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The Importance of Muscle Capillarization for Optimizing Satellite Cell Plasticity

Joshua P. Nederveen[1], Milan W. Betz[2], Tim Snijders[2], and Gianni Parise[3]

[1]Department of Pediatrics, Faculty of Health Sciences, McMaster University Medical Centre, Hamilton, ON, Canada; [2]NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, Netherlands; and [3]Department of Kinesiology, Faculty of Sciences, McMaster University, Hamilton, ON, Canada

NEDERVEEN, J.P., M.W. BETZ, T. SNIJDERS, and G. PARISE. The importance of muscle capillarization for optimizing satellite cell plasticity. *Exerc. Sport Sci. Rev.*, Vol. 49, No. 4, pp. 284–290, 2021. *Satellite cells are essential for skeletal muscle regeneration, repair, and adaptation. The activity of satellite cells is influenced by their interactions with muscle-resident endothelial cells. We postulate that the microvascular network between muscle fibers plays a critical role in satellite cell function. Exercise-induced angiogenesis can mitigate the decline in satellite cell function with age.* **Key Words:** satellite cells, capillarization, endothelial cells, skeletal muscle, regeneration

Key Points

- Resident satellite cells are indispensable for muscle repair, regeneration, and the maintenance of skeletal muscle tissue.
- Growing evidence suggests an important and reciprocal relation between muscle satellite cells and endothelial cells, owing to their close spatial proximity and important cell-cell interactions.
- Observations of reduced muscle mass with advanced age occur concomitant with decline in muscle satellite and endothelial cell function and content.
- Training-induced angiogenesis occurs alongside improved satellite cell function in aged muscle.

INTRODUCTION

Skeletal muscle is the most abundant tissue in the body, accounting for ~40% of body mass, and is responsible for whole-body locomotion, breathing, maintenance of homeostasis, and fuel utilization (1). Muscle is a metabolically active tissue requiring sufficient delivery of oxygen, nutrients, and blood-borne growth factors. Skeletal muscle also is remarkably dynamic, exhibiting plasticity and regenerative capacity, and is capable of adapting to a variety of stimuli. In part, the plasticity of skeletal muscle is achieved through the activity of the muscle-resident stem cell pool, termed "satellite cells" (SC).

Since their initial discovery by two groups independently in the same year (2,3), muscle SC have been extensively studied for their contribution to skeletal muscle regeneration, maintenance of muscle mass, exercise-driven adaptation, and in relation to a variety of disease states (4).

Skeletal muscle fibers are multinucleate syncytia, with resident myonuclei existing in a postmitotic state. Postnatal muscle is therefore dependent on myonuclear replacement or accretion, which is facilitated by resident muscle SC (5). These cells, typically quiescent "at rest," become activated after a stimulus such as muscle damage. The SC myogenic program is governed by the up- or downregulation of the paired box transcription factor 7 (Pax7) and a transcriptional network referred to as the myogenic regulatory factors. Muscle SC are the primary source of new myonuclei during periods of adaptation, repair, or regeneration and with the ability to self-renew to maintain their basal population (4). This ability of the muscle SC to self-renew and return to a state of quiescence in the niche, between the sarcolemma and basal lamina, is a key aspect of "stemness" and a requirement for the preservation of regenerative capacity (4).

The orchestration of the myogenic program and associated transcriptional factors has been shown to be influenced extensively by a plethora of cytokines and growth factors (4). Situated near the anatomical "niche" of the muscle SC, but outside the basal lamina, are capillaries composed of a monolayer of endothelial cells (ECs), often with pericapillary contractile cells called pericytes. Skeletal muscle is highly vascularized, with individual myofibers interlaced with an expansive network of microvasculature. This network responds by matching increases in energy and metabolic demand with an increase in blood flow to facilitate metabolite removal and oxygen and nutrient delivery. Besides this critical role, there is growing evidence of the close spatial association (6) and interaction (7,8) between muscle SC and the microvascular

network. The interplay between muscle SC and capillaries, local EC, and pericytes may play a critical role in the distribution of these factors necessary for optimal SC function — and represents a growing interest in the field of muscle and exercise physiology. We hypothesize that reduced myofiber capillarization may be a limiting factor for optimal muscle SC function and thereby may limit muscle plasticity in both healthy young and older adult after exercise. The close spatial proximity and anatomical relation between muscle SC and EC may be altered with aging (described in Fig. 1). This review will provide an update on recent research investigating the relation between the myofiber microvascular network, muscle adaptation, and, specifically, SC in both animal and human muscle tissue. Furthermore, the review will focus on the importance of the relation between the microvasculature and SC and how it may be implicated in the development of age-related sarcopenia. Finally, we address exciting next steps and future directions.

Satellite and ECs in Muscle Regeneration

Muscle SC are unequivocally required for skeletal muscle tissue regeneration, evidenced by data from animal models. Skeletal muscle that has been experimentally depleted of the resident SC population fails to regenerate after injury-induced necrosis (9). Fiber necrosis, a hallmark feature that distinguishes regeneration from muscle repair (10), is followed by the reestablishment of fiber cytostructural proteins (11) and eventual complete muscle tissue recovery. Although muscle resident SC are the primary driver of the regenerative process after fiber necrosis, there are a number of resident cells that respond to muscle injury, including but not limited to mesenchymal progenitors/fibroadipogenic progenitors, pericytes (12), and ECs (8). These cells, with a particular focus on ECs, have been shown to contribute to muscle regeneration, repair, and overall muscle health.

During the early stages of the regenerative process in muscle, there is substantial angiogenesis and revascularization at the site of injury with extensive branching and anastomosis (13), driven by the concomitant activation and proliferation of EC populations. When revascularization is blunted, the recovery of muscle remains incomplete (14), and conversely, when vascularization is enhanced, regeneration seems improved (15), suggesting that both EC and SC populations are essential for regeneration.

Satellite and EC interaction

A growing body of evidence supports the notion that the microvasculature is vital to the regulation of muscle SC function, and not only by the delivery of oxygen and nutrients but also by delivering signaling factors. Christov *et al.* (7) identified a
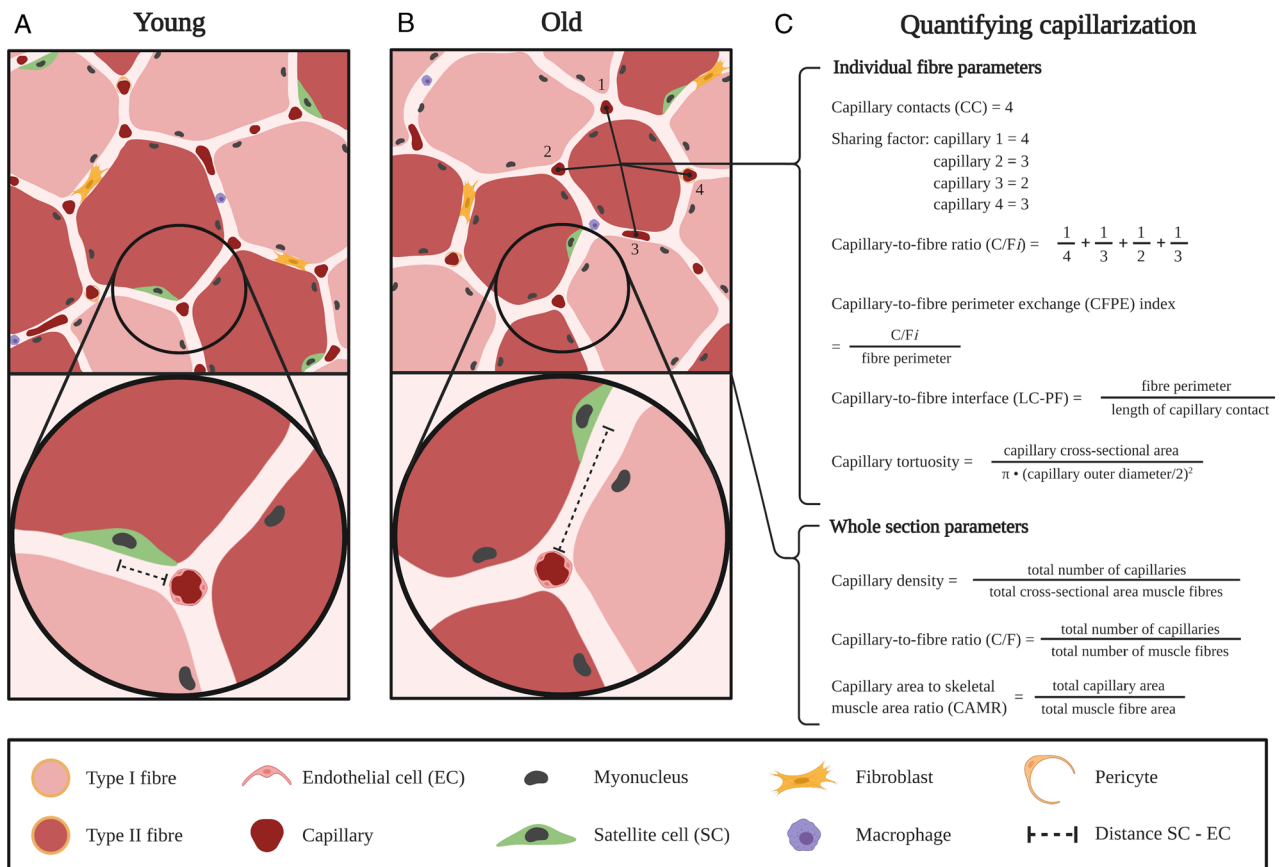
Figure 1. Representation of a muscle fiber cross section in a young (A) and older (B) individual. After a stimulus such as exercise or myofibrillar damage, SC exit the quiescent state and become activated, proliferate, and differentiate by fusing with the damaged muscle fiber. The cross section of an aged muscle fiber (B) is reduced in size and has lower capillarization and fewer SC, predominately in type II fibers. The expanded schematic indicates that muscle SC are located at a further distance from the nearest EC and capillary. We hypothesize that the increased distance reduces cell-to-cell interaction between these cell populations and represents an important mechanism underlying the reduced number of activated SC after a stimulus in older as compared with younger individuals. Muscle-resident cell populations including but not limited to fibroblasts, macrophages, and pericytes exhibit similar interactions with muscle SC. The accurate assessment of muscle capillarization (C), is important for the assessment of muscle perfusion, oxygen exchange, and the spatial relation with muscle SC.

strong correlation between the number of SC and capillaries associated with a myofiber. *In vitro*, coculture experiments demonstrated that ECs enhance myogenic cell proliferation and growth (7,16), whereas myogenic cells are concomitantly proangiogenic (7). Indeed, as myogenic cells progress through differentiation (*i.e.*, from myocytes to myotubes), they stimulate capillary elongation and lumenization (16). These cellular interactions are driven by a plethora of paracrine growth factors including vascular endothelial growth factor (VEGF), insulin-like growth factor 1, platelet-derived growth factor–BB, hepatocyte growth factor, and basic fibroblast growth factor (17). VEGF, the main regulator of angiogenesis and revascularization in adult muscle, has received particular attention in relation to muscle regeneration, as it is secreted from myogenic cells during differentiation (7,18), promotes myogenic cell migration, and protects against myogenic cell apoptosis (17). Evidence suggests that mature capillary EC, *in vivo*, do not produce VEGF but instead express VEGF receptors R1 (Flt-1) and R2 (Flk-1), which also are present on myogenic cells (19). Together, this points to VEGF as a coregulatory factor for both the process of vascular remodeling and concurrent regeneration (17). Consistent with this hypothesis, administration of VEGF complementary DNA via adeno-associated virus (19), recombinant VEGF (20), or a VEGF-coated collagen matrix (21) to injured muscle has been shown to enhance muscle SC proliferation and regeneration *in vivo*. In contrast, conditional deletion of VEGF leads to capillary loss and apoptosis, with a large number of apoptotic cells identified as ECs and muscle SC (22).

More recently, Verma *et al.* (2018) elegantly demonstrated the intimate relation (7,18) between EC and SC through a VEGF-mediated signaling pathway. By using a fluorescent genetic labeling model combined with confocal microscopy, Verma *et al.* (2018) revealed that ~40% of muscle SC were in direct contact with capillary ECs in the tibialis anterior muscle, with ~80% being in direct contact within the murine. soleus, confirming previous observation of a close spatial relation in mice and humans (6,7). Using transcriptome analysis on isolated muscle ECs and SC, Verma *et al.* confirmed that VEGFA (an isoform) is highly expressed in quiescent and active muscle SC (8). A model of muscle SC–specific VEGFA deletion (VEGF$^{\Delta/\Delta}$) resulted in a greater distance between SC and capillary EC *in vivo*, and a reduced ability to facilitate EC migration *in vitro* (8). The SC-VEGF$^{\Delta/\Delta}$ animals also exhibited fewer SC in the early response to cardiotoxin-induced injury. Conversely, muscle SC isolated from animals that expressed twofold greater VEGF (VEGF^{Hyper}) than their wild-type counterparts revealed a significantly shorter anatomical distance between SC and ECs (8). Furthermore, muscle SC demonstrated greater VEGF expression after acute injury via cardiotoxin. Together, these findings provide further support of a functional relation between muscle SC and EC facilitated by VEGF signaling. Although not within the scope of this review, the muscle itself has long been described as a "secretory organ" (23). Many growth factors and cytokines have been shown to be integral for SC function (4) and can be transduced through the muscle-resident capillary network (10,24). Together, muscle capillarization does not only seem to facilitate direct EC-SC interaction but also allow for distribution of growth factor gradients from other cell types.

The importance of vascularization for regeneration in advanced age

In advanced age, the regenerative capacity for muscle is severely reduced (10). There is still considerable debate to whether SC dysfunction with aging is intrinsic (25) or related to the extrinsic environment (26), such as the delivery of key circulating signaling factors. Regardless of mechanism, there seems to be an age-related impairment in the regenerative process in rodent muscles after injury (10). Previous work examining the early response to muscle injury suggests that aged rodents are not able to reestablish muscle cross-sectional area (CSA) 30 d after injury (27,28). We observed that aged (24-month) animals exhibit an impaired regenerative response to cardiotoxin injection (27). However, this was rescued in aged animals that exercise trained for 8 wk before injury (27). Interestingly, we observed that there was greater capillarization in the trained aged animals as compared with their aged sedentary counterparts in line with previous data, suggesting that revascularization earlier in the regenerative response may facilitate improved recovery (14). Taken together, muscle capillarization seems to be a factor in regeneration with aging (10,27).

The Satellite Cell–Capillary Relation in Humans

Observations of a satellite cell–capillary relation *in vitro* or using animal models has more recently been supported by a limited number of studies in human skeletal muscle. We (6,29) and others (7) have shown a close anatomical relationship between SC and capillaries with activated (*i.e.*, nonquiescent or proliferating) SC situated closer to capillaries than their quiescent counterparts.

In human experimental models, muscle biopsies are typically taken at rest and hours/days/weeks after a bout of exercise/damage (*e.g.*, unaccustomed eccentric muscle contraction). We (30) and others (31) have shown that the SC pool expands significantly in response to a single bout of eccentric contraction–induced muscle damage (*e.g.*, 300 eccentric contractions). Significant changes in muscle SC content become detectable as early as ~24 h and peaks around 72 h postdamage (32), which may also occur in a fiber type–specific manner (30). In models of electrically evoked eccentric contraction–induced muscle damage, muscle SC content may peak and remain elevated as long as 192 h (8 d) of postinjury recovery (33).

Recently, we observed that myofiber perfusion may be critical for SC activation in response to a muscle-damaging stimulus. We found that healthy young men with a higher degree of muscle fiber capillarization had a greater activation and expansion of the muscle SC pool in response to a bout of voluntary eccentric contraction–induced muscle damage (30). Taken together, these human data highlight the close anatomical proximity and the correlative relation between muscle SC and ECs during the muscle repair response. The spatial proximity and interaction between muscle SC and ECs for the maintenance of SC content in skeletal muscle is highlighted when the relation becomes "dysregulated." Besides a variety of disease states, research has emerged on the apparent strong relation between the number and function of resident muscle SC and fiber capillarization during age-related sarcopenia (see hypothetical model presented in Fig. 2).
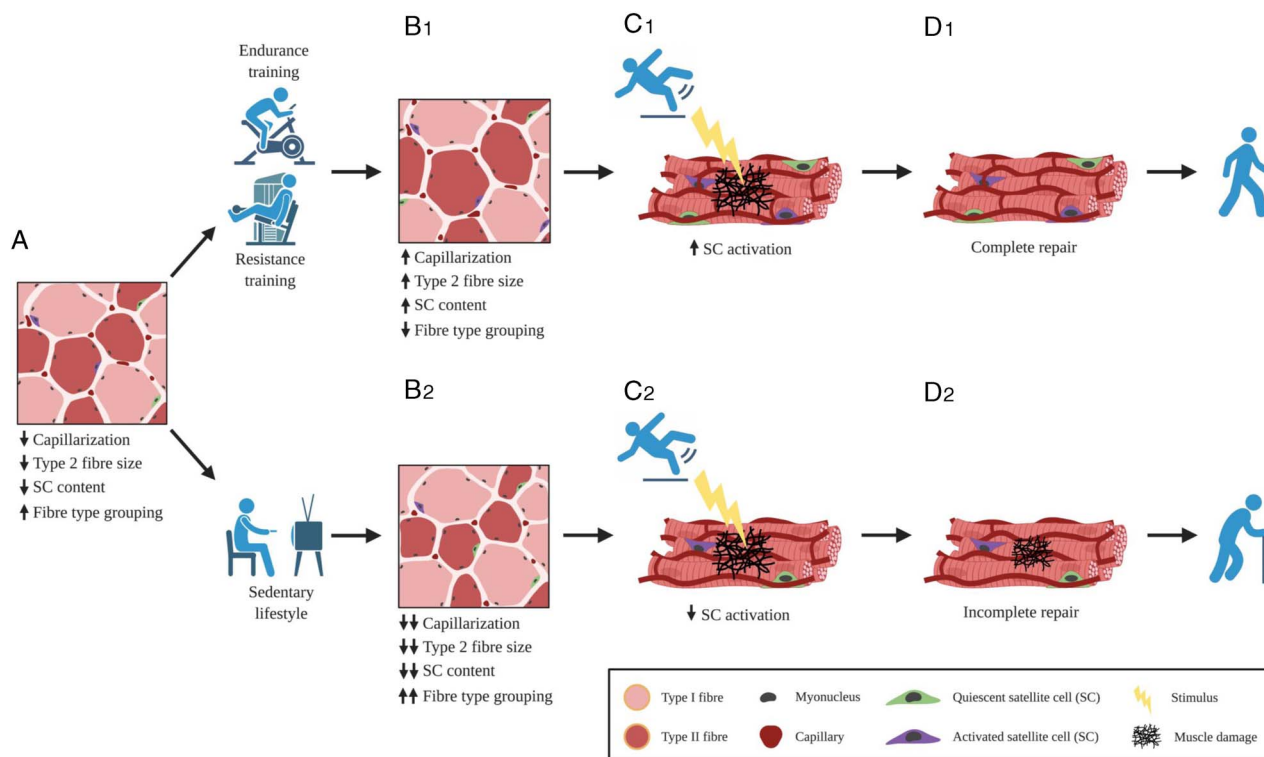
Figure 2. Hypothetical model for the role of exercise training to improve aged muscle. A. Graphical representation of aged human skeletal muscle, exhibiting a loss of type II muscle CSA, increased incidence of fiber type grouping (*i.e.*, loss of normal "checkerboard" pattern), decreased indices of capillarization, and reduced muscle SC content. B_1. Voluntary exercise or physical activity, which leads to an improved microvascular network that can support muscle hypertrophy and increase SC activation and function. After a stimulus such as muscle damage or injury (C_1), the exercise training–induced improvements in capillarization facilitate an enhanced "responsiveness" of muscle SC (including greater activation and expansion of the SC pool), ultimately leading to a more complete muscle repair and return to normal function (D_1). Alternatively, sedentary lifestyle with reduced physical activity (B_2) leads to an exacerbated loss of muscle CSA, capillarization, and SC function with aging. After an injurious stimulus (C_2), sedentary aged muscle with reduced capillarization and SC function may exhibit a slower or "incomplete" repair process (D_2), including the possible decrease in muscle quality (*i.e.*, infiltration of fat or fibrosis), incomplete restoration of CSA, and, ultimately, the prevention of a return to normal function and strength.

Aging, Capillarization, and SC

At the level of the muscle fiber, aging is characterized by a preferential loss of type II myofiber CSA, which is accompanied by a type II fiber–specific reduction in SC number and function (34,35). This observation led to the hypothesis that loss in muscle SC number may be an important contributing factor in the loss of muscle mass with age. Conversely, a conditional SC ablation model (*i.e.*, Pax7^{CreER}-DTA mouse model) suggested that SC are not required to maintain muscle mass/fiber size during aging in sedentary mice (36). In a more recent study, the same research group reported that muscle SC do fulfill a key role in maintaining physical function and myofiber hypertrophy during lifelong activity (37). It is widely accepted that throughout life, human skeletal muscle experiences repeated anabolic and catabolic challenges, and collectively, these events are influential in the ultimate development of age-related sarcopenia. Given the fact that it is impossible to track an individual throughout an entire lifespan, limb immobilization has been used as a model of a short-term (*e.g.*, 5 or 14 d) disuse-induced muscle atrophy. Work using this model has reported a reduction in regenerative potential of skeletal muscle with aging in humans (35). Indeed, after 14 d of single leg knee immobilization followed by 30 d of reloading (*i.e.*, resistance training), young men were able to recover muscle size and a short-term increase in muscle SC content, whereas older men showed incomplete recovery with limited change in the SC pool size (35).

Optimizing SC number has, therefore, been hypothesized to be an important strategy in resisting the onset and development of age-related sarcopenia, which is often exacerbated during times of inactivity (Fig. 2). This may be particularly true for type II fibers as they seem to be preferentially targeted by the sarcopenic process.

Beyond the observed loss of muscle SC content, SC function also seems to decline with aging. In humans, muscle SC function is typically evaluated in muscle biopsies taken at rest and at subsequent time points after a single bout of exercise. The difference in SC pool size and activation status between rest and each postexercise time point is used as a proxy measure of SC function *in vivo*. We demonstrated that type II myofiber SC pool expansion was delayed, with a blunted activation response, in the first ~72 h after a single bout of resistance exercise (38,39) in older adults. Performing eccentric contractions or a bout of resistance exercise allows for the evaluation of myofiber repair or a growth response, respectively, but is not a true reflection of regeneration (40). Karlsen *et al.* were the first to evaluate the muscle SC response in young and older adults during myofiber regeneration (41). In this study, biopsies were taken at rest and 9 d after a single session of electrically stimulated forced eccentric contractions, which resulted in the appearance of necrosis and regenerating fibers. This study reported that there were no significant differences in the change in muscle SC content between young and older individuals during muscle

regeneration. Taking all three models (*i.e.*, eccentric contractions, resistance exercise, and electrical stimulation), these data suggest that during human aging, muscle SC remain functional, but their response or reactivity may be diminished. Impairments in SC function have been suggested as a contributing factor to the development of sarcopenia or reduced muscle adaptive response to exercise training or injury recovery observed with aging. An increasing body of evidence suggests that this may be a result of reduced myofiber capillarization and, in turn, the dysregulation of delivery of circulating factors critical to SC function (10,24,42,43).

Parallel with the loss of muscle SC and fiber CSA, there also seems to be a significant loss of microvasculature with aging (44). We (6) and others (45,46) have observed that there is a selective loss of type II fiber–associated capillaries in aged men as compared with their younger counterparts. Besides the morphology of the microvasculature, vascular function also seems impaired in older adults. Arterial blood flow is reduced in older compared with young adults, both in the postabsorptive and the postprandial state independent of muscle mass (47). This age-related impairment in arterial blood flow is likely related to several factors, including chronic vasoconstriction, lower oxygen demand, and decreased endothelial wall function in older adults (47). Older adults consequently demonstrate a reduced microvascular blood flow response to both exercise and nutrition compared with younger adults, which may also be related to lower myofiber capillarization. This is in keeping with evidence reporting a relation between low muscle capillarization, reduced insulin sensitivity (46), reduced delivery of amino acids, and "anabolic resistance" with aging. Altogether, the age-related impairment in microvascular morphology, but also arterial and microvascular blood flow to the muscle fibers, likely limits the delivery of systemic factors, which has been hypothesized to be a contributing factor in impaired SC function in older adults.

Vascularization during human skeletal muscle adaptation across the lifespan

We (29) and others (48,49) have reported increases in muscle fiber capillarization after resistance training in young adults. Yet, there is less agreement in the literature regarding the angiogenic response after resistance exercise training in older adults (50). Although some studies do (48,51), others do not (52) report an increase in myofiber capillarization after prolonged resistance training in older adults. This discrepancy may be attributed to different exercise intensities (*e.g.*, differing percentage of one-repetition maximum) or exercise volume (*e.g.*, total sets and reps of work performed) used in the various studies.

Given the importance of capillarization as a part of the adaptation to exercise and SC function, the accurate determination of capillarization in skeletal muscle is critical. Although not the focus of the review, there are a variety of methodologies used for the assessment of muscle vascularization (Fig. 1C), and we refer the interested reader to literature regarding quantitation of capillaries in human biopsies (53,54). We have extensively examined the capillary-to-fiber perimeter exchange (CFPE) index, as it reflects the capacity for muscle fiber perfusion by taking the fiber perimeter into account (55) and thus may represent a more accurate evaluation of myofiber capillarization and relevant blood flow. Importantly, CFPE may be a limiting factor for myofiber growth during prolonged resistance exercise

training in older adults (52). This is supported by the observation that older adults with low CFPE at baseline showed no increase in muscle CSA after resistance training, whereas older adults with high baseline CFPE were able to increase type II myofiber CSA. These improvements were mirrored by the response of the muscle SC pool, with only those with the highest CFPE demonstrating an expansion of the SC pool. Furthermore, we have shown that prolonged exercise training can improve the acute SC response to a single bout of resistance exercise in young (29) and older men (56), respectively. In older adults, the improvement in the acute SC response was mainly associated with an exercise-induced increase in muscle fiber capillarization and reduction in SC distance to its nearest capillary. Altogether, these observations provide further support for the hypothesis that the muscle fiber capillary network may be a limiting factor for SC function and subsequent muscle fiber hypertrophy in human skeletal muscle tissue. However, it cannot be excluded that factors (*e.g.*, changes in signaling proteins, neuromuscular adaptations) that change with aging or after exercise training may be of equal importance for impaired muscle SC function in older adults.

SUMMARY AND FUTURE PERSPECTIVES

Evidence clearly supports that there is an intimate relation between muscle SC, ECs, and associated microvasculature. These relation play a key role in muscle homeostasis and fiber adaptation in both healthy young and aging muscle tissue. Although a growing number of studies support this hypothesis, demonstrating true causality in human skeletal muscle tissue remains challenging. The field must continue to adopt new techniques and approaches to better understand the intricate relation between SC and capillaries.

Skeletal muscle also exhibits extensive heterogeneity, not only in fiber type distribution but also capillary distribution. With growing use of automated analysis to accelerate or improve the detection of the capillary–to–muscle fiber interface (57,58), high-throughput histological analysis may continue to improve our understanding of how individual myofibers are supplied by the microcirculation — without relying on biophysical transport models. Taken together, the identification of capillary domains represents a novel model for further exploration of the relation between muscle SC and muscle capillarization (59).

Aerobic exercise training is currently the most effective way to induce remodeling and expansion of the microvascular network in a relatively short period in humans. Hence, it would be of interest to assess whether aerobic exercise training–induced increases in capillarization may have subsequent improvements on the function of other resident cell populations integral to the microvasculature, such as pericytes. Whether improvements in pericyte function influence muscle SC and, subsequently, muscle fiber regeneration, repair and reconditioning would be of significant interest.

Alternatively, utilization of aerobic exercise training as a model may not be ideal as it does not specifically target microvascular remodeling in isolation. Repeated exercise stimulus provides both benefits to the local muscle milieu and also at the systemic level as well. Similarly, blood flow restriction training (*i.e.*, low-load training with vascular occlusion) has been shown to increase indicators of capillarization (60). Further, similar strength benefits as traditional resistance training

have been observed, although blood flow restriction training also promotes local factors known to facilitate muscle SC proliferation, such as VEGF. Instead, by examining animal models that rely on the local, unilateral reduction in functional capillary density, we may be able to better isolate the effect of the microvascular network on muscle SC.

Acknowledgments

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