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

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

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12 interpreted results of experiments; J.P.N., prepared figures; J.P.N., G.P. drafted manuscript;  
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19

20

21 **ABSTRACT**

22 Skeletal muscle satellite cells (SC) play an important role in muscle adaptation. In untrained  
23 individuals, SC content and activation status has been observed to increase in response to a  
24 single bout of exercise. Muscle fiber characteristics change considerably when resistance  
25 exercise is performed chronically, but whether training status affects the activity of SC in  
26 response to a single bout of exercise remains unknown. We examined the changes in SC content  
27 and activation status following a single bout of resistance exercise, prior to and following a 16wk  
28 progressive resistance training (RT) program in fourteen young (25±3yr) men. Before and after  
29 RT, percutaneous biopsies from the vastus lateralis muscle were taken prior to a single bout of  
30 resistance exercise and after 24 and 72h of post-exercise recovery. Muscle fiber size,  
31 capillarization, and SC response were determined by immunohistochemistry. Following RT,  
32 there was a greater activation of SC after 24h in response to a single bout of resistance exercise  
33 (Pre:1.4±0.3,24h:3.1±0.3 Pax7<sup>+</sup>/MyoD<sup>+</sup> cells/100 fibers) as compared to before RT  
34 (Pre:1.4±0.3,24h:2.2±0.3 Pax7<sup>+</sup>/MyoD<sup>+</sup> cells/100 fibers,  $p<0.05$ ); no difference was observed  
35 72h post-exercise. Following 16wk of RT, MyoD mRNA expression increased from basal to 24h  
36 after the single bout of exercise ( $p<0.05$ ); this change was not observed prior to training.  
37 Individual capillary-to-fiber ratio (C/Fi) increased in both type I (1.8±0.3 to 2.0±0.3 C/Fi,  
38  $p<0.05$ ) and type II (1.7±0.3 to 2.2±0.3 C/Fi,  $p<0.05$ ) fibers in response to RT. Following RT,  
39 enhanced activation of SC in response to resistance exercise is accompanied by increases in  
40 muscle fiber capillarization.

41

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

43

44

## 45 INTRODUCTION

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

61 The progression of SC through the myogenic program is orchestrated by a transcriptional  
62 network collectively known as the myogenic regulatory factors (i.e., MyoD, Myf5, Myogenin  
63 and MRF4). There is relatively little known regarding adaptation in the myogenic program  
64 following exercise-training. In addition, various regulatory factors such as hepatocyte growth  
65 factor (HGF), interleukin 6 (IL-6), myostatin, insulin-like growth factor-1 (IGF-1) have been  
66 shown to be key regulators in the process of activation, proliferation and/or differentiation (21-  
67 23, 26). Some of these factors are produced locally by skeletal muscle (27, 39). As an ‘endocrine

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

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

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

## 87 **METHODS**

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

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

101 ***Muscle biopsy sampling.*** Percutaneous needle biopsies were taken, after an (~10h) overnight  
102 fast, from the mid-portion of the *vastus lateralis* under local anesthetic using a 5 mm Bergstrom  
103 needle adapted for manual suction (2). Subjects had not participated in any physical activity for  
104 at least 96 hours before the biopsy collection prior to the bout of resistance exercise in the  
105 untrained condition (i.e., prior to resistance training) and the trained condition (i.e., following  
106 resistance training). The muscle biopsy procedure was repeated under the same fasted condition  
107 (~10h) 24h and 72h following the single bout of resistance exercise detailed below. Incisions for  
108 the repeated muscle biopsy sampling were spaced approximately 3 cm apart to minimize any  
109 effect of the previous biopsy. Upon excision, muscle samples were immediately mounted in  
110 optimal cutting temperature (OCT) compound, frozen in liquid nitrogen-cooled isopentane, and  
111 stored at -80° C until further analyses.

112 **Exercise Training.** Exercise training was performed four times per week, divided into two upper  
113 and two lower body sessions under strict supervision as described previously (24). The lower  
114 body session consisted of five exercises: leg press, leg extension, leg curl, calf press and plank  
115 exercise. The upper body session consisted of six exercises: chest press, shoulder press, lat pull  
116 down, row, biceps curl and triceps extension. Training progressed from two sets performed at  
117 70% of 1 repetition maximum (RM) to four sets performed at 85% of 1RM, with the final set  
118 performed to the point of momentary muscle exhaustion. At the conclusion of each workout, and  
119 on the mornings of non-training days, participants consumed a beverage containing 30 g of whey  
120 protein, 25.9 g of carbohydrates and 3.4 g of fat (Musashi p30, Notting Hill Victoria, Australia).

121  
122 **Single bout of resistance exercise.** To determine the impact of resistance exercise on SC content  
123 and activation status in relation to RT, participants performed a single bout of resistance exercise  
124 both prior to and following 16 wks of RT. In short, the participants completed four sets of eight  
125 repetitions each at 80% of 1RM on leg press (Maxam, Hamilton, Ontario), leg extension  
126 (Atlantis, Laval, Quebec), calf press and leg curl (Hur, Kokkola Finland). The single bout of  
127 exercise was performed at the same relative intensity both prior to and following RT. The final  
128 set of each exercise was performed to volitional failure (1). A resting period of 2 min between  
129 sets was allowed. All participants were verbally encouraged during the exercise session to  
130 complete the entire protocol. Prior to and following the resistance exercise, a 5 min warm up was  
131 performed on a cycle ergometer.

132 **Immunofluorescence.** Muscle cross sections (7 $\mu$ m) were prepared from unfixed OCT embedded  
133 samples, allowed to air dry for 30 minutes and stored at -80°C. Samples were stained with  
134 antibodies against appropriate primary and secondary antibodies, found in Table 1, as previously



135 described (25). Nuclei were labelled with DAPI (4',6-diamidino-2-phenylindole) (1:20000,  
136 Sigma-Aldrich, Oakville, ON, Canada), prior to cover slipping with fluorescent mounting media  
137 (DAKO, Burlington, ON, Canada). The staining procedures were verified using negative  
138 controls, in order to ensure appropriate specificity of staining. Slides were viewed with the Nikon  
139 Eclipse *Ti* Microscope (Nikon Instruments, Inc. USA), equipped with a high-resolution  
140 Photometrics CoolSNAP HQ2 fluorescent camera (Nikon Instruments, Melville, NY, USA).  
141 Images were captured and analyzed using the Nikon NIS Elements AR 3.2 software (Nikon  
142 Instruments, Inc., USA). All images were obtained with the 20x objective, and  $\geq 200$  muscle  
143 fibers/subject/time point were included in the analyses for SC content/activation status (i.e.,  
144 Pax7<sup>+</sup>/MyoD<sup>-</sup> or Pax7<sup>+</sup>/MyoD<sup>+</sup>), and fiber cross sectional area (CSA), and perimeter. The  
145 activation status of SCs was determined via the colocalization of Pax7<sup>+</sup> and DAPI  
146 (Pax7<sup>+</sup>/MyoD<sup>-</sup>) and/or the co-localization of Pax7, MyoD and DAPI (i.e., Pax7<sup>+</sup>/MyoD<sup>+</sup>). Slides  
147 were blinded for both group and time point. The quantification of muscle fiber capillaries was  
148 performed on 50 muscle fibers/subject/time point (30). Based on the work of Hepple *et al.* (15),  
149 quantification of; i) capillary contacts (CC; the number of capillaries around a fiber), ii) the  
150 capillary-to-fiber ratio on an individual fiber basis ( $C/Fi$ ), iii) the number of fibers sharing each  
151 capillary (i.e., the sharing factor) and iv) the capillary density (CD) was performed. The CD was  
152 calculated by using the cross sectional area ( $\mu\text{m}^2$ ) as the reference space. The capillary-to-fiber  
153 perimeter exchange index (CFPE) was calculated as an estimate of the capillary-to-fiber surface  
154 area (15). The SC-to-capillary distance measurements were performed on all SC that were  
155 enclosed by other muscle fibers, and has been described previously as well as in Fig 1. (25). All  
156 immunofluorescent analysis were completed in a blinded fashion.

157 **RNA Isolation.** RNA was isolated from 15–25 mg of muscle using the Trizol/RNeasy method.  
158 All samples were homogenized with 1 mL of Trizol Reagent (Life Technologies, Burlington,  
159 ON, Canada), in Lysing Maxtrix D tubes (MP Biomedicals, Solon, OH, USA), with the  
160 FastPrep-24 Tissue and Cell Homogenizer (MP Biomedicals, Solon, OH, USA) for a duration of  
161 40 sec at a setting of 6 m/sec. Following five minute room temperature incubation, homogenized  
162 samples were stored at -80°C for one month until further processing. After thawing on ice, 200  
163 ml of chloroform (Sigma-Aldrich, Oakville, ON, Canada) was added to each sample, mixed  
164 vigorously for 15 sec, incubated at RT for 5 min, and spun at 12000 g for 10 min at 4°C. The  
165 RNA (aqueous) phase was purified using the E.Z.N.A. Total RNA Kit 1 (Omega Bio-Tek,  
166 Norcross, GA, USA) as per manufacturer's instructions. RNA concentration (ng/ml) and purity  
167 (260/280) was determined with the Nano-Drop 1000 Spectrophotometer (Thermo Fisher  
168 Scientific, Rockville, MD, USA). RNA integrity was determined using the Agilent 2100  
169 Bioanalyzer (Agilent Technologies, Toronto, ON, Canada). Samples were reverse transcribed  
170 using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA,  
171 USA) in 20 µl reaction volumes, as per manufacturer's instructions, using an Eppendorf  
172 Mastercycler epGradient Thermal Cycler (Eppendorf, Mississauga, ON, Canada) to obtain  
173 cDNA for gene expression analysis.

174 **Quantitative real time RT-PCR.** All QPCR reactions were run in duplicate in 25 µl volumes  
175 containing RT Sybr Green qPCR Master Mix (Qiagen Sciences, Valencia, CA, USA), prepared  
176 with the epMotion 5075 Eppendorf automated pipetting system (Eppendorf, Mississauga, ON,  
177 Canada), and carried out using an Eppendorf Realplex2 Master Cycler egradient (Eppendorf,  
178 Mississauga, ON, Canada). Primers are listed in Table 2 and were re-suspended in 1X TE buffer  
179 (10mM Tris-HCl and 0.11 mM EDTA) and stored at -20°C prior to use. Messenger RNA

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

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

199

## 200 **RESULTS**

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

203 a significant increase in muscle fiber CSA in a larger cohort (1). Analysis of this subset of  
204 subjects resulted in similar statistically significant changes to those observed in the larger cohort  
205 previously reported (1). The percentage of type II muscle fibers was significantly greater than  
206 type I fibers ( $p < 0.05$ , Table 3); muscle fiber type distribution did not change with RT. Following  
207 16 wks of RT, there was a significant increase in both type I and type II ~~fiber~~ muscle fiber CSA  
208 and perimeter ( $p < 0.05$ , Table 3). Furthermore, following 16 weeks of RT, type II muscle fiber  
209 CSA was greater than type I ( $p < 0.05$ , Table 3).

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

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

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

224 **UTSR:** Response to a single bout of exercise resulted in total Pax7<sup>+</sup> cells/100 myofiber  
225 remaining unchanged at 24h ( $11.9 \pm 0.9$  cells/100 myofiber) but increased significantly at 72h

226 (15.2 ± 1.3 cells/100 myofiber) compared to Pre (11.8 ± 1.1 cells/100 myofiber) (p<0.05, Fig.  
227 2A). Pax7<sup>+</sup>/MyoD<sup>+</sup> cells/100 myofiber were significantly higher at 24h (2.2 ± 0.3 cells/100  
228 myofiber) and 72h (2.3 ± 0.4 cells/100 myofiber) after the single bout of exercise as compared to  
229 Pre (1.4 ± 0.3 cells/100 myofiber) (p<0.05, Fig. 2B). Pax7<sup>+</sup>/MyoD<sup>-</sup> cells/100 myofiber did not  
230 change from Pre (10.4 ± 1.0 cells/100 myofiber) to 24h (9.7 ± 0.8 cells/100 myofiber), but was  
231 trending towards significance at 72h (12.9 ± 1.2 cells/100 myofiber) after the single bout of  
232 exercise (p = 0.06, Fig. 2C).

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

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

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

247 **UTSR:** Pax7<sup>+</sup>/MyoD<sup>+</sup> cells were closer to their nearest capillary compared to  
248 Pax7<sup>+</sup>/MyoD<sup>-</sup> 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<sup>+</sup>/MyoD<sup>-</sup> or Pax7<sup>+</sup>/MyoD<sup>+</sup> (p>0.05, Figure 3A) at 72h post-exercise. Prior to resistance  
251 training, there was no difference in the distance of Pax7<sup>+</sup>/MyoD<sup>+</sup> or Pax7<sup>+</sup>/MyoD<sup>-</sup> 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<sup>+</sup>/MyoD<sup>+</sup> cells were located closer to the nearest capillary compared to  
255 Pax7<sup>+</sup>/MyoD<sup>-</sup> 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<sup>+</sup>/MyoD<sup>+</sup>  
259 cells were again located closer to their nearest capillary compared to Pax7<sup>+</sup>/MyoD<sup>-</sup> cells (p<0.05,  
260 Figure 3B). Following 16 wks resistance training, there was no difference in the distance of  
261 Pax7<sup>+</sup>/MyoD<sup>+</sup> or Pax7<sup>+</sup>/MyoD<sup>-</sup> 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).

272 However, MyoD mRNA expression was no longer increased 72h post-exercise recovery  
273 compared to Pre (1.2-fold change) ( $p>0.05$ , Fig. 4A). Myf5 mRNA expression was increased at  
274 both 24h (2.0-fold) and 72h (1.5-fold) post-exercise compared to Pre ( $p<0.05$ , Fig 4C). MRF4  
275 mRNA expression did not significantly increase from basal levels at 24h (1.2-fold change) or at  
276 72h post-exercise (1.2-fold change).

277

## 278 **DISCUSSION**

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

283 Activation, proliferation and/or differentiation of SC are important events in the post-  
284 exercise recovery period to support muscle fiber adaptation. Accordingly, SC number is  
285 increased substantially in the days following a single bout of resistance exercise (36). More  
286 importantly, a greater proportion of SC are in the active state following exercise, as defined by  
287 the co-localization of MyoD with Pax7 (23, 37). In the present study, prior to exercise training,  
288 there was an ~35% increase in active SC (MyoD<sup>+</sup>/Pax7<sup>+</sup>) 24h following a single bout of  
289 resistance exercise. However, there was a significantly greater increase in active SC (~55%) at  
290 the same time point following 16 wks of RT. Consistent with this observation, we observed an  
291 increase in MyoD gene expression (~1.4 fold from Pre) 24h post exercise following RT as  
292 compared to no change in the untrained status response. These findings suggest an enhanced SC  
293 activation following 16 wks of RT. We suggest that this is an adaptive response to chronic  
294 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



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

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

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

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

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

367

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376

377 **References**

- 378 1. **Bellamy LM, Joanisse S, Grubb A, Mitchell CJ, McKay BR, Phillips SM, Baker S,**  
379 **and Parise G.** The acute satellite cell response and skeletal muscle hypertrophy following  
380 resistance training. *PloS one* 9: e109739, 2014.
- 381 2. **Bergstrom J.** Percutaneous needle biopsy of skeletal muscle in physiological and clinical  
382 research. *Scandinavian journal of clinical and laboratory investigation* 35: 609-616, 1975.
- 383 3. **Brack AS and Rando TA.** Intrinsic changes and extrinsic influences of myogenic stem  
384 cell function during aging. *Stem cell reviews* 3: 226-237, 2007.
- 385 4. **Chazaud B, Sonnet C, Lafuste P, Bassez G, Rimaniol AC, Poron F, Authier FJ,**  
386 **Dreyfus PA, and Gherardi RK.** Satellite cells attract monocytes and use macrophages as a  
387 support to escape apoptosis and enhance muscle growth. *The Journal of cell biology* 163: 1133-  
388 1143, 2003.
- 389 5. **Christov C, Chretien F, Abou-Khalil R, Bassez G, Vallet G, Authier FJ, Bassaglia Y,**  
390 **Shinin V, Tajbakhsh S, Chazaud B, and Gherardi RK.** Muscle satellite cells and endothelial  
391 cells: close neighbors and privileged partners. *Molecular biology of the cell* 18: 1397-1409,  
392 2007.
- 393 6. **Clark MG.** Impaired microvascular perfusion: a consequence of vascular dysfunction  
394 and a potential cause of insulin resistance in muscle. *American journal of physiology*  
395 *Endocrinology and metabolism* 295: E732-750, 2008.
- 396 7. **Clark MG, Wallis MG, Barrett EJ, Vincent MA, Richards SM, Clerk LH, and**  
397 **Rattigan S.** Blood flow and muscle metabolism: a focus on insulin action. *American journal of*  
398 *physiology Endocrinology and metabolism* 284: E241-258, 2003.
- 399 8. **Coggan AR, Spina RJ, King DS, Rogers MA, Brown M, Nemeth PM, and Holloszy**  
400 **JO.** Skeletal muscle adaptations to endurance training in 60- to 70-yr-old men and women.  
401 *Journal of applied physiology (Bethesda, Md : 1985)* 72: 1780-1786, 1992.
- 402 9. **Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, and Rando TA.**  
403 Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* 433:  
404 760-764, 2005.
- 405 10. **Emslie-Smith AM and Engel AG.** Microvascular changes in early and advanced  
406 dermatomyositis: a quantitative study. *Annals of neurology* 27: 343-356, 1990.
- 407 11. **Gavin TP, Drew JL, Kubik CJ, Pofahl WE, and Hickner RC.** Acute resistance  
408 exercise increases skeletal muscle angiogenic growth factor expression. *Acta physiologica*  
409 *(Oxford, England)* 191: 139-146, 2007.
- 410 12. **Green H, Goreham C, Ouyang J, Ball-Burnett M, and Ranney D.** Regulation of fiber  
411 size, oxidative potential, and capillarization in human muscle by resistance exercise. *The*  
412 *American journal of physiology* 276: R591-596, 1999.
- 413 13. **Groen BB, Hamer HM, Snijders T, van Kranenburg J, Frijns D, Vink H, and van**  
414 **Loon LJ.** Skeletal muscle capillary density and microvascular function are compromised with  
415 aging and type 2 diabetes. *Journal of applied physiology (Bethesda, Md : 1985)* 116: 998-1005,  
416 2014.
- 417 14. **Hather BM, Tesch PA, Buchanan P, and Dudley GA.** Influence of eccentric actions on  
418 skeletal muscle adaptations to resistance training. *Acta physiologica Scandinavica* 143: 177-185,  
419 1991.
- 420 15. **Hepple RT, Mackinnon SL, Goodman JM, Thomas SG, and Plyley MJ.** Resistance  
421 and aerobic training in older men: effects on VO<sub>2</sub>peak and the capillary supply to skeletal  
422 muscle. *Journal of applied physiology (Bethesda, Md : 1985)* 82: 1305-1310, 1997.

- 423 16. **Hepple RT and Mathieu-Costello O.** Estimating the size of the capillary-to-fiber  
424 interface in skeletal muscle: a comparison of methods. *Journal of applied physiology (Bethesda,*  
425 *Md : 1985)* 91: 2150-2156, 2001.
- 426 17. **Kadi F, Charifi N, Denis C, Lexell J, Andersen JL, Schjerling P, Olsen S, and Kjaer**  
427 **M.** The behaviour of satellite cells in response to exercise: what have we learned from human  
428 studies? *Pflugers Archiv : European journal of physiology* 451: 319-327, 2005.
- 429 18. **Livak KJ and Schmittgen TD.** Analysis of relative gene expression data using real-time  
430 quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif)* 25: 402-408,  
431 2001.
- 432 19. **McCall GE, Byrnes WC, Dickinson A, Pattany PM, and Fleck SJ.** Muscle fiber  
433 hypertrophy, hyperplasia, and capillary density in college men after resistance training. *Journal*  
434 *of applied physiology (Bethesda, Md : 1985)* 81: 2004-2012, 1996.
- 435 20. **McHugh MP.** Recent advances in the understanding of the repeated bout effect: the  
436 protective effect against muscle damage from a single bout of eccentric exercise. *Scandinavian*  
437 *journal of medicine & science in sports* 13: 88-97, 2003.
- 438 21. **McKay BR, De Lisio M, Johnston AP, O'Reilly CE, Phillips SM, Tarnopolsky MA,**  
439 **and Parise G.** Association of interleukin-6 signalling with the muscle stem cell response  
440 following muscle-lengthening contractions in humans. *PloS one* 4: e6027, 2009.
- 441 22. **McKay BR, O'Reilly CE, Phillips SM, Tarnopolsky MA, and Parise G.** Co-  
442 expression of IGF-1 family members with myogenic regulatory factors following acute  
443 damaging muscle-lengthening contractions in humans. *The Journal of physiology* 586: 5549-  
444 5560, 2008.
- 445 23. **McKay BR, Ogborn DI, Bellamy LM, Tarnopolsky MA, and Parise G.** Myostatin is  
446 associated with age-related human muscle stem cell dysfunction. *FASEB journal : official*  
447 *publication of the Federation of American Societies for Experimental Biology* 26: 2509-2521,  
448 2012.
- 449 24. **Mitchell CJ, Churchward-Venne TA, Bellamy L, Parise G, Baker SK, and Phillips**  
450 **SM.** Muscular and systemic correlates of resistance training-induced muscle hypertrophy. *PloS*  
451 *one* 8: e78636, 2013.
- 452 25. **Nederveen JP, Joannis S, Snijders T, Ivankovic V, Baker SK, Phillips SM, and**  
453 **Parise G.** Skeletal muscle satellite cells are located at a closer proximity to capillaries in healthy  
454 young compared with older men. *Journal of Cachexia, Sarcopenia and Muscle: n/a-n/a*, 2016.
- 455 26. **O'Reilly C, McKay B, Phillips S, Tarnopolsky M, and Parise G.** Hepatocyte growth  
456 factor (HGF) and the satellite cell response following muscle lengthening contractions in  
457 humans. *Muscle & nerve* 38: 1434-1442, 2008.
- 458 27. **Pedersen BK and Febbraio MA.** Muscle as an endocrine organ: focus on muscle-  
459 derived interleukin-6. *Physiological reviews* 88: 1379-1406, 2008.
- 460 28. **Pizza FX, Baylies H, and Mitchell JB.** Adaptation to eccentric exercise: neutrophils and  
461 E-selectin during early recovery. *Canadian journal of applied physiology = Revue canadienne*  
462 *de physiologie appliquee* 26: 245-253, 2001.
- 463 29. **Pizza FX, Davis BH, Henrickson SD, Mitchell JB, Pace JF, Bigelow N, DiLauro P,**  
464 **and Naglieri T.** Adaptation to eccentric exercise: effect on CD64 and CD11b/CD18 expression.  
465 *Journal of applied physiology (Bethesda, Md : 1985)* 80: 47-55, 1996.
- 466 30. **Porter MM, Koolage CW, and Lexell J.** Biopsy sampling requirements for the  
467 estimation of muscle capillarization. *Muscle & nerve* 26: 546-548, 2002.

- 468 31. **Proctor DN, Sinning WE, Walro JM, Sieck GC, and Lemon PW.** Oxidative capacity  
469 of human muscle fiber types: effects of age and training status. *Journal of applied physiology*  
470 (*Bethesda, Md : 1985*) 78: 2033-2038, 1995.
- 471 32. **Rasmussen BB, Fujita S, Wolfe RR, Mittendorfer B, Roy M, Rowe VL, and Volpi E.**  
472 Insulin resistance of muscle protein metabolism in aging. *FASEB journal : official publication of*  
473 *the Federation of American Societies for Experimental Biology* 20: 768-769, 2006.
- 474 33. **Rhoads RP, Johnson RM, Rathbone CR, Liu X, Temm-Grove C, Sheehan SM,**  
475 **Hoying JB, and Allen RE.** Satellite cell-mediated angiogenesis in vitro coincides with a  
476 functional hypoxia-inducible factor pathway. *American journal of physiology Cell physiology*  
477 296: C1321-1328, 2009.
- 478 34. **Serrano AL, Baeza-Raja B, Perdiguero E, Jardi M, and Munoz-Canoves P.**  
479 Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy. *Cell*  
480 *metabolism* 7: 33-44, 2008.
- 481 35. **Sironi M, Breviario F, Proserpio P, Biondi A, Vecchi A, Van Damme J, Dejana E,**  
482 **and Mantovani A.** IL-1 stimulates IL-6 production in endothelial cells. *Journal of immunology*  
483 (*Baltimore, Md : 1950*) 142: 549-553, 1989.
- 484 36. **Snijders T, Nederveen JP, McKay BR, Joannis S, Verdijk LB, van Loon LJ, and**  
485 **Parise G.** Satellite cells in human skeletal muscle plasticity. *Frontiers in physiology* 6: 283,  
486 2015.
- 487 37. **Snijders T, Verdijk LB, Smeets JS, McKay BR, Senden JM, Hartgens F, Parise G,**  
488 **Greenhaff P, and van Loon LJ.** The skeletal muscle satellite cell response to a single bout of  
489 resistance-type exercise is delayed with aging in men. *Age (Dordrecht, Netherlands)* 36: 9699,  
490 2014.
- 491 38. **Snijders T, Nederveen JP, Joannis S, Leenders M, Verdijk LB, van Loon LJ,**  
492 **and Parise G.** Muscle fibre capillarization is a critical factor in muscle fibre hypertrophy during  
493 resistance exercise training in older men. *Journal of Cachexia, Sarcopenia and Muscle*,  
494 doi: 10.1002/jcsm.12137, 2016.
- 495 39. **Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, and Klarlund Pedersen**  
496 **B.** Production of interleukin-6 in contracting human skeletal muscles can account for the  
497 exercise-induced increase in plasma interleukin-6. *The Journal of physiology* 529 Pt 1: 237-242,  
498 2000.
- 499 40. **Timmerman KL, Lee JL, Dreyer HC, Dhanani S, Glynn EL, Fry CS, Drummond**  
500 **MJ, Sheffield-Moore M, Rasmussen BB, and Volpi E.** Insulin stimulates human skeletal  
501 muscle protein synthesis via an indirect mechanism involving endothelial-dependent vasodilation  
502 and mammalian target of rapamycin complex 1 signaling. *The Journal of clinical endocrinology*  
503 *and metabolism* 95: 3848-3857, 2010.
- 504 41. **Toth KG, McKay BR, De Lisio M, Little JP, Tarnopolsky MA, and Parise G.** IL-6  
505 induced STAT3 signalling is associated with the proliferation of human muscle satellite cells  
506 following acute muscle damage. *PloS one* 6: e17392, 2011.
- 507 42. **Velloso CP.** Regulation of muscle mass by growth hormone and IGF-I. *British journal of*  
508 *pharmacology* 154: 557-568, 2008.
- 509 43. **Verdijk LB, Koopman R, Schaart G, Meijer K, Savelberg HH, and van Loon LJ.**  
510 Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly.  
511 *American journal of physiology Endocrinology and metabolism* 292: E151-157, 2007.

- 512 44. **Vincent HK and Vincent KR.** The effect of training status on the serum creatine kinase  
513 response, soreness and muscle function following resistance exercise. *International journal of*  
514 *sports medicine* 18: 431-437, 1997.
- 515 45. **Yan SF, Tritto I, Pinsky D, Liao H, Huang J, Fuller G, Brett J, May L, and Stern D.**  
516 Induction of interleukin 6 (IL-6) by hypoxia in vascular cells. Central role of the binding site for  
517 nuclear factor-IL-6. *The Journal of biological chemistry* 270: 11463-11471, 1995.

518

519 **Figure Legend**

520 Figure 1

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

524 Figure 2

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

533 Figure 3

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

539

540 Figure 4

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

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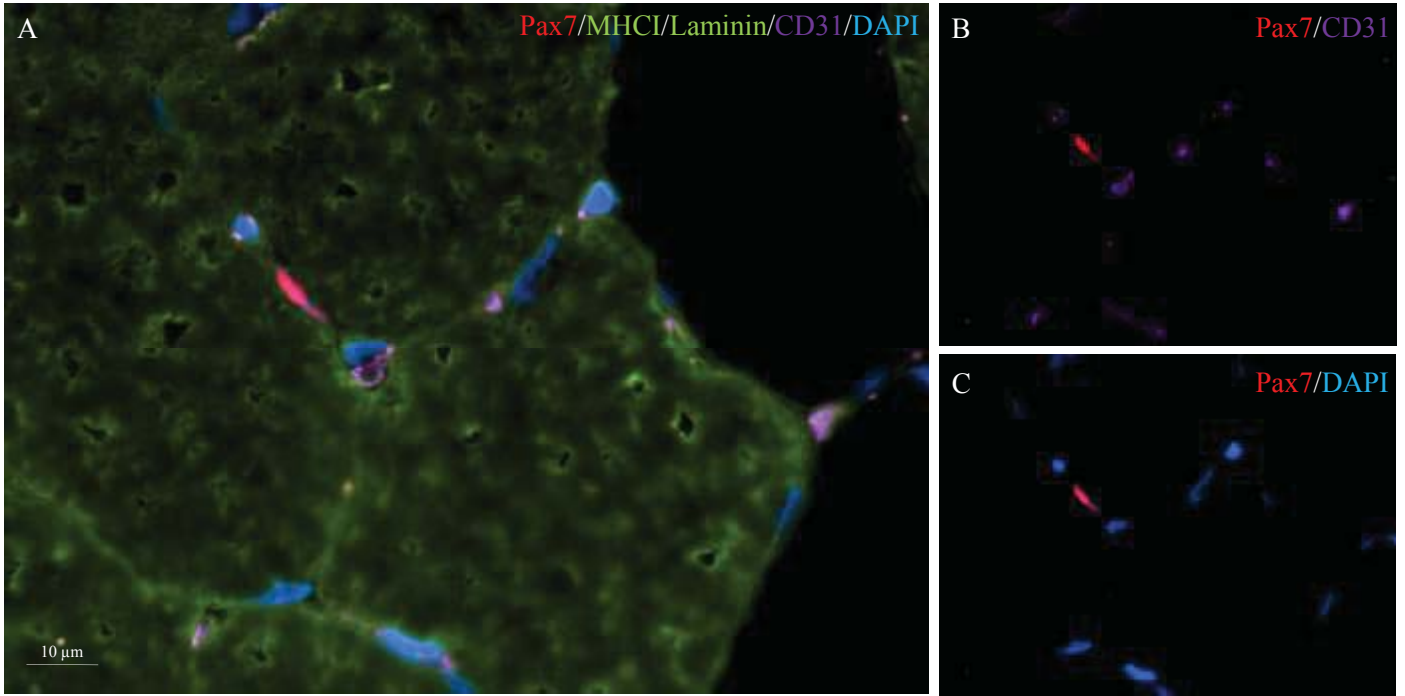


Figure 1

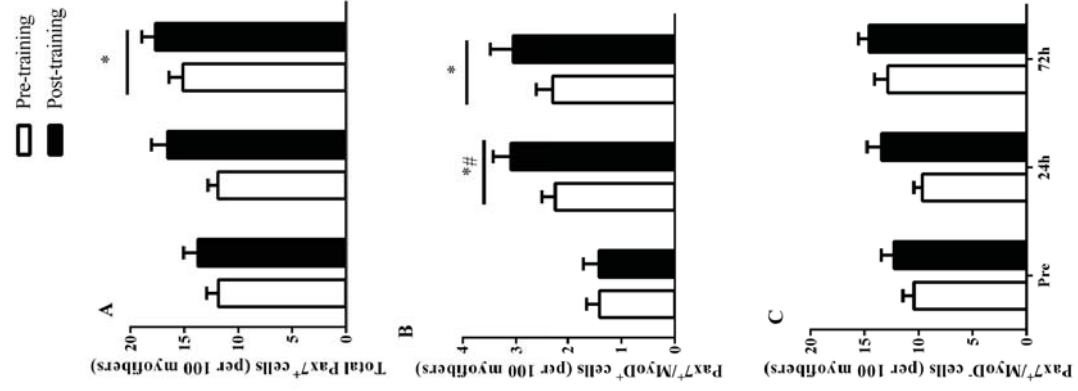


Figure 2

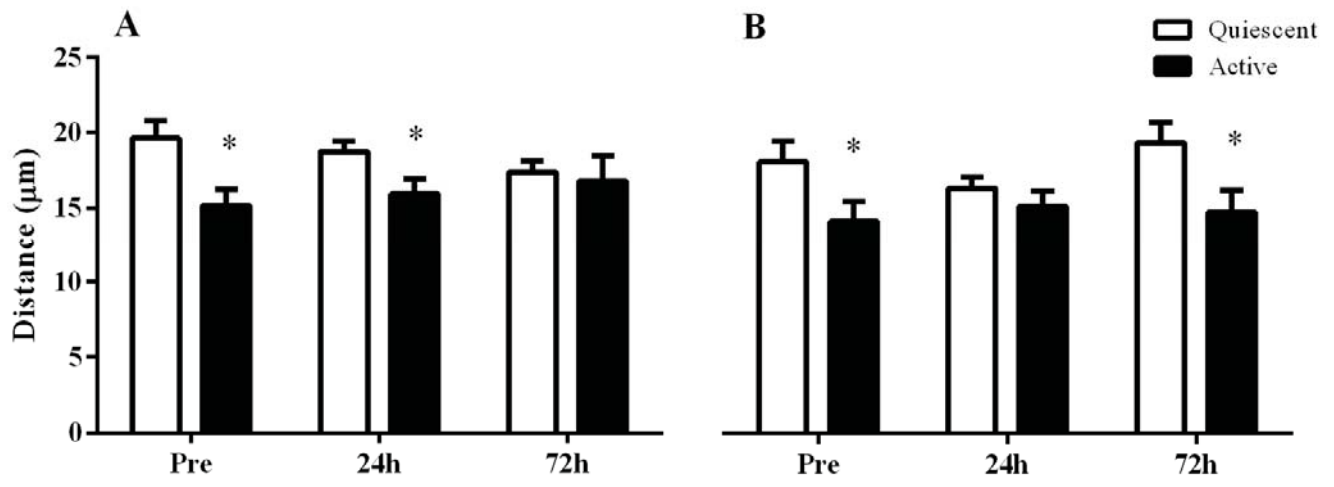


Figure 3



**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')
<i>Myf5</i>	5' - ATGGACGTGATGGATGGCTG -3'	GCGGCACAAACTCGTCCCCAA
<i>MyoD</i>	5'- GGTCCCTCGCGCCCAAAAGAT-3'	CAGTTCTCCCGCCTCTCCTAC
<i>MRF4</i>	5' - CCCCTTCAGCTACAGACCCAA-3'	CCCCCTGGAATGATCGGAAAC
$\beta$ -2- <i>m</i>	5' -ATGAG TATGCCTGCCGTGTGA-3'	GGCATCTTCAAACCTCCATG

Table 2. *MyoD*, myogenic determination factor; *Myf5*, myogenic factor-5; *MRF4*, myogenic regulatory factor-4;  $\beta$ -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 ( $\mu\text{m}^2$ )	I	5621 $\pm$ 409	6263 $\pm$ 413 <sup>#</sup>
	II	5771 $\pm$ 381 <sup>*</sup>	7725 $\pm$ 519 <sup>**#</sup>
Fiber perimeter ( $\mu\text{m}^2$ )	I	294 $\pm$ 9	309 $\pm$ 11 <sup>#</sup>
	II	319 $\pm$ 10 <sup>*</sup>	359 $\pm$ 18 <sup>**#</sup>
Fiber type distribution (fiber %)	I	33 $\pm$ 3	38 $\pm$ 2
	II	67 $\pm$ 3 <sup>*</sup>	62 $\pm$ 2 <sup>*</sup>

Table 3. \*, significant difference between fiber types ( $p < 0.05$ ); #; significant effect of exercise training ( $p < 0.05$ ). Mean  $\pm$  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/Fi)	I	1.71 ± 0.08	1.94 ± 0.03#
	II	1.64 ± 0.09	2.07 ± 0.09#
Capillary density (capillaries x mm <sup>-2</sup> )	I	586 ± 32	640 ± 54
	II	383 ± 34*	400 ± 33*
CFPE (capillaries x 1000 μm <sup>-1</sup> )	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 <sup>+</sup> cells per 100 myofibers)	I	10.9 ± 0.8	13.4 ± 0.6 <sup>#</sup>
	II	11.9 ± 0.8 <sup>*</sup>	15.6 ± 0.9 <sup>*#</sup>
SC distance to capillary (μm)	I	15.2 ± 1.0	13.9 ± 0.7
	II	16.8 ± 0.7 <sup>*</sup>	15.9 ± 0.9 <sup>*</sup>

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