .0001$) and leg press (384 ± 179 vs. 523 ± 209; $p < .0001$) increased from post AC to post RT (Figure 3G,H).

3.9 | Leg fat-free mass

Leg fat-free mass (g) increased post RT (9901 ± 2495) relative to both baseline (9135 ± 2420; $p < .001$) and post AC (9174 ± 2427; $p < .001$) (Figure 3I).

3.10 | SC content

SC content (Pax7+ cells/100 fibers) increased from post AC to post RT (effect of time) in type I (6±3 vs. 8±5; $p<.05$) (Figure 4E) and type II (4±2 vs. 8±4; $p<.001$) (Figure 4F) fibers. Type I fiber-associated SC content was also greater in the AC+RT leg (8±5) compared to the RT leg (5±2; $p<.05$).

3.11 | Myonuclear content

Myonuclear content (myonuclei/1000 μm^2) tended to increase from post AC (0.7±0.2) to post RT (0.9±0.3; $p = .06$) (effect of time) and was greater in the AC+RT leg (0.9±0.3) compared to the RT leg (0.8±0.1; $p<.05$) (Figure 4G).

3.12 | Correlations

Type II CFPE at post AC was significantly and positively correlated to the change in type II fiber CSA with RT ($r = 0.35$, $p<.05$) (Figure 5A). In addition, the change in SC content from post AC to post RT was significantly and positively correlated to the change in type II fiber CSA from post AC to post RT ($r = 0.33$, $p<.05$) (Figure 5B).

3.13 | SC activation

More quiescent SC (*Pax7+/MyoD-*; cells/100 fibers) specific to type I fibers were observed in the AC+RT leg (6.2±2.8) compared to the RT leg (4.5±2.1; $p<.01$) (Figure 6G), where quiescent SC in type II fibers tended to increase from post AC (4.3±2.5) to post RT (5.8±3.5; $p = .09$) (effect of time; Figure 6J). Activated SC (*Pax7+/MyoD+*; cells/100 fibers) content increased from post AC to post RT in type I (0.7±0.9 vs. 2.5±1.8; $p<.001$) (Figure 6H) and type II (0.5±0.8 vs. 1.5±0.9; $p<.01$) (Figure 6K) fibers. Additionally, differentiating SC (*Pax7-/MyoD+*; cells/100 fibers) content increased from post AC to post RT in type II fibers (0.3±0.4 vs. 0.9±0.6; $p<.0001$) (Figure 6L) and tended to increase in type I fibers (0.5±1.4 vs. 1.0±0.7; $p = .08$) (Figure 6I).

3.14 | High vs. low responder to AT and RT outcomes

Individual leg responses to AC and RT were stratified to examine the association between "high" and "low" responders and CSA, SC content, and CFPE. Responders were categorized by the highest ($n = 10$) and lowest values ($n = 10$) for each variable. High responders of type

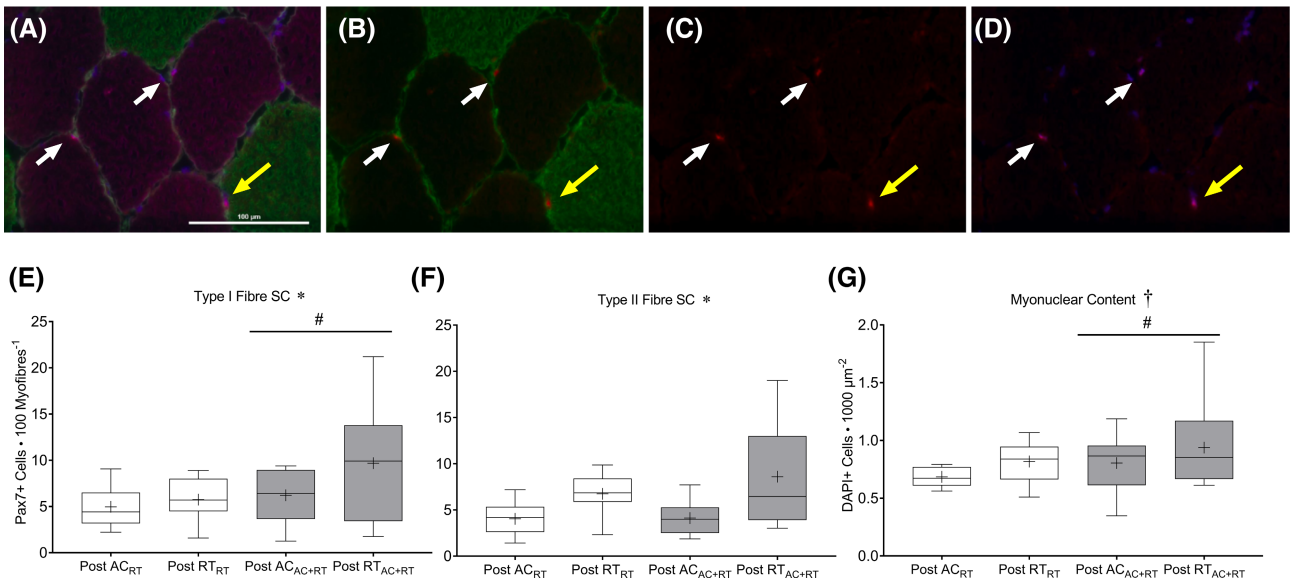

FIGURE 4 Fiber type-specific satellite cell content and myonuclear content per area. (A) Representative image of an immunofluorescent stain containing MHCI (green), laminin (green), MHCII (pink), Pax7 (red), and DAPI (blue) on a muscle cross section. (B–D) Channel views of MHCI/Laminin/Pax7 (B), Pax7 (C), and Pax7/DAPI (D). Scale bars = 100 µm. Type I satellite cell is denoted by the yellow arrow and type II satellite cells by the white arrows. (E and F) Characterization of satellite cell pool expansion specific to type 1 (E) and type II (F) fibers, and myonuclear content per area (G) post aerobic conditioning (post AC) and post resistance training (post RT) in the unconditioned (RT) and aerobically conditioned (AC+RT) legs. Data are expressed as box and whisker plots, where + represents the mean, horizontal lines represent the first quartile (bottom), median (middle), and third quartile (top), and error bars represent the minimum (bottom) and maximum (top) values. *$p<.05$, effect of time (post AC vs. post RT). †$p = .07$, effect of time. #$p<.05$, group difference (RT vs. AC+RT).

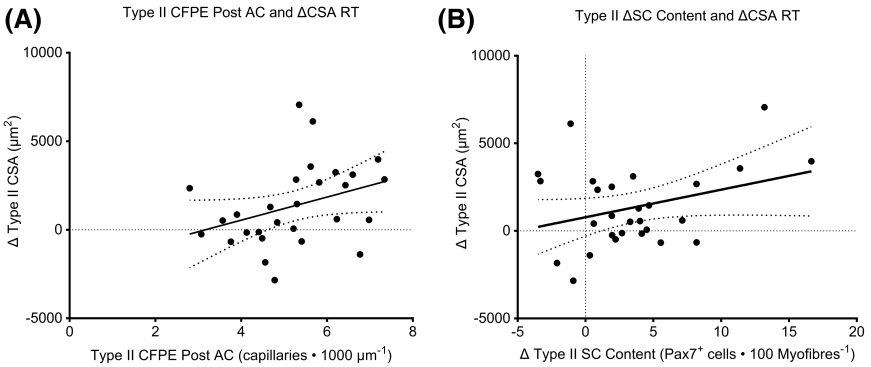

FIGURE 5 Muscle size, satellite cell, and capillarization correlations. (A) Relationship between type II muscle fiber-specific capillary to fiber perimeter exchange ratio post aerobic conditioning (post AC) and the change in cross-sectional area with resistance training in both the unconditioned (RT) and aerobically conditioned (AC + RT) legs ($r = 0.35$). (B) Relationship between type II muscle fiber-specific satellite cell pool expansion with resistance training and the change in cross-sectional area with resistance training in both the RT and AC + RT legs ($r = 0.33$).

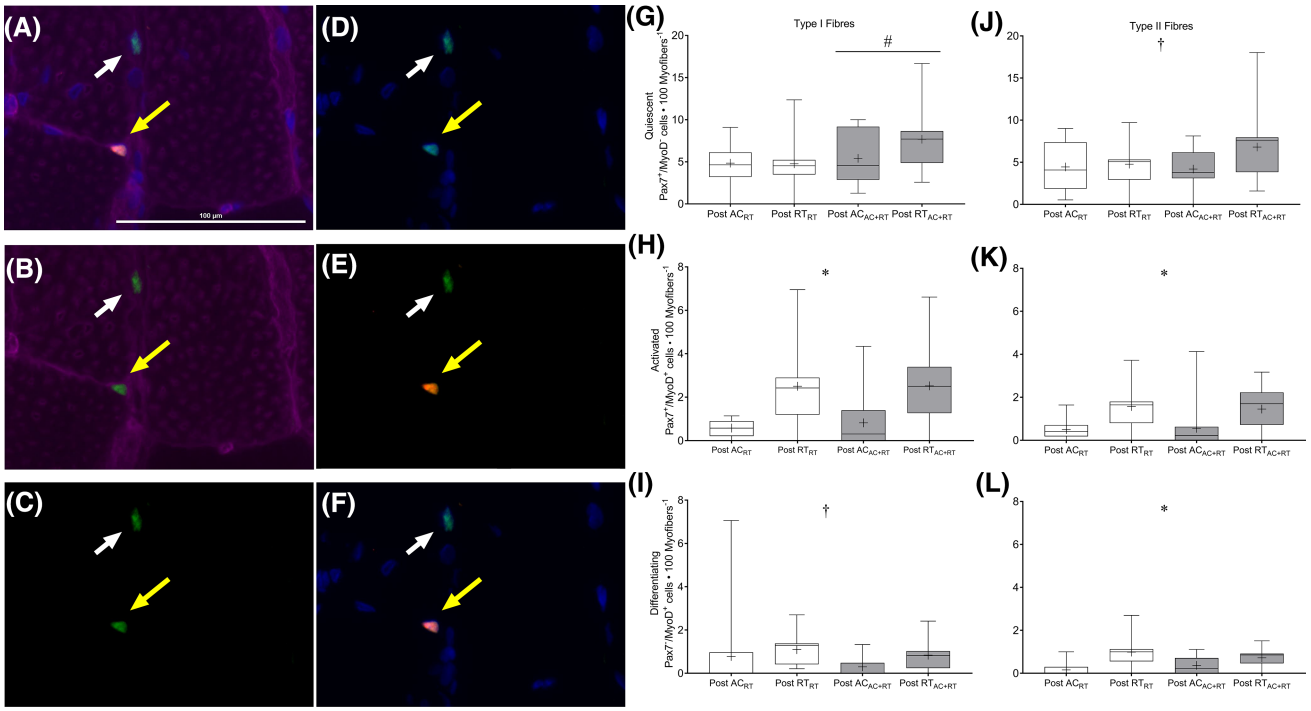

FIGURE 6 Fiber type-specific satellite cell activation status post aerobic conditioning (post AC) and post resistance training (post RT) in the unconditioned (RT) and aerobically conditioned (AC + RT) legs. (A) Representative image of an immunofluorescent stain containing Pax7 (red), MyoD (green), MHCII (pink), WGA (pink), and DAPI (blue) on a muscle cross section. (B–F) Channel views of MyoD/MHCII (B), MyoD (C), MyoD/DAPI (D), MyoD/Pax7 (E), MyoD/Pax7/DAPI (F) satellite cells. Scale bar = 100 μm. The $Pax7^{-}/MyoD^{+}$ cell is denoted by the white arrows and the $Pax7^{+}/MyoD^{+}$ cell by the yellow arrows. (G–L) Characterization of satellite cell activation status post AC and post RT in the unconditioned (RT) and aerobically conditioned (AC + RT) legs specific to type I (G–I) and type II (J–L) fibers for quiescent (G and J), activated (H and K), and differentiating (I and L) satellite cells. $^{*}p < .05$, effect of time (post AC vs. post RT). $^{\dagger}.05 < p < .10$, effect of time. $^{\#}p < .05$, group difference (RT vs. AC + RT).

I SC content (ΔSC content from post AC to post RT) exhibited a greater change in type I fiber CSA (μm^2) from post AC to post RT compared to their low responder counterparts (*high* 1099 ± 1183 vs. *low* 38 ± 661; $p = .06$) (Figure 7A). High responders of type I and type II fiber CSA (ΔCSA-RT) had greater type I (*high* 6.99 ± 1.39 vs. *low* 5.93 ± 1.01; $p = .06$) and type II (*high*; 6.15 ± 0.69 vs. *low*; 4.69 ± 0.95; $p < .05$) fiber CFPE (capillaries · 1000 μm^{-1}) at post AC compared to their low responder counterparts (Figure 7B). Additionally, individuals identified to have "high" CFPE post AC also had a greater change in both type I (*high*; 1047 ± 1741 vs. *low*; −23 ± 807; $p < .05$) and

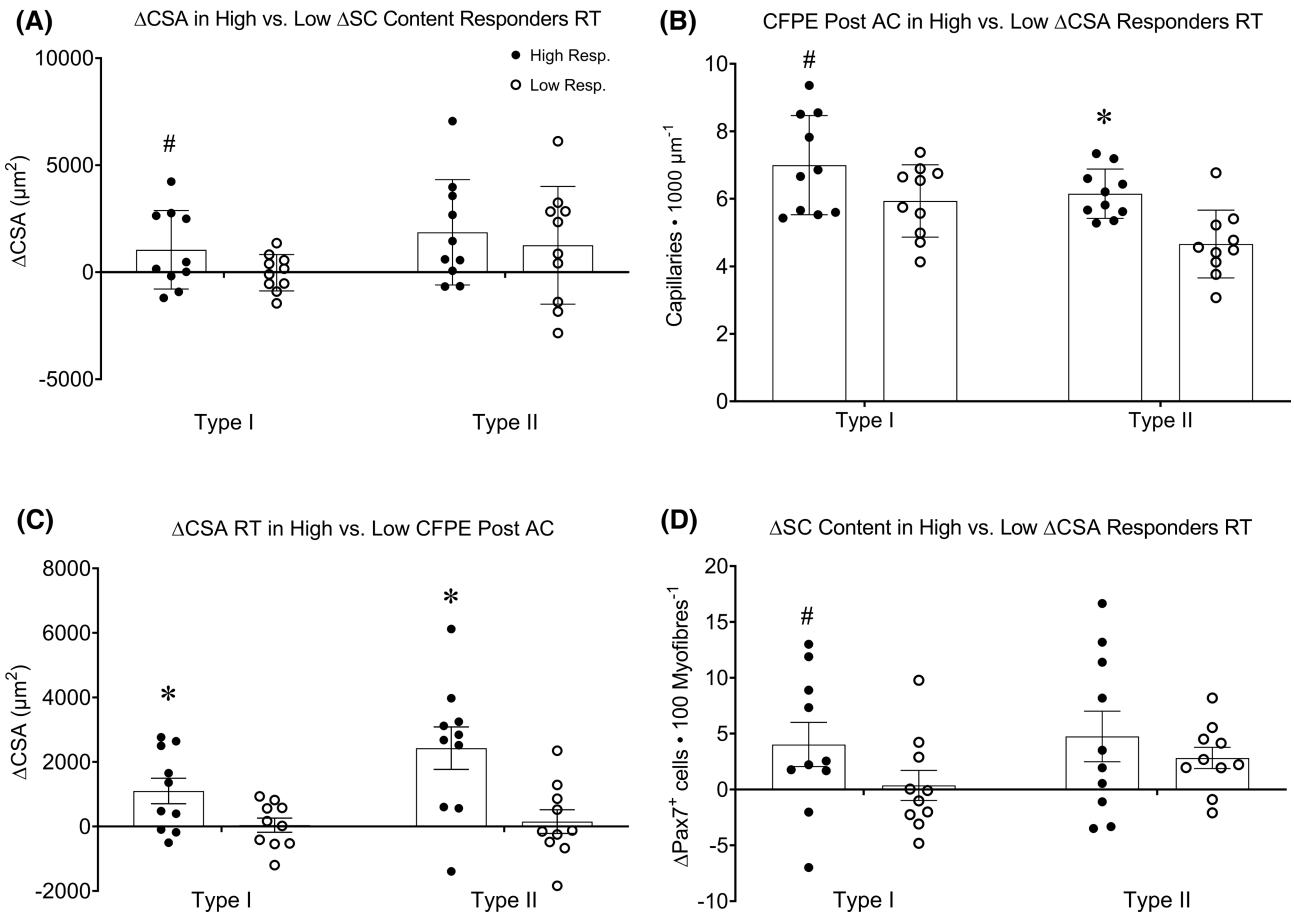

FIGURE 7 High vs. low responder analyses of (A) Change in CSA (post AC to post RT) of type I and II fibers sorted based on the change in SC content (post AC to post RT). (B) CFPE of type I and II fibers at the post AC sorted based on the change in CSA (post AC to post RT). (C) Change in CSA (post AC to post RT) of type I and II fibers sorted based on CFPE at post AC. (D) Change in SC content (post AC to post RT) of type I and II fibers sorted based on the change in CSA (post AC to post RT). *$p < .05$, group difference (high vs. low responders). # $p = .06$, group difference.

type II (*high*; 2425 ± 1970 vs. *low*; 149 ± 401; $p < .01$) fiber CSA (μm^2) from post AC to post RT (Figure 7C). Lastly, high type I fiber CSA responders (ΔType I CSA from post AC to post RT) exhibited a greater change in type I SC content from post AC to post RT (*high*; 4.02 ± 5.92 vs. *low*; 0.36 ± 4.04 Pax7+ cells/100 fibers; $p = .06$) (Figure 7D).

4 | DISCUSSION

In the current investigation, we observed that AC increased capillarization in the AC + RT leg and augmented CSA following RT. Specifically, we show a preferential elevation of capillarization relative to fiber size in the AC + RT leg. Subsequently, when RT was performed, fiber CSA was augmented in the AC + RT leg compared to the RT leg. Furthermore, SC pool expansion following RT was augmented in the AC + RT compared to the RT leg. Our data support our hypothesis that AC augments RT-induced skeletal muscle adaptations, and have further supported our previous thesis that muscle capillarization may be a critical factor in supporting these hypertrophic adaptations. Individuals with a greater capacity for muscle perfusion appear more likely to experience significant RT-induced hypertrophy. Collectively, these results may have implications for the development of training programs or therapeutic strategies for maximizing muscle growth with RT.

In the present study, our 6-week unilateral AC was sufficient to increase aerobic capacity specific to the AC + RT leg and remained elevated compared to the RT leg following 10 weeks of bilateral RT. Our AC protocol also increased outcomes related to muscle capillarization with elevated type I (+20%) and type II (+21%) fiber CFPE in the AC + RT leg following AC, where type I CFPE was greater in the AC + RT leg compared to the RT leg following AC. The increased measures of muscle capillarization are consistent with previous literature which have reported 10–30% increases in untrained individuals following 6–8 weeks of moderate-intensity AT.[32,34] Unexpectedly,

we observed an increase in C/Fi in the RT leg (+27%) following AC which may have resulted due to a systemic increase of pro-angiogenic factors, such as VEGF, generated from the AC + RT leg during AC or perhaps an unforeseen elevation in blood flow to the RT leg during AC could have elevated local signaling factors.[32,38] Indeed, rises in circulating VEGF protein have been observed 1 h following moderate-intensity aerobic exercise.[39,40] Furthermore, increases in endurance capacity and peak blood flow of a control contralateral limb has been observed following 5 weeks of unilateral upper-body arm ergometry further highlighting the potential cross-education effects between the exercised and control limbs.[28] Although an increase C/Fi was observed in both the AC + RT and RT legs following unilateral AC, when made relative to their respective fiber perimeter to assess muscle perfusion (CFPE), the difference between the AC + RT and RT legs became more apparent. Therefore, CFPE is likely a more representative measure of capillarization compared to C/Fi and has been more closely linked to both aerobic measures and other measures of vascular perfusion.[37]

RT is well-known to cause increases in skeletal muscle strength and hypertrophy.[15,41,42] In the present study, 10 weeks of bilateral lower-body RT-increased fiber CSA, leg fat-free mass, and strength. Our lab and others[16,18,43] have previously shown that skeletal muscle capillarization associated with an elevated aerobic capacity may be a contributing factor to influence muscle mass maintenance and growth, as it is essential for the delivery of oxygen, nutrients, and growth factors to the muscle, stimulating muscle protein synthesis, and turnover of damaged proteins.[16,18,43] To corroborate these findings, we demonstrate that the AC + RT leg (which had elevated muscle capillarization) augmented muscle hypertrophy to a greater extent than the RT leg when analyzed independently, which brought forth differences in comparisons that the original statistical methods were not powerful enough to detect. Consistent with previous work, the degree of muscle capillarization appeared to have an underlying influence on fiber size and potentially skeletal muscle's ability to respond to an RT stimulus.[16,18] The mechanism by which AC augments muscle accretion following RT is unknown, and while we acknowledge that AC induces a number of skeletal muscle adaptions,[44] we chose capillarization as one of the important contributors to the SC response and muscle hypertrophy, although other AC-induced adaptations may also play important roles. We propose that altered capillary perfusion, in addition to enhanced regulation of the SC pool may play an influential role on muscle's ability to adapt and cope with the stress associated with chronic RT.

To further characterize the impact of capillary perfusion on accretion of muscle mass following RT, individual leg data were split into high or low responder groups based on fiber type-specific CFPE following AC. "High" CFPE following AC was associated with greater type I and type II fiber hypertrophy following RT. Conversely, participants with greater type I and type II fiber hypertrophy following RT had greater CFPE following AC. Together, these data further demonstrate the proposed importance of capillary perfusion to support muscle hypertrophy.

SCs are well-known for their role in myofibre maintenance and repair, and increase in content and activity following hypertrophy.[15,45–48] However, the influence of AC on the SC pool in humans is not fully elucidated. In the present study, no change in type I or type II SC content was observed in the AC + RT or RT legs following AC, which is consistent with recent work from our lab.[22] Interestingly, while SC content increased in both type I and type II fibers following RT, type I fiber SC content was greater in the AC + RT leg compared to the RT leg. This type I-specific SC pool expansion may be the result of altered microvascular perfusion following AC. Greater perfusion of type I fibers in AC + RT compared to the RT leg indicates a greater potential for blood flow and signaling delivery to surrounding SC and may help explain why type I fiber CSA increased in the AC + RT but not the RT leg following RT.

Consistent with previous work, we observed a significant, positive relationship between the change in type II SC content and muscle hypertrophy following RT.[14,15] When individual leg data were split into high and low changes in SC content across the RT period, those with the greatest type I SC expansion following RT had greater type I fiber hypertrophy compared to the low type I SC expansion group. Conversely, those with the greatest type I fiber hypertrophy observed the greatest increases in type I SC content. Taken together these data support the notion that SC content is important to myofiber hypertrophy.[15,16]

The current investigation reported no differences in basal (resting) SC activity status following AC. Joanisse and colleagues[21,22] reported more activated and differentiating SC in response to 6 weeks of AT. The disparate results between the present study and Joanisse et al.[21] findings may be attributed to participant demographic studied and/or the study design, where the authors examined a subgroup of obese women and utilized sprint interval training as the training modality. Joanisse et al.[22] demonstrated an increase in the number of activated and differentiating SC following moderate-intensity AT in young individuals, contradicting what was observed in the present study. It is important to note that differences in activation status were compared between legs following AC and baseline values were not considered. Therefore, an increase in SC activation could have occurred that may have been washed out in the contralateral leg due to systemic factors "priming" the SC pool to activate.[49] It is also

possible that a higher intensity of AC may evoke an SC response similar to resistance exercise as high-intensity interval training has been demonstrated to be nearly as effective as resistance exercise for increasing SC activation.[50] We did observe an increase in type I and type II SC activation and differentiation was observed following RT, with no differences between legs. It is likely that the increase in SC activation and differentiation is due to the remodeling of muscle fibers in response to the exercise stimulus.[21,22]

It is generally accepted that SC contributes to muscle hypertrophy induced via RT. Enhancing microvascular perfusion may alter SC dynamics and support their response to exercise-induced adaptations. Here we demonstrate that AC can augment skeletal muscle hypertrophy following RT, likely attributed (in part) to elevated microvascular perfusion and enhanced SC regulation.

AUTHOR CONTRIBUTIONS

Aaron C. Q. Thomas and Gianni Parise conceptualized the study design. Aaron C. Q. Thomas, Alex Brown, Aidan A. Hatt, Katherine Manta, and Anamaria Costa-Parke supervised exercise training and performed exercise testing. Aaron C. Q. Thomas, Chris McGlory, Stuart M. Phillips, and Dinesh Kumbhare collected tissue. Aaron C. Q. Thomas, Alex Brown, Aidan A. Hatt, Katherine Manta, Anamaria Costa-Parke, and Michael Kamal analyzed data. Aaron C. Q. Thomas, Alex Brown, Sophie Joanisse, and Gianni Parise interpreted results. Aaron C. Q. Thomas and Alex Brown prepared figures. Aaron C. Q. Thomas, Alex Brown, and Aidan A. Hatt drafted the manuscript. Aaron C. Q. Thomas, Alex Brown, Aidan A. Hatt, Katherine Manta, Anamaria Costa-Parke, Michael Kamal, Sophie Joanisse, Chris McGlory, Stuart M. Phillips, Dinesh Kumbhare, and Gianni Parise edited and revised manuscript and approved for submission.

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DISCLOSURES

Dr. Phillips reports grants from US National Dairy Council, Dairy Farmers of Canada, Roquette Freres, National Science, and Engineering Research Council, Canadian Institutes for Health Research during the conduct of the study; personal fees from US National Dairy Council, non-financial support from Enhanced Recovery, outside the submitted work; In addition, Dr. Phillips has a patent Canadian 3052324 issued to Exerkine, and a patent US 20200230197 pending to Exerkine but reports no financial gains.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article. Raw values for the data presented in this study can be found in Supplementary 2.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.