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Original Article

Aging and the Skeletal Muscle Angiogenic Response to Exercise in Women

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Abstract

Whether aging lowers skeletal muscle basal capillarization and angiogenesis remains controversial. To investigate the effects of aging on skeletal muscle capillarization, eight young (YW) and eight aged (AW) women completed 8 weeks of exercise training. The response and relationships of muscle capillarization, interstitial vascular endothelial growth factor (VEGF), and microvascular blood flow to aerobic exercise training were investigated. Vastus lateralis biopsies were obtained before and after exercise training for the measurement of capillarization. Muscle interstitial VEGF protein and microvascular blood flow were measured at rest and during submaximal exercise at PRE, 1-WK, and 8-WKS by microdialysis. Exercise training increased (20%–25%) capillary contacts of type I, IIA, and IIB fibers in YW and AW. Interstitial VEGF protein was higher in AW than YW at rest and was higher in YW than AW during exercise independent of training status. Differences in muscle capillarization were not explained by secreted VEGF nor were differences in VEGF explained by microvascular blood flow. These results confirm that aging (57–76 years age range) does not impair the muscle angiogenic response to exercise training, although sex differences may exist in similarly trained women and men.

Key Words: Aging—Angiogenesis—Exercise training—Skeletal muscle—VEGF

Even in healthy aging, there are many physiologic changes, including reductions in maximal oxygen consumption (Vo_2MAX) (1–4), insulin sensitivity (5–7), and strength (8,9). Specifically in women, aging is associated with reductions in skeletal muscle mitochondrial capacity (1), fiber size (1,3), capillarization (1,3), and microvascular blood flow (10). In aged (AW) compared with young women (YW), muscle fiber size is smaller and there are fewer capillaries per fiber of type II, but not type I muscle fibers (3). Fewer capillaries per type IIA and per IIB fiber have been observed in aged men (4,11,12); however, reports in aged rodents suggest that capillarization may be increased (13), maintained (14,15), or reduced (16,17). Whether there are fewer capillaries surrounding both type II fiber subtypes (IIA and IIB) with aging in women is unknown.

Exercise training is a well-known stress that can counteract many physiologic decrements observed in aged individuals. In aged men and women, aerobic exercise training increases skeletal muscle capillarization (angiogenesis), an important adaptive response that increases oxygen diffusive capacity to muscle fibers (4,18,19).

Exercise-induced angiogenesis of both type I and II fibers is similar in young (YM) and aged (AM) men, although the initial differences in capillaries surrounding type IIA and IIB fibers between YM and AM persisted after training (12). It is unknown whether the angiogenic response to exercise is similar for both fiber types in AW and YW.

Vascular endothelial growth factor (VEGF) is an important regulator of basal skeletal muscle capillarization and exercise-induced angiogenesis (20,21). In men, acute exercise-induced skeletal muscle interstitial VEGF protein was lower in aged compared with young (40 vs 70 pg/mL) and greater exercise-induced interstitial VEGF protein was associated with more capillaries surrounding type I, IIA, and IIB fibers (4). These results suggest that activity-induced increases in the secretion of VEGF protein may be an important regulator of skeletal muscle capillarization. Comparing results from our previous reports (3,4), basal skeletal muscle VEGF protein is about 25% lower in women compared with men and about 25% lower in aged compared with young individuals such that skeletal muscle VEGF protein is greatest in YM, similar in AM and YW, and lowest in AW. If basal VEGF is lowest in AW and the VEGF response to acute exercise is impaired in AW compared with YW (3), then exercise training-induced angiogenesis might be impaired in AW compared with YW.

The regulation of exercise-induced secretion of VEGF is complex. Moderate, but not high-intensity, exercise increases VEGF secretion (22). Passive leg movement without muscular contraction increases the secretion of VEGF (23). Exercise and passive leg movement increase (24,25), whereas aging lowers muscle microvascular blood flow (10). Whether VEGF secretion is regulated by muscle microvascular blood flow and whether this contributes to age-associated lower exercise-induced VEGF secretion is unknown.

There are two prominent theories on the regulation of muscle capillarization: (i) capillarization is scaled to fiber size (26); and (ii) capillarization is scaled to metabolic demand, specifically mitochondrial capacity (27). The effects of aerobic exercise training on the relationship between muscle capillarization and fiber size is poorly understood. In the current report, we hypothesized that: (i) the relationship between muscle capillarization and fiber size would be altered by exercise training; (ii) in AW, compared with YW, the angiogenic response to exercise would be impaired and would be associated with low exercise-induced secreted VEGF protein; and (iii) low exercise-induced age-associated secretion of VEGF protein would be associated with low microvascular blood flow.

Methods

Subjects

Eight sedentary young (YW; range 20–29 years) and eight sedentary aged (AW; range 57–76 years) women volunteered to participate in the study after receiving written and verbal explanations of the content and intent of the study in accordance with the University & Medical Center Institutional Review Board. All subjects were healthy nonsmokers, with no history of cardiopulmonary disease. Subject characteristics are listed in Table 1. Subjects were carefully prescreened to preclude participation by individuals with overt cardiovascular disease. Subjects taking medications for cardiovascular disease were excluded. Sedentary subjects were defined as participating in less than 1 hour of strenuous physical activity per week. To minimize the potential for estradiol influences in the current study, YW were studied during the menses phase of the menstrual cycle or during the low estradiol phase of their oral contraceptive treatment. All AW were postmenopausal. We have shown previously that this design results in similar estrogen levels on the day of testing in YW and AW (3).

Table 1. Subject Characteristics Before the Initiation of Aerobic Exercise Training

	Young	Aged
Age, y	23 ± 1	65 ± 2*
Height, m	1.67 ± 0.02	1.60 ± 0.03
Mass, kg	62.6 ± 3.1	64.9 ± 4.0
Body fat (%)	24.5 ± 1.8	29.5 ± 1.1*
Fat-free mass, kg	46.4 ± 2.1	45.2 ± 3.5
Heart rate at maximum exercise (bpm)	187 ± 4	159 ± 4*

Notes: *Significant effect of age. Mean ± *SE*. *N* = 8/group.

Vo_2max and Body Composition

Maximal oxygen consumption (Vo_2MAX) was measured on an electronically braked cycle ergometer (Lode, Excalibur Sport, Groningen, Netherlands) by open circuit spirometry (True Max 2400, Parvo Medics, Salt Lake City, UT) before (PRE) and after the 8-week (8-WKS) exercise training program. The test began with a 5-min warm-up at 50 W for YW and 25 W for AW. After the warm-up, the workload was increased 25 W every 2 minutes until volitional fatigue. Before the initiation of exercise training, body density (D_b) was determined via hydrostatic weighing. Residual volume was measured by oxygen dilution (28). Body fat percentage was determined from D_b based on the two-compartment model (29).

Exercise Training

Subjects were enrolled into an 8-week aerobic exercise training program. Cycle ergometer exercise was performed at a workload that elicited a heart rate equivalent to 65% of Vo_2MAX as determined by the initial exercise test. Heart rate was monitored throughout each exercise training bout and the exercise workload was routinely increased during the training program to maintain an exercise heart rate equivalent to 65% of Vo_2MAX. During the first week, subjects trained every day for 1 hour/session. During weeks 2–8, subjects exercised 4 days per week for 1 hour/session.

Submaximal Exercise and Muscle Biopsies

Before the initiation (PRE) and after 1 week (1-WK) and 8 weeks (8-WKS) of the exercise training program, subjects completed 40 minutes of acute cycle ergometer exercise (20 minutes exercise at 30% of Vo_2MAX, 5 minutes rest, and 20 minutes of exercise at 65% of Vo_2MAX). Before the commencement of the acute exercise bout, a muscle biopsy was obtained from the vastus lateralis (PRE and 8-WKS) and microdialysis probes (PRE, 1-WK, and 8-WKS) were inserted in the contralateral leg for the collection of muscle interstitial dialysate for the measurement of microvascular blood flow (at 8-WKS AW only) and VEGF protein. After a 60-minute equilibration period, dialysate from the muscle interstitial space was collected for 60 minutes at rest (REST) and during the acute exercise bout (EX). At 8-WKS, the biopsy was obtained 16 hours after the last exercise training bout. Legs for each procedure were alternated between biopsies and microdialysis for a given visit, and for microdialysis between visits. Microdialysis samples for microvascular blood flow were stored at 4°C and analyzed within 48 hours, whereas samples for VEGF protein were stored at –80°C until analysis. The biopsy sample was oriented in an OCT-tragacanth mixture, frozen in liquid

nitrogen cooled isopentane, and stored at –80°C until processing for the measurement of muscle morphometry and capillarization.

Microdialysis, Microvascular Blood Flow, and VEGF Protein Analysis

For microvascular blood flow, a 20-kDa pore size microdialysis probe (CMA 20 Elite, CMA Microdialysis, North Chelmsford, MA) was inserted into the vastus lateralis and perfused with 10 mmol/L ethanol in 0.9% saline using a CMA/102 microinfusion pump (CMA/Microdialysis, Stockholm, Sweden) as previously described (10). Ethanol concentrations of the perfusate and dialysate were analyzed using an enzymatic–fluorometric method based on the conversion of ethanol and nicotinamide adenine dinucleotide (NAD) to acetaldehyde and reduced NAD (NADH) (30). The ethanol outflow-to-inflow ratio was calculated as $(\text{Ethanol})_{\text{dialysate}}/(\text{Ethanol})_{\text{perfusate}}$, which is inversely related to local blood flow in a nonlinear fashion, and was converted to blood flow units ($\text{mL} \times \text{min}^{-1} \times 100\,\text{g}^{-1}$) using the equations of Wallgren and colleagues (31). The microdialysis–ethanol technique reflects the microvascular blood flow in the local environment of the microdialysis probe rather than gross blood flow through the limb or muscle bed.

For VEGF secretion, a second microdialysis probe with a 100-kDa pore size (CMA/20, CMA Microdialysis, North Chelmsford, MA) was inserted percutaneously into the vastus lateralis. This probe was perfused with Ringer's solution using a CMA/102 microinfusion pump (CMA/Microdialysis, Stockholm, Sweden). A commercial VEGF enzyme-linked immunosorbent assay kit was used according to the manufacturer's instructions (R&D Systems, Minneapolis, MN).

Morphometry, Morphology, and Histochemistry

Muscle tissue from the muscle biopsies was sectioned to a thickness of 10 μm on a cryostat, mounted on slides, and kept at –20°C until fixation. For two YW and two AW, PRE and 8-WKS samples were lost due to a cryostat malfunction, thus morphometry data reflects an $N = 6$ for both YW and AW. Serial sections were stained for capillaries using the double stain technique (32) as modified by Porter and colleagues (33) and for fiber type by use of a myosin ATPase stain (34) as described previously (4). The myosin ATPase stain identifies muscle fibers as type I, IIA, or IIB fibers (34). Despite the fact that human skeletal muscle identified as type IIB by histochemical analysis actually expresses type IIX (35), in the present report, fiber types were identified using the myosin ATPase stain nomenclature used in the original work. Representative photomicrographs of muscle stained for capillaries and fiber type are in Figure 1.

Muscle sections were viewed under a light microscope (Nikon 400) and a digital image taken of the section (Nikon Coolpix 990) as described previously (36). Capillaries were quantified manually from

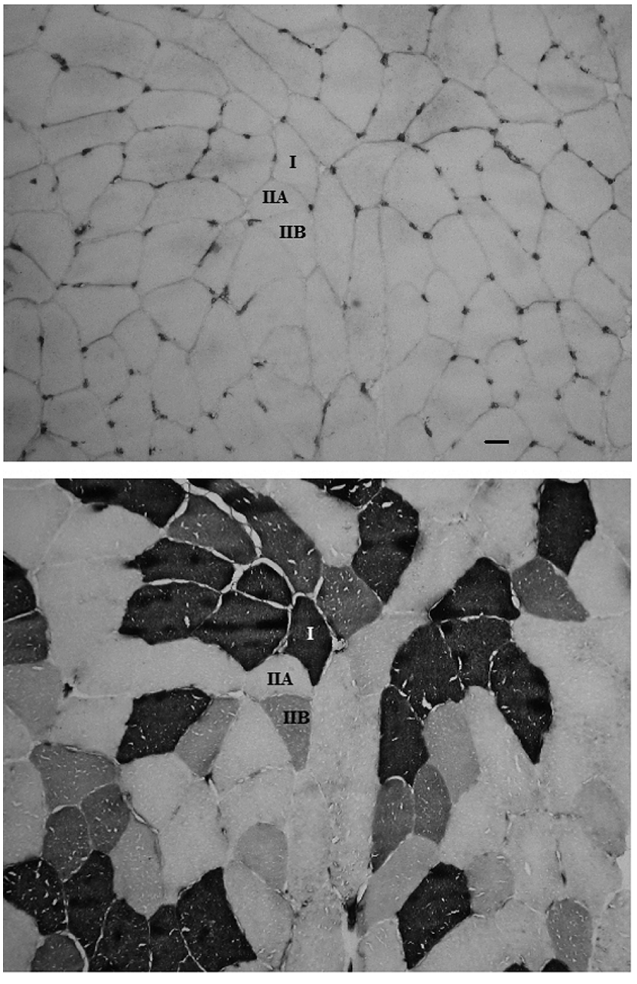


Figure 1. Representative photomicrograph of muscle stained for capillaries (top) and fiber type (bottom). Capillaries appear as dark between fibers. Letters identify fiber types and a given letter identifies the same fiber in the serial sections. Bar = 50 μm.

the digital image on individual fibers. The following indexes were measured: (i) the number of capillaries around a fiber (capillary contacts [CC]), (ii) the capillary-to-fiber ratio on an individual-fiber basis (C/F_i), and (iii) the number of fibers sharing each capillary (sharing factor). Capillary density was calculated by using the fiber as the reference space. Capillary-to-fiber perimeter exchange index (CFPE) was calculated as an estimate of the capillary-to-fiber surface area. Quantification of the capillary supply was performed on at least 50 fibers by randomly selecting a fiber in an artifact-free region. Fiber cross-sectional area (FCSA) and perimeter were measured with the image-analysis system and commercial software (SigmaScan, Jandel Scientific) calibrated to transform the number of pixels (viewed on a computer monitor) into micrometers from an image of the myosin ATPase stain.

Statistical Treatment

For Vo_2MAX and muscle fiber characteristics, a two-way mixed-plot factorial analysis of variance (Age × Training Status [PRE and 8-WKS]) with repeated measures on training status was used. For microvascular blood flow, a two-way mixed-plot factorial analysis of variance (Age + Training Status × Acute Exercise [REST/EX]) with repeated measures on training status was used. For interstitial VEGF protein, a three-way mixed-plot factorial analysis of variance (Age × Training Status × Acute Exercise) was performed with repeated measures on training status and acute exercise. After a significant *F* ratio, a Bonferroni post hoc analysis was used. Unpaired Student's *t* tests were used to compare differences in all other variables between YW and AW. Linear regression was performed to investigate associations: (i) at PRE between REST interstitial VEGF protein and fiber type–specific CC; (ii) at PRE between EX interstitial VEGF protein and fiber type–specific CC at PRE; and (iii) at PRE, 1-WK, and 8-WKS between REST or EX interstitial VEGF protein and the change in fiber type–specific CC between PRE and 8-WKS. The rationale for selectively performing these regressions was to investigate: (i) whether resting interstitial VEGF protein explained individual differences in muscle capillarization in untrained individuals; (ii) whether exercise-induced interstitial VEGF protein explained individual differences in muscle capillarization in untrained individuals; and (iii) whether rest or exercise-induced interstitial VEGF protein during an exercise training program explained individual differences in exercise-induced angiogenesis. Linear regression was also used to investigate associations between VEGF and microvascular blood flow at rest or during exercise at each specific time point PRE, 1-WK, and 8-WKS. Data were normally distributed (D'Agostino & Pearson normality test). Significance was established at $p \leq .05$ for all statistical sets and data reported are mean ± *SE*.

Results

AW were ~40 years older than YW (Table 1). There were no differences in height, mass, or fat-free mass between groups. Body fat (%) was higher and heart rate at maximum exercise was lower in AW compared with YW. As anticipated, absolute and relative Vo_2MAX were lower in AW compared with YW (Table 2). There was a main effect of 8 weeks of aerobic exercise training to increase Vo_2MAX in YW and AW.

There were no differences in muscle FCSA between YW and AW either before or after training (Table 3). There was no difference in fiber type percentage or % area of fiber types between YW and AW. Exercise training decreased type IIB fiber type percentage and IIB % area in YW and AW.

Muscle CC and C/F_i surrounding type I fibers were ~35% higher and capillary density of type IIA fibers ~25% greater in AW compared with YW independent of training status (Table 4). Exercise training increased capillarization (CC, C/F_i, and CFPE) ~20–25% of type I, IIA, and IIB fiber types in AW and YW, and there was no difference in the angiogenic response to exercise training between YW and AW. Exercise training also increased type I capillary density ~20% in YW and AW.

When the means of each woman were analyzed, linear regression revealed significant correlations between FCSA and C/F_i of type I ($r = .689$) and type IIA ($r = .593$) and trend of type IIB ($r = .575$; $p = .051$) in untrained (Figure 2). When analyzed by age group, there was a significant correlation only between FCSA and C/F_i of type IIA fiber in AW ($r = .865$). After completion of the aerobic exercise training, there were no significant correlations identified between FCSA and C/F_i of any fiber type whether analyzed with all women or by age group, suggesting that aerobic exercise training alters the relationship between capillarization and fiber size.

To further investigate the potential regulatory role of muscle fiber size on capillarization (26), the cross-sectional area of individual fibers was plotted against the fibers' individual capillary-to-fiber ratio for type I and II fibers for YW and AW before and after training (Figure 3). There was a significant relationship between FCSA and C/F_i for YW and AW for type I and II fibers before and after training. When comparing the y-intercept and slope within a given fiber type, exercise training increased the y-intercept (~50%) without changing the slope of either fiber type in YW and AW. With aging, the y-intercept was lower and the slope was greater for both fiber types independent of training status.

REST and EX interstitial VEGF protein for YW and AW at PRE, 1-WK, and 8-WKS are shown in Figure 4. There were no interactions between age, training status (PRE, 1-WK, 8-WKS), and acute exercise (REST/EX) (Age × Training Status × Acute Exercise; $p = .579$), age and training status (Age × Training Status; $p = .840$), or between training status and acute exercise (Training Status × Acute Exercise; $p = .636$). There was a significant interaction between age and acute exercise (Age × Acute Exercise; $p = .025$). Acute exercise increased interstitial VEGF protein in YW and AW independent of training status (PRE, 1-WK, or 8-WKS), but differences were present with AW REST > YW REST and YW EX > AW EX. There were no associations between PRE REST or EX interstitial VEGF protein and PRE fiber type–specific CC. There were no associations between REST and EX interstitial VEGF protein at any time point (PRE, 1-WK, or 8-WKS) and the change in fiber type–specific CC.

Muscle microvascular blood flow at rest and during exercise was monitored using microdialysis. There was a Main Effect of acute exercise to increase microvascular blood flow (Figure 5). There was no difference at rest or exercise between YW and AW at any exercise training status time point. Linear regression was performed to identify whether microvascular blood flow was predictive of secreted VEGF either at rest or during exercise at each time point. In contrast to our hypothesis, there were no relationships identified ($p > .10$) between interstitial VEGF protein and microvascular blood flow at any training status time point either at rest or during acute exercise.

Discussion

The principal finding from the current report is that even when basal skeletal muscle capillarization is well maintained in AW, 8 weeks of aerobic exercise training promotes angiogenesis in both AW and YW. Interestingly, angiogenesis occurred around type I, IIA, and IIB

Table 2. Absolute and Relative Maximal Oxygen Consumption (Vo_2max) Before (PRE) and After 8 Weeks (8-WKS) of Aerobic Exercise Training in Young Women (YW) and Aged Women (AW)

	YW PRE	YW 8-WKS	AW PRE	AW 8-WKS
Absolute Vo_2max (L/min)*,†	2.19 ± 0.13	2.48 ± 0.14	1.55 ± 0.13	1.62 ± 0.14
Relative Vo_2max (mL/kg/min)*,†	35.4 ± 2.3	39.1 ± 2.3	24.5 ± 2.7	25.4 ± 2.3

Notes: Exercise training increased absolute and relative Vo_2max. Absolute and relative Vo_2max were lower in aged. There were no significant interactions.

*Main effect of exercise training.

†Main effect of age. Mean ± *SE*. *N* = 8/group.

Table 3. Skeletal Muscle Fiber Type Characteristics Before (PRE) and After 8 Weeks (8-WKS) of Aerobic Exercise Training in Young Women (YW) and Aged Women (AW)

	YW PRE	YW 8-WKS	AW PRE	AW 8-WKS
Fiber area, μm²				
Type I	3,313 ± 88	3,336 ± 142	3,599 ± 263	3,865 ± 268
Type IIA	3,738 ± 331	3,690 ± 388	2,906 ± 321	4,079 ± 635
Type IIB	2,924 ± 149	2,854 ± 189	2,280 ± 298	2,752 ± 219
Fiber perimeter, μm				
Type I	237 ± 5	243 ± 4	253 ± 10	262 ± 10
Type IIA	252 ± 11	248 ± 12	229 ± 13	262 ± 12
Type IIB	232 ± 8	222 ± 7	202 ± 13	221 ± 8
Fiber type (%)				
Type I	45.2 ± 8.3	52.7 ± 3.7	47.8 ± 8.1	51.3 ± 5.0
Type IIA	29.7 ± 4.9	35.2 ± 2.9	35.9 ± 6.9	35.9 ± 3.8
Type IIB*	25.1 ± 6.7	12.1 ± 1.4	16.3 ± 4.1	12.8 ± 3.4
Area of fibers (%)				
Type I	45.3 ± 7.9	51.8 ± 4.2	54.1 ± 9.0	53.0 ± 4.8
Type IIA	33.1 ± 5.5	38.4 ± 3.1	33.6 ± 7.4	37.1 ± 3.9
Type IIB*	21.6 ± 5.7	9.9 ± 1.6	12.2 ± 3.0	9.8 ± 2.8

Notes: There were no significant interactions identified between age and training status (Age × Training Status) for any measured outcome variable. There were no significant interactions.

*Main effect of exercise training.

†Main effect of age. Mean ± *SE*. *N* = 6/group.

fibers in response to exercise training in both YW and AW, which is in contrast to the angiogenesis of only type I and IIA in similarly trained young and aged men (12). In addition, there were no relationships identified between muscle interstitial VEGF and microvascular blood flow, suggesting that differences in interstitial VEGF are not explained by differences in microvascular blood flow.

There is little data on muscle capillarization in AW and even less on exercise-induced angiogenesis. Previously, we reported fewer capillaries surrounding type II, but not type I muscle fibers in AW compared with YW (3). In the current report, we planned to expand upon this finding to identify within the type II muscle fiber population (IIA and IIB) in women whether there are fewer muscle capillaries surrounding both IIA and IIB fibers as has been previously reported in aged versus young men (4,12,19). Surprisingly, capillarization of type I fibers was greater and that of II fibers was not different in AW compared with YW. Thus, in relative terms, AW had greater basal capillarization than previous aged cohorts in which fiber type–specific capillarization has been reported (4,12,19) even in women (3). Although all of the subjects in the current investigation

Table 4. Skeletal Muscle Capillarization Before (PRE) and After 8 Weeks (8-WKS) of Aerobic Exercise Training in Young Women (YW) and Aged Women (AW)

	YW PRE	YW 8-WKS	AW PRE	AW 8-WKS
Capillary contacts				
Type I*,†	3.31 ± 0.18	3.71 ± 0.18	4.19 ± 0.49	5.12 ± 0.46
Type IIA*	3.14 ± 0.28	3.58 ± 0.16	3.19 ± 0.43	4.53 ± 0.58
Type IIB*	2.73 ± 0.23	3.38 ± 0.20	2.63 ± 0.38	3.78 ± 0.43
Individual capillary-to-fiber ratio				
Type I*,†	1.22 ± 0.05	1.43 ± 0.07	1.58 ± 0.22	1.94 ± 0.23
Type IIA*	1.13 ± 0.10	1.34 ± 0.07	1.17 ± 0.18	1.68 ± 0.26
Type IIB*	0.95 ± 0.09	1.26 ± 0.07	0.94 ± 0.15	1.34 ± 0.17
Sharing factor				
Type I	2.84 ± 0.06	2.76 ± 0.09	2.84 ± 0.07	2.81 ± 0.08
Type IIA	2.91 ± 0.05	2.77 ± 0.09	2.91 ± 0.07	2.89 ± 0.07
Type IIB	2.93 ± 0.03	2.83 ± 0.10	2.90 ± 0.05	3.03 ± 0.07
Capillary density, capillaries × mm^{-2}				
Type I*	426 ± 22	510 ± 38	475 ± 51	569 ± 51
Type IIA	325 ± 30	394 ± 38	437 ± 39	463 ± 72
Type IIB	365 ± 25	476 ± 43	463 ± 63	514 ± 84
CFPE, capillaries × 1,000 μm^{-1}				
Type I*	5.24 ± 0.16	6.26 ± 0.42	6.17 ± 0.73	7.66 ± 0.68
Type IIA*	4.50 ± 0.31	5.47 ± 0.37	5.04 ± 0.52	6.38 ± 0.80
Type IIB*	4.30 ± 0.33	5.77 ± 0.43	4.67 ± 0.61	5.85 ± 0.77

Notes: There were no significant interactions identified between age and training status (age × training status) for any measured outcome variable. CFPE, capillary-to-fiber perimeter exchange index.

*Main effect of exercise training. There were no significant interactions.

†Main effect of age. Mean ± *SE*. *N* = 6/group.

were sedentary at baseline, one possibility for the greater capillarization in AW could be greater informal, unstructured physical activity than YW, which was not measured.

There was an increase in capillarization of type IIB fibers with training in women that was not observed in similarly exercise-trained men (4). One possibility for this discrepancy might be greater recruitment of type IIB fibers during the exercise training in women, but not men. Recruitment of type IIB fibers would result in fiber type adaptation to a more oxidative phenotype and thus transition from IIB to IIA. Consistent with this, the percentage of type IIB fibers significantly decreased ~10% in women with training, whereas we did not observe a change in men (~0% pre- to posttraining) (12). Waters and colleagues (37) reported that increases in muscle capillarization precede shifts in fiber type with voluntary wheel running in mice. However, fibers that remain the most glycolytic (type IIb + II d/x in mice) did not demonstrate an angiogenic response. Although it is not possible to determine the fiber type origin or concomitant angiogenesis that would precede fiber type shifting, the current findings highlight a pool of type IIB fibers around which angiogenesis occurs in response to exercise training but in which fiber type shifting to type IIA does not subsequently proceed. Greater type IIB capillarization has been reported in highly trained aged men compared with fit younger and older men (19). However, further research is necessary to identify why an identical, moderate intensity, aerobic exercise training program produces angiogenesis of type IIB fibers in women, but not in men (12).

Croley and colleagues (3) reported smaller type II FCSA in AW that was correlated with fewer capillaries and as a result had proposed that the loss of capillaries of type II muscle fibers could contribute to muscle fiber atrophy in aged. In the current report, there was a significant relationship between capillaries and FCSA of type IIA in aged untrained

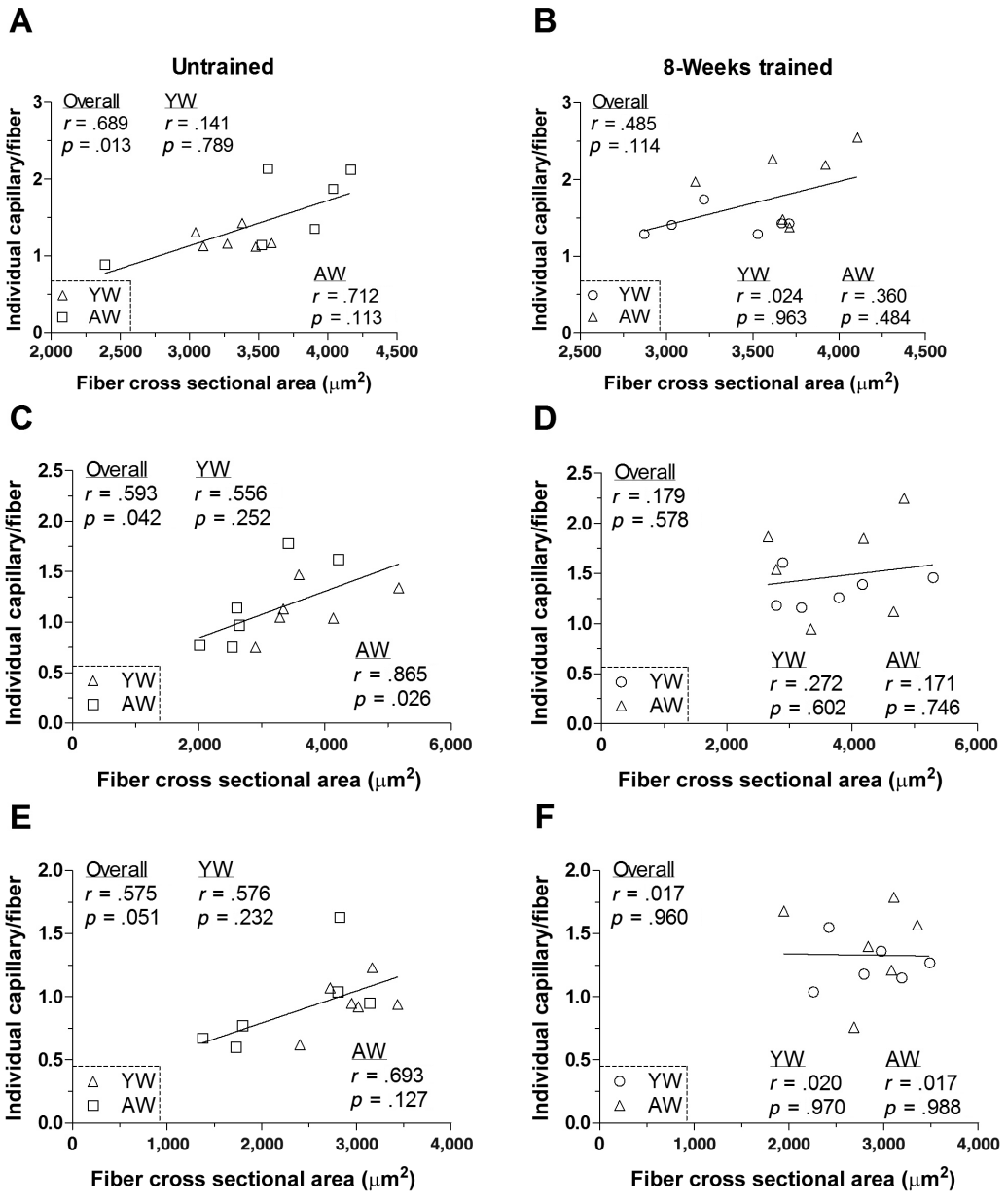
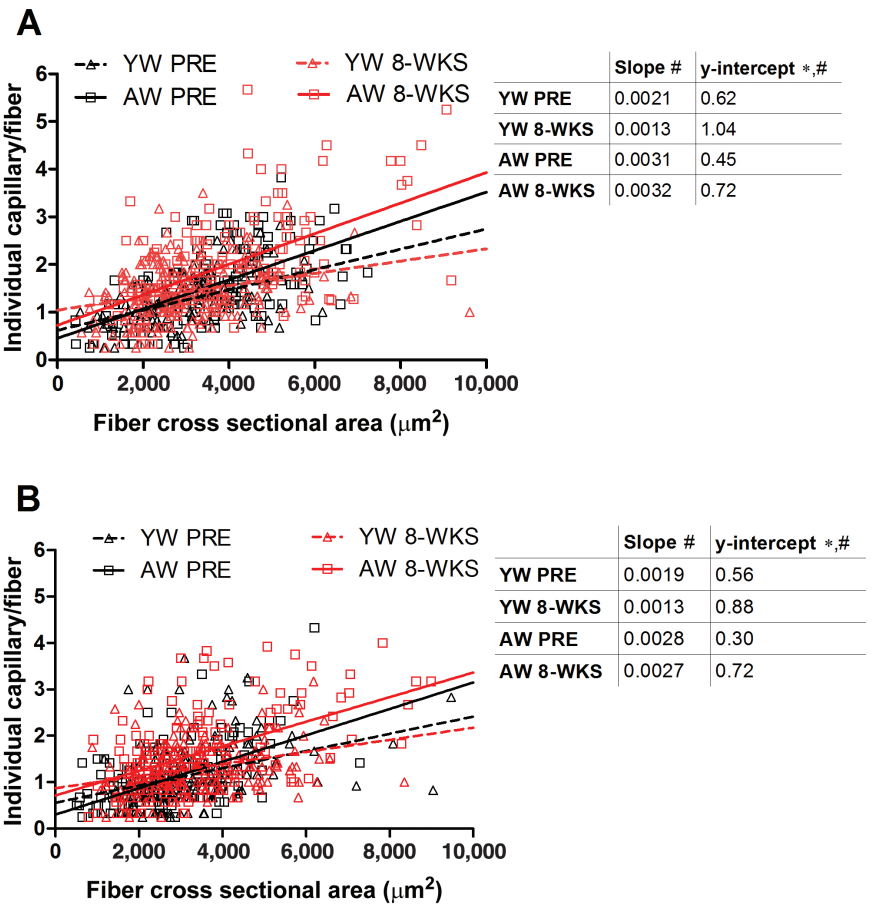


Figure 2. Linear regression between mean muscle fiber cross-sectional area (FCSA) and capillary/fiber (C/F_i) in young (YW) and aged (AW) women in type I (**A**, **B**); type IIA (**C**, **D**); type IIB (**E**, **F**) before (A, C, E) and after (B, D, F) exercise training.

women suggesting that the regulation of muscle fiber size of this intermediate fiber type is closely associated with muscle capillarization with aging. This relationship was not present after endurance training. It should be noted that the smaller (~20%, but not significant) FCSA of type IIA and IIB in the AW compared with YW before training was improved by endurance training such that FCSA was similar between YW and AW after training. Increases in FCSA of type I and IIA fibers in aged men and women with endurance training have been reported previously (18), although we did not observe an increase in FCSA of any fiber type in aged men using a similar training protocol as the current report (12). Recently, Murias and colleagues (38) also reported no increase in FCSA with endurance training in aged men. Why an identical exercise training program increases FCSA in AW, but not aged men, requires further investigation.

There are two prominent theories on the regulation of muscle capillarization: (i) capillarization is scaled to fiber size (26); and (ii) capillarization is scaled to metabolic demand, specifically mitochondrial capacity (27). Linear regression was performed on individual muscle fiber CSA to capillary ratio by age group and training status to investigate the regulation of muscle capillarization (26). It was reasoned that differences in the y-intercept (capillary ratio at 0 mm^2 FCSA) reflect inherent differences in regulation by metabolism, whereas differences in slope reflect differences in regulation by fiber size. Consistent with lower muscle oxidative capacity with aging (18), the y-intercepts of both type I and II fibers were lower in AW compared with YW independent of training status. With aging, the slope was greater, suggesting that muscle capillarization is more sensitive to changes (either positive or negative) in FCSA independent of training status. The y-intercepts were increased with exercise training independent of age and without a change in slope, consistent with exercise training increasing muscle oxidative capacity (18). These findings suggest



	Slope #	y-intercept *,#
YW PRE	0.0021	0.62
YW 8-WKS	0.0013	1.04
AW PRE	0.0031	0.45
AW 8-WKS	0.0032	0.72

	Slope #	y-intercept *,#
YW PRE	0.0019	0.56
YW 8-WKS	0.0013	0.88
AW PRE	0.0028	0.30
AW 8-WKS	0.0027	0.72

Figure 3. Linear regression between individual fiber cross-sectional area (FCSA) and individual capillary/fiber (C/F_i) ratio before (UT) and after (TR) aerobic exercise training in young (YW) and aged (AW) women of type I (**A**) and II (**B**) fibers. For each regression analysis, there was a significant ($p < .001$) relationship between FCSA and C/F_i. When comparing the y-intercept and slope within a given fiber type, exercise training increased the y-intercept (~50%) without changing the slope of either fiber type in YW and AW. With aging, the y-intercept was lower and the slope was greater of both fiber types independent of training status. There were no significant interactions. *Main effect of exercise training. #Main effect of age.

that fundamental changes occur in muscle with aging that alter the important interaction between muscle fiber size, mitochondria, and capillarization.

Previously, it was reported that muscle capillarization and interstitial VEGF protein during acute exercise are lower in untrained aged men compared with young men, and a significant positive correlation was identified between exercise interstitial VEGF protein and capillarization (12). It was suggested that exercise interstitial VEGF protein might therefore be an important regulator of basal capillarization. In the current report, lower exercise interstitial VEGF protein in AW compared with YW was also observed, yet capillarization was similar or higher depending on fiber type–specific capillarization in AW compared with YW. As a result, linear regression identified a negative relationship between exercise interstitial VEGF protein and capillarization in YW and AW. Combined, these results demonstrate that aging lowers the VEGF response to the stress of exercise, but that individual differences in VEGF present in humans may not directly explain differences in either basal or exercise-induced capillarization. Skeletal muscle VEGF is critical for basal and exercise-induced angiogenesis, as inhibition of muscle-specific VEGF expression lowers basal muscle capillarization and inhibits exercise-induced angiogenesis (20,21). However, the maintained angiogenic response to exercise demonstrated in aged men and women despite low, relative to young, increases in exercise interstitial VEGF protein suggest that a minimum VEGF response is necessary and that a greater increase does not result in a more robust angiogenic response. Consistent with this conclusion, recent work suggests exercise-induced skeletal muscle angiogenesis is a temporally coordinated process of pro- and antiangiogenic regulators where VEGF is important, but not the only regulator of muscle capillarization (39).

The regulation of muscle interstitial VEGF is poorly understood. The 165-kDa isoform of VEGF ($VEGF_{165}$) can bind to the extracellular matrix where it can be released by proteolytic cleavage via plasmin, a process likely mediated by the combined effects of numerous cells (40). Skeletal muscle tissue plasmin activator (tPA) protein content is higher in older compared with younger men (41). In addition, recent work suggests that VEGF can be prestored in vesicles in skeletal muscle that translocate to the plasma membrane (42). Whether these mechanisms may contribute to higher resting interstitial VEGF in AW and a lower response to exercise remains to be investigated.

In response to exercise, dialysate from the muscle interstitial space contains factors that promote endothelial cell proliferation and migration, including VEGF and other angiogenic factors (43). Increases in muscle interstitial VEGF occur in response to passive leg movement and moderate intensity exercise, but not high intensity exercise demonstrating complex regulation of muscle VEGF secretion (22,23). Mechanistically, exercise-induced VEGF secretion is regulated by adenosine (44), but not prostanoids (43). In the current

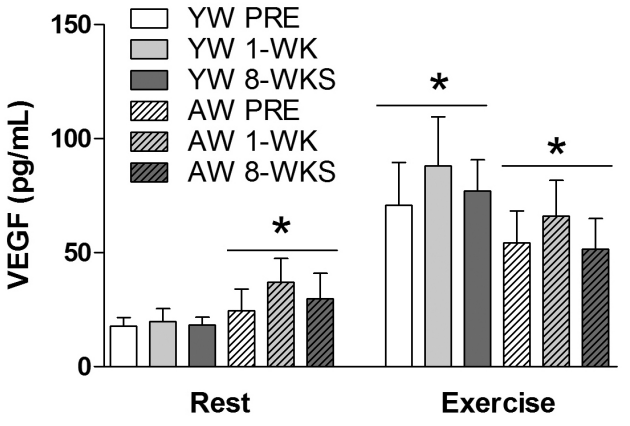


Figure 4. Interstitial VEGF protein before (PRE; white bars), after 1 week (1-WK; light grey bars), and after 8 weeks (8-WKS; dark grey bars) of aerobic exercise training at rest and in response to acute aerobic exercise in young (YW; open bars) and aged (AW; hatched bars) women. There was no interaction between age, training status (PRE, 1-WK, 8-WKS), and acute exercise (rest/exercise) (Age × Training Status × Acute Exercise; $p = .579$). There were no interactions between age and training status (Age × Training Status; $p = .840$) or between training status and acute exercise (Training Status × Acute Exercise; $p = .636$). There was a significant interaction between age and acute exercise (Age × Acute Exercise; $p = .025$) with aged greater than young at rest, but young greater than aged during exercise. *Significantly different than all other groups. Mean ± *SE*. $N = 8$/group.

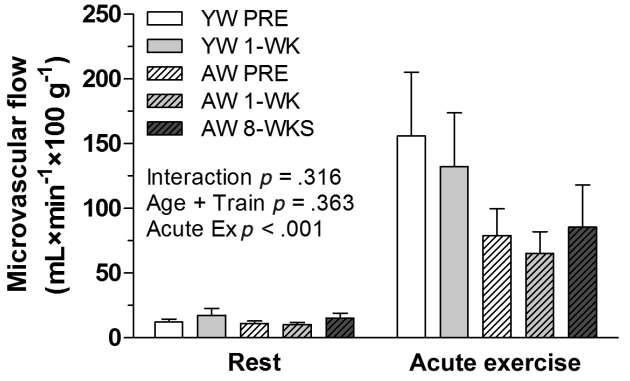


Figure 5. Skeletal muscle microvascular blood flow before (PRE; white bars), after 1 week (1-WK; light grey bars), and after 8 weeks (8-WKS; dark grey bars) of aerobic exercise training at rest and in response to acute aerobic exercise in young (YW; open bars) and aged (AW; hatched bars) women. Mean ± *SE*. $N = 8$/group.

report, we investigated whether muscle VEGF secretion was related to muscle microvascular blood flow, which is lower in AW compared with YW in part due to low endothelial nitric oxide synthase (10). It was reasoned that differences in VEGF secretion with aging might be due to differences in movement of fluid in the interstitial space. However, we did not observe any relationship between muscle microvascular blood flow and interstitial VEGF either at rest or during exercise, suggesting that interstitial fluid flow does not regulate VEGF secretion.

It must be noted that the AW in the current study represent healthy individuals because they did not present with overt cardiovascular disease. Aging is the leading risk factor for cardiovascular disease and cardiovascular disease prognosis is worse in aged patients, potentially the result of reduced neovascular potential (45). The detrimental impact of aging on CVD increases with advancing age such that those older than 80 years demonstrate a higher frequency of chronic heart failure, angina, myocardial infarction, and multivessel heart disease (46). In skeletal muscle, resistance exercise-induced hypertrophy is maintained in septuagenarian (47), but not octogenarian women (48). Our finding that skeletal muscle exercise-induced angiogenesis is preserved in AW (57–76 years) does not preclude the possibility that aging might impair angiogenesis in older individuals (75+ years).

In summary, the current report demonstrated that in contrast to our hypothesis, the angiogenic response to 8 weeks of endurance exercise training is preserved in AW compared with YW when fat-free mass is the same in both groups. Interestingly, an exercise-induced increase in capillarization of type IIB fibers occurred in women, which was not observed in similarly trained men (12), suggesting a possible difference in muscle regulation between sexes perhaps due to hormonal changes resulting from menopause. Given that exercise-induced interstitial VEGF is lower in aged compared with young women and yet angiogenesis is well maintained suggests that a minimum interstitial VEGF response is necessary for the increases in muscle capillarization induced by endurance exercise training.

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