



## Lower capillarization, VEGF protein, and VEGF mRNA response to acute exercise in the vastus lateralis muscle of aged vs. young women

Authors:

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Abstract

Lower capillarization, VEGF protein, and VEGF mRNA response to acute exercise in the vastus lateralis muscle of aged vs. young women. *J Appl Physiol* 99: 1872–1879, 2005. First published July 14, 2005; doi:10.1152/jappphysiol.00498.2005.—In humans, the majority of studies demonstrate an age-associated reduction in the number of capillaries surrounding skeletal muscle fibers; however, recent reports in rats suggest that muscle capillarization is well maintained with advanced age. In sedentary and trained men, aging lowers the number of capillaries surrounding type II, but not type I, skeletal muscle fibers. The fiber type-specific effect of aging on muscle capillarization is unknown in women. Vascular endothelial growth factor (VEGF) is important in the basal maintenance of skeletal muscle capillarization, and lower VEGF expression is associated with increased age in nonskeletal muscle tissue of women. Compared with young women (YW), we hypothesized that aged women (AW) would demonstrate 1) lower muscle capillarization in a fiber type-specific manner and 2) lower VEGF and VEGF receptor expression at rest and in response to acute exercise. Nine sedentary AW (70 + 8 yr) and 11 YW (22 + 3 yr) had vastus lateralis muscle biopsies obtained before and at 4 h after a submaximal exercise bout for the measurement of morphometry and VEGF and VEGF receptor expression. In AW compared with YW, muscle capillary contacts were lower overall (YW: 2.36 + 0.32 capillaries; AW: 2.08 + 0.17 capillaries), specifically in type II (YW: 2.37 + 0.39 capillaries; AW: 1.91 + 0.36 capillaries) but not type I fibers (YW: 2.36 + 0.34 capillaries; AW: 2.26 + 0.24 capillaries). Muscle VEGF protein was 35% lower at rest, and the exercise-induced increase in VEGF mRNA was 50% lower in AW compared with YW. There was no effect of age on VEGF receptor expression. These results provide evidence that, in the vastus lateralis of women, 1) capillarization surrounding type II muscle fibers is lower in AW compared with YW and 2) resting VEGF protein and the VEGF mRNA response to exercise are lower in AW compared with YW.

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In humans, the majority of studies demonstrate an age-associated reduction in the number of capillaries surrounding skeletal muscle fibers; however, recent reports in rats suggest that muscle capillarization is well maintained with advanced age. In sedentary and trained men, aging lowers the number of capillaries surrounding type II, but not type I, skeletal muscle fibers. The fiber type-specific effect of aging on muscle capillarization is unknown in women. Vascular endothelial growth factor (VEGF) is important in the basal maintenance of skeletal muscle capillarization, and lower VEGF expression is associated with increased age in nonskeletal muscle tissue of women. Compared with young women (YW), we hypothesized that aged women (AW) would demonstrate 1) lower muscle capillarization in a fiber type-specific manner and 2) lower VEGF and VEGF receptor expression at rest and in response to acute exercise. Nine sedentary AW (70 + 8 yr) and 11 YW (22 + 3 yr) had vastus lateralis muscle biopsies obtained before and at 4 h after a submaximal exercise bout for the measurement of morphometry and VEGF and VEGF receptor expression. In AW compared with YW, muscle capillary contacts were lower overall (YW: 2.36 + 0.32 capillaries; AW: 2.08 + 0.17 capillaries), specifically in type II (YW: 2.37 + 0.39 capillaries; AW: 1.91 + 0.36 capillaries) but not type I fibers (YW: 2.36 + 0.34 capillaries; AW: 2.26 + 0.24 capillaries). Muscle VEGF protein was 35% lower at rest, and the exercise-induced increase in VEGF mRNA was 50% lower in AW compared with YW. There was no effect of age on VEGF receptor expression. These results provide evidence that, in the vastus lateralis of women, 1) capillarization surrounding type II muscle fibers is lower in AW compared with YW and 2) resting VEGF protein and the VEGF mRNA response to exercise are lower in AW compared with YW.

kinase insert domain-containing receptor; Flt-1; fiber cross-sectional area; vascular endothelial growth factor

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BY THE YEAR 2030, IT IS PREDICTED that there will be 70 million people 65 yr or older in the United States (38). Among the many changes associated with advanced age in humans are significant changes in skeletal muscle structure and function, including sarcopenia (13, 42), lower oxidative capacity (13, 33), and fewer capillaries surrounding muscle fibers (13, 41, 42). These adverse changes are not present in all muscles and may affect only certain muscle fiber types within a given

muscle. For example, aging can lower citrate synthase activity in the gastrocnemius muscle but not the vastus lateralis muscle of aged men (33). In addition, aging can lower muscle fiber cross-sectional area (FCSA) of type II, but not type I, fibers in humans (13, 42). In the only known report investigating fiber type-specific muscle capillarization in humans (13), capillary-to-fiber ratio (C/F) was lower surrounding type IIA and IIB fibers but not type I fibers of aged compared with young men. Interestingly, recent reports demonstrate that muscle capillarization is well maintained in Fisher 344 (F344) and F344 X Brown Norway rats (31, 40, 48). Thus age-associated reductions in muscle capillarization may be present predominantly surrounding type II muscle fibers in humans. Reductions in capillarization may be detrimental in the aged as skeletal muscle capillarization is an important determinant of maximal oxygen consumption ( $\dot{V}O_{2\max}$ ), insulin sensitivity, and FCSA in humans (27, 28). To our knowledge, in aged women (AW) compared with young women (YW), there is only a single report demonstrating lower overall skeletal muscle (gastrocnemius) capillarization (13) and no report on fiber type-specific effects of aging on muscle capillarization. Thus the effects of aging on capillarization in muscles other than the gastrocnemius and on fiber type-specific capillarization in women are unknown.

It is well known that endurance exercise training promotes skeletal muscle angiogenesis in humans (5, 7, 12, 28). Endurance exercise training can also increase muscle capillarization in aged humans (12, 28, 42). However, debate exists on whether the angiogenic response to endurance exercise training is similar between young and aged humans. In response to a 20-wk endurance-training program (1 h/day; 4 days/wk at 70–80%  $\dot{V}O_{2\max}$ ), young men (22 yr) demonstrated a significant increase in the number of capillaries in contact with both type I and IIa fibers (14, 42). In contrast, aged men (62 yr) did not demonstrate an increase in capillary contacts (CC) with any fiber type (14), suggesting that the angiogenic response to exercise training may be at least attenuated in aged muscle. This study by Denis et al. (14) included aged men who were fairly active at the beginning of the study; thus results from this study must be interpreted with caution. A subsequent study by this same group did demonstrate significant increases in individual C/F of type I and IIa fibers, but not type IIb/x fibers, in response to 14 wk of endurance training in aged men (8). Proctor et al. (42) demonstrated similar C/F surrounding type I but lower C/F surrounding type IIA and IIB fibers in aged compared with young untrained and trained men.

In young mouse skeletal muscle, inhibition of endogenous vascular endothelial growth factor (VEGF) production reduces the skeletal muscle capillary supply by 64% and promotes skeletal muscle cell apoptosis demonstrating that VEGF is important in basal skeletal muscle capillary regulation, which in turn is important in the basal maintenance of skeletal muscle mass (51). In human skeletal muscle, acute exercise increases VEGF mRNA (20, 21, 26, 44), whereas exercise training increases VEGF protein levels (25, 26). In rats, inhibition of VEGF prevents increases in vessel density in response to exercise training, demonstrating that VEGF is important in skeletal muscle angiogenesis (3). Together, these data demonstrate that VEGF is important for the maintenance and expansion of skeletal muscle capillarization.

Aging lowers VEGF mRNA expression in breast tissue (22) and fibroblasts (37) in humans, whereas aging lowers VEGF protein in the kidney (36) and carotids bodies (16) in rats. In skeletal muscle, the VEGF mRNA response to the same relative intensity of acute resistance exercise is similar in young and aged men (35), whereas the VEGF mRNA and protein responses to severe ischemia are lower in aged mice (46). The VEGF mRNA response to the same relative intensity of acute aerobic exercise is preserved in 24- vs. 6-mo-old F344 rats (48). Whether muscle VEGF expression is lower at rest and in response to acute aerobic exercise in AW is unknown. Compared with YW, we hypothesized that AW would demonstrate 1) lower muscle capillarization in a fiber type-specific manner and 2) lower VEGF and VEGF receptor expression at rest and in response to acute exercise.

## METHODS

**Subjects.** Eleven sedentary YW (range 18–28 yr) and nine sedentary AW (range 60–85 yr) volunteered to participate in the study after written and verbal explanations of the content and intent of the study were given, in accordance with the University and 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. One AW was on hormone replacement therapy. Sedentary subjects were defined as participating in <1 h of strenuous physical activity per week.

**$\dot{V}O_{2\max}$  and body composition.**  $\dot{V}O_{2\max}$  was measured on an electronically braked cycle ergometer (Lode, Excaliber Sport, Groningen, The Netherlands) by open-circuit spirometry (True Max 2400, Parvo Medics, Salt Lake City, UT). The test began with a 5-min warm-up at 50 W for YW and 20 W for AW. After the warm-up, the workload

Table 1. Subject characteristics

	Young	Aged	P Value
Age, yr	22+3	70+8	<0.001
Height, cm	165.0+4.6	161.2+6.3	0.141
Mass, kg	62.4+10.0	61.5+8.0	0.821
Body fat, %	25.0+6.3	25.2+1.7	0.800
$\dot{V}O_{2\max}$ , ml O <sub>2</sub> ·kg <sup>-1</sup> ·min <sup>-1</sup>	28.0+4.8	17.6+4.5	<0.001
$\dot{V}O_{2\max}$ , ml O <sub>2</sub> ·kg FFM <sup>-1</sup> ·min <sup>-1</sup>	35.8+5.2	22.5+5.8	<0.001
Estradiol, pg/ml	45.4+39.4	31.6+19.7	0.363
Race (Caucasian/African American/ Native Hawaiian/Asian)	7/2/1/1	9/0/0/0	

Values are means + SD.  $\dot{V}O_{2\max}$ , maximal oxygen consumption; FFM, fat-free mass.

was increased 25 W for YW and 20 W for AW every 2 min until volitional fatigue. Body density was determined via hydrostatic weighing, and body fat percent was determined from body density based on a two-compartment model.

**Submaximal exercise and muscle biopsies.** At least 1 wk after the  $\dot{V}O_{2\max}$  test, subjects completed 45 min of cycle ergometer exercise (25 min exercise, 5 min rest, and 20 min of exercise) at 50%  $\dot{V}O_{2\max}$ . Before commencement of exercise and at 4 h postexercise, a muscle biopsy was obtained from the vastus lateralis for the measurement of mRNA and protein. The resting and postexercise muscle biopsy samples were obtained from alternate legs. Samples were stored at -80°C until analysis. A section of the resting biopsy sample was oriented in an optimal cutting temperature (OCT)-tragacanth mixture, frozen in liquid nitrogen-cooled isopentane, and stored at -80°C until processing for the measurement of muscle morphometry and capillarization.

The postexercise biopsy was obtained at 4 h postexercise, as we have previously shown that VEGF mRNA is increased to a similar level at 2 and 4 h after the completion of a single acute systemic cycle ergometer exercise bout in young men (20). Because it is possible that the VEGF mRNA response to acute exercise may be delayed in aged compared with young humans, we chose to obtain a single biopsy at 4 h and not 2 h postexercise.

**Estradiol.** Estradiol has been shown to modulate VEGF expression in human vascular smooth muscle cells (6). To minimize the potential for estradiol influences in the present study, YW were studied during the menses phase of the menstrual cycle. Six YW were taking an oral contraceptive and were studied during the low-estradiol phase of their oral contraceptive treatment. All AW were postmenopausal. Plasma estradiol was measured from a venous sample obtained before the submaximal exercise bout by a chemiluminescent enzyme immunoassay (Access, Beckman Coulter, Brea, CA).

**Morphometric and morphological analysis.** At -20°C, muscle tissue mounted in OCT-tragacanth was sectioned to a thickness of 10  $\mu$ m on a cryostat, mounted on slides, and kept until fixation. Sections were stained for capillaries using the Rosenblatt method (47), which simultaneously provides fiber typing (type I and II) and capillary visualization. There is no difference in the number of capillaries visualized with frozen biopsy samples using the Rosenblatt technique and the number visualized in tissue sections prepared from perfusion-fixed muscle (29). In human skeletal muscle, type I fibers stain more intensely than type II fibers (10).

Muscle sections were viewed under a light microscope (Nikon 400), and a digital image was taken of the section (Nikon Coolpix 990), as previously described (20). Capillaries were quantified manually from the digital image on individual fibers in an artifact-free region. The following indexes were measured (28): 1) the number of capillaries around a fiber (CC), 2) the C/F on an individual-fiber basis, and 3) the number of fibers sharing each capillary (sharing factor). Capillary density (CD) was calculated by using the fiber as the reference space. Capillary-to-fiber perimeter exchange index was calculated as an estimate of the capillary-to-fiber surface area. Quantification of the capillaries was performed on at least 50 fibers by randomly selecting a fiber in an artifact-free region. FCSA and fiber 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.

**RNA isolation and Northern analysis.** Total cellular RNA was isolated from each sample, and standard Northern blot analysis was performed for VEGF, KDR, and Flt-1 mRNA as previously described (20). Blots were exposed to XAR-5 X-ray film (Eastman Kodak, New Haven, CT) by use of a Cronex Lightning Plus screen at -80°C. Autoradiographs were quantitated by densitometry within the linear range of signals and normalized to rJ-actin mRNA levels.

**Protein isolation and analysis.** A portion of the muscle biopsy sample was homogenized in RIPA (1X PBS, 1% Igepal, 0.5% sodium

deoxycholate, 0.1% SDS with protease inhibitors) as previously described (20). Total protein was measured by bicinchoninic acid (Bio-Rad, Hercules, CA). For each sample, VEGF (from 50 µg of total protein), KDR (from 125 µg of total protein), and Flt-1 (from 50 µg of total protein) were measured in duplicate. Commercial VEGF, KDR, and Flt-1 ELISA kits were used according to the manufacturer's instructions (R&D Systems, Minneapolis, MN).

*Statistical treatment.* For mRNA and protein, a two-way mixed-plot factorial analysis of variance (age X exercise level) was used. Following a significant *F* ratio, a Bonferroni post hoc analysis was used. Unpaired Student's *t*-tests were used to compare differences in

muscle morphometry was performed from only 10 YW and 8 AW. In three YW, both rest and 4-h postexercise mRNA samples were lost during isolation. Significance was established at *P* < 0.05 for all statistical sets, and data are reported as means + SD.

## RESULTS

*Subject characteristics.* AW subjects were ~50 yr older than YW (Table 1). Despite the difference in age, groups were well matched for height, weight, and body composition. As anticipated,  $\dot{V}O_{2\max}$  was lower in AW compared with YW. Estradiol was not different between groups.

*Muscle morphology.* There was no difference in type I FCSA between groups, but type II FCSA was smaller in AW compared with YW (Table 2). There was no difference in type I fiber percentage, but the area percentage of type I fibers was greater in AW compared with YW.

Overall, there were fewer capillaries surrounding muscle fibers in AW compared with YW (Table 3). When analyzed by fiber type, the overall difference resulted from fewer muscle capillaries associated with type II muscle fibers in AW compared with YW. Interestingly, neither CD nor capillary-to-fiber perimeter exchange index was different between YW and AW, suggesting that the loss of muscle capillaries with aging is similar in magnitude to the loss of FCSA and fiber perimeter such that these relative measures of muscle capillarization remain unchanged with aging. Consistent with this, linear regression revealed significant relationships between C/F on an individual-fiber basis and FCSA for type II fibers when all subjects were examined together (Fig. 1). When analyzed within groups, a significant relationship was only observed in type II fibers of AW.

*VEGF and VEGF receptor expression.* Results for VEGF, KDR, and Flt-1 mRNA at rest and in response to acute systemic exercise are in Fig. 2. The expression of mRNA was analyzed in the vastus lateralis muscle at rest and at 4 h after a single, 45-min submaximal exercise bout. There were no

Table 2. Fiber cross-sectional area comparison

	Young	Aged	<i>P</i> Value
Overall fiber cross-sectional area, µm <sup>2</sup>	4,808±1,415	4,060±760	0.198
Type I	4,852±1,484	5,017±1,038	0.794
Type II	4,784±1,477	3,407±893	0.034
Overall fiber perimeter	292±45	268±23	0.188
Type I	290±47	297±32	0.727
Type II	262±35	232±26	0.027
Type I fibers, %	35.5±9.1	42.9±14.2	0.199
Area of type I fibers, %	35.8±10.0	52.2±14.4	0.011

Values are means + SD.

Table 3. Capillary comparison among groups

	Young	Aged	<i>P</i> Value
Overall capillary contacts	2.36±0.32	2.08±0.17	0.042
Type I	2.36±0.34	2.26±0.24	0.465
Type II	2.37±0.39	1.91±0.36	0.020
Overall individual capillary-to-fiber ratio	0.86±0.12	0.76±0.08	0.061
Type I	0.84±0.15	0.83±0.13	0.884
Type II	0.87±0.13	0.68±0.15	0.009
Overall share factor	2.87±0.14	2.81±0.36	0.601
Type I	2.92±0.18	2.86±0.27	0.575
Overall capillary density, capillaries/mm <sup>2</sup>	190±51	190±26	0.969
Type I	187±55	170±31	0.468
Type II	277±61	285±65	0.709
Overall CFPE, capillaries/1,000 µm	2.99±0.48	2.87±0.24	0.541
Type I	3.01±0.62	2.87±0.37	0.584
Type II	3.01±0.50	2.79±0.41	0.320

Values are means + SD. CFPE, capillary-to-fiber perimeter exchange index.

differences in VEGF, KDR, or Flt-1 mRNA at rest between groups. Systemic exercise increased VEGF mRNA in YW (8-fold), and this response was significantly lower in AW (3.8-fold). Systemic exercise increased both KDR and Flt-1 mRNA independent of age.

VEGF, KDR, and Flt-1 protein at rest and in response to acute exercise are in Fig. 3. VEGF protein was significantly lower in AW compared with YW independent of exercise condition. Flt-1 protein was significantly reduced by acute exercise independent of age. KDR protein was unaltered by age or exercise.

## DISCUSSION

The major findings of the present study in the vastus lateralis muscle of women are 1) capillarization surrounding type II muscle fibers is lower in AW compared with YW; 2) VEGF protein is 35% lower at rest in AW compared with YW; and 3) the VEGF mRNA response to exercise is 50% lower in AW compared with YW. To our knowledge, these are the first findings demonstrating lower fiber type-specific muscle capillarization, lower muscle VEGF protein, and a lower muscle VEGF mRNA response to acute aerobic exercise in AW compared with YW.

*Subjects.* Groups were well matched for height, weight, and body composition. Consistent with previous reports, aerobic capacity was lower in AW (13). As groups, both YW and AW were in the lowest 10th percentile in aerobic capacity (1). These age-adjusted values are based on treadmill exercise, whereas cycle ergometry was used in the current report.  $\dot{V}O_{2\max}$  is 3–5 ml O<sub>2</sub>·kg<sup>-1</sup>·min<sup>-1</sup> lower during cycle ergometry compared with treadmill exercise (19). When adjusting for this difference, both YW and AW groups were in the 20th percentile for their age, respectively.

*Aging and skeletal muscle capillarization.* Our finding of a loss of capillaries surrounding muscle fibers in the vastus lateralis of AW compared with YW is consistent with a previous finding in gastrocnemius muscle (13). It could be hypothesized that a greater loss of capillaries would be present in the vastus lateralis than the gastrocnemius since the gastrocnemius is used frequently during common everyday activities such as walking. However, citrate synthase activity is lowered by advanced age in gastrocnemius, but not the vastus lateralis,

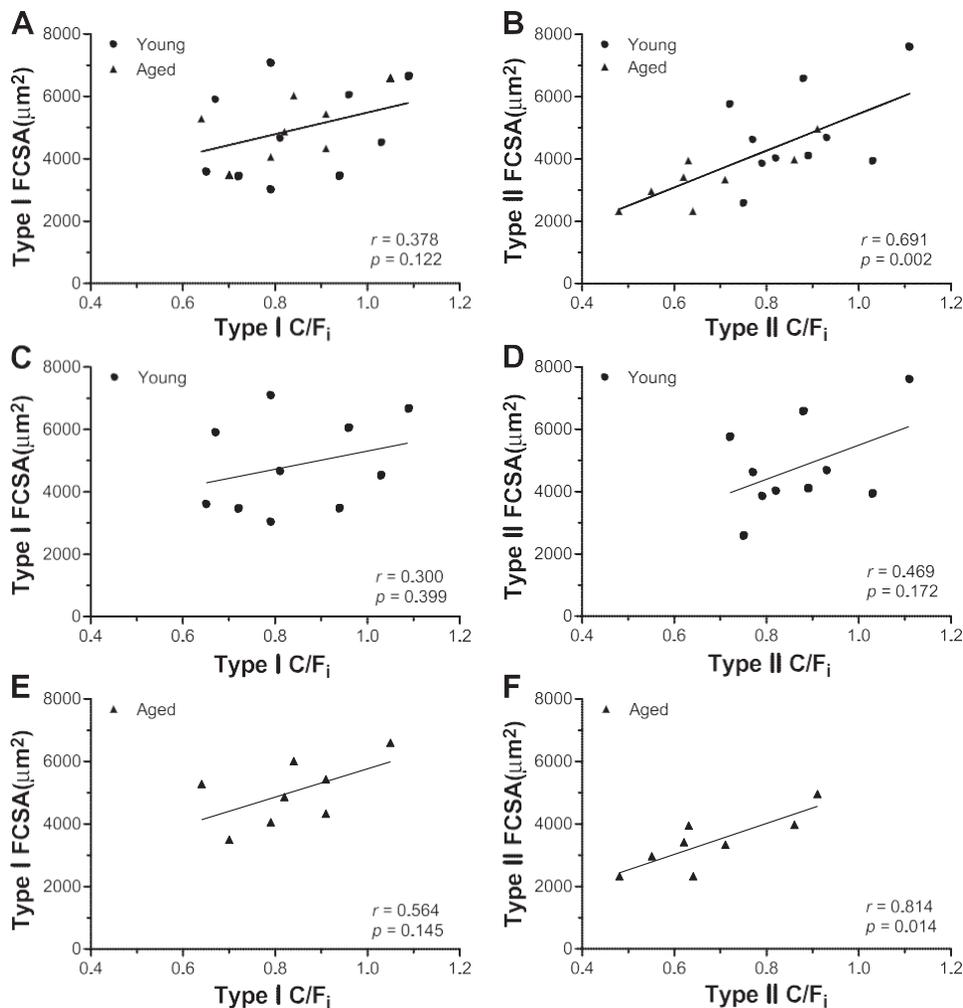


Fig. 1. Linear regression between muscle capillary-to-fiber ratio on an individual-fiber basis (C/F<sub>i</sub>) and fiber cross-sectional area (FCSA). A: type I fibers for all subjects. B: type II fibers for all subjects. C: type I fibers for young subjects. D: type II fibers for young subjects. E: type I fibers for aged subjects. F: type II fibers for aged subjects.

suggesting that more distal muscles may be more greatly affected by aging than more proximal muscles (33). In the present report, C/F on an individual-fiber basis and CC were ~12% lower in AW, whereas Coggan et al. (13) reported an ~35% reduction in C/F and CC. Thus differences in the magnitude of the loss of muscle capillaries between the present report and Coggan et al. could possibly be due to greater age-associated loss of muscle capillaries in more distal muscles.

To our knowledge, the present findings are the first report demonstrating a fiber type-specific loss of muscle capillaries in AW. In men, aging results in an ~25% loss of muscle capillaries surrounding type II fibers, with no significant loss surrounding type I fibers (42). Combined, these data suggest that type II fibers may be more susceptible to the age-associated loss of muscle capillaries than type I fibers. It should be noted that, because of the nature of human muscle biopsies, our results could be influenced by fiber-type grouping in aged muscle (15).

Recent data suggests that muscle capillarization is well preserved in F344 X Brown Norway (31, 40) and F344 (48) rats with aging. Animal models are attractive in aging research in that genetic differences between animals are minimized and successful subject recruitment issues are not relevant. Several factors likely influence group results from human aging studies and make definitive statements more complex. These factors

include differences in physical activity levels (9, 14, 34), differences in the number of men and women studied in each group (34), and the lack of statistical analyses (4, 23). Except for a single report (14), a review of the human aging literature suggests that muscle C/F is lower in aged compared with young subjects whether statistical differences were present or not (4, 9, 13, 23, 34, 41, 42). We are aware of only three reports that have adequately controlled participant enrollment to study sedentary young and aged individuals (13, 41, 42). In each of these reports, muscle C/F is statistically lower in aged compared with young subjects. In addition, Frontera et al. (18) demonstrated that, from the age of 65 to 77 yr, there is a 22% reduction in muscle capillarization without a loss of muscle FCSA. Therefore, the vast majority of data in humans suggests that aging lowers muscle capillarization, which is in contrast to recent reports in rats (31, 40, 48).

There are two prominent theories on the regulation of muscle capillarization. The first suggests that muscle capillarization is primarily scaled to fiber size (2), whereas the second suggests that muscle capillarization is scaled to metabolic demand (32). Although aging resulted in the loss of muscle capillaries, the magnitude of the loss was similar to the losses in FCSA and fiber perimeter such that there was no difference in either CD or capillary-to-fiber perimeter exchange index between YW and AW. It is impossible to determine from our

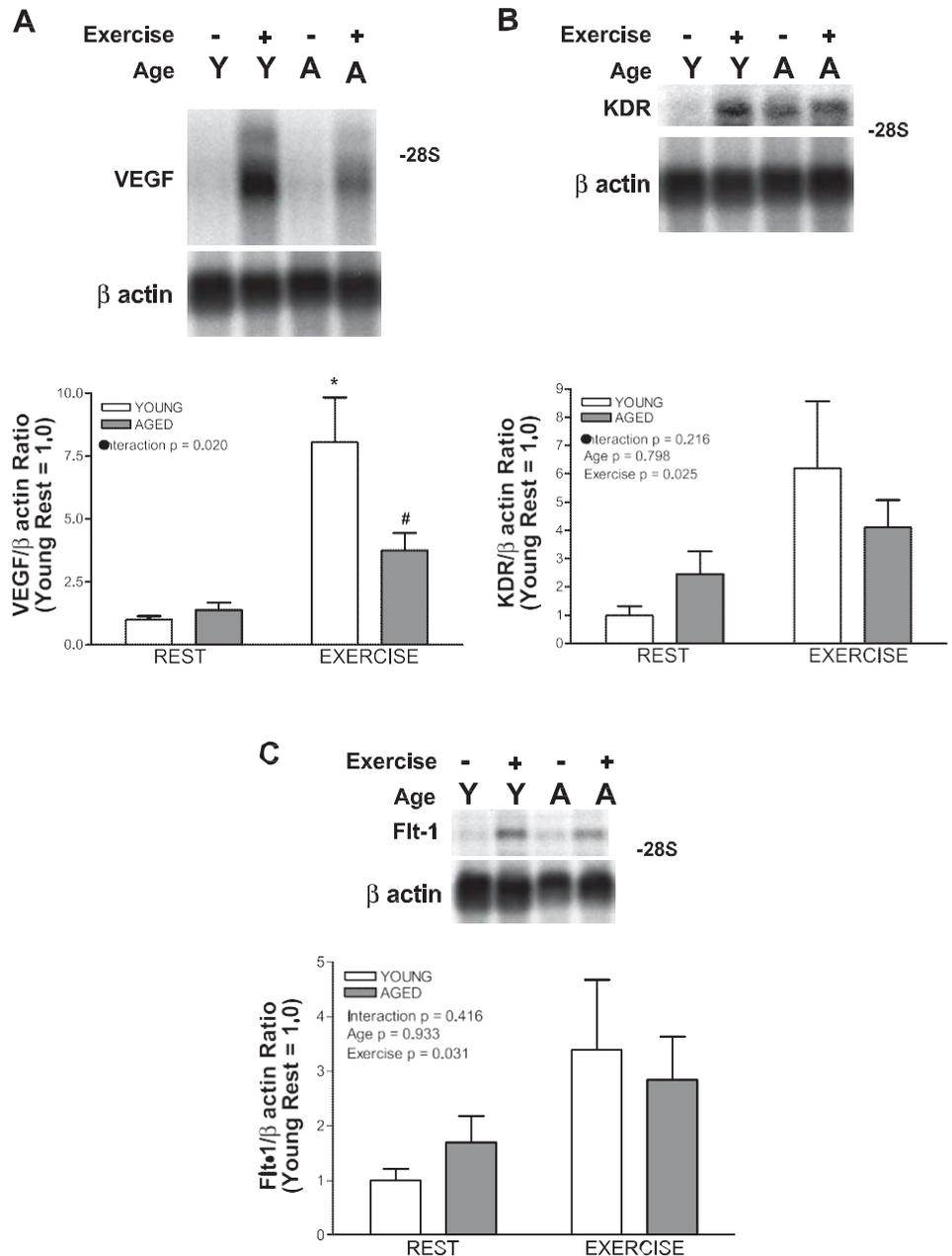


Fig. 2. Representative Northern blot and quantitative densitometry for the ratio of vascular endothelial growth factor (VEGF; A), KDR (B), and Flt-1 mRNA (C) mRNA to  $\beta$ -actin mRNA (B) in skeletal muscle of young (Y) and aged (A) women at rest and 4 h after the completion of 45 min of cycle ergometer exercise. Young rest data were normalized to young + rest to allow for comparisons. VEGF was significantly increased by exercise 8-fold in young women. In aged women, the VEGF mRNA response to acute exercise was significantly lower (3.8-fold increase). KDR and Flt-1 mRNA were significantly increased by exercise independent of age. There were no differences in KDR or Flt-1 mRNA between young and aged women. Values are means  $\pm$  SD;  $n = 8$  for young and 9 for aged women. \*Signifi different from all other conditions. #Signifi different from aged + rest ( $P < 0.05$ ).

data which mechanism predominates in the regulation of human muscle capillarization. It is interesting, however, that the relationship between muscle capillarization and muscle fiber size is maintained (no difference in CD). Many mechanisms have been proposed to contribute to age-associated sarcopenia, including motoneuron denervation, mitochondria-induced apoptosis, changes in anabolic hormones, and increased rates of proteolysis (recently reviewed in Refs. 15, 30, 39). Given that the loss of endogenous VEGF results in capillary rarefaction and, therefore, indirectly results in muscle nuclei apoptosis (a likely mechanism of muscle atrophy) in transgenic mice (51), an additional mechanism for age-associated sarcopenia could be that aging reduces VEGF, resulting in the loss of muscle capillaries, which in turn promotes muscle atrophy. This theory remains to be experimentally tested.

*Aging, resting VEGF, and resting VEGF receptor expression.* We had hypothesized that resting muscle VEGF expression would be lower in AW. In the present report, there was no difference in resting VEGF mRNA between young and aged muscle, but resting VEGF protein was 35% lower in aged muscle, suggesting that lower VEGF may contribute to lower muscle capillarization in AW. Lower VEGF protein and a concomitantly lower muscle capillarization are consistent with a recent report from Tang et al. (51) demonstrating that VEGF is important in basal maintenance of muscle capillarization, where inactivation of endogenous skeletal muscle VEGF production decreased capillarization by 64%. To further identify whether VEGF protein levels may in part regulate muscle capillarization, we analyzed the relationship between muscle capillarization and VEGF protein from individuals in the

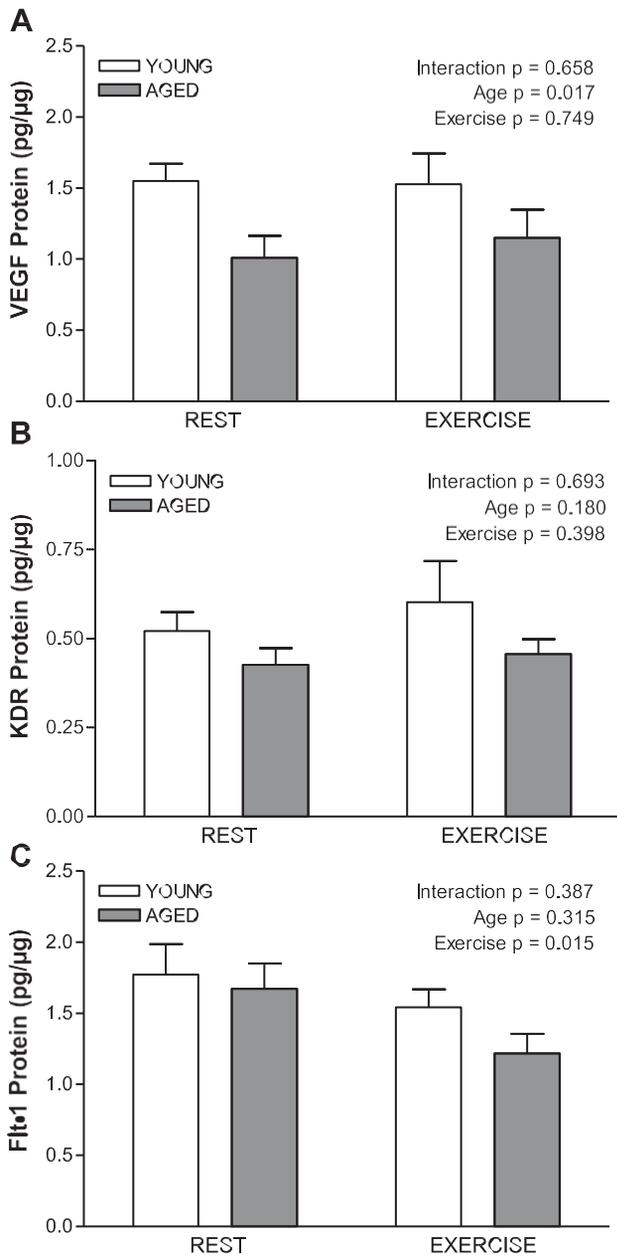


Fig. 3. Skeletal muscle protein for VEGF (A), KDR (B), and Flt-1 (C) at rest and 4 h after the completion of a 45-min acute submaximal exercise bout. Muscle VEGF was significantly lower in aged compared with young women, whereas Flt-1 was lowered by exercise. KDR was unaltered by age or exercise. Values are means + SD;  $n = 11$  for young and 9 for aged women.

present report and our laboratory's previous report from lean and obese young men (21). Linear regression revealed a strong relationship between resting VEGF protein levels and overall CC, consistent with the suggestion that VEGF likely contributes to the regulation of muscle capillarization in humans (Fig. 4).

A divergence between resting VEGF mRNA and protein was observed in aged muscle. Two possibilities for the divergence of VEGF mRNA and protein levels in aged muscle are 1) a reduction in VEGF translation may be present in aged muscle and 2) VEGF protein degradation may be accelerated in aged muscle. The translation of VEGF is complicated because of the long 5' untranslated region of VEGF mRNA, which is

incompatible with efficient ribosomal scanning (49). To overcome this, the 5' untranslated region of VEGF mRNA contains an internal ribosome entry site that maintains efficient translation of VEGF mRNA under conditions of stress, such as hypoxia, when overall protein synthesis is reduced (49). Aged human muscle does demonstrate increased activation of several stress-associated MAPK signaling cascade proteins (ERK1/2, p90RSK, Mnk 1, p38 MAPK, and JNK/SAPK), which may be involved in alterations in the regulation of translation in aged muscle (53). We are unaware of any studies investigating specific VEGF protein translation or degradation rates in aged tissue.

The biological activity of VEGF occurs predominantly through binding of VEGF to the VEGF receptors KDR and Flt-1. It is currently hypothesized that most angiogenic activity occurs through KDR, whereas Flt-1 acts as a negative (angiostatic) regulator of angiogenesis (17). Lower capillarization in aged tissue therefore could be the result of either lower KDR or increased Flt-1 expression. There were no differences in KDR or Flt-1 mRNA and protein between young and aged muscle, suggesting that lower VEGF receptor expression does not play an important role in the lower capillarization observed in AW.

*Aging, exercise, VEGF, and VEGF receptor expression.* As hypothesized, the VEGF mRNA response to acute aerobic exercise was 50% lower in aged human muscle. It was also hypothesized that aging would reduce the VEGF receptor response to exercise; however, both KDR and Flt-1 mRNA were increased by exercise independent of age. Our finding that the VEGF mRNA response to acute exercise is lower in aged muscle is significant, as the angiogenic response to exercise training may be attenuated in aged compared with young human muscle (14, 42).

Exercise is a well-known stress to muscle that results in intracellular hypoxia (43, 45) and angiogenesis (5, 7, 12, 28). Consistent with this, acute exercise increases VEGF mRNA (20, 24, 44), whereas exercise training increases VEGF protein in human muscle (25, 26). Interestingly, the skeletal muscle VEGF mRNA response to resistance exercise is similar in young and aged men (35). The discrepancy between our data and those of Jozsi et al. (35) may likely reflect different VEGF regulatory mechanisms involved in resistance vs. aerobic ex-

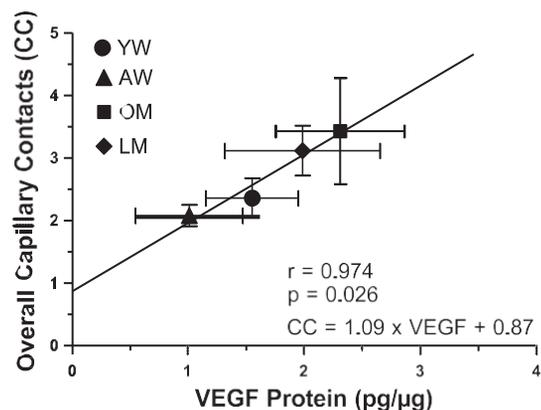


Fig. 4. Linear regression between VEGF protein and overall capillary contacts (CC). Values are means + SD. YW, young women; AW, aged women; LM, lean young men (previously reported in Ref. 21); OM, obese young men (previously reported in Ref. 21).

ercise. A likely mediator of resistance exercise-induced VEGF mRNA expression may be activation of Akt by hypertrophic stimuli (50). Likewise, aerobic exercise-induced VEGF expression has been proposed to involve hypoxia (52). A lower VEGF mRNA response to ischemia-induced hypoxia has been observed in aged mouse muscle (46), whereas a similar muscle VEGF mRNA response to the same relative acute aerobic exercise intensity between young and aged F344 rats has been recently reported (48). Thus the age-attenuated VEGF mRNA response to the same relative aerobic exercise intensity appears to be unique to humans.

Several studies have demonstrated an angiogenic response to endurance exercise training in aged individuals (11, 12, 28, 42); however, these studies employed either a cross-sectional design of young and aged untrained and trained individuals (11, 42) or an experimental design utilizing aged individuals only (12, 28). In the only known report investigating the angiogenic response to the same relative intensity exercise training program in young and aged subjects, angiogenesis was observed in young but not aged individuals (14). These results must be viewed with some caution, because the aged individuals were fairly active before the initiation of the training. However, if the pattern of a lower VEGF mRNA response to acute exercise persists throughout an exercise-training program, our results of a lower VEGF mRNA response to the same relative exercise intensity are consistent with an aging-related impairment of the angiogenic response to exercise training.

In summary, we have demonstrated in the vastus lateralis muscle of AW that capillarization surrounding type II muscle fibers, resting VEGF protein, and the exercise-induced increase in VEGF mRNA are lower compared with YW. Whether a lower VEGF mRNA response to acute exercise contributes to a lower angiogenic response to exercise training in AW remains to be determined.

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#### REFERENCES

1. **ACSM.** *ACSM's Guidelines for Exercise Testing and Prescription.* Baltimore, MD: American College of Sports Medicine, 2000.
2. **Ahmed SK, Egginton S, Jakeman PM, Mannion AF, and Ross HF.** Is human skeletal muscle capillary supply modelled according to fibre size or fibre type? *Exp Physiol* 82: 231–234, 1997.
3. **Amaral SL, Papanek PE, and Greene AS.** Angiotensin II and VEGF are involved in angiogenesis induced by short-term exercise training. *Am J Physiol Heart Circ Physiol* 281: H1163–H1169, 2001.
4. **Andersen JL.** Muscle fibre type adaptation in the elderly human muscle. *Scand J Med Sci Sports* 13: 40–47, 2003.
5. **Andersen P and Henriksson J.** Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. *J Physiol* 270: 677–690, 1977.
6. **Bausero P, Ben-Mahdi MH, Mazucattelli JP, Bloy C, and Perrot-Applanat M.** Vascular endothelial growth factor is modulated in vascular muscle cells by estradiol, tamoxifen, and hypoxia. *Am J Physiol Heart Circ Physiol* 279: H2033–H2042, 2000.
7. **Brodal P, Ingjer F, and Hermansen L.** Capillary supply of skeletal muscle fibers in untrained and endurance-trained men. *Am J Physiol Heart Circ Physiol* 232: H705–H712, 1977.
8. **Charifi N, Kadi F, Feasson L, Costes F, Geysant A, and Denis C.** Enhancement of microvessel tortuosity in the vastus lateralis muscle of old men in response to endurance training. *J Physiol* 554: 559–569, 2004.
9. **Chilibeck PD, Paterson DH, Cunningham DA, Taylor AW, and Noble EG.** Muscle capillarization O<sub>2</sub> diffusion distance, and  $\dot{V}_{O_2}$  kinetics in old and young individuals. *J Appl Physiol* 82: 63–69, 1997.
10. **Chilibeck PD, Jeon J, Weiss C, Bell G, and Burnham R.** Histochemical changes in muscle of individuals with spinal cord injury following functional electrical stimulated exercise training. *Spinal Cord* 37: 264–268, 1999.
11. **Coggan AR, Spina RJ, Rogers MA, King DS, Brown M, Nemeth PM, and Holloszy JO.** Histochemical and enzymatic characteristics of skeletal muscle in master athletes. *J Appl Physiol* 68: 1896–1901, 1990.
12. **Coggan AR, Spina RJ, King DS, Rogers MA, Brown M, Nemeth PM, and Holloszy JO.** Skeletal muscle adaptations to endurance training in 60- to 70-yr-old men and women. *J Appl Physiol* 72: 1780–1786, 1992.
13. **Coggan AR, Spina RJ, King DS, Rogers MA, Brown M, Nemeth PM, and Holloszy JO.** Histochemical and enzymatic comparison of the gastrocnemius muscle of young and elderly men and women. *J Gerontol* 47: 71–76, 1992.
14. **Denis C, Chatard JC, Dormois D, Linossier MT, Geysant A, and Lacour JR.** Effects of endurance training on capillary supply of human skeletal muscle on two age groups (20 and 60 years). *J Physiol* 81: 379–383, 1986.
15. **Deschenes MR.** Effects of aging on muscle fibre type and size. *Sports Med* 34: 809–824, 2004.
16. **Di Giulio C, Bianchi G, Cacchio M, Macri MA, Ferrero G, Rapino C, Verratti V, Piccirilli M, and Artese L.** Carotid body HIF-1 $\alpha$ , VEGF and NOS expression during aging and hypoxia. *Adv Exp Med Biol* 536: 603–610, 2003.
17. **Ferrara N.** Molecular and biological properties of vascular endothelial growth factor. *J Mol Med* 77: 527–543, 1999.
18. **Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, and Roubenoff R.** Aging of skeletal muscle: a 12-yr longitudinal study. *J Appl Physiol* 88: 1321–1326, 2000.
19. **Gavin TP and Stager JM.** The effect of exercise modality on exercise-induced hypoxemia. *Respir Physiol* 115: 317–323, 1999.
20. **Gavin TP, Robinson CB, Yeager RC, England JA, Nifong LW, and Hickner RC.** Angiogenic growth factor response to acute systemic exercise in human skeletal muscle. *J Appl Physiol* 96: 19–24, 2004.
21. **Gavin TP, Stallings HW III, Zwetsloot KA, Westerkamp LM, Ryan NA, Moore RA, Pofahl WE, and Hickner RC.** Lower capillary density but no difference in VEGF expression in obese vs. lean young skeletal muscle in humans. *J Appl Physiol* 98: 315–321, 2005.
22. **Greb R, Maier I, Wallwiener D, and Kiesel L.** Vascular endothelial growth factor A (VEGF-A) mRNA expression levels decrease after menopause in normal breast tissue but not in breast cancer lesions. *Br J Cancer* 81: 225–231, 1999.
23. **Grimby G, Danneskiold-Samsøe B, Hvid K, and Saltin B.** Morphology and enzymatic capacity in arm and leg muscles in 78–81 year old men and women. *Acta Physiol Scand* 115: 125–134, 1982.
24. **Gustafsson T, Puntchart A, Kaijser L, Jansson E, and Sundberg CJ.** Exercise-induced expression of angiogenesis-related transcription and growth factors in human skeletal muscle. *Am J Physiol Heart Circ Physiol* 276: H679–H685, 1999.
25. **Gustafsson T, Bodin K, Sylven C, Gordon A, Tyni-Lenne R, and Jansson E.** Increased expression of VEGF following exercise training in patients with heart failure. *Eur J Clin Invest* 31: 362–366, 2001.
26. **Gustafsson T, Knutsson A, Puntchart A, Kaijser L, Nordqvist AC, Sundberg CJ, and Jansson E.** Increased expression of vascular endothelial growth factor in human skeletal muscle in response to short-term one-legged exercise training. *Pflügers Arch* 444: 752–759, 2002.
27. **Hedman A, Berglund L, Essen-Gustavsson B, Reneland R, and Lithell H.** Relationships between muscle morphology and insulin sensitivity are improved after adjustment for intra-individual variability in 70-year-old men. *Acta Physiol Scand* 169: 125–132, 2000.
28. **Hepple RT, Mackinnon SL, Goodman JM, Thomas SG, and Plyley MJ.** Resistance and aerobic training in older men: effects on  $\dot{V}_{O_2}$  peak and the capillary supply to skeletal muscle. *J Appl Physiol* 82: 1305–1310, 1997.
29. **Hepple RT and Mathieu-Costello O.** Estimating the size of the capillary-to-fiber interface in skeletal muscle: a comparison of methods. *J Appl Physiol* 91: 2150–2156, 2001.

30. **Hepple RT.** Sarcopenia—a critical perspective. *Sci Aging Knowledge Environ* 46: pe31, 2003.
31. **Hepple RT and Vogell JE.** Anatomic capillarization is maintained in relative excess of fiber oxidative capacity in some skeletal muscles of late middle-aged rats. *J Appl Physiol* 96: 2257–2264, 2004.
32. **Hoppeler H, Mathieu O, Krauer R, Claassen H, Armstrong RB, and Weibel ER.** Design of the mammalian respiratory system. VI. Distribution of mitochondria and capillaries in various muscles. *Respir Physiol* 44: 87–111, 1981.
33. **Houmard JA, Weidner ML, Gavigan KE, Tyndall GL, Hickey MS, and Alshami A.** Fiber type and citrate synthase activity in the human gastrocnemius and vastus lateralis with aging. *J Appl Physiol* 85: 1337–1341, 1998.
34. **Jakobsson F, Borg K, and Edstrom L.** Fibre-type composition, structure and cytoskeletal protein location of fibres in anterior tibial muscle. Comparison between young adults and physically active aged humans. *Acta Neuropathol (Berl)* 80: 459–468, 1990.
35. **Jozsi AC, Dupont-Versteegden EE, Taylor-Jones JM, Evans WJ, Trappe TA, Campbell WW, and Peterson CA.** Aged human muscle demonstrates an altered gene expression profile consistent with an impaired response to exercise. *Mech Ageing Dev* 120: 45–56, 2000.
36. **Kang DH, Anderson S, Kim YG, Mazzalli M, Suga S, Jefferson JA, Gordon KL, Oyama TT, Hughes J, Hugo C, Kerjaszki D, Schreiner GF, and Johnson RJ.** Impaired angiogenesis in the aging kidney: vascular endothelial growth factor and thrombospondin-1 in renal disease. *Am J Kidney Dis* 37: 601–611, 2001.
37. **Kim H, Lee DK, Choi JW, Kim JS, Park SC, and Youn HD.** Analysis of the effect of aging on the response to hypoxia by cDNA microarray. *Mech Ageing Dev* 124: 941–949, 2003.
38. **Lakatta EG and Levy D.** Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises. Part I: aging arteries: a “set up” for vascular disease. *Circulation* 107: 139–146, 2003.
39. **Leeuwenburgh C.** Role of apoptosis in sarcopenia. *J Gerontol A Biol Sci Med Sci* 58: 999–1001, 2003.
40. **Mathieu-Costello O, Ju Y, Trejo-Morales M, and Cui L.** Greater capillary-fiber interface per fiber mitochondrial volume in skeletal muscles of old rats. *J Appl Physiol* 99: 281–289, 2005.
41. **Parizkova J, Eiselt E, Sprynarova S, and Wachtlova M.** Body composition, aerobic capacity, and density of muscle capillaries in young and old men. *J Appl Physiol* 31: 323–325, 1971.
42. **Proctor DN, Sinning WE, Walro JM, Sieck GC, and Lemon PW.** Oxidative capacity of human muscle fiber types: effects of age and training status. *J Appl Physiol* 78: 2033–2038, 1995.
43. **Richardson RS, Noyszewski EA, Kendrick KF, Leigh JS, and Wagner PD.** Myoglobin O<sub>2</sub> desaturation during exercise. Evidence of limited O<sub>2</sub> transport. *J Clin Invest* 96: 1916–1926, 1995.
44. **Richardson RS, Wagner H, Mudaliar SR, Henry R, Noyszewski EA, and Wagner PD.** Human VEGF gene expression in skeletal muscle: effect of acute normoxic and hypoxic exercise. *Am J Physiol Heart Circ Physiol* 277: H2247–H2252, 1999.
45. **Richardson RS, Newcomer SC, and Noyszewski EA.** Skeletal muscle intracellular Po<sub>2</sub> assessed by myoglobin desaturation: response to graded exercise. *J Appl Physiol* 91: 2679–2685, 2001.
46. **Rivard A, Fabre JE, Silver M, Chen D, Murohara T, Kearney M, Magner M, Asahara T, and Isner JM.** Age-dependent impairment of angiogenesis. *Circulation* 99: 111–120, 1999.
47. **Rosenblatt JD, Kuzon WM, Pyley MJ, Pynn BR, and McKee NH.** A histochemical method for the simultaneous demonstration of capillaries and fiber type in skeletal muscle. *Stain Technol* 62: 85–92, 1987.
48. **Rossiter HB, Howlett RA, Holcombe HH, Entin PL, Wagner H, and Wagner PD.** Age is no barrier to muscle structural, biochemical, and angiogenic adaptations to training up to 24 months in female rats. *J Physiol* 565: 993–1005, 2005.
49. **Stein I, Itin A, Einat P, Skaliter R, Grossman Z, and Keshet E.** Translation of vascular endothelial growth factor mRNA by internal ribosome entry: implications for translation under hypoxia. *Mol Cell Biol* 18: 3112–3119, 1998.
50. **Takahashi A, Kureishi Y, Yang J, Luo Z, Guo K, Mukhopadhyay D, Ivashchenko Y, Branellec D, and Walsh K.** Myogenic Akt signaling regulates blood vessel recruitment during myofiber growth. *Mol Cell Biol* 22: 4803–4814, 2002.
51. **Tang K, Breen EC, Gerber HP, Ferrara NM, and Wagner PD.** Capillary regression in vascular endothelial growth factor-deficient skeletal muscle. *Physiol Genomics* 18: 63–69, 2004.
52. **Wagner PD.** Skeletal muscle angiogenesis. A possible role for hypoxia. *Adv Exp Med Biol* 502: 21–38, 2001.
53. **Williamson D, Gallagher P, Harber M, Hollon C, and Trappe S.** Mitogen-activated protein kinase (MAPK) pathway activation: effects of age and acute exercise on human skeletal muscle. *J Physiol* 547: 977–987, 2003.