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Title of article

Altered muscle satellite cell activation following 16 weeks of resistance training in young men

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ABSTRACT

Skeletal muscle satellite cells (SC) play an important role in muscle adaptation. In untrained individuals, SC content and activation status has been observed to increase in response to a single bout of exercise. Muscle fiber characteristics change considerably when resistance exercise is performed chronically, but whether training status affects the activity of SC in response to a single bout of exercise remains unknown. We examined the changes in SC content and activation status following a single bout of resistance exercise, prior to and following a 16wk progressive resistance training (RT) program in fourteen young (25±3yr) men. Before and after RT, percutaneous biopsies from the vastus lateralis muscle were taken prior to a single bout of resistance exercise and after 24 and 72h of post-exercise recovery. Muscle fiber size, capillarization, and SC response were determined by immunohistochemistry. Following RT, there was a greater activation of SC after 24h in response to a single bout of resistance exercise (Pre:1.4±0.3,24h:3.1±0.3 $Pax7^+/MyoD^+$ cells/100 fibers) as compared to before RT (Pre:1.4±0.3,24h:2.2±0.3 $Pax7^+/MyoD^+$ cells/100 fibers, $p<0.05$); no difference was observed 72h post-exercise. Following 16wk of RT, MyoD mRNA expression increased from basal to 24h after the single bout of exercise ($p<0.05$); this change was not observed prior to training. Individual capillary-to-fiber ratio (C/F*i*) increased in both type I (1.8±0.3 to 2.0±0.3 C/F*i*, $p<0.05$) and type II (1.7±0.3 to 2.2±0.3 C/F*i*, $p<0.05$) fibers in response to RT. Following RT, enhanced activation of SC in response to resistance exercise is accompanied by increases in muscle fiber capillarization.

KEY WORDS: muscle stem cells, Pax7, MyoD, capillaries, perfusion

INTRODUCTION

The activation, proliferation and/or differentiation of satellite cells (SC) are important events in post-exercise recovery leading to muscle fiber adaptation, remodeling and repair. Following a single bout of damage (21, 22) or resistance exercise (37) in humans, expansion of the SC pool is observed by 24h, peaking at 72h post-exercise (36). Irrespective of the model employed, these aforementioned studies (21, 22, 37) were primarily performed on exercise-naïve participants. Presumably then, the typically observed increase in SC content may be a result of general stress rather than a refined adaptive response to an exercise bout. It is well established that repeated bouts of exercise result in markedly reduced indices of muscle damage and stress following subsequent bouts (20). Similarly, exercise-trained individuals typically demonstrate an attenuated damage or stress response to a habitual exercise challenge (28, 29, 44), suggesting that adaptation has occurred. However, whether the acute SC response following a single bout of exercise is altered in exercise-trained individuals (i.e., individuals who are accustomed to the exercise stimulus) as compared to exercise-naïve individuals following a single exercise session remains unknown. Consequently, comparing the change in SC content in the untrained and trained state following a single bout of exercise can provide insight to the nature of adaptation.

The progression of SC through the myogenic program is orchestrated by a transcriptional network collectively known as the myogenic regulatory factors (i.e., MyoD, Myf5, Myogenin and MRF4). There is relatively little known regarding adaptation in the myogenic program following exercise-training. In addition, various regulatory factors such as hepatocyte growth factor (HGF), interleukin 6 (IL-6), myostatin, insulin-like growth factor-1 (IGF-1) have been shown to be key regulators in the process of activation, proliferation and/or differentiation (21-23, 26). Some of these factors are produced locally by skeletal muscle (27, 39). As an ‘endocrine

organ', skeletal muscle tissue produces and releases various cytokines that act in a paracrine, autocrine, or endocrine fashion (27). Consistent with this notion, it has been shown that the systemic environment plays a critical role in SC function (3, 9). Although regulatory signals may originate locally, they may also be derived from other organs and the broader circulatory system (42). Therefore, it has been hypothesized that muscle fiber capillarization may play an important role in the regulation of SC (5).

In healthy young men, RT is sufficient to promote capillarization (11). The increase in capillary number, induced by training, likely reflects the necessity to match the demand for oxygen (15) and nutrients (6, 7) to support growing/adapting muscle fibers. Furthermore, the increase in capillary number is larger as compared to the increase in muscle fiber size, leading to a greater number of capillaries per area muscle, which suggests a more efficient perfusion of the muscle fiber following prolonged resistance exercise training (14). Whether increased muscle fiber capillarization influences SC regulation in healthy young adults remains unknown.

We assessed the activation of the SC pool in response to a single bout of resistance exercise in a group of healthy young men prior to (untrained state response; UTSR) and following (trained state response; TSR) 16 weeks of resistance training (RT). We hypothesized that, following RT there would be an augmented activation of muscle SC in response to a single bout of resistance exercise and that this would be associated with enhanced muscle fibre perfusion.

METHODS

Participants. Fourteen healthy young men (YM: 25 ± 3 yr; mean ± SEM) were recruited to participate in this study. All participants were recreationally active with no formal weight

training experience in the previous 6 months. The participants in this study were a subset of a larger project investigating the adaptation of skeletal muscle tissue to prolonged resistance exercise training in healthy young men and included data relating to fiber cross sectional area, strength changes with training and expansion of the quiescent satellite cell pool (1, 24). The participant selection for the present study was based upon the availability of tissue for all time points for which to perform immunohistochemical analysis. Exclusion criteria included smoking, diabetes, the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and/or statins, and history of respiratory disease and/or any major orthopaedic disability. The study was approved by the Hamilton Health Sciences Integrated Research Ethics Board, and conformed to the guidelines outlined in the Declaration of Helsinki. Participants gave their informed written consent prior to their inclusion to the study.

Muscle biopsy sampling. Percutaneous needle biopsies were taken, after an (~10h) overnight fast, from the mid-portion of the *vastus lateralis* under local anesthetic using a 5 mm Bergstrom needle adapted for manual suction (2). Subjects had not participated in any physical activity for at least 96 hours before the biopsy collection prior to the bout of resistance exercise in the untrained condition (i.e., prior to resistance training) and the trained condition (i.e., following resistance training). The muscle biopsy procedure was repeated under the same fasted condition (~10h) 24h and 72h following the single bout of resistance exercise detailed below. Incisions for the repeated muscle biopsy sampling were spaced approximately 3 cm apart to minimize any effect of the previous biopsy. Upon excision, muscle samples were immediately mounted in optimal cutting temperature (OCT) compound, frozen in liquid nitrogen–cooled isopentane, and stored at -80° C until further analyses.

Exercise Training. Exercise training was performed four times per week, divided into two upper and two lower body sessions under strict supervision as described previously (24). The lower body session consisted of five exercises: leg press, leg extension, leg curl, calf press and plank exercise. The upper body session consisted of six exercises: chest press, shoulder press, lat pull down, row, biceps curl and triceps extension. Training progressed from two sets performed at 70% of 1 repetition maximum (RM) to four sets performed at 85% of 1RM, with the final set performed to the point of momentary muscle exhaustion. At the conclusion of each workout, and on the mornings of non-training days, participants consumed a beverage containing 30 g of whey protein, 25.9 g of carbohydrates and 3.4 g of fat (Musashi p30, Notting Hill Victoria, Australia).

Single bout of resistance exercise. To determine the impact of resistance exercise on SC content and activation status in relation to RT, participants performed a single bout of resistance exercise both prior to and following 16 wks of RT. In short, the participants completed four sets of eight repetitions each at 80% of 1RM on leg press (Maxam, Hamilton, Ontario), leg extension (Atlantis, Laval, Quebec), calf press and leg curl (Hur, Kokkola Finland). The single bout of exercise was performed at the same relative intensity both prior to and following RT. The final set of each exercise was performed to volitional failure (1). A resting period of 2 min between sets was allowed. All participants were verbally encouraged during the exercise session to complete the entire protocol. Prior to and following the resistance exercise, a 5 min warm up was performed on a cycle ergometer.

Immunofluorescence. Muscle cross sections (7μm) were prepared from unfixed OCT embedded samples, allowed to air dry for 30 minutes and stored at -80˚C. Samples were stained with antibodies against appropriate primary and secondary antibodies, found in Table 1, as previously

described (25). Nuclei were labelled with DAPI (4',6-diamidino-2-phenylindole) (1:20000, Sigma-Aldrich, Oakville, ON, Canada), prior to cover slipping with fluorescent mounting media (DAKO, Burlington, ON, Canada). The staining procedures were verified using negative controls, in order to ensure appropriate specificity of staining. 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, and ≥ 200 muscle fibers/subject/time point were included in the analyses for SC content/activation status (i.e., $Pax7^{+}/MyoD^{-}$ or $Pax7^{+}/MyoD^{-}$), and fiber cross sectional area (CSA), and perimeter. The activation status of SCs was determined via the colocalization of Pax7+ and DAPI ($Pax7^{+}/MyoD^{-}$) and/or the co-localization of Pax7, MyoD and DAPI (i.e., $Pax7^{+}/MyoD^{+}$). Slides were blinded for both group and time point. The quantification of muscle fiber capillaries was performed on 50 muscle fibers/subject/time point (30). Based on the work of Hepple *et al.* (15), quantification of; i) capillary contacts (CC; the number of capillaries around a fiber), ii) the capillary-to-fiber ratio on an individual fiber basis (C/F*i*), iii) the number of fibers sharing each capillary (i.e., the sharing factor) and iv) the capillary density (CD) was performed. The CD was calculated by using the cross sectional area (μm^2) as the reference space. The capillary-to-fiber perimeter exchange index (CFPE) was calculated as an estimate of the capillary-to-fiber surface area (15). The SC-to-capillary distance measurements were performed on all SC that were enclosed by other muscle fibers, and has been described previously as well as in Fig 1. (25). All immunofluorescent analysis were completed in a blinded fashion.

RNA Isolation. RNA was isolated from 15–25 mg of muscle using the Trizol/RNeasy method. All samples were homogenized with 1 mL of Trizol Reagent (Life Technologies, Burlington, ON, Canada), in Lysing Maxtrix D tubes (MP Biomedicals, Solon, OH, USA), with the FastPrep-24 Tissue and Cell Homogenizer (MP Biomedicals,Solon, OH, USA) for a duration of 40 sec at a setting of 6 m/sec. Following five minute room temperature incubation, homogenized samples were stored at -80°C for one month until further processing. After thawing on ice, 200 ml of chloroform (Sigma-Aldrich, Oakville, ON, Canada) was added to each sample, mixed vigorously for 15 sec, incubated at RT for 5 min, and spun at 12000 g for 10 min at 4°C. The RNA (aqueous) phase was purified using the E.Z.N.A. Total RNA Kit 1 (Omega Bio-Tek, Norcross, GA, USA) as per manufacturer's instructions. RNA concentration (ng/ml) and purity (260/280) was determined with the Nano-Drop 1000 Spectrophotometer (Thermo Fisher Scientific, Rockville, MD, USA). RNA integrity was determined using the Agilent 2100 Bioanalyzer (Agilent Technologies, Toronto, ON, Canada). Samples were reverse transcribed using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) in 20 µl reaction volumes, as per manufacturer's instructions, using an Eppendorf Mastercycler epGradient Thermal Cycler (Eppendorf, Mississauga, ON, Canada) to obtain cDNA for gene expression analysis.

Quantitative real time RT-PCR. All QPCR reactions were run in duplicate in 25 µl volumes containing RT Sybr Green qPCR Master Mix (Qiagen Sciences, Valencia, CA, USA), prepared with the epMotion 5075 Eppendorf automated pipetting system (Eppendorf, Mississauga, ON, Canada), and carried out using an Eppendorf Realplex2 Master Cycler epgradient (Eppendorf, Mississauga, ON, Canada). Primers are listed in Table 2 and were re-suspended in 1X TE buffer (10mM Tris–HCl and 0.11 mM EDTA) and stored at −20◦C prior to use. Messenger RNA

expression was calculated using the $2^{-\Delta\Delta Ct}$ method, and fold changes from baseline were calculated using the $\Delta\Delta C_t$ method (18). Gene expression was normalized to the housekeeping gene Beta-2-microglobulin (β2M). Expression of β2M did not differ between time points.

Statistical Analysis. Statistical analysis was performed using Sigma Stat 3.1.0 analysis software (Systat Software, Chicago, IL, USA). To assess the long-term changes in muscle fiber characteristics in response to 16 wks of RT, two way ANOVA was performed with time (pre- and post-exercise training) and fiber type (type I and II) as within subject factors, appropriate post-hoc analysis was performed if interactions were detected. Separate one-way repeated measures ANOVA, with time (Pre, 24 and 72 h) as a within factor, were performed to assess the following; the acute change in satellite cell activity status (i.e., $Pax7^+/MyoD^-$ and/or $Pax7^+/MyoD^+$ cells); the acute change in distance of activated SC to nearest capillary following a single bout of resistance type exercise; the acute change in MRF mRNA expression, prior to and following 16 wks of RT. In the one-way repeated measures ANOVA design for the acute SC response, post-exercise time points were only compared with baseline and Bonferonni corrections were applied to account for multiple comparisons. In addition, to assess the difference in the acute SC response prior to and following 16 wks of exercise training, a paired sample Student's *t*-test was utilized to compare the change in SC content and activation status (Pre vs 24h, and Pre vs 72h), prior to and following 16 wks of RT. Statistical significance was accepted at $p < 0.05$. All results were presented as means ± standard error of the mean (SEM).

RESULTS

Muscle fiber CSA and fiber-type distribution. Muscle fiber CSA was significantly greater in type II compared to type I, both prior to and following RT ($p<0.05$, Table 3). We previously reported

a significant increase in muscle fiber CSA in a larger cohort (1). Analysis of this subset of subjects resulted in similar statistically significant changes to those observed in the larger cohort previously reported (1). The percentage of type II muscle fibers was significantly greater than type I fibers ($p<0.05$, Table 3); muscle fiber type distribution did not change with RT. Following 16 wks of RT, there was a significant increase in both type I and type II ~~fiber~~ muscle fiber CSA and perimeter ($p<0.05$, Table 3). Furthermore, following 16 weeks of RT, type II muscle fiber CSA was greater than type I ($p<0.05$, Table 3).

Muscle fiber capillarization. There was greater CC (the number of capillaries around a fiber), C/F*i* ratio (capillary-to-fiber ratio), CFPE (capillary-to-fiber perimeter exchange index), and CD (capillary density) in type I compared to type II muscle fibers ($p<0.05$, Table 4). In both type I and type II muscle fibers, CFPE, C/F*i* ratio, was significantly greater following RT (all $p<0.05$, Table 4). In contrast, no differences in type I and type II muscle fiber CC and CD were observed with RT.

Fiber type specific satellite cell content and distance to nearest capillary. In resting muscle, SC content was greater in type II than type I muscle fibers ($p<0.05$, Table 5) both prior to and following RT, as previously reported (1). Type II-associated SC were located at a greater distance to their nearest capillary as compared to type I-associated SC ($p<0.05$, Table 5) both prior to and following RT. Both the number of type I- and type II-associated SC increased following RT ($p<0.05$, Table 5). There was no change in distance to the nearest capillary from either type I- or type II-associated SC following 16 wks RT (Table 5).

Satellite cell content and activation status in response to an acute bout of exercise.

UTSR: Response to a single bout of exercise resulted in total $Pax7^+$ cells/100 myofiber remaining unchanged at 24h (11.9 ± 0.9 cells/100 myofiber) but increased significantly at 72h

(15.2 ± 1.3 cells/100 myofiber) compared to Pre (11.8 ± 1.1 cells/100 myofiber) ($p<0.05$, Fig. 2A). $Pax7^+/MyoD^+$ cells/100 myofiber were significantly higher at 24h (2.2 ± 0.3 cells/100 myofiber) and 72h (2.3 ± 0.4 cells/100 myofiber) after the single bout of exercise as compared to Pre (1.4 ± 0.3 cells/100 myofiber) ($p<0.05$, Fig. 2B). $Pax7^+/MyoD^-$ cells/100 myofiber did not change from Pre (10.4 ± 1.0 cells/100 myofiber) to 24h (9.7 ± 0.8 cells/100 myofiber), but was trending towards significance at 72h (12.9 ± 1.2 cells/100 myofiber) after the single bout of exercise ($p = 0.06$, Fig. 2C).

TSR: In response to a single bout of resistance exercise of the same relative intensity following 16 wks of RT, total $Pax7^+$ cells/100 myofiber were unchanged 24h (16.6 ± 1.5 cells/100 myofiber) and increased significantly at 72h (17.7 ± 1.3 cells/100 myofiber) compared to Pre (13.7 ± 1.4 cells/100 myofiber) ($p<0.05$, Fig. 2A). $Pax7^+/MyoD^+$ cells/100 myofiber were significantly increased at 24h (3.1 ± 0.2 cells/100 myofiber) and 72h (3.1 ± 0.4 cells/100 myofiber) after the single bout of exercise as compared to Pre (1.4 ± 0.4 cells/100 myofiber) ($p<0.05$, Fig 2B). $Pax7^+/MyoD^-$ cells/100 myofiber were unchanged from Pre (12.3 ± 1.2 cells/100 myofiber) to 24h (13.5 ± 1.3 cells/100 myofiber), but was trending towards significance at 72h (14.6 ± 1.0 cells/100 myofiber) after the single bout of exercise ($p = 0.08$, Fig. 2C).

UTSR v. TSR: In comparing the UTSR and TSR responses we discovered that there was a greater change in the number of $Pax7^+/MyoD^+$ cells from Pre to 24h post-exercise recovery compared to UTSR (Fig. 2B).

*Distance of SC to nearest capillary in response to an acute bout of resistance exercise**.***

UTSR: $Pax7^+/MyoD^+$ cells were closer to their nearest capillary compared to $Pax7^+/MyoD^-$ cells both prior to the single bout of exercise (Pre) and at 24h post-recovery

249 ($p<0.05$, Figure 3A). There were no difference in distance to the nearest capillary from SC that
250 were $Pax7^+/MyoD^-$ or $Pax7^+/MyoD^+$ ($p>0.05$, Figure 3A) at 72h post-exercise. Prior to resistance
251 training, there was no difference in the distance of $Pax7^+/MyoD^+$ or $Pax7^+/MyoD^-$ cells to the
252 nearest capillary 24h or 72h following a single bout of exercise in comparison to the Pre
253 distance.

254 ***TSR:*** $Pax7^+/MyoD^+$ cells were located closer to the nearest capillary compared to
255 $Pax7^+/MyoD^-$ cells prior to the single bout of exercise ($p<0.05$, Figure 3B). However, at 24h
256 post-recovery, the difference in distance between SC and its nearest capillary was abolished,
257 such that there was no difference between the two SC populations (Figure 3B). At 72h, there was
258 a re-establishment of the relationship observed at the Pre time point, such that $Pax7^+/MyoD^+$
259 cells were again located closer to their nearest capillary compared to $Pax7^+/MyoD^-$ cells ($p<0.05$,
260 Figure 3B). Following 16 wks resistance training, there was no difference in the distance of
261 $Pax7^+/MyoD^+$ or $Pax7^+/MyoD^-$ cells to the nearest capillary 24h or 72h following a single bout
262 of exercise as compared to baseline measurements.

263 *MRF genes in response to an acute bout of resistance exercise.*

264 ***UTSR:*** In response to a single bout of exercise, MyoD mRNA expression did not
265 increase from basal levels at 24h (1.1-fold change) or 72h post-exercise recovery (1.8-fold
266 change), compared to Pre (Fig 4A). MRF4 mRNA expression did not significantly increase from
267 basal expression at 24h (1.2-fold change) or at 72h post-exercise recovery (1.3-fold change) (Fig
268 4B). Myf5 mRNA expression did not significantly increase from basal expression at 24h (1.4-
269 fold change) or at 72h post-exercise recovery (1.1-fold change) (Fig 4C).

270 ***TSR:*** Following 16wk of RT, a single bout of exercise resulted in MyoD mRNA
271 expression increased 1.4-fold from basal levels at 24h post-exercise recovery ($p<0.05$, Fig. 4A).

However, MyoD mRNA expression was no longer increased 72h post-exercise recovery compared to Pre (1.2-fold change) ($p>0.05$, Fig. 4A). Myf5 mRNA expression was increased at both 24h (2.0-fold) and 72h (1.5-fold) post-exercise compared to Pre ($p<0.05$, Fig 4C). MRF4 mRNA expression did not significantly increase from basal levels at 24h (1.2-fold change) or at 72h post-exercise (1.2-fold change).

DISCUSSION

In the present study we observed an altered activation of the SC pool in response to a single bout of exercise following 16 wks of RT. We speculate that increased capillarization as a result of 16 wks of exercise training may be an important factor for enhancing SC activation in the post-exercise period.

Activation, proliferation and/or differentiation of SC are important events in the post-exercise recovery period to support muscle fiber adaptation. Accordingly, SC number is increased substantially in the days following a single bout of resistance exercise (36). More importantly, a greater proportion of SC are in the active state following exercise, as defined by the co-localization of MyoD with Pax7 (23, 37). In the present study, prior to exercise training, there was an ~35% increase in active SC ($MyoD^{+}/Pax7^{+}$) 24h following a single bout of resistance exercise. However, there was a significantly greater increase in active SC (~55%) at the same time point following 16 wks of RT. Consistent with this observation, we observed an increase in MyoD gene expression (~1.4 fold from Pre) 24h post exercise following RT as compared to no change in the untrained status response. These findings suggest an enhanced SC activation following 16 wks of RT. We suggest that this is an adaptive response to chronic exercise training that allows for an augmented post-exercise response to acute exercise. To better

295 understand the nature of this observation to an acute bout of exercise following training, we
296 examined whether enhanced SC activation following RT in young men was accompanied by
297 changes in muscle fiber capillarization.

298 Skeletal muscle fiber perfusion is essential for the delivery of oxygen, growth factors and
299 macronutrients to skeletal muscle fibers. Inadequate muscle fiber perfusion has been suggested
300 to play a role in 'anabolic resistance' and impaired nutritive flow in various populations (13, 32,
301 40). In order to meet increased metabolic demand and to support continuous muscle hypertrophy
302 during resistance exercise, an increase in muscle capillarization may be required. Consistent with
303 this notion, muscle fiber capillarization has been reported to increase significantly in response to
304 RT in healthy young men (12, 14, 19). In agreement, we report a ~13% increase in C/Fi in type I
305 and a ~26% increase in type II muscle fibers. Furthermore, we observed an increase in type I
306 (~10%) and type II (~17%) CFPE index. As CFPE is regarded as a proxy measure of
307 microvascular perfusion (16), an increase in CFPE suggests improved delivery of circulating
308 nutrients and/or growth factors. Therefore, increases in muscle fiber vascularization and/or the
309 reorganization of the microvascular bed following RT may result in enhanced supply of
310 circulating growth factors during the post-exercise period that could influence the SC response.

311 There are many growth factors that may play a role in regulating SC function (e.g., IL-6,
312 IGF-1, Myostatin, HGF) (17). Therefore, an increase in muscle fiber perfusion may result in
313 enhanced exposure of SC to regulatory growth factors in circulation (4, 5). We and others have
314 reported an anatomical relationship between muscle SC and capillaries (5) and have also noted
315 that activated SC are closer to capillaries than quiescent SC (5, 25) suggesting that proximity of a
316 SC to a capillary could be an important factor for SC function. Accordingly, it has been
317 hypothesized that SC content (5, 10) and/or activation status (4, 5, 25) may be related to muscle

fiber capillarization. In the present study, activated SC cells were located in closer proximity to capillaries compared to quiescent SC at baseline (Pre; prior to the single bout of resistance exercise) in both the UTSR and the TSR condition. We were unable to observe any direct or significant correlation between the increase in muscle capillarization and the altered acute SC response in the TSR. However, we observed that the temporal-spatial relationship between both quiescent and active SC and the nearest capillary had been changed in response to a single bout of exercise at 24h following 16 wk RT. These small changes may be indicative of an adaptive response of the spatial relationship between SC and capillaries following chronic training. Whether the small changes in the relationship between active and/or quiescent SC and the distance to the nearest capillary can explain the enhanced activation of SC in response to a single bout of exercise following 16 wks of RT remains unknown and requires further study. Furthermore, SC activation status was not determined in a fiber type specific manner, and future studies should address this issue.

While we observed an increase in capillarization following RT that accompanied an altered SC response to resistance exercise, there remains an incomplete understanding of how the SC response to a stimulus is initiated. Indeed, there is evidence to suggest that numerous cytokines and growth factors produced by skeletal muscle and/or the microvasculature may stimulate SC in an autocrine/paracrine fashion rather than through circulation. IL-6, previously reported to have a role in SC regulation (34, 41), is produced locally by contracting muscles (39). Interestingly, cell types such as endothelial cells within the muscle have also produce IL-6 under certain conditions (35, 45), as well as IGF-1 and HGF (5). Given the established spatial relationship between capillaries and SC, it would stand to reason that cellular cross-talk between endothelial cells and SC may influence angiogenesis (5, 33). Indeed, Chazaud et al. (2003)

reported that human muscle progenitor cells undergoing differentiation produce VEGF, a key factor for angiogenesis (4). Taken together, these findings indicate that the relationship between microvascular capillaries and SC may be predicated not only on the exposure to systemic factors, but also the immediate paracrine cross-talk between endothelial cells and SC. Future studies should address whether cytokines released from skeletal muscle or the microvasculature stimulate the SC response through autocrine/paracrine pathways, or exposure to endocrine-derived signals delivered through the microvasculature, or some combination of both.

Given the increased muscle perfusion following 16 wks of RT, we speculate that SC may have received enhanced input from circulating growth factors and more rapidly initiated the myogenic program and migratory function of SC leading to a loss in the observed anatomical relationship between SC and capillaries in the rested state and early activated state following exercise. While we do not find a significant correlation between the altered (post-RT) response and the increase in capillarization, recent work might lead us to speculate that capillarization may play a role in resistance training adaptation. Indeed, Snijders et al. (2016) recently observed that capillarization was linked to changes in muscle cross-sectional area following resistance training in older men. The study observed that individuals who started with a higher muscle fiber capillarization at baseline had a greater muscle hypertrophy following resistance training in older men. Taken together, the changes in SC activation that accompany the increases in muscle capillarization following long term RT warrant further study into the relationship between capillaries and the SC pool. In compromised populations, such as older adults, who can have a relatively reduced muscle capillarization (8, 31) and reduced muscle mass (43), an impaired SC activation in response to exercise has been observed (23, 37). Furthermore, it would be interesting to investigate whether increasing muscle fiber capillarization would result in an

augmented SC response during the post-exercise period in older adults. In conclusion, we observed that an altered activation of the SC pool in response to a single bout of resistance exercise is accompanied by increased capillarization following 16 wks RT.

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Figure Legend

Figure 1

Fig. 1 Fiber type specific staining with muscle capillaries. (***A***) Representative image of a MHCI/laminin/CD31/Pax7/DAPI stain of a muscle cross section. Channel views of ***(B***) CD31/Pax7 (***C***) Pax7/DAPI.

Figure 2

Fig. 2 Characterization of the activity status of SC following a single bout of resistance exercise prior to (UTSR; open bars) and following 16 weeks of RT (TSR; filled bars). Quantification of these cell populations as total number of $Pax7^+$ SC (***A***) number of $MyoD^{+/}Pax7^+$ (active SC; ***B***), number of $MyoD^{-/}Pax7^+$ (quiescent SC; ***C***) per 100 myofiber, prior to, 24h and 72h post-exercise recovery. *; time effect versus Pre ($p<0.05$), bar indicates that effect of time is present for both prior to and following 16 wks of RT. #; indicates a significantly greater ($p<0.05$) increase with time TSR vs UTSR. Mean ± SEM. SC: satellite cell.

Figure 3

Fig. 3 Distance between activated ($MyoD^{+/}Pax7^+$) and quiescent ($MyoD^{-/}Pax7^+$) SC to nearest capillary following a single bout of exercise prior to as compared to following 16 wks of RT. Response to resistance exercise prior to 16 wks RT exercise (UTSR; ***A***) and following (TSR; ***B)***. *; significantly different compared to active SC within time point ($p<0.05$), Mean ± SEM. SC: satellite cell.

Figure 4

Fig. 4 Relative expression of MyoD mRNA (A), MRF4 mRNA (B), Myf5 mRNA (C) expression in response to a single bout of exercise prior to (UTSR; open bars) compared to following 16 wks of RT (TSR; filled bars), expressed as fold change from Pre. Data are normalized to Beta-2-microglobulin. *; significantly different compared to Pre ($p<0.05$), Mean ± SEM.

549

550

551

552

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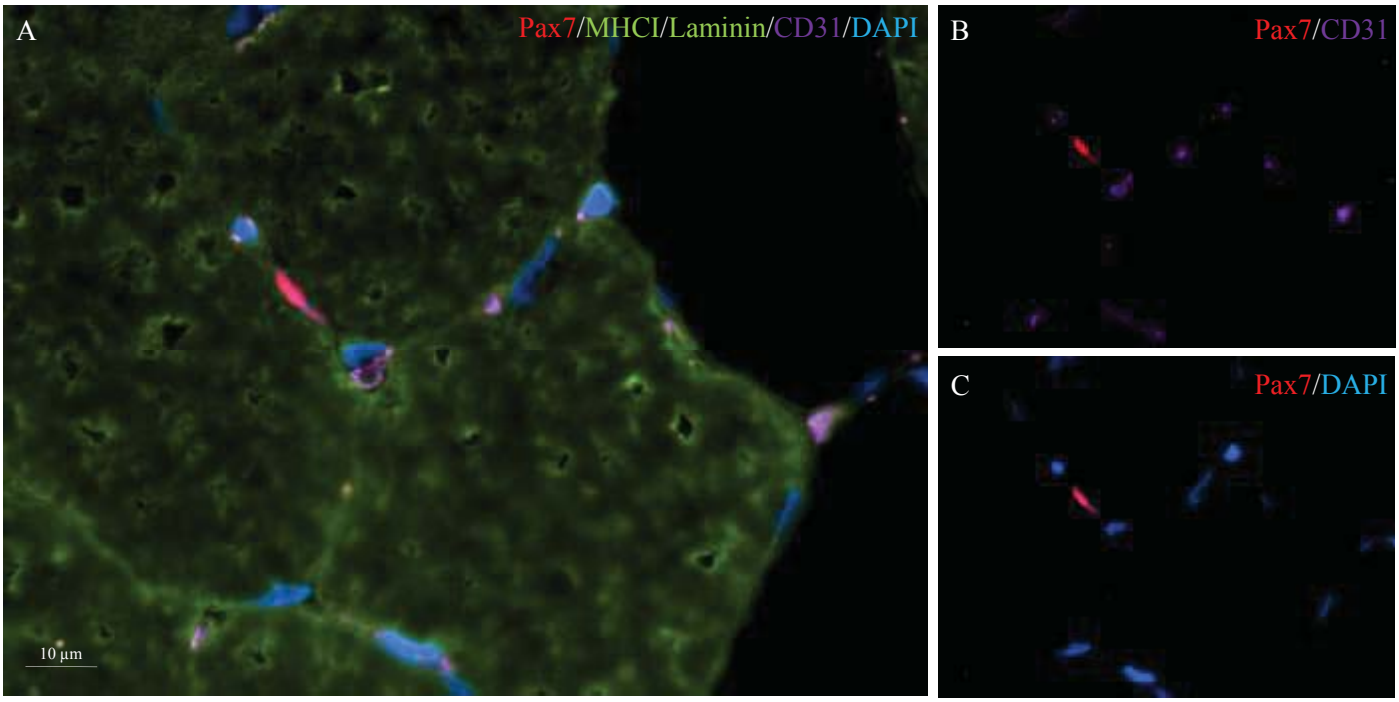

Figure 1

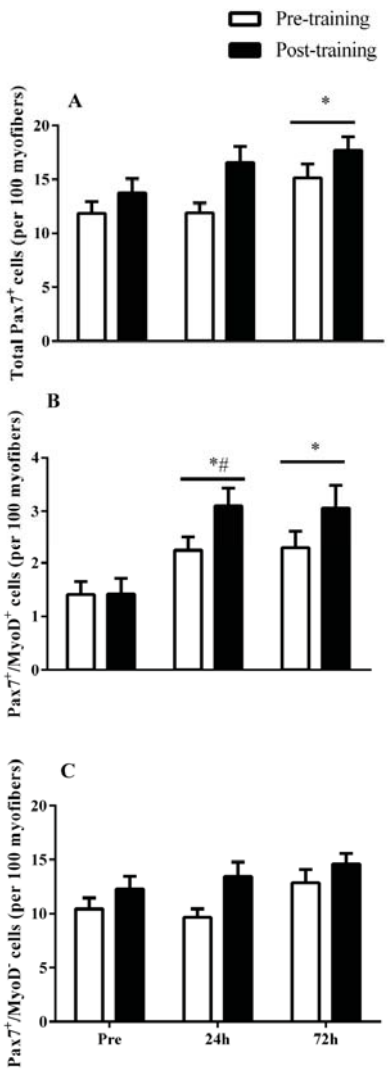
Pre-training
Post-training
A
Total Pax7+ cells (per 100 myofibers)
20
15
10
5
0
*
B
Pax7+/MyoD+ cells (per 100 myofibers)
4
3
2
1
0
*#
*
C
Pax7+/MyoD- cells (per 100 myofibers)
20
15
10
5
0
Pre
24h
72h

Figure 2

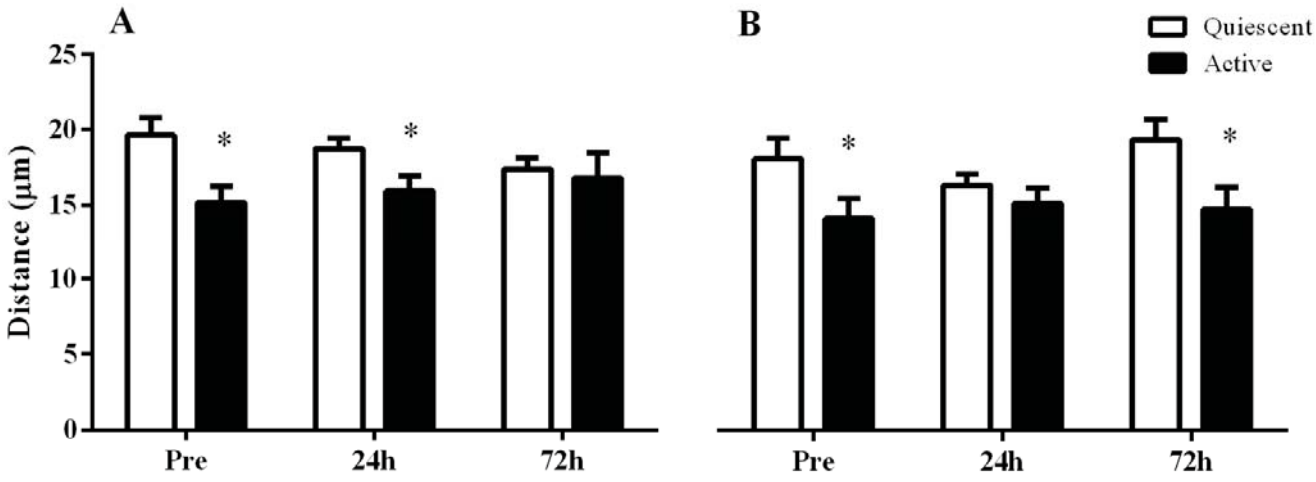
A
B
Quiescent
Active
Distance (μm)
25
20
15
10
5
0
*
*
*
*
Pre
24h
72h
Pre
24h
72h

Figure 3

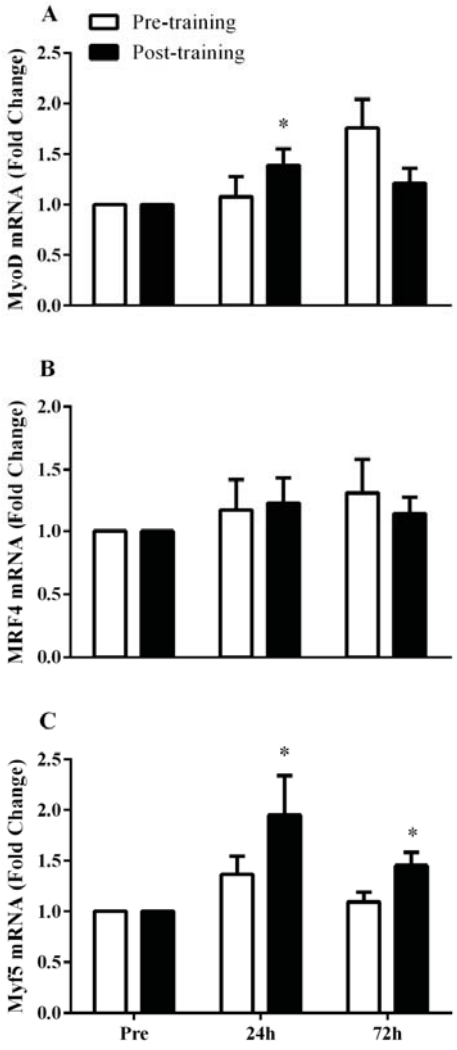
A
Pre-training
Post-training
MyoD mRNA (Fold Change)
2.5
2.0
1.5
1.0
0.5
0.0
*
B
MRF4 mRNA (Fold Change)
2.0
1.5
1.0
0.5
0.0
C
Myf5 mRNA (Fold Change)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
Pre
24h
72h

Figure 4

Table 1. Antibody information

Antibody	Species	Source	Clone	Primary	Secondary
Anti-Pax7	Mouse	DSHB	Pax7	1:1	Alexa 594, 488 goat-anti mouse 1:500
Anti-laminin	Rabbit	Abcam	ab11575	1:500	Alexa Fluor 488, 647 goat anti-rabbit, 1:500
Anti-MHCI	Mouse	DSHB	A4.951 Slow isoform	1:1	Alexa Fluor 488 goat anti-mouse, 1:500
Anti-CD31	Rabbit	Abcam	ab28364	1:30	Alexa Fluor 647 goat anti-rabbit, 1:500
Anti-MyoD	Mouse	Dako	5.8A	1:50	goat anti-mouse biotinylated secondary antibody, 1:200; streptavidin-594 fluorochrome, 1:250

Table 1. Detailed information on primary and secondary antibodies and dilutions used for immunofluorescent staining of the frozen muscle cross sections.

Table 2. Primer sequences for quantitative real-time PCR

Gene Name	Forward Sequence (5′-3′)	Reverse Sequence (5′-3′)
Myf5	5′ - ATGGACGTGATGGATGGCTG -3′	GCGGCACAAACTCGTCCCCAA
MyoD	5′- GGTCCCTCGCGCCCAAAAGAT-3′	CAGTTCTCCCGCCTCTCCTAC
MRF4	5′ - CCCCTTCAGCTACAGACCCAA-3′	CCCCCTGGAATGATCGGAAAC
β-*2-m*	5′ -ATGAG TATGCCTGCCGTGTGA-3′	GGCATCTTCAAACCTCCATG

Table 2. *MyoD*, myogenic determination factor; *Myf5*, myogenic factor-5; *MRF4*, myogenic regulatory factor-4; *β-2-m*, beta-2-microglobulin

Table 3. Skeletal muscle fibre characteristics prior to and following 16 weeks of resistance exercise training in young men

	Fiber type	Pre	Post
Fiber area (μm^2)			
	I	5621± 409	6263± 413$^{\#}$
	II	5771 ± 381*	7725 ± 519$^{*\#}$
Fiber perimeter (μm^2)			
	I	294 ± 9	309 ± 11$^{\#}$
	II	319 ± 10*	359 ± 18$^{*\#}$
Fiber type distribution (fiber %)			
	I	33 ± 3	38 ± 2
	II	67 ± 3*	62 ± 2*

Table 3. *; significant difference between fiber types (*p<0.05)* #; significant effect of exercise training (*p<0.05)*. Mean ± SEM

Table 4: Skeletal muscle fiber capillarization characteristics prior to and following 16 weeks of resistance exercise training in young men

	Fiber type	Pre	Post
Capillary contacts			
	I	3.18 ± 0.17	3.78 ± 0.22
	II	2.12 ± 0.16*	2.95 ± 0.21*
Individual capillary-to-fiber ratio (C/F*i*)			
	I	1.71 ± 0.08	1.94 ± 0.03$^{\#}$
	II	1.64 ± 0.09	2.07 ± 0.09$^{\#}$
Capillary density (capillaries x mm^{-2})			
	I	586 ± 32	640 ± 54
	II	383 ± 34*	400 ± 33*
CFPE (capillaries x 1000 μm^{-1})			
	I	5.89 ± 0.21	6.45 ± 0.22$^{\#}$
	II	5.07 ± 0.19*	5.95 ± 0.18$^{*\#}$

Table 4. *; Significantly different compared with type I muscle fibers (*p<0.05)* #; significant effect for exercise training (*p<0.05)*. Mean ± SEM. CFPE: capillary to fiber perimeter exchange index.

Table 5: Fiber type associated SC content and distance to nearest capillary prior to and following 16 weeks of resistance exercise training in young men

	Fiber type	Pre	Post
SC ($Pax7^+$ cells per 100 myofibers)			
	I	10.9 ± 0.8	13.4 ± 0.6$^{\#}$
	II	11.9 ± 0.8*	15.6 ± 0.9$^{*\#}$
SC distance to capillary (µm)			
	I	15.2 ± 1.0	13.9 ± 0.7
	II	16.8 ± 0.7*	15.9 ± 0.9*

*; significant effect of fiber type (*p<0.05)* #; significant effect for exercise training (*p<0.05)*. Mean ± SEM. SC: satellite cell