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The influence of capillarization on satellite cell pool expansion and activation following exercise-induced muscle damage in healthy young men

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Conflict of Interest

There are no conflict of interests.

ABSTRACT:

Factors that determine the skeletal muscle satellite cell (SC) response remain incompletely understood. It is known, however, that SC activation status is closely related to the anatomical relationship between SC and muscle capillaries. We investigated the impact of muscle fibre capillarization on the expansion and activation status of SC following a muscle damaging exercise protocol in healthy young men. Twenty-nine young men (21±0.5 yrs) performed 300 unilateral eccentric contractions (180 deg·s^{-1}) of the knee extensors. Percutaneous muscle biopsies from the vastus lateralis and blood samples from the antecubital vein were taken prior to (Pre) and at 6h, 24h, 72h and 96h of post-exercise recovery. A comparison was made between subjects who had a relative low mixed muscle capillary-to-fibre perimeter exchange index (CFPE; Low group) and high mixed muscle CFPE index (High group) at baseline. Type I and type II muscle fibre size, myonuclear content, capillarization, and SC response were determined via immunohistochemistry. Overall, there was a significant correlation ($r= 0.39$; $p<0.05$) between the expansion of SC content (change in total Pax7^{+} cells/100 myofibre) 24h following eccentric exercise and mixed muscle CFPE index. There was a greater increase in activated SC (MyoD^{+}/Pax7^{+} cells) in the High as compared to the Low CFPE group 72h following eccentric exercise ($p<0.05$). The current study provides further evidence that muscle fibre capillarization may play an important role in the activation and expansion of the SC pool during the process of skeletal muscle repair.

INTRODUCTION:

Skeletal muscle satellite cells (SC) are indispensable for muscle regeneration and repair following injury (Lepper *et al.*, 2011; McCarthy *et al.*, 2011; Sambasivan *et al.*, 2011). In response to a physiological cue (e.g. exercise), SC activate, proliferate and differentiate donating nuclei to existing muscle fibres to aid in repair/adaptation or return to a state of quiescence to replenish the basal SC pool (Bentzinger *et al.*, 2012; Yin *et al.*, 2013). The process of SC activation through terminal differentiation is orchestrated by a transcriptional network, known as the myogenic regulatory factors (MRFs), and is collectively referred to as the myogenic program. Expansion of the SC pool following a single bout of exercise or muscle fibre contraction-induced damage has been well characterized in humans (McKay *et al.*, 2008; McKay *et al.*, 2009; McKay *et al.*, 2012; McKay *et al.*, 2013; Bellamy *et al.*, 2014; Nederveen *et al.*, 2017) with appreciable expansion occurring by 24h and peaking 72h post-stimulus (Snijders *et al.*, 2015).

A number of cytokines and growth factors including, but not limited to, interleukin-6 (IL-6), insulin-like growth factor-1 (IGF-1), myostatin and hepatocyte growth factor (HGF) are known regulators of SC progression through the myogenic program (McKay *et al.*, 2008; O'Reilly *et al.*, 2008; McKay *et al.*, 2009). Many of these factors are produced by skeletal muscle in its function as an 'endocrine organ' (Steensberg *et al.*, 2000; Pedersen & Febbraio, 2008), or by other organs, tissues or cells (Velloso, 2008) and then delivered to the SC niche via the vasculature. Therefore, delivery of these factors to the SC niche may be a requirement of the myogenic response. Indeed, the importance of extrinsic factors in regulating SC function has been demonstrated using parabiotic pairings of old and young rodents (Conboy *et al.*, 2005; Brack & Rando, 2007).

Muscle capillaries function as the delivery mechanism for oxygen, fuel, cytokines and growth factors that may regulate SC, but may also act as an important modulator of the SC response. We and others have reported an anatomical relationship between muscle SC and the microvasculature, with activated SC situated geographically closer to capillaries than quiescent SC (Christov *et al.*, 2007; Nederveen *et al.*, 2016; Nederveen *et al.*, 2017). Consequently, it has been proposed that SC content (Emslie-Smith & Engel, 1990; Christov *et al.*, 2007) and/or SC activation status (Chazaud *et al.*, 2003; Christov *et al.*, 2007; Nederveen *et al.*, 2016) may be related to the extent of muscle fibre capillarization as a result of exposure of SC to circulating factors or direct communication between endothelial cells and SC during muscle repair (Chazaud *et al.*, 2003; Ochoa *et al.*, 2007; Joanisse *et al.*, 2017). However, to what extent the muscle fibre microvascular bed may dictate the acute muscle SC response during muscle repair in humans remains unknown. Therefore, in the present study, we assessed the expansion and activation status of the SC pool following a single bout of exercise-induced muscle fibre damage in a group of healthy young men with varying degrees of muscle fibre capillarization. We hypothesized that individuals with a greater degree of muscle fibre capillarization would demonstrate a more rapid and pronounced SC response following a single bout of eccentric exercise.

METHODS

Participants. Twenty nine healthy young men (YM: 22 ± 0.5 yr; mean ± SEM) were recruited to participate in this study. Exclusion criteria included smoking, diabetes, the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and/or statins, and a history of respiratory disease and/or any major orthopaedic disability. Subjects were told to refrain from exercising throughout the duration of the study, and refrain from the use of NSAIDs (Mackey *et al.*, 2016).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 inclusion in the study.

VO_2peak test and anthropometric measurements. During an initial visit to the laboratory participants performed a VO_{2peak} test on a cycle ergometer (model: H-300-R Lode; Lode B.V., Groningen, The Netherlands) and had anthropometric measurements recorded. The VO_{2peak} test consisted of load-less pedaling for one minute, followed by a step-wise increase to 50 watts (W) for two minutes. After the increase to 50 watts, work rate was increased by 30 W/min until the participant reached volitional fatigue (determined by the inability of the participant to maintain a minimum cadence of 60 RPM). Gas exchange was collected throughout the test using a metabolic cart (Moxus, AEI Technologies, Pittsburgh, PA) and VO_{2peak} was calculated using the highest 30 second average VO_2 during the final stage of the ramp protocol. Work rate (WR) was collected continuously throughout the test and peak aerobic power (WR_{peak}) was calculated using the average WR from the last 30 seconds of the test.

Muscle biopsy sampling. Percutaneous needle biopsies were taken, after an (~10h) overnight fast (Pre), from the mid-portion of the *vastus lateralis* under local anesthetic using a 5 mm Bergstrom needle adapted for manual suction. Subjects had not participated in any physical activity for at least 96 hours before muscle biopsy collection prior to the single bout of eccentric exercise. The muscle biopsy procedure was repeated at 6h, and in the fasted condition (~10h) 24h, 72h and 96h of post-exercise recovery.. 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, while another part was directly frozen in liquid nitrogen, and stored at -80° C until further analyses.

Blood sampling. Blood samples were obtained from the antecubital vein immediately prior the muscle biopsy sampling procedure before and after 6h, 24h, 72h and 96h of the single bout of eccentric exercise. Blood (~10 mL) samples were collected in EDTA containing tubes and centrifuged at 1500 rpm for 10 min at 4 °C. Aliquots of plasma were frozen in liquid nitrogen and stored at −80°C. Plasma samples were analyzed for IL-6 protein and creatine kinase activity using commercially available Enzyme-Linked ImmunoSorbant Assay (ELISA) (R & D Systems, Inc., USA) and activity assay kits (Abcam Inc., Canada), respectively, following the manufacturer's instructions. Statistics were performed on the raw values, and expressed as a percentage change from baseline.

Eccentric Muscle Damage Protocol. Maximal isokinetic unilateral muscle-lengthening contractions of the quadriceps were performed using the Biodex dynamometer (Biodex-System 3, Biodex Medical Systems, Inc., USA) at 180 deg s^{-1}. For each subject, one leg was selected randomly to perform the exercise protocol described below. Movement at the shoulders, hips and thigh were restrained with straps in order to isolate the knee extensors during the protocol. Immediately prior to the intervention, subjects underwent a brief familiarization with the equipment, involving 5–10 submaximal lengthening contractions of the leg to be exercised. Subjects were required to perform 30 sets of 10 maximal knee extensions with 1 min rest between sets, for a total of 300 lengthening contractions. During each set, investigators provided verbal encouragement for the subjects to complete and exert maximal force during each contraction. This protocol has been previously shown to induce a significant level of skeletal muscle damage (Beaton *et al.*, 2002).

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

appropriate primary and secondary antibodies against specific antigens, found in Table 1, as previously described (Nederveen *et al.*, 2016). 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 fibres/subject/time point were included in the analyses for SC content/activation status (i.e., $Pax7^{+}/MyoD^{-}$ or $Pax7^{+}/MyoD^{-}$), and fibre 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., $Pax7^{+}/MyoD^{+}$). Cell membranes were labelled with Peroxidase conjugated Wheat Germ Agglutinin (WGA) (1 ug/mL, Vector PL-1026, Burlington, ON, Canada) and realized with a substrate kit (Vector, SK-4700, Burlington, ON, Canada) as per manufacturer's instructions. Slides were blinded for both group and time point. The quantification of muscle fibre capillaries was performed on 50 muscle fibres/subject/time point (Porter *et al.*, 2002). Based on the work of Hepple *et al.* (Hepple, 1997; Hepple & Mathieu-Costello, 2001), quantification of; i) capillary contacts (CC; the number of capillaries around a fibre), ii) the capillary-to-fibre ratio on an individual fibre basis (C/F*i*), iii) the number of fibres 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-fibre perimeter exchange index (CFPE) was calculated as an

estimate of the capillary-to-fibre surface area (Hepple, 1997). The SC-to-capillary distance measurements were performed on all SC that were enclosed by other muscle fibres, and has been described previously (Nederveen *et al.*, 2016). All immunofluorescent analysis were completed in a blinded fashion.

RNA Isolation. RNA was isolated from 15–25 mg of muscle tissue 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 a 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).

Reverse Transcription. 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 RT-PCR. All quantitative RTPCR 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 Supplementary Table 1 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 (Livak & Schmittgen, 2001). Briefly, Ct values were first normalized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Supplementary Table 1). Ct values normalized to GAPDH were expressed as delta-Cts (ΔCt). ΔCt values were then normalized to Pre values, expressed as delta-delta Cts (ΔΔCt). Values were then transformed out of the logarithmic scale using the formula: fold change = $2^{-\Delta\Delta Ct}$ (Livak & Schmittgen, 2001). Thus, mRNA values are expressed as a fold change from Pre (mean ±sem). GAPDH expression was not different from Pre at any of the post-intervention time-points.

Statistical analysis. Statistical analysis was performed using Sigma Stat 3.1.0 analysis software (Systat Software, Chicago, IL, USA). *Stratification of individuals based on capillarization*. To assess the impact of muscle fibre capillarization on the muscle SC response following a single bout of eccentric exercise and subsequent muscle damage, participants were assigned into one of two groups (n = 10 per group) based on mixed muscle fibre capillarization (corrected for capillary sharing factor and muscle fibre perimeter, also known as capillary-to-fibre perimeter exchange (CFPE) index) for non-correlative statistical analysis. This resulted in a group with a relatively low (Low; CFPE: 5.2 ± 0.5 capillaries • 1000 μm^{-1}) and relatively high (High; CFPE: 7.6 ± 1.0 capillaries • 1000 μm^{-1}) mixed muscle fibre CFPE index. Stratification of participants resulted in a ‘middle’ group (n = 9) who were not used in non-correlative statistical analysis,

with the intent to create a clear separation between the Low and High group. *Baseline comparisons*. Comparisons of participant demographics between the High CPFE and the Low CFPE groups are found in Table 2, and were performed via a Student's *t* test. Baseline comparisons of muscle fibre type specific characteristics between the High CFPE and the Low CFPE group were performed using a two-way ANOVA (group x fibre type). *Response to eccentric contractions.* One-way repeated measures ANOVA were performed separately for each of the 'Overall' group, for the High CFPE group and for the Low CFPE group, with time (Pre, 6h, 24h and 72h and 96h) as a within group factor. In these one-way repeated measures ANOVA design for the acute response, post-exercise time points were only compared with baseline (Pre) and Bonferonni corrections were applied to account for multiple comparisons. These tests were performed to assess the following; the acute change in SC activity status (i.e., $Pax7^{+}/MyoD^{+}$ cells); the acute change in SC content (i.e., mixed muscle, type I and/or type II $Pax7^{+}$ cells); the acute change in plasma IL-6 content; the acute change in plasma creatine kinase activity; the acute change in quadriceps muscle force production and the acute change in MRF mRNA expression, following the bout of eccentric exercise induced muscle damage. For correlations, Pearson's correlation analyses were performed where appropriate between indices of muscle fibre capillarization and the SC response following eccentric exercise. Statistical significance was accepted at $p < 0.05$. All results were presented as means ± standard error of the mean (SEM).

RESULTS

Subject characteristics

Overall: Complete subject characteristics are reported in Table 2.

Low vs. High CFPE group: There were no differences in age or height between the groups (Table 2). There was a significant difference in bodyweight ($p<0.05$, Table 2) and a trend for BMI ($p = 0.06$, Table 2) between the groups. Both the VO_{2max} (mL/kg/min) and W_{peak} (W) was significantly greater in the High as compared to the Low group ($p<0.05$; Table 2). There was no significant difference in force production prior to single bout eccentric exercise in the High (272.6 ± 8.8 N·m) compared to the Low (314.4 ± 13.3 N·m) group (Table 2).

Indices of muscle damage following repeated eccentric contractions.

Overall: Following eccentric contractions, force production (N·m) was significantly reduced at 6h (253 ± 15 N·m), 24h (233 ± 16 N·m), 72h (261± 18 N·m) and 96h (270 ± 17 N·m), as compared to Pre (319 ± 14 N·m) ($p<0.05$; Supplementary Fig 1A). Following the eccentric contraction protocol, plasma creatine kinase activity was significantly increased at 24h (103.7 ± 8.2 mU/mL, $p<0.05$; Supplementary Fig 1C) compared to Pre (75.4 ± 6.1 mU/mL), and returned back to baseline at 72h (76.3 ± 6.7 mU/mL) and 96h (79.1 ± 6.6 mU/mL)

High CFPE vs Low CFPE group: In the High group, force production was significantly reduced at 6h (211± 15 N·m), 24h (196 ± 17 N·m), 72h (230 ± 21 N·m) as compared to Pre (272 ± 15 N·m and was back at baseline levels again at 96h (241 ± 23 N·m) ($p<0.05$; Supplementary Fig 1B). In the Low group, force production was significantly reduced at 6h (248 ± 29 N·m), 24h (215 ± 34 N·m), 72h (242 ± 37 N·m) and 96h (257 ± 32 N·m) as compared to Pre (314 ± 23 N·m) ($p<0.05$; Supplementary Fig 1B). In the High group, plasma creatine kinase activity was significantly increased 24h (93.3 ± 13.8 mU/mL, $p<0.05$) following eccentric exercise, but was not significantly different at 72h (56.0 ± 5.4 mU/mL) and 96h (66.4 ± 8.6 mU/mL) as compared to Pre (60.3 ± 8.4 mU/mL) ($p<0.05$; Supplementary Fig 1D). In the Low group, plasma creatine

kinase activity was significantly increased 24h (107.9 ± 10.7 mU/mL, $p<0.05$) following eccentric exercise, but was not significantly different at 72h (92.9 ± 12.4 mU/mL) and 96h (91.1 ± 14.3 mU/mL) as compared to Pre (88.4 ± 11.2 mU/mL) ($p<0.05$; Supplementary Fig 1D). Prior to the intervention, there were no significant differences in creatine kinase activity in the High compared to the Low group; there were no differences in creatine kinase activity changes following eccentric exercise.

Skeletal muscle fibre characteristics

Overall: Muscle fibre CSA was significantly greater in type II (7500 ± 355 um^2) compared to type I fibres (6326 ± 205 um^2, $p<0.05$). Muscle fibre perimeter was significantly greater in type II (326 ± 6 um) compared to type I fibres (306 ± 5 um, $p<0.05$). The number of myonuclei per fibre was not different between type II as compared to type I (3.7 ± 0.2 vs 3.6 ± 0.2 myonuclei/fibre, respectively). Myonuclear domain size was significantly greater in type II as compared to type I muscle fibres (2019 ± 88 vs 1805 ± 53 um^2, $p<0.05$, respectively). Muscle C/Fi (2.08 ± 0.1 vs. 1.94 ± 0.1 capillaries per fibre), CFPE index (6.86 ± 0.2 vs. 6.03 ± 0.23 capillaries per fibre · 1000^{-1}, and CD (655 ± 26 vs. 478 ± 29 capillaries/ mm^2) was significantly greater in type I compared to type II muscle fibres, respectively ($p<0.05$). SC distance to nearest capillary was significantly greater in type II compared to type I muscle fibres (15.8 ± 0.7 vs 13.9 ± 0.7 um, respectively, $p<0.05$) at baseline (Pre). Total $Pax7^+$ cell distance to nearest capillary at baseline was negatively correlated to mixed muscle CFPE index ($r = -0.49$, $p<0.05$) across all participants. Type II muscle fibre associated SC distance to nearest capillary at baseline was negatively correlated to type II fibre CFPE index ($r = -0.51$, $p<0.05$; Figure 4D).

High CFPE vs Low CFPE group: Type II muscle fibre CSA and perimeter were significantly greater compared with type I muscle fibre in both groups, with no difference between group

($p<0.05$; Table 3). Interestingly, the proportion of type I muscle fibres was significantly higher in the High group compared with the Low group ($p<0.05$, Table 3). Both type I and type II muscle fibre C/Fi, CFPE index, and CC were significantly higher in the High as compared to the Low group ($p<0.05$; Table 3). There were no significant differences ($p>0.05$) in type I or type II myonuclei per fibre and/or myonuclear domain size between groups.

SC distance to nearest capillary across both type I and type II was significantly lower in the High group as compared to the Low group ($p<0.05$, Table 3).

Mixed muscle SC response

Overall: Following the eccentric contraction protocol, total mixed muscle $Pax7^+$ cells/100 myofibre tended to increase significantly at 6h (14.4 ± 1.1 cells/100 myofibre ($p = 0.056$), increased significantly at 24h (14.9 ± 1.1 cells/100 myofibre; $p<0.05$) and 72h (15.8 ± 1.0 cells/100 myofibre; $p<0.05$) compared to Pre (11.8 ± 0.7 cells/100 myofibre, Supplementary Table 2). The change in total mixed muscle $Pax7^+$ cells/100 myofibre between Pre and 24h ($r = 0.39$, $p<0.05$; Figure 1G) was positively correlated to mixed muscle CFPE index across all participants. The activation status of the SC pool was assessed by colocalizing SC with MyoD before and after the eccentric contraction protocol. Mixed muscle $Pax7^+/MyoD^+$ cells/100 myofibres were significantly elevated at 6h (1.8 ± 0.3 cells/100 myofibre, $p<0.05$), 24h (2.2 ± 0.2 cells/100 myofibre, $p<0.05$), 72h (1.9 ± 0.4 cells/100 myofibre, $p<0.05$) and 96h (1.1 ± 0.2 cells/100 myofibre, $p<0.05$) as compared to Pre (0.4 ± 0.1; Figure 2F). The change in total mixed muscle $MyoD^+/Pax7^+$ cells/100 myofibre between Pre and 6h ($r = 0.40$, $p<0.05$; Figure 1G) and Pre and 72 ($r = 0.37$, $p<0.05$; Figure 1H) was positively correlated to mixed muscle CFPE index across all participants.

High vs. Low CFPE Group: Prior to the intervention, there were no differences in mixed muscle total Pax7+ cells/100 myofibre ($p>0.05$) between the High (11.0 ± 1.2 cells/100 myofibre) and the Low (11.9 ± 1.3 cells/100 myofibre) group. Compared to baseline, total mixed muscle Pax7+ cells/100 fibre were significantly increased at 6h ($p<0.05$); 24h ($p<0.05$) and 72h ($p<0.05$) after the single bout of eccentric exercise in the High group (Figure 1F, Supplementary Table 3). In contrast, total mixed muscle Pax7+ cells/100 myofibre was only significantly increased at 72h ($p<0.05$) during post-exercise recovery in the Low group compared to baseline (Figure 1F, Supplementary Table 3). There was a significantly greater increase in mixed muscle total Pax7+ cells/100 myofibres from Pre to 6h ($p<0.05$); Pre to 24h ($p<0.05$) and a trend for Pre to 72h ($p=0.052$) following eccentric contractions in the High compared with the Low Group.

Prior to the intervention, there were no differences in total MyoD+/Pax7+ cells/100 myofibre in mixed muscle ($p>0.05$) between the High (0.3 ± 0.2 cells/100 myofibre) and the Low (0.2 ± 0.1 cells/100 myofibre) groups. Mixed muscle Pax7+/MyoD+ cells/100 myofibres were significantly higher in the High group at 6h ($p<0.05$), 24h ($p<0.05$), 72h ($p<0.05$), and 96h ($p<0.05$) as compared to Pre (Figure 2F, Supplementary Table 3). In the Low group, Pax7+/MyoD+ cells/100 myofibres in mixed muscle were only significantly elevated at 24h ($p<0.05$) as compared to Pre (Figure 2F, Supplementary Table 3) In comparing the Low and the High mixed muscle SC activation (Pax7+/MyoD+ cells) response to eccentric exercise, we observed that there was a significantly greater increase in the number of mixed muscle Pax7+/MyoD+ cells/100 myofibre from Pre to 6h, and from Pre to 72h post-exercise recovery in the High as compared to the Low group ($p<0.05$, Figure 2F).

Type I and type II muscle fibre SC response

Overall: Prior to the intervention, there was no significant difference between type I-associated (11.5 ± 0.9 cells/100 myofibre) and type II-associated Pax7+ cells/100 myofibres (11.8 ± 1.0 cells/100 myofibre) across all participants ($p>0.05$, Supplementary Table 2). Type I-associated Pax7+ cells/100 myofibres remained unchanged at 6h, 24h, 96h and trended towards a significant increase at 72h ($p=0.09$), as compared to Pre (Supplementary Table 2).

Type II-associated Pax7+ cells/100 myofibres remained unchanged at 6h and 24h but increased significantly at 72h ($p<0.05$), returning to basal levels at 96h as compared to Pre (Figure 3A).

The change in type II-associated Pax7+ cells/100 myofibre between Pre and 6h ($r = 0.45$, $p<0.05$; Figure 3B) and Pre and 24h ($r = 0.42$, $p<0.05$; Figure 3C) following eccentric exercise was positively correlated with type II CFPE index across all participants.

The change in type II associated SC Pax7+ cells/100 myofibre from Pre to 24h ($r = -0.37$, $p<0.05$; Figure 4B) following eccentric exercise was negatively correlated to type II SC distance to nearest capillary at baseline across all participants. There were no relationships between type I associated SC and type I SC distance to nearest capillary at baseline across all participants.

High vs. Low CFPE Group: Prior to the intervention, there were no differences in type I-associated Pax7+ cells/100 myofibre or type II-associated Pax7+ cells/100 myofibre between Low and High groups. ($p>0.05$; Table 3). Type I Pax7+ cells/100 myofibre was not significantly changed at 6h, 24h, 72h, or 96h as following eccentric contractions compared to Pre in the High ($p>0.05$) or Low group ($p>0.05$, Supplementary Table 3). In comparing the Low group to the High group following eccentric exercise, there were no differences between Type I Pax7+/100 myofibres between Pre and any post-exercise time point. Type II Pax7+ cells/100 myofibre were

significantly increased at 6h ($p<0.05$) 24h ($p<0.05$) and 72h ($p<0.05$) following eccentric contractions in the High group, as compared to Pre (Figure 3A, Supplementary Table 3).

In the Low group, Type II Pax7+ cells/100 fibre was only significantly elevated at 72h ($p<0.05$), as compared to Pre (Figure 3A, Supplementary Table 3). In comparing the Low and the High muscle fibre type specific SC response to eccentric exercise, we observed that there was a greater change in the number of Type II Pax7+ cells/100 myofibres from Pre to 6h, and from Pre to 24h post-exercise in the High group as compared to the Low ($p<0.05$, Figure 3A, Supplementary Table 3).

SC distance to nearest capillary response following eccentric

Overall: SC distance to nearest capillary in mixed muscle fibres and/or type I/II-associated SC did not change in response to the single bout of eccentric exercise.

High vs. Low CFPE Group: Type I SC distance to nearest capillary did not change ($p>0.05$) following eccentric contractions in either the High (Pre: 12.2 ± 0.8 ; 6h: 13.0 ± 0.9; 24h: 12.4 ± 0.8; 72h: 12.6 ± 0.7; 96h: 11.9 ± 1.2 μm) or the Low group (Pre: 13.9 ± 0.9; 6h: 12.1 ± 0.7; 24h: 17.0 ± 1.1; 72h: 17.5 ± 1.8; 96h: 15.0 ± 0.9 μm) as compared to baseline values. Type II SC distance to nearest capillary did not change ($p>0.05$) following eccentric contractions in either the High (Pre: 13.3 ± 0.8 ; 6h: 13.1 ± 1.3; 24h: 15.1 ± 1.2; 72h: 16.0 ± 1.0; 96h: 12.5 ± 1.3 μm) or the Low group (Pre: 17.3 ± 0.9; 6h: 17.0 ± 1.0; 24h: 16.9 ± 0.8; 72h: 20.0 ± 1.5; 96h: 16.8 ± 1.1 μm) as compared to baseline values.

Myogenic regulatory factor response

Overall: MyoD, MRF4 and Myogenin mRNA expression were significantly increased at 6h (2.2-, 1.8- and 4.4-fold change, respectively), 24h (1.4-, 2.1- and 4.0-fold change, respectively), 72h (1.6-, 1.6-, and 2.0-fold change, respectively) and 96h (1.4-, 2.3- and 1.9-fold change,

respectively) after the single bout of eccentric exercise. Myf5 mRNA expression was only significantly increased at 24h (1.6-fold change), 72h (1.6-fold change) and 96h (1.9-fold change) following exercise.

High vs. Low CFPE Group: In comparing the Low and the High myogenic gene mRNA expression in response to eccentric exercise, we observed that there was a greater change in MyoD mRNA expression from Pre to 72h in the High group as compared to the Low group ($p<0.05$, Supplementary Table 4). We also observed that there was a trend for a smaller increase in Myogenin mRNA gene expression from Pre to 24h, in the High group as compared to the Low ($p=0.055$).

Cytokine response to repeated eccentric contractions

Overall: Plasma IL-6 concentrations were significantly increased at 6h (2.2 ± 0.2 pg/mL, $p<0.05$, Supplementary Figure 1E) and 24h (1.6 ± 0.1 pg/mL, $p<0.05$) but at 72h (1.2 ± 0.1 pg/mL) was not different anymore from Pre (1.1 ± 0.1 pg/mL). The change in plasma IL-6 between Pre and 6h ($r = 0.42$, $p<0.05$, Supplementary Figure 2A), as well as Pre and 72h ($r = -0.42$, $p<0.05$) following eccentric contractions was negatively correlated to mixed muscle CFPE index across all participants.

High CFPE vs Low CFPE group: Prior to the intervention, there were no significant differences in plasma IL-6 concentrations in the High compared to the Low group. In the High group, plasma IL-6 concentration was significantly increased at 6h (1.9 ± 0.3 pg/mL, $p<0.05$) and 24h (1.8 ± 0.2 pg/mL, $p<0.05$) but was not significantly different at 72h (1.2 ± 0.2 pg/mL) as compared to Pre (1.2 ± 0.2 pg/mL). In the Low group, plasma IL-6 concentration was significantly increased at 6h (2.7 ± 0.2 pg/mL, $p<0.05$) and 24h (1.7 ± 0.1 pg/mL, $p<0.05$) but

was not significantly different at 72h (1.3 ± 0.1 pg/mL) as compared to Pre (1.2 ± 0.2 pg/mL). In comparing the IL-6 to eccentric exercise in the High CFPE as compared to the Low CFPE group, we observed that there was a greater change in plasma IL-6 concentrations from Pre to 6h in the Low group as compared to the High group (Supplementary Figure 2B; $p<0.05$).

DISCUSSION:

In the present study, we observed that there was an enhanced expansion and activation of the SC pool in individuals with high as compared to low capacity for muscle perfusion following eccentric contractions. Therefore muscle fibre capillarization may be a critical factor for the activation and expansion of the SC pool in response to muscle damage in humans. Furthermore, individuals with a higher capacity for muscle perfusion experienced a more rapid force recovery following muscle damage as compared to the group with a lower capacity for muscle perfusion.

SC are indispensable for the repair and/or regeneration of damaged muscle in rodents (Lepper *et al.*, 2011; McCarthy *et al.*, 2011; Sambasivan *et al.*, 2011). In humans, a single bout of high-velocity eccentric contractions results in increased plasma creatine kinase, reduced force production and myofibrillar ultrastructual damage (Beaton *et al.*, 2002; Clarkson & Hubal, 2002; Paulsen *et al.*, 2012). Consequently, eccentric contractions are an effective tool for expansion of the muscle SC pool (Crameri *et al.*, 2004; Dreyer *et al.*, 2006; McKay *et al.*, 2008; McKay *et al.*, 2009; Cermak *et al.*, 2013) though the degree of expansion is dependent on many factors (Snijders *et al.*, 2015). However, the specific factors that determine the degree of activation and expansion of the SC pool are not well understood. In agreement with previous literature, we report that there is an expansion in the SC pool (as determined by total $Pax7^{+}$ cells/100 myofibre) and an increase in SC pool activation (as determined by $MyoD^{+}/Pax7^{+}$ cells/100 myofibre) in the

days following a single bout of eccentric contractions. To better understand factors that determine the degree of activation and expansion of the SC pool we examined whether muscle fibre capillarization may be a determining factor following an acute bout of eccentric contractions in young men. Skeletal muscle capillarization and perfusion is necessary for the delivery of oxygen, growth factors and macronutrients to muscle fibres and resident cell populations alike. We and others have previously already reported an anatomical relationship between muscle SC and capillaries (Christov *et al.*, 2007; Nederveen *et al.*, 2016; Nederveen *et al.*, 2017), suggesting that the proximity of SC to their nearest capillary may be a determining factor in their activation status (Christov *et al.*, 2007; Nederveen *et al.*, 2016). In the present study, there was a positive correlation between the expansion of the total SC pool 24h post-eccentric exercise and mixed muscle CFPE, an index of muscle perfusion, suggesting that the greatest SC pool size expansion was experienced by subjects with the highest capacity for muscle fibre perfusion. When participants were retrospectively divided based on their mixed muscle CFPE index into a High CFPE and Low CFPE group, we observed that there was a greater expansion of the total $Pax7^+$ SC pool in the group with high CFPE (High; CFPE index 7.6 ± 1.0 capillaries · 1000 μm^{-1}) as compared to low CFPE (Low; CFPE index 5.2 ± 0.5 capillaries · 1000 μm^{-1}). This observation was made 6h (~48% vs. ~1% $Pax7^+$ cells/100 myofibre, respectively) and 24h (~73% vs. ~10% $Pax7^+$ cells/100 myofibre, respectively) post-eccentric contractions. Work by Christov and colleagues (2007) supports these findings as they observed a correlation between fibre capillarization and SC content in human deltoid muscle in the resting state, regardless of muscle fibre type. Furthermore, we observed that the High group had a greater activation of the SC pool at 6h (~750% vs. ~450% $MyoD^+/Pax7^+$ cells/100 myofibre, respectively) and 72h (~750% vs. ~300% ± $MyoD^+/Pax7^+$ cells/100 myofibre,

respectively). Interestingly, although the degree of muscle damage was similar between groups (assessed by increases creatine kinase activity, reduction in force production), we observed that the force production returned to baseline again at 96h post-exercise recovery in the High group, whereas this was not the case in the Low group. Together with the greater activation and expansion of the SC pool size observed in the High group during post-exercise recovery these data indicate that individuals with a high CFPE index have an accelerated muscle fibre recovery response following an acute bout of damaging exercise.

In the present study we observed that participants in the High group had a significantly greater percentage of type I muscle fibres as compared with the Low group (~56% vs ~34% Type I fibres, respectively). Type I muscle fibres are more oxidative, associated with more capillaries and/or are perfused to a greater degree than their type II counterparts. Considering that muscle fibre capillaries are shared amongst a mosaic of fibre types in humans, a greater percentage of type I muscle fibres may result in enhanced perfusion of neighboring type II muscle fibres. A greater association with shared muscle fibre capillaries amongst the muscle fibre type mosaic may contribute to not only a greater type II muscle fibre perfusion, but also contribute to a closer proximity between type II Pax7+ SC and the nearest capillary observed in the High group. Consistent with this notion, we observed a negative correlation between type II muscle fibre CFPE index and type II Pax7+ SC distance to their nearest capillary. Considering that SC distance to nearest capillary in type II muscle fibres was negatively correlated with a greater change in type II Pax7+ cells/100 myofibre from Pre to 24h following eccentric exercise, we propose that the link between a greater SC activation/expansion in response to muscle fibre damage may be the reduced spatial proximity to microvascular capillaries. In line with this, we have previously observed that type II SC are located at a further distance from capillaries in older

men as compared to their young counterparts (Nederveen JCSM). Older men typically exhibit an impaired expansion and/or activation response to exercise (McKay *et al.*, 2012; Snijders *et al.*, 2014), as well as lower basal SC content (Verdijk 2007) concomitant with a loss of muscle capillarization (Proctor 2005). Taken together, these data support a relationship between muscle capillarization and functional SC in humans.

We also observed that enhanced capacity for muscle perfusion (i.e., muscle CFPE) or a reduction in the distance of a SC to its nearest capillary was associated with an enhanced activation and expansion of the SC pool in response to eccentric contraction-induced muscle fibre damage in mixed, type I and type II muscle fibres. Previously, we reported that following resistance training, there was an increase in muscle capillarization and also an enhanced activation of SC in response to an acute bout of resistance exercise (Nederveen *et al.*, 2017). Taken together, this suggests that muscle capillarization and the ability of the SC pool to activate and expand following exercise/damage are closely linked. Indeed, it is now well established that activated SC are found at closer proximity to capillaries than their quiescent counterparts (Chazaud *et al.*, 2003; Christov *et al.*, 2007; Nederveen *et al.*, 2016). However, the specific cues for induction of the myogenic program in response to muscle fibre damage remain to be elucidated. The process of SC activation, proliferation and/or differentiation is regulated by a multitude of cytokines and growth factors (e.g., IL-6, IGF-1, myostatin, HGF) (Kadi *et al.*, 2005). Considering that CFPE index is regarded as a proxy measure of microvascular perfusion (Hepple & Mathieu-Costello, 2001; Weber *et al.*, 2006), variations in CPFE index could modify delivery of circulating nutrients and/or growth factors and presumably change the local environment of a SC post exercise/damage (Conboy *et al.*, 2005; Brack & Rando, 2007). In this capacity, few growth factors have been as extensively investigated as the cytokine IL-6, a well

characterized member of the interleukin family. IL-6 is known to respond to various forms of exercise (Pedersen & Febbraio, 2008), but importantly known to play a role in SC function (Toth *et al.*, 2011; McKay *et al.*, 2013). Furthermore, elevations of IL-6 concentration has been shown to be associated specifically with SC proliferation in response to muscle fibre injury (Pedersen & Febbraio, 2008; Toth *et al.*, 2011; McKay *et al.*, 2013). In the current study, we observed that individuals with a lower mixed muscle CFPE index had a greater increase in plasma concentration of IL-6 from Pre to 6h following eccentric exercise. In line with this, we observe that the Low as compared to the High group had a greater change in circulating IL-6 from the Pre to 6h (increase of ~163% vs. ~66%, respectively). Interestingly, the greater plasma concentration of IL-6 observed in the Low group occurred simultaneously with a lesser activation of muscle SC (i.e., $MyoD^{+}/Pax7^{+}$ cells/100 myofibre) over this same time period. Diminished activation of SC in the Low group despite an elevated systemic plasma IL-6 response in comparison to the High group suggests that there may be other mechanisms that regulate the impact of systemic IL-6 concentration upon SC activation and/or proliferation. Previous work has established that the presence of IL-6 can reduce endothelial signalling in some physiological situations (Yuen *et al.*, 2009). Increased plasma IL-6 concentrations may have implications for an increased local SC niche concentration, and may therefore interfere with the observed cellular cross-talk between SC and endothelial cells (Chazaud *et al.*, 2003; Ochoa *et al.*, 2007). Previous work suggests that IL-6 is produced by various resident cell types such as macrophages (Zhang *et al.*, 2013), fibroblasts (Joe *et al.*, 2010) or endothelial cells (Sironi *et al.*, 1989; Yan *et al.*, 1995), the exercising muscle (Steensberg *et al.*, 2000; Pedersen & Febbraio, 2008) as well as SC themselves (Kami & Senba, 2002). Future work should continue to address the paracrine and autocrine functions of increased IL-6 within the local SC niche.

Given the positive relationship between muscle capillarization and the activation and expansion of the SC pool we conclude that the SC response is modulated by cross-talk with endothelial cells within the microvasculature, exposure to circulating signals, or a combination of both. In the future, attention should be focused on study populations who are compromised with respect to muscle capillarization Coggan *et al.*, 1992; Proctor *et al.*, 1995), and/or impaired SC content at rest and in response to exercise (McKay *et al.*, 2012; Snijders *et al.*, 2014) such as in the elderly. These future studies may provide insight into whether the blunted post-exercise SC response in elderly individuals can be improved by increasing muscle fibre capillarization. In conclusion, the present study shows that skeletal muscle fibre capillarization is a significant factor for muscle SC activation and pool size expansion, thereby accelerating the muscle repair response following muscle damage in healthy young men.

Figure Legends

Figure 1

Fig. 1 Fibre type specific satellite cell staining with muscle capillaries. (***A***) Representative image of a MHCI/laminin/CD31/Pax7/DAPI stain of a muscle cross section. Channel views of ***(B***) Merge (***C***) Pax7/DAPI ***(D***) Pax7/CD31 (***E***) Pax7/MHCI/Laminin (***F***) Characterization of the expansion of the total mixed muscle satellite cell (SC) pool before and after 6h, 24h, 72h and 96h following eccentric contractions in the group with a High capillary to fibre exchange (CFPE) index and the group with Low CFPE index. *; Significantly different compared with Pre ($p<0.05$), bar indicates that effect of time is present for both groups. #; indicates a significantly greater increase with time High vs Low group ($p<0.05$). Data are expressed as mean ± sem. Relationship between the expansion of the total SC pool and mixed muscle CFPE following (***G***) Δ24h post-eccentric exercise ($r = 0.39$, $p<0.05$) and (***H***) Δ72h-post exercise ($r = 0.15$, $p>0.05$) across all participants.

Figure 2

Fig. 2 Mixed muscle staining of satellite cell (SC) activation with muscle capillaries. (***A***) Representative image of a CD31/Pax7/MyoD/DAPI stain of a muscle cross section). Channel view of ***(B***) Pax7/DAPI (***C***) MyoD/DAPI ***(D***) Pax7/CD31 (***E***) MyoD/CD31 (***F***) Characterization of the activation status of the SC pool before and after 6h, 24h, 72h and 96h following eccentric contractions in the group with a High capillary to fibre exchange (CFPE) index and the group with Low CFPE index. *; Significantly different compared with Pre ($p<0.05$), bar indicates that effect of time is present for both High and Low group. #; indicates a significantly greater increase with time High vs Low group ($p<0.05$). Data are expressed as mean ± sem. Relationship between the activation of the SC pool ($\Delta MyoD^{+}/Pax7^{+}$ cells) and mixed muscle CFPE following (***G***) Δ6h post-eccentric exercise ($r = 0.40$, $p<0.05$) and (***H***) Δ72h-post exercise ($r = 0.37$, $p<0.05$) across all participants.

Figure 3

Fig. 3 Characterization of the expansion of type II fibre-associated satellite cell (SC) pool following eccentric contractions before and after 6h, 24h, 72h and 96h following eccentric contractions in the group with a High capillary to fibre exchange (CFPE) index and the group with Low CFPE index. (***A***) *; Significantly different compared with Pre ($p<0.05$), bar indicates that effect of time is present for both groups. #; indicates a significantly greater increase with time High vs Low group ($p<0.05$). Data are expressed as mean ± sem. Relationship between the expansion of the type II SC pool and type II CFPE following (***B***) Δ6h post-eccentric exercise ($r = 0.45$, $p<0.05$) and (***C***) Δ24h-post exercise ($r = 0.42$, $p<0.05$) across all participants.

Figure 4

Fig. 4 Relationship between the expansion of the satellite cell (SC) pool from Pre to 24h post-eccentric exercise in a fibre type specific manner and fibre type specific CFPE for (***A***) type I-associated SC ($r = 0.05$, $p = 0.79$) and (***B***) type II-associated SC ($r = -0.39$, $p<0.05$) across all participants. Relationship between fibre type specific Capillary to fibre exchange (CFPE) index and distance of $Pax7_+$ SC to nearest capillary following eccentric exercise prior to eccentric damage for (***C***) type I-associated SC ($r = -0.22$, $p = 0.15$) and (***D***) type II-associated SC ($r = -0.51$, $p<0.05$) across all participants.

Supplementary Figures

Supplementary Figure 1

Sup.1 (**A**) Characterization of the force production (nm) response following eccentric contractions in the overall group. *; Significantly different compared with Pre ($p<0.05$) (**B**) in the group with a high capillary to fibre exchange (CFPE) index and the group with low CFPE index. *; Significantly different compared with Pre in the Low CFPE group ($p<0.05$), bar indicates that effect of time is present for both groups. (**C**) Characterization of the creatine kinase activity (CKA; mU/mL) response following eccentric contractions in the overall group *; Significantly different compared with Pre ($p<0.05$) (**D**) in the group with a high capillary to fibre exchange (CFPE) index and the group with low CFPE index. . Bar indicates significantly different compared with Pre ($p<0.05$), present for both group

Supplementary Figure 2

Sup.2 (**A**) Relationship between the change IL-6 concentrations (expressed as a percentage) from Pre to 6h post-eccentric exercise and mixed muscle CFPE ($r = -0.42$, $p<0.05$) across all participants. (**B**) Characterization of the IL-6 (pg/mL) response following eccentric contractions in the overall group *; significantly different compared with Pre ($p<0.05$) **(C**) Relationship between the change IL-6 concentrations (expressed as a percentage) from Pre to 72h post-eccentric exercise and mixed muscle CFPE ($r = -0.43$, $p<0.05$) across all participants. (**D**) Characterization of the IL-6 response (expressed as a percentage) in the group with a high capillary to fibre exchange (CFPE) index and the group with Low CFPE index. Bar indicates significantly different compared with Pre ($p<0.05$), present for both group. #; indicates a significantly greater change with time High as compared to the Low CFPE group ($p<0.05$).

REFERENCES

Beaton LJ, Tarnopolsky MA & Phillips SM. (2002). Variability in estimating eccentric contraction-induced muscle damage and inflammation in humans. *Canadian journal of applied physiology = Revue canadienne de physiologie appliquee* **27,** 516-526.

Bellamy LM, Joanisse S, Grubb A, Mitchell CJ, McKay BR, Phillips SM, Baker S & Parise G. (2014). The acute satellite cell response and skeletal muscle hypertrophy following resistance training. *PloS one* **9,** e109739.

Bentzinger CF, Wang YX & Rudnicki MA. (2012). Building muscle: molecular regulation of myogenesis. *Cold Spring Harbor perspectives in biology* **4**.

Brack AS & Rando TA. (2007). Intrinsic changes and extrinsic influences of myogenic stem cell function during aging. *Stem cell reviews* **3,** 226-237.

Cermak NM, Snijders T, McKay BR, Parise G, Verdijk LB, Tarnopolsky MA, Gibala MJ & Van Loon LJ. (2013). Eccentric exercise increases satellite cell content in type II muscle fibers. *Medicine and science in sports and exercise* **45,** 230-237.

Chazaud B, Sonnet C, Lafuste P, Bassez G, Rimaniol AC, Poron F, Authier FJ, Dreyfus PA & Gherardi RK. (2003). Satellite cells attract monocytes and use macrophages as a support to escape apoptosis and enhance muscle growth. *The Journal of cell biology* **163,** 1133-1143.

Christov C, Chretien F, Abou-Khalil R, Bassez G, Vallet G, Authier FJ, Bassaglia Y, Shinin V, Tajbakhsh S, Chazaud B & Gherardi RK. (2007). Muscle satellite cells and endothelial cells: close neighbors and privileged partners. *Molecular biology of the cell* **18,** 1397-1409.

Clarkson PM & Hubal MJ. (2002). Exercise-induced muscle damage in humans. *American journal of physical medicine & rehabilitation* **81,** S52-69.

Coggan AR, Spina RJ, King DS, Rogers MA, Brown M, Nemeth PM & Holloszy JO. (1992). Histochemical and enzymatic comparison of the gastrocnemius muscle of young and elderly men and women. *Journal of gerontology* **47,** B71-76.

Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL & Rando TA. (2005). Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* **433,** 760-764.

Crameri RM, Langberg H, Magnusson P, Jensen CH, Schroder HD, Olesen JL, Suetta C, Teisner B & Kjaer M. (2004). Changes in satellite cells in human skeletal muscle after a single bout of high intensity exercise. *The Journal of physiology* **558,** 333-340.

Dreyer HC, Blanco CE, Sattler FR, Schroeder ET & Wiswell RA. (2006). Satellite cell numbers in young and older men 24 hours after eccentric exercise. *Muscle & nerve* **33,** 242-253.

Emslie-Smith AM & Engel AG. (1990). Microvascular changes in early and advanced dermatomyositis: a quantitative study. *Annals of neurology* **27,** 343-356.

Hepple RT. (1997). A new measurement of tissue capillarity: the capillary-to-fibre perimeter exchange index. *Canadian journal of applied physiology = Revue canadienne de physiologie appliquee* **22,** 11-22.

Hepple RT & Mathieu-Costello O. (2001). Estimating the size of the capillary-to-fiber interface in skeletal muscle: a comparison of methods. *Journal of applied physiology (Bethesda, Md : 1985)* **91,** 2150-2156.

Joanisse S, Nederveen JP, Snijders T, McKay BR & Parise G. (2017). Skeletal Muscle Regeneration, Repair and Remodelling in Aging: The Importance of Muscle Stem Cells and Vascularization. *Gerontology* **63,** 91-100.

Joe AW, Yi L, Natarajan A, Le Grand F, So L, Wang J, Rudnicki MA & Rossi FM. (2010). Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nature cell biology* **12,** 153-163.

Kadi F, Charifi N, Denis C, Lexell J, Andersen JL, Schjerling P, Olsen S & Kjaer M. (2005). The behaviour of satellite cells in response to exercise: what have we learned from human studies? *Pflugers Archiv : European journal of physiology* **451,** 319-327.

Kami K & Senba E. (2002). In vivo activation of STAT3 signaling in satellite cells and myofibers in regenerating rat skeletal muscles. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* **50,** 1579-1589.

Lepper C, Partridge TA & Fan CM. (2011). An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. *Development (Cambridge, England)* **138,** 3639-3646.

Livak KJ & Schmittgen TD. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif)* **25,** 402-408.

Mackey AL, Rasmussen LK, Kadi F, Schjerling P, Helmark IC, Ponsot E, Aagaard P, Durigan JL & Kjaer M. (2016). Activation of satellite cells and the regeneration of human skeletal muscle are expedited by ingestion of nonsteroidal anti-inflammatory medication. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **30,** 2266-2281.

McCarthy JJ, Mula J, Miyazaki M, Erfani R, Garrison K, Farooqui AB, Srikuea R, Lawson BA, Grimes B, Keller C, Van Zant G, Campbell KS, Esser KA, Dupont-Versteegden EE & Peterson CA. (2011). Effective fiber hypertrophy in satellite cell-depleted skeletal muscle. *Development (Cambridge, England)* **138,** 3657-3666.

McKay BR, De Lisio M, Johnston AP, O'Reilly CE, Phillips SM, Tarnopolsky MA & Parise G. (2009). Association of interleukin-6 signalling with the muscle stem cell response following muscle-lengthening contractions in humans. *PloS one* **4,** e6027.

McKay BR, O'Reilly CE, Phillips SM, Tarnopolsky MA & Parise G. (2008). Co-expression of IGF-1 family members with myogenic regulatory factors following acute damaging muscle-lengthening contractions in humans. *The Journal of physiology* **586,** 5549-5560.

McKay BR, Ogborn DI, Baker JM, Toth KG, Tarnopolsky MA & Parise G. (2013). Elevated SOCS3 and altered IL-6 signaling is associated with age-related human muscle stem cell dysfunction. *American journal of physiology Cell physiology* **304,** C717-728.

McKay BR, Ogborn DI, Bellamy LM, Tarnopolsky MA & Parise G. (2012). Myostatin is associated with age-related human muscle stem cell dysfunction. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **26,** 2509-2521.

Nederveen JP, Joanisse S, Snijders T, Ivankovic V, Baker SK, Phillips SM & Parise G. (2016). Skeletal muscle satellite cells are located at a closer proximity to capillaries in healthy young compared with older men. *J Cachexia Sarcopenia Muscle*.

Nederveen JP, Snijders T, Joanisse S, Wavell CG, Mitchell CJ, Johnston LM, Baker SK, Phillips SM & Parise G. (2017). Altered muscle satellite cell activation following 16 wk of resistance training in young men. *American journal of physiology Regulatory, integrative and comparative physiology* **312,** R85-R92.

O'Reilly C, McKay B, Phillips S, Tarnopolsky M & Parise G. (2008). Hepatocyte growth factor (HGF) and the satellite cell response following muscle lengthening contractions in humans. *Muscle & nerve* **38,** 1434-1442.

Ochoa O, Sun D, Reyes-Reyna SM, Waite LL, Michalek JE, McManus LM & Shireman PK. (2007). Delayed angiogenesis and VEGF production in CCR2-/- mice during impaired skeletal muscle regeneration. *American journal of physiology Regulatory, integrative and comparative physiology* **293,** R651-661.

Paulsen G, Mikkelsen UR, Raastad T & Peake JM. (2012). Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exercise immunology review* **18,** 42-97.

Pedersen BK & Febbraio MA. (2008). Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiological reviews* **88,** 1379-1406.

Porter MM, Koolage CW & Lexell J. (2002). Biopsy sampling requirements for the estimation of muscle capillarization. *Muscle & nerve* **26,** 546-548.

Proctor DN, Sinning WE, Walro JM, Sieck GC & Lemon PW. (1995). Oxidative capacity of human muscle fiber types: effects of age and training status. *Journal of applied physiology (Bethesda, Md : 1985)* **78,** 2033-2038.

Sambasivan R, Yao R, Kissenpfennig A, Van Wittenberghe L, Paldi A, Gayraud-Morel B, Guenou H, Malissen B, Tajbakhsh S & Galy A. (2011). Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. *Development (Cambridge, England)* **138,** 3647-3656.

Sironi M, Breviario F, Proserpio P, Biondi A, Vecchi A, Van Damme J, Dejana E & Mantovani A. (1989). IL-1 stimulates IL-6 production in endothelial cells. *Journal of immunology (Baltimore, Md : 1950)* **142,** 549-553.

Snijders T, Nederveen JP, McKay BR, Joanisse S, Verdijk LB, van Loon LJ & Parise G. (2015). Satellite cells in human skeletal muscle plasticity. *Frontiers in physiology* **6,** 283.

Snijders T, Verdijk LB, Smeets JS, McKay BR, Senden JM, Hartgens F, Parise G, Greenhaff P & van Loon LJ. (2014). The skeletal muscle satellite cell response to a single bout of resistance-type exercise is delayed with aging in men. *Age (Dordrecht, Netherlands)* **36,** 9699.

Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B & Klarlund Pedersen B. (2000). Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *The Journal of physiology* **529 Pt 1,** 237-242.

Toth KG, McKay BR, De Lisio M, Little JP, Tarnopolsky MA & Parise G. (2011). IL-6 induced STAT3 signalling is associated with the proliferation of human muscle satellite cells following acute muscle damage. *PloS one* **6,** e17392.

Velloso CP. (2008). Regulation of muscle mass by growth hormone and IGF-I. *British journal of pharmacology* **154,** 557-568.

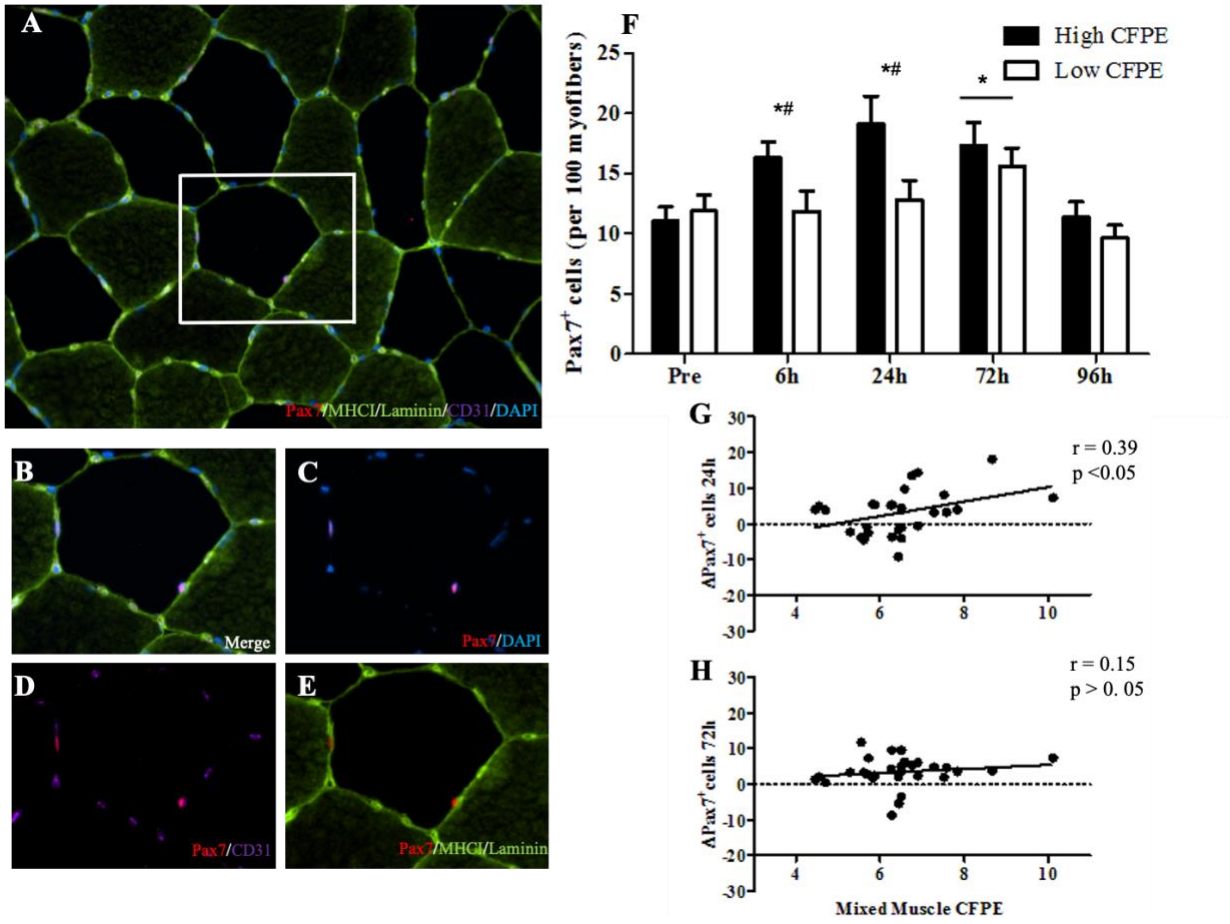
Weber MA, Krakowski-Roosen H, Delorme S, Renk H, Krix M, Millies J, Kinscherf R, Kunkele A, Kauczor HU & Hildebrandt W. (2006). Relationship of skeletal muscle perfusion measured by contrast-enhanced ultrasonography to histologic microvascular density. *Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine* **25,** 583-591.

Yan SF, Tritto I, Pinsky D, Liao H, Huang J, Fuller G, Brett J, May L & Stern D. (1995). Induction of interleukin 6 (IL-6) by hypoxia in vascular cells. Central role of the binding site for nuclear factor-IL-6. *The Journal of biological chemistry* **270,** 11463-11471.

Yin H, Price F & Rudnicki MA. (2013). Satellite cells and the muscle stem cell niche. *Physiological reviews* **93,** 23-67.

Yuen DY, Dwyer RM, Matthews VB, Zhang L, Drew BG, Neill B, Kingwell BA, Clark MG, Rattigan S & Febbraio MA. (2009). Interleukin-6 attenuates insulin-mediated increases in endothelial cell signaling but augments skeletal muscle insulin action via differential effects on tumor necrosis factor-alpha expression. *Diabetes* **58,** 1086-1095.

Zhang C, Li Y, Wu Y, Wang L, Wang X & Du J. (2013). Interleukin-6/signal transducer and activator of transcription 3 (STAT3) pathway is essential for macrophage infiltration and myoblast proliferation during muscle regeneration. *The Journal of biological chemistry* **288,** 1489-1499.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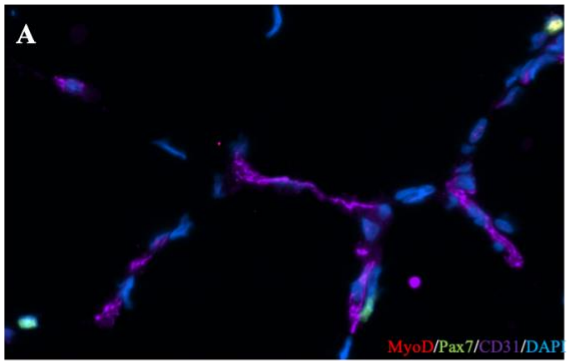
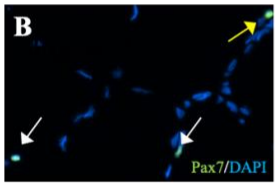
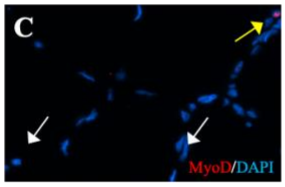
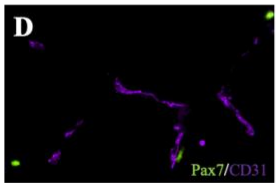
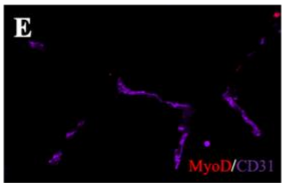
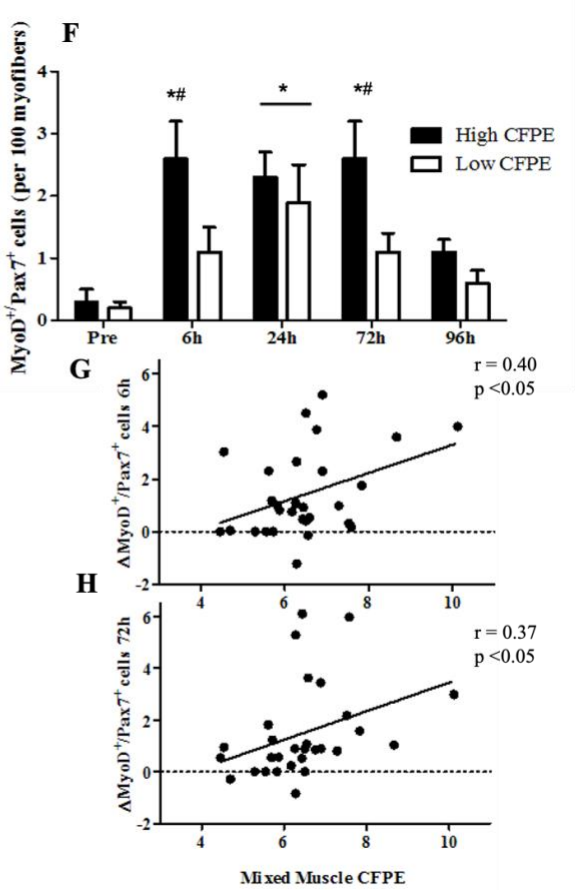

A
MyoD/Pax7/CD31/DAPI

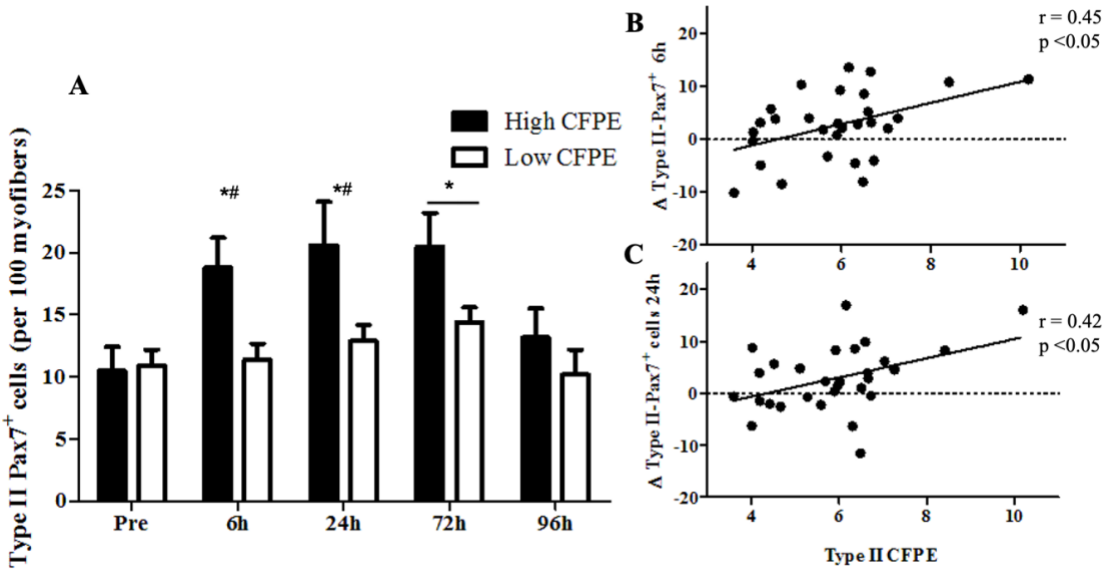

B
Pax7/DAPI

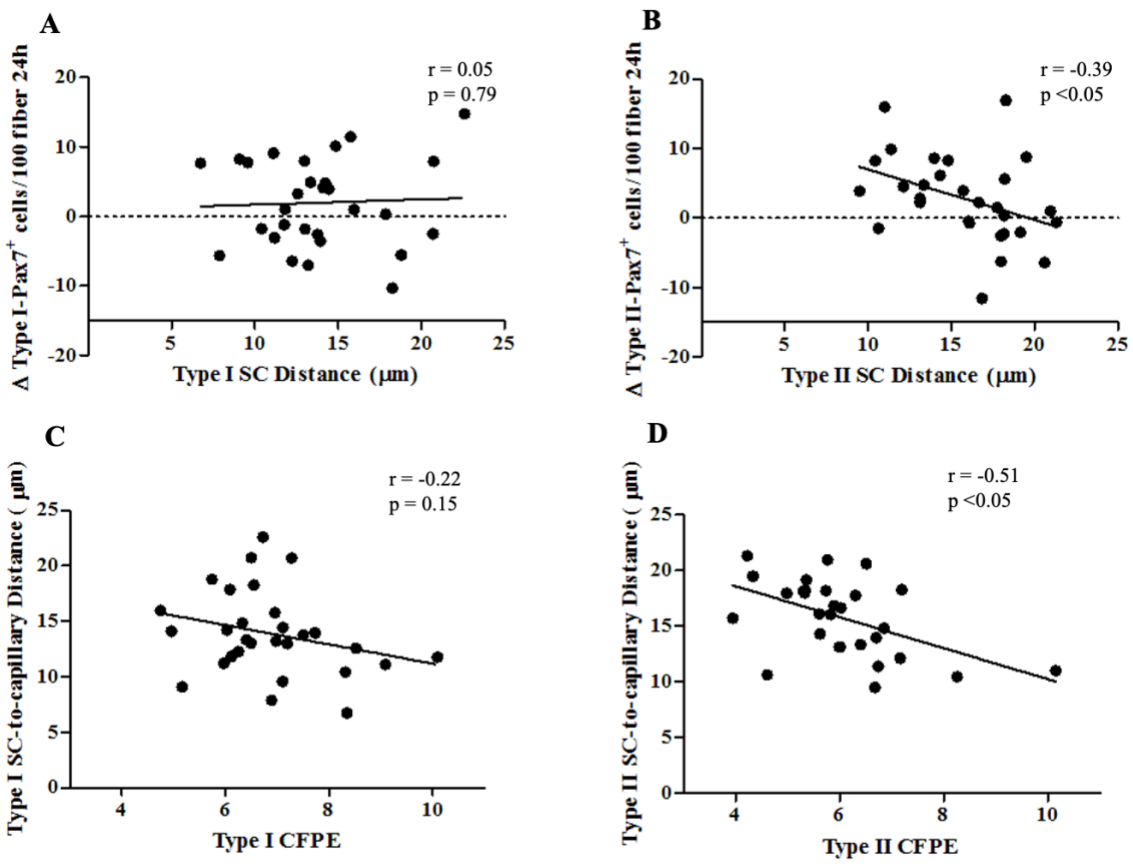

C
MyoD/DAPI


D
Pax7/CD31

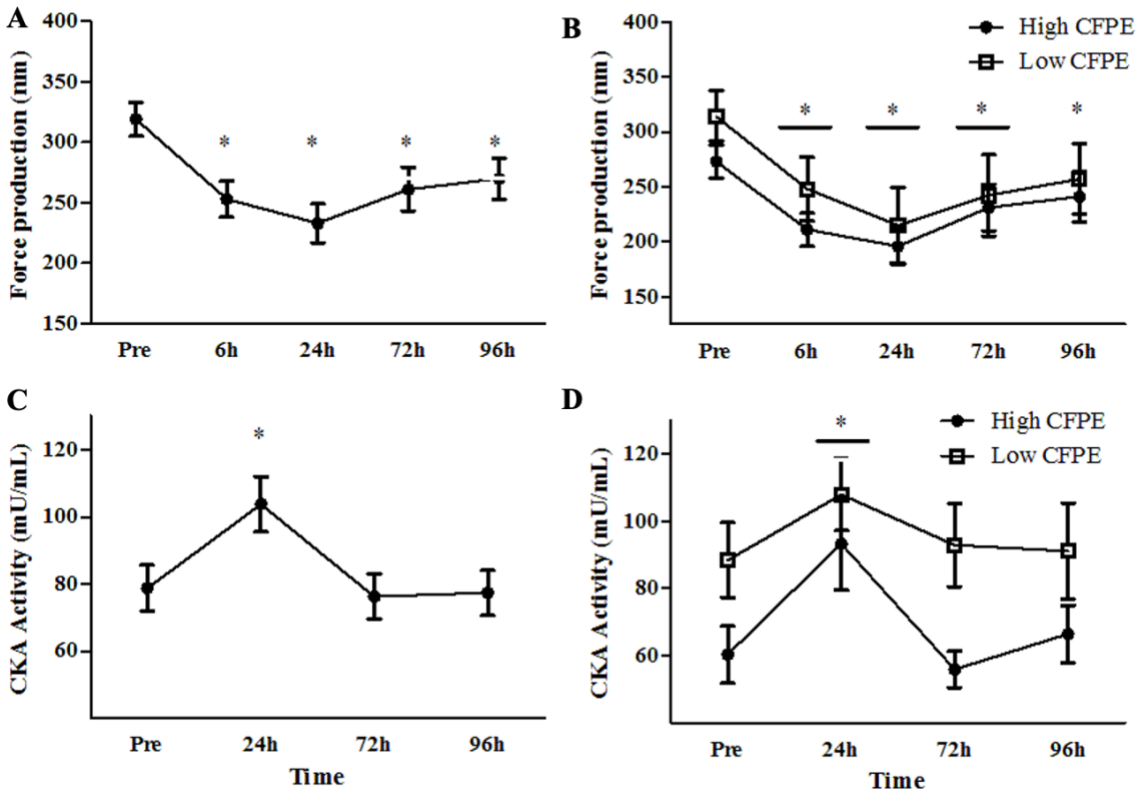

E
MyoD/CD31


F
MyoD+/Pax7+ cells (per 100 myofibers)
*#
*
*#
High CFPE
Low CFPE
Pre
6h
24h
72h
96h
G
r = 0.40
p <0.05
ΔMyoD+/Pax7+ cells 6h
H
r = 0.37
p <0.05
ΔMyoD+/Pax7+ cells 72h
Mixed Muscle CFPE


A
High CFPE
Low CFPE
Type II Pax7+ cells (per 100 myofibers)
25
20
15
10
5
0
*#
*#
*
Pre
6h
24h
72h
96h
B
r = 0.45
p <0.05
Δ Type II-Pax7+ 6h
20
10
0
-10
-20
4
6
8
10
C
r = 0.42
p <0.05
Δ Type II-Pax7+ cells 24h
Type II CFPE


A
r = 0.05
p = 0.79
Δ Type I-Pax7+ cells/100 fiber 24h
Type I SC Distance (µm)
B
r = -0.39
p <0.05
Δ Type II-Pax7+ cells/100 fiber 24h
Type II SC Distance (µm)
C
r = -0.22
p = 0.15
Type I SC-to-capillary Distance (µm)
Type I CFPE
D
r = -0.51
p <0.05
Type II SC-to-capillary Distance (µm)
Type II CFPE

Supplementary
Figure 1



Supplementary
Figure 2