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

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3 Authors and institutions

- 4 Joshua P. Nederveen¹, Tim Snijders¹, Sophie Joanisse¹, Christopher G. Wavell¹, Cameron J.
- 5 Mitchell¹, Leeann M. Johnston¹, Steven K. Baker³, Stuart M. Phillips¹ and Gianni Parise^{1,2*}
- 6 Departments of ¹Kinesiology, ²Medical Physics & Applied Radiation Sciences, and ³Medicine,
- 7 McMaster University, Hamilton, Ontario, Canada, L8S 4L8

8 Author contributions

- 9 J.P.N., T.S., S.J., C.W., C.J.M., L.M., J., S.K.B., S.M.P., G.P., conceived and designed the
- 10 experiments; C.J.M., L.M.J., S.K.B., S.M.P., G.P., collected samples; J.P.N., T.S., S.J., C.W.,
- 11 L.M.J., performed experiments; J.P.N., T.S., S.J., G.P., analyzed data; J.P.N., T.S., S.J., G.P.,
- interpreted results of experiments; J.P.N., prepared figures; J.P.N., G.P. drafted manuscript;
 J.P.N., T.S., S.J., C.W., C.J.M., L.M.,J., S.K.B., S.M.P., G.P., approved final version of
 manuscript.

15 Corresponding author

*Departments of Kinesiology and Medical Physics and Applied Radiation Sciences, McMaster
University, Hamilton, Ontario, Canada L8S 4L8. E-mail: pariseg@mcmaster.ca; Telephone: 905
525 9140 ext. 27353

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21 ABSTRACT

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

40 muscle fiber capillarization.

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

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45 INTRODUCTION

The activation, proliferation and/or differentiation of satellite cells (SC) are important 46 events in post-exercise recovery leading to muscle fiber adaptation, remodeling and repair. 47 Following a single bout of damage (21, 22) or resistance exercise (37) in humans, expansion of 48 the SC pool is observed by 24h, peaking at 72h post-exercise (36). Irrespective of the model 49 employed, these aforementioned studies (21, 22, 37) were primarily performed on exercise-naïve 50 51 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 52 53 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 54 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 exercise is altered in exercise-trained individuals (i.e., individuals who are accustomed to the 57 exercise stimulus) as compared to exercise-naïve individuals following a single exercise session 58 remains unknown. Consequently, comparing the change in SC content in the untrained and 59 trained state following a single bout of exercise can provide insight to the nature of adaptation. 60

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.

87 METHODS

88 *Participants*. Fourteen healthy young men (YM: 25 ± 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 larger project investigating the adaptation of skeletal muscle tissue to prolonged resistance 91 exercise training in healthy young men and included data relating to fiber cross sectional area, 92 strength changes with training and expansion of the quiescent satellite cell pool (1, 24). The 93 participant selection for the present study was based upon the availability of tissue for all time 94 points for which to perform immunohistochemical analysis. Exclusion criteria included smoking, 95 diabetes, the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and/or statins, and history of 96 respiratory disease and/or any major orthopaedic disability. The study was approved by the 97 98 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 99 their inclusion to the study. 100

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

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

121

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

135 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 136 (DAKO, Burlington, ON, Canada). The staining procedures were verified using negative 137 controls, in order to ensure appropriate specificity of staining. Slides were viewed with the Nikon 138 Eclipse Ti Microscope (Nikon Instruments, Inc. USA), equipped with a high-resolution 139 Photometrics CoolSNAP HQ2 fluorescent camera (Nikon Instruments, Melville, NY, USA). 140 Images were captured and analyzed using the Nikon NIS Elements AR 3.2 software (Nikon 141 Instruments, Inc., USA). All images were obtained with the 20x objective, and \geq 200 muscle 142 fibers/subject/time point were included in the analyses for SC content/activation status (i.e., 143 Pax7⁺/MyoD⁻ or Pax7⁺/MyoD⁻), and fiber cross sectional area (CSA), and perimeter. The 144 activation status of SCs was determined via the colocalization of Pax7+ and DAPI 145 (Pax7⁺/MyoD⁻) and/or the co-localization of Pax7, MyoD and DAPI (i.e., Pax7⁺/MyoD⁺). Slides 146 were blinded for both group and time point. The quantification of muscle fiber capillaries was 147 performed on 50 muscle fibers/subject/time point (30). Based on the work of Hepple et al. (15), 148 quantification of; i) capillary contacts (CC; the number of capillaries around a fiber), ii) the 149 capillary-to-fiber ratio on an individual fiber basis (C/Fi), iii) the number of fibers sharing each 150 capillary (i.e., the sharing factor) and iv) the capillary density (CD) was performed. The CD was 151 calculated by using the cross sectional area (μm^2) as the reference space. The capillary-to-fiber 152 153 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 immunofluorescent analysis were completed in a blinded fashion. 156

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

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 epgradient (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 $2^{-\Delta\Delta Ct}$ 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 (β 2M). Expression of β 2M did not differ between time points.

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

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 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).

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⁺ cells/100 myofiber 225 remaining unchanged at 24h (11.9 \pm 0.9 cells/100 myofiber) but increased significantly at 72h 226 (15.2 \pm 1.3 cells/100 myofiber) compared to Pre (11.8 \pm 1.1 cells/100 myofiber) (p<0.05, Fig. 227 2A). Pax7⁺/MyoD⁺ cells/100 myofiber were significantly higher at 24h (2.2 \pm 0.3 cells/100 228 myofiber) and 72h (2.3 \pm 0.4 cells/100 myofiber) after the single bout of exercise as compared to 229 Pre (1.4 \pm 0.3 cells/100 myofiber) (p<0.05, Fig. 2B). Pax7⁺/MyoD⁻ cells/100 myofiber did not 230 change from Pre (10.4 \pm 1.0 cells/100 myofiber) to 24h (9.7 \pm 0.8 cells/100 myofiber), but was 231 trending towards significance at 72h (12.9 \pm 1.2 cells/100 myofiber) after the single bout of 232 exercise (p = 0.06, Fig. 2C).

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

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).

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

247 *UTSR:* $Pax7^+/MyoD^+$ cells were closer to their nearest capillary compared to 248 $Pax7^+/MyoD^-$ cells both prior to the single bout of exercise (Pre) and at 24h post-recovery (p<0.05, Figure 3A). There were no difference in distance to the nearest capillary from SC that were $Pax7^+/MyoD^-$ or $Pax7^+/MyoD^+$ (p>0.05, Figure 3A) at 72h post-exercise. Prior to resistance training, there was no difference in the distance of $Pax7^+/MyoD^+$ or $Pax7^+/MyoD^-$ cells to the nearest capillary 24h or 72h following a single bout of exercise in comparison to the Pre distance.

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

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

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

277

278 **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-283 exercise recovery period to support muscle fiber adaptation. Accordingly, SC number is 284 increased substantially in the days following a single bout of resistance exercise (36). More 285 importantly, a greater proportion of SC are in the active state following exercise, as defined by 286 the co-localization of MyoD with Pax7 (23, 37). In the present study, prior to exercise training, 287 there was an $\sim 35\%$ increase in active SC (MyoD⁺/Pax7⁺) 24h following a single bout of 288 resistance exercise. However, there was a significantly greater increase in active SC (~55%) at 289 the same time point following 16 wks of RT. Consistent with this observation, we observed an 290 291 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 292 activation following 16 wks of RT. We suggest that this is an adaptive response to chronic 293 294 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, we296 examined whether enhanced SC activation following RT in young men was accompanied by297 changes in muscle fiber capillarization.

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

There are many growth factors that may play a role in regulating SC function (e.g., IL-6, IGF-1, Myostatin, HGF) (17). Therefore, an increase in muscle fiber perfusion may result in enhanced exposure of SC to regulatory growth factors in circulation (4, 5). We and others have reported an anatomical relationship between muscle SC and capillaries (5) and have also noted that activated SC are closer to capillaries than quiescent SC (5, 25) suggesting that proximity of a SC to a capillary could be an important factor for SC function. Accordingly, it has been 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 318 capillaries compared to quiescent SC at baseline (Pre; prior to the single bout of resistance 319 exercise) in both the UTSR and the TSR condition. We were unable to observe any direct or 320 significant correlation between the increase in muscle capillarization and the altered acute SC 321 response in the TSR. However, we observed that the temporal-spatial relationship between both 322 quiescent and active SC and the nearest capillary had been changed in response to a single bout 323 of exercise at 24h following 16 wk RT. These small changes may be indicative of an adaptive 324 response of the spatial relationship between SC and capillaries following chronic training. 325 326 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 327 bout of exercise following 16 wks of RT remains unknown and requires further study. 328 Furthermore, SC activation status was not determined in a fiber type specific manner, and future 329 studies should address this issue. 330

While we observed an increase in capillarization following RT that accompanied an 331 altered SC response to resistance exercise, there remains an incomplete understanding of how the 332 SC response to a stimulus is initiated. Indeed, there is evidence to suggest that numerous 333 cytokines and growth factors produced by skeletal muscle and/or the microvasculature may 334 stimulate SC in an autocrine/paracrine fashion rather than through circulation. IL-6, previously 335 reported to have a role in SC regulation (34, 41), is produced locally by contracting muscles (39). 336 337 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 338 relationship between capillaries and SC, it would stand to reason that cellular cross-talk between 339 340 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 endocrinederived 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 348 349 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 350 relationship between SC and capillaries in the rested state and early activated state following 351 exercise. While we do not find a significant correlation between the altered (post-RT) response 352 and the increase in capillarization, recent work might lead us to speculate that capillarization 353 may play a role in resistance training adaptation. Indeed, Snijders et al. (2016) recently observed 354 that capillarization was linked to changes in muscle cross-sectional area following resistance 355 training in older men. The study observed that individuals who started with a higher muscle fiber 356 357 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 358 capillarization following long term RT warrant further study into the relationship between 359 360 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 361 activation in response to exercise has been observed (23, 37). Furthermore, it would be 362 363 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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375 **Conflict of Interest -** There are no conflict of interests.

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377 References

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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 sports medicine 18: 431-437, 1997. 514 515 45. Yan SF, Tritto I, Pinsky D, Liao H, Huang J, Fuller G, Brett J, May L, and Stern D. Induction of interleukin 6 (IL-6) by hypoxia in vascular cells. Central role of the binding site for 516 517 nuclear factor-IL-6. The Journal of biological chemistry 270: 11463-11471, 1995. 518 **Figure Legend** 519 520 Figure 1 521 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. 522 Channel views of (B) CD31/Pax7 (C) Pax7/DAPI. 523 Figure 2 524 525 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 526 (TSR; filled bars). Quantification of these cell populations as total number of 527 $Pax7^+$ SC (A) number of MyoD^{+/}Pax7⁺ (active SC; B), number of MyoD^{-/}Pax7⁺ 528 (quiescent SC; C) per 100 myofiber, prior to, 24h and 72h post-exercise recovery. 529 *; time effect versus Pre (p < 0.05), bar indicates that effect of time is present for 530 531 both prior to and following 16 wks of RT. #; indicates a significantly greater (p<0.05) increase with time TSR vs UTSR. Mean \pm SEM. SC: satellite cell. 532 533 Figure 3 Fig. 3 Distance between activated ($MvoD^{+/}Pax7^{+}$) and quiescent ($MvoD^{-/}Pax7^{+}$) 534 SC to nearest capillary following a single bout of exercise prior to as compared to 535 following 16 wks of RT. Response to resistance exercise prior to 16 wks RT 536 exercise (UTSR; A) and following (TSR; B). *; significantly different compared 537 to active SC within time point (p < 0.05), Mean \pm SEM. SC: satellite cell. 538 539 Figure 4 540 Fig. 4 Relative expression of MyoD mRNA (A), MRF4 mRNA (B), Myf5 mRNA 541 (C) expression in response to a single bout of exercise prior to (UTSR; open bars) 542 compared to following 16 wks of RT (TSR; filled bars), expressed as fold change 543 from Pre. Data are normalized to Beta-2-microglobulin. *; significantly different 544 compared to Pre (p < 0.05), Mean \pm SEM. 545 546 547 548



Figure 1





Figure 3



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 Anti-MyoD	Rabbit Mouse	Abcam Dako	ab28364 5.8A	1:30 1:50	Alexa Fluor 647 goat anti-rabbit, 1:500 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	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
Name		
Myf5	5' - ATGGACGTGATGGATGGCTG -3'	GCGGCACAAACTCGTCCCCAA
MyoD	5'- GGTCCCTCGCGCCCAAAAGAT-3'	CAGTTCTCCCGCCTCTCCTAC
MRF4	5' - CCCCTTCAGCTACAGACCCAA-3'	CCCCCTGGAATGATCGGAAAC
β- <i>2-m</i>	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

	Fiber type	Pre	Post
Γ^{1} (²)			
Fiber area (µm ⁻)			
	Ι	5621 ± 409	$6263 \pm 413^{\#}$
	II	$5771 \pm 381^{*}$	$7725 \pm 519^{*\#}$
Fiber perimeter (μm^2)			
	Ι	294 ± 9	$309 \pm 11^{\#}$
	II	$319 \pm 10^{*}$	$359 \pm 18^{*\#}$
Fiber type distribution (fiber %)			
	I	33 ± 3	38 ± 2
	Î	$67 \pm 3^*$	$62 \pm 2^*$

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

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

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

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

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.

Fiber type	Pre	Post
Ι	10.9 ± 0.8	$13.4 \pm 0.6^{\#}$
II	$11.9 \pm 0.8^{*}$	$15.6 \pm 0.9^{*\#}$
Ι	15.2 ± 1.0	13.9 ± 0.7
II	$16.8 \pm 0.7^{*}$	$15.9 \pm 0.9^{*}$
	Fiber type I II I	Fiber type Pre I 10.9 ± 0.8 II $11.9 \pm 0.8^*$ I 15.2 ± 1.0 II $16.8 \pm 0.7^*$

 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

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