No difference in the skeletal muscle angiogenic response to aerobic exercise training between young and aged men

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Ischaemia-induced skeletal muscle angiogenesis is impaired in aged compared with young mice. In humans, vascular endothelial growth factor (VEGF) mRNA and protein following an acute exercise bout are lower in aged compared with young untrained men. We hypothesized that exercise-induced skeletal muscle angiogenesis would be attenuated in aged compared with young men. In eight aged (mean age: 64 years) and six young (mean age: 25 years) sedentary men, muscle biopsies were obtained from the vastus lateralis prior to (Pre), after 1 week and after 8 weeks of an aerobic exercise training program for the measurement of capillarization and VEGF mRNA. Dialysate VEGF protein collected from the muscle interstitial space was measured at rest and during submaximal exercise at Pre, 1 week and 8 weeks. Exercise training increased capillary contacts (CC) and capillary-to-fibre perimeter exchange index (CFPE) of type I and IIA fibres similarly in young and aged. The CC of type IIA and IIB fibres was lower in aged compared with young independent of training status. Exercise-induced interstitial VEGF protein was lower in aged compared with young independent of training status. In untrained, greater exercise-induced interstitial VEGF protein during exercise was associated with greater type I, IIA and IIB CC. Exercise training increased VEGF mRNA similarly in young and aged. These results demonstrate that the angiogenic response to aerobic exercise training is not altered during the ageing process in humans. In addition, muscular activity-associated increases in interstitial VEGF protein may play an important role in the maintenance of skeletal muscle capillarization across the life span.

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In general it is believed that ageing reduces the ability of an organism to respond to different types of stress (Rivard *et al.* 2000). For example, the angiogenic response to hindlimb ischaemia is impaired in aged compared with young mice (Rivard *et al.* 1999; Shimada *et al.* 2004; Yu *et al.* 2006). Exercise training, a well-known physiological stressor, increases skeletal muscle capillarization, known as angiogenesis, in young (Andersen & Henriksson, 1977; Brodal *et al.* 1977; Ingjer, 1979) and aged individuals (Coggan *et al.* 1992*a*; Hepple *et al.* 1997). Cross sectional data suggest that habitual exercise training increases the number of capillary contacts (CC) of type I and IIA muscle fibres similarly in young and aged men (Proctor *et al.* 1995). In the only known report investigating the angiogenic response to the same relative intensity exercise training program in young and aged, angiogenesis was observed in young, but not aged individuals (Denis *et al.* 1986). The results from Denis *et al.* must be viewed with caution, however, as the aged individuals were fairly active prior to the initiation of the training (Denis *et al.* 1986). Whether the skeletal muscle angiogenic response to aerobic exercise training is similar in previously sedentary young and aged men is unknown.

Vascular endothelial growth factor (VEGF) is a predominantly endothelial cell-specific, heparin-binding, 45 kDa homodimeric glycoprotein mitogen and is an important regulator of basal skeletal muscle capillarization as well as exercise-induced angiogenesis (Amaral *et al.* 2001; Tang *et al.* 2004; Wagner *et al.* 2006). In men, skeletal muscle VEGF mRNA and protein are lower at rest and in response to acute exercise in aged compared with young (Ryan *et al.* 2006). A lower VEGF response to exercise might lead to reduced angiogenic potential with advanced age. During exercise, skeletal muscle interstitial VEGF

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Table 1. Subject characteristics prior to the initiation of aerobic exercise training

	Young	Aged
Age (years)	24 ± 1	64 ± 2#
Height (m)	1.80 ± 0.02	1.79 ± 0.02
Mass (kg)	96.4 ± 6.7	85.6 ± 3.8
Body fat (%)	22.6 ± 2.6	25.4 ± 2.2
Fat free mass (FFM) (kg)	74.7 ± 6.0	63.6 ± 2.4
Race (Caucasian/African American)	5/1	8/0

Mean ± S.E.M. $N =$ 6 for young and 8 for aged. #Significantly different.

protein is increased (Hoffner *et al.* 2003). This increase in interstitial VEGF protein is probably an important step in VEGF binding to the VEGF receptors located on the abluminal surface of endothelial cells (MacGabhann *et al.* 2006; MacGabhann & Popel, 2006). Whether ageing lowers interstitial VEGF protein at rest or during exercise is unknown. In the current report it was hypothesized that in skeletal muscle exercise-induced angiogenesis would be attenuated in aged compared with young men due to lower exercise-induced interstitial VEGF protein resulting from lower exercise-induced VEGF mRNA expression (Fig. 1).

Methods

Subjects

Six sedentary young (YM; range 19–30 years) and eight sedentary aged (AM; range 56–74 years) men 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 and Medical Center Institutional Review Board. All subjects were healthy non-smokers, with no history of cardiopulmonary disease. Subject characteristics are listed in Table 1. Subjects were carefully pre-screened 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 h of strenuous physical activity per week. Originally eight young subjects were recruited for the study; however, two subjects dropped out after the initial (Pre) experiment. The data for these two subjects were used only in the linear regression analysis between interstitial VEGF protein and muscle capillarization prior to (Pre) the commencement of exercise training.

$\dot{V}_{O_2max}$ and body composition

Maximal oxygen consumption ($\dot{V}_{O_2max}$) 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, USA) prior to (Pre) and after the 8 week (8 weeks) exercise training program. The test began with a 5 min warm-up at 125 W for YM and 50 W for AM. Following the warm-up, the workload was increased 25 W for YM or 20 W for AM every 2 min until volitional fatigue. Prior to the initiation of exercise training, body density (D_b) was determined via hydrostatic weighing. Residual volume was measured by oxygen dilution (Wilmore *et al.* 1980). Body fat percentage (%BF) was determined from D_b based upon the two-compartment model (Siri, 1961).

Exercise training

Subjects were enrolled in an 8 week aerobic exercise training program. Cycle ergometer exercise was performed at a workload that elicited a heart rate equivalent to 65% of $\dot{V}_{O_2max}$ as determined by the initial exercise test. Heart

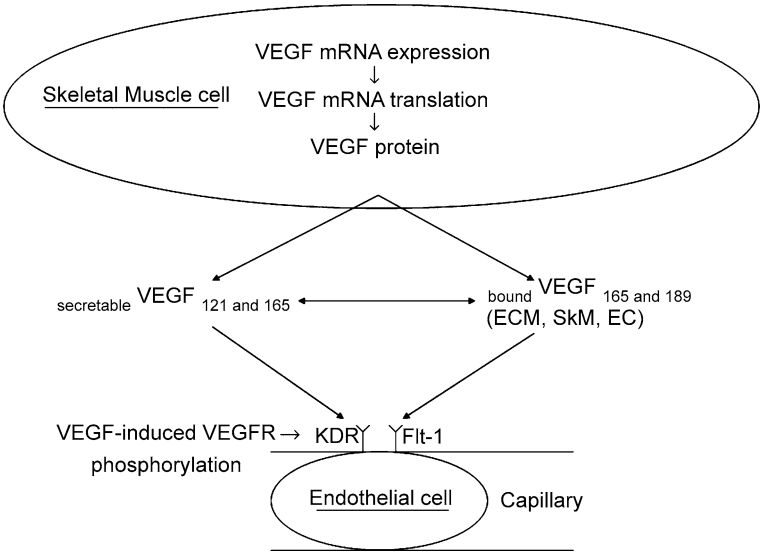

Figure 1. Schematic of skeletal muscle with skeletal muscle cells (SkM), endothelial cells (EC), extracellular matrix (EM) and VEGF expression depicted
In response to acute exercise, skeletal muscle VEGF mRNA and protein and skeletal muscle interstitial VEGF protein are increased. The increase in interstitial VEGF during exercise is presumed to lead to an increase in VEGF receptor (VEGFR) phosphorylation on endothelial cells and resultant angiogenesis. In the current report it was hypothesized that in aged compared with young men, exercise-induced skeletal muscle angiogenesis would be attenuated due to lower exercise-induced interstitial VEGF protein resulting from lower exercise-induced VEGF mRNA expression.

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 $\dot{V}_{O_2max}$. During the first week, subjects trained every day for 1 h per session. During weeks 2–8, subjects exercised 4 days per week for 1 h per session.

Sub-maximal exercise and muscle biopsies

Prior to the initiation (Pre) and after 1 week and 8 weeks of the exercise training program, subjects completed 40 min of acute cycle ergometer exercise (20 min exercise at 30% of $\dot{V}_{O_2max}$, 5 min rest, and 20 min of exercise at 65% of $\dot{V}_{O_2max}$) (Fig. 2). Prior to the commencement of the acute exercise bouts at Pre, 1 week and 8 weeks, a muscle biopsy was obtained from the vastus lateralis and a microdialysis probe was inserted in the contralateral leg for the collection of muscle interstitial dialysate for the measurement of VEGF protein. Dialysate from the muscle interstitial space was collected at rest (Rest) and during the acute exercise bout (Ex). At 1 week and 8 weeks, the biopsy was obtained 18 h after the last exercise training bout. Legs for each procedure were alternated between biopsies and microdialysis and between visits. Biopsy and microdialysis samples were stored at −80°C until analysis. A section of the biopsy sample was orientated in an optimal cutting temperature compound (OCT) tragacanth gum mixture, frozen in liquid nitrogen-cooled isopentane, and stored at −80°C until processing for the measurement of muscle morphometry and capillarization.

Morphometry, morphology, and histochemistry

Muscle tissue from the muscle biopsies (Pre and 8 weeks) was sectioned to a thickness of 10 μm on a cryostat, mounted on slides and kept at −20°C until fixation. Serial sections were stained for capillaries using the double stain technique (Qu *et al.* 1997) as modified by Porter *et al.* (2002) and for fibre type by use of a myosin ATPase stain (Brooke & Kaiser, 1970) as previously described (Ryan *et al.* 2006). The myosin ATPase stain identifies muscle fibres as type I, IIA or IIB fibres (Brooke & Kaiser, 1970). In spite of the fact that human skeletal muscle identified as type IIB by histochemical analysis actually expresses type IIx (Smerdu *et al.* 1994), in the present report fibre types were identified using the myosin ATPase stain nomenclature used in the original work.

Muscle sections were viewed under a light microscope (Nikon 400) and a digital image taken of the section (Nikon Coolpix 990) as previously described (Gavin *et al.* 2004). Capillaries were quantified manually from the digital image on individual fibres. The following indexes were measured: (1) the number of capillaries around a fibre (capillary contacts (CC)), (2) the capillary-to-fibre ratio on an individual-fibre basis (C/F_i), and (3) the number of fibres sharing each capillary (sharing factor (SF)). Capillary density (CD) was calculated by using the fibre as the reference space. Capillary-to-fibre perimeter exchange index (CFPE) was calculated as an estimate of the capillary-to-fibre surface area. Quantification of the capillary supply was performed on at least 50 fibres by randomly selecting a fibre in an artifact-free region. Fibre cross-sectional area 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 microns from an image of the myosin ATPase stain.

Microdialysis and VEGF protein analysis

Prior to the initiation (Pre) and after 1 week and 8 weeks of the exercise training program, a microdialysis probe with a 100 kDa pore size (CMA/20 no. 830 9671, CMA Microdialysis, North Chelmsford, MA, USA) was inserted percutaneously into the vastus lateralis. The probe was perfused with Ringer solution using a CMA/102 microinfusion pump (CMA/Microdialysis, Stockholm, Sweden). Interstitial dialysis samples were collected in 150 μl polyethylene collection vials and stored at −80°C until VEGF protein analysis. A commercial VEGF ELISA kit was used according to the manufacturer's instructions (R & D Systems, Minneapolis, MN, USA).

RNA isolation and real-time PCR

Approximately 30 mg of muscle was homogenized and RNA was isolated by use of an RNeasy fibrous tissue mini kit (Qiagen, Inc., Valencia, CA, USA). RNA was quantified fluorometrically using RiboGreen RNA quantification kit (Molecular Probes, Eugene, OR, USA) and 500 ng was reverse transcribed into first strand cDNA using MultiScribe RT in the High-capacity cDNA archive kit (Applied Biosystems (AB), Foster City, CA, USA).

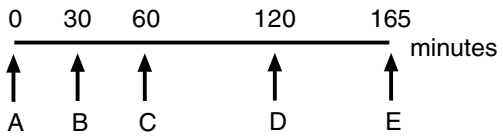

Figure 2. Time line (minutes) of the activities for each submaximal acute exercise testing day which was completed at Pre, 1 week and 8 weeks

A, insertion of the microdialysis probe. B, muscle biopsy. C, begin collection of resting microdialysis for interstitial VEGF protein. D, end collection of resting microdialysis for interstitial VEGF protein, begin collection of exercise microdialysis dialysate for VEGF protein, and commence cycle ergometer exercise. E, end collection of exercise microdialysis for interstitial VEGF protein and end cycle ergometer exercise. Muscle biopsies were obtained 18 h following the completion of the previous days exercise training bout.

Real-time PCR was conducted in duplicate on 25 ng of cDNA per reaction in 50 μl reaction volumes using TaqMan Universal PCR Master Mix with commercially available (AB) primer and probe sets for human VEGF (product no.: Hs00173626_m1). Real-time PCR was run for one cycle (50°C for 2 min, 95°C for 10 min) immediately followed by 40 cycles (95°C for 15 s, 60°C for 1 min). Fluorescence was measured after each of the repeated cycles. RNA samples were normalized to 18S rRNA (eukaryotic 18S PDAR primer-limited VIC/TAMRA, AB, product no. 4310893E) multiplexed during the analysis of each specific gene.

Statistical treatment

For $\dot{V}_{O_2max}$, muscle fibre characteristics and VEGF mRNA a two-way mixed-plot factorial analysis of variance (age × training status (Pre, 1 week, 8 weeks)) with repeated measures on training status was used. For interstitial VEGF protein, a three-way mixed-plot factorial analysis of variance (age × training status × activity level (Rest/Ex)) was performed with repeated measures on training status and activity level. Following a significant F ratio, a Bonferroni *post hoc* analysis was used. Student's unpaired t tests were used to compare differences in all other variables between YM and AM. Linear regression was performed to investigate associations: (1) at Pre, 1 week and 8 weeks between Rest or Ex interstitial VEGF protein and the change in fibre-type-specific CC between Pre and 8 weeks; (2) at Pre between Rest interstitial VEGF protein and fibre-type-specific CC; and (3) at Pre between Ex interstitial VEGF protein and fibre-type-specific CC at Pre. The rationale for selectively performing these regressions was to investigate: (1) if rest or exercise-induced interstitial VEGF protein during an exercise training program explained individual differences in exercise-induced angiogenesis; (2) if resting interstitial VEGF protein explained individual differences in muscle capillarization in untrained individuals; and (3) if exercise-induced interstitial VEGF protein explained individual differences in muscle capillarization in untrained individuals. Significance was established at $P \leq 0.05$ for all statistical sets and data reported are mean ± S.E.M.

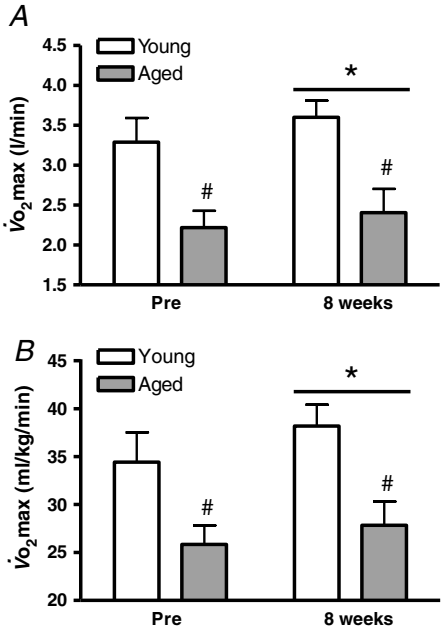

Figure 3. Absolute (*A*) and relative (*B*) maximal oxygen consumption ($\dot{V}_{O_2max}$) prior to (Pre) and after 8 weeks of aerobic exercise training in young and aged men
Exercise training increased absolute and relative $\dot{V}_{O_2max}$. Absolute and relative $\dot{V}_{O_2max}$ were lower in aged. *Main effect of exercise. #Main effect of age. Mean ± S.E.M. $N = 6$ for young and 8 for aged.

Results

AM were ∼40 years older than YM (Table 1). There were no differences in height, mass, body fat (%) or fat-free mass (FFM) between groups. As anticipated, absolute and relative $\dot{V}_{O_2max}$ values were lower in AM compared with YM (Fig. 3). Eight weeks of aerobic exercise training increased $\dot{V}_{O_2max}$ similarly in YM and AM.

Muscle fibre cross-sectional area of type IIA and IIB was ∼30% lower in AM compared with YM independent of training status, while there was no effect of exercise training on fibre cross-sectional area of any fibre type (Table 2). There was no difference in fibre type percentage between YM and AM, but the percentage of type I fibres tended ($P = 0.08$) to be greater (11%) in AM compared with YM. The percentage area of type I fibres was 16% greater and type IIA fibres tended ($P = 0.06$) to be lower (11%) in AM compared with YM independent of training status. There was no effect of exercise training on fibre type percentage or the percentage area of any fibre type.

Muscle capillarization (CC and C/F_i) surrounding type IIA and IIB fibres was ∼25% lower in AM compared with YM independent of training status (Table 3). Exercise training increased capillarization (CC, C/F_i and CFPE) ∼25% of type I and IIA in YM and ∼18% of type I and IIA in AM. There was no difference in the angiogenic response to exercise training between YM and AM. Exercise training decreased the sharing factor of type IIB fibres 10% in YM, but not in AM. Exercise training increased CD of type IIA fibres. There was no effect of age on CFPE or CD of any fibre type.

Rest and Ex interstitial VEGF protein for YM and AM at Pre, 1 week and 8 weeks are in Fig. 4. There were no interactions between age, training status (Pre, 1 week, 8 weeks), and activity level (Rest/Ex) (age × training status × activity level; $P = 0.301$), age and training status (age × training status; $P = 0.518$), or between training status and activity level (training status × activity level; $P = 0.847$). There was a significant interaction between

Table 2. Skeletal muscle fibre type characteristics prior to (Pre) and after 8 weeks (8 weeks) of aerobic exercise training in young and aged men

	Young Pre	Young 8 weeks	Aged Pre	Aged 8 weeks
Fibre area (μm^2)				
Type I	5757 ± 704	5690 ± 260	5091 ± 482	5613 ± 604
Type IIA	7360 ± 730	7265 ± 459	5164 ± 520#	5578 ± 615#
Type IIB	5933 ± 682	6016 ± 431	4272 ± 461#	4125 ± 615#
Fibre perimeter (μm)				
Type I	316 ± 21	314 ± 11	297 ± 14	304 ± 18
Type IIA	359 ± 19	359 ± 14	299 ± 14#	308 ± 19#
Type IIB	331 ± 26	334 ± 15	274 ± 19#	271 ± 24#
Fibre type (%)				
Type I	45.2 ± 4.9	40.3 ± 5.5	55.8 ± 4.6	51.7 ± 4.2
Type IIA	37.4 ± 2.8	42.4 ± 6.9	31.4 ± 2.9	35.9 ± 4.4
Type IIB	17.5 ± 3.6	17.3 ± 3.4	12.7 ± 3.7	12.3 ± 3.3
Area of fibres (%)				
Type I	40.8 ± 4.6	36.2 ± 5.2	56.5 ± 5.5#	53.1 ± 4.8#
Type IIA	43.4 ± 2.7	47.4 ± 7.1	32.4 ± 3.4	37.1 ± 5.3
Type IIB	15.8 ± 3.5	16.4 ± 3.0	11.1 ± 3.2	9.8 ± 3.1

There were no significant interactions identified between age and training status (age × training status) for any measured outcome variable. #Main effect of age. Mean ± S.E.M. $N = 6$ for young and 8 for aged.

Table 3. Skeletal muscle capillarization prior to (Pre) and after 8 weeks of aerobic exercise training in young and aged men

	Young Pre	Young 8 weeks	Aged Pre	Aged 8 weeks
Capillary contacts				
Type I	3.84 ± 0.29	4.77 ± 0.31*	3.61 ± 0.37	4.24 ± 0.23*
Type IIA	4.00 ± 0.32	5.11 ± 0.33*	3.19 ± 0.37#	3.81 ± 0.35*,#
Type IIB	3.69 ± 0.27	4.15 ± 0.24	3.26 ± 0.28#	2.75 ± 0.38#
Individual capillary-to-fibre ratio				
Type I	1.42 ± 0.12	1.87 ± 0.15*	1.39 ± 0.15	1.65 ± 0.11*
Type IIA	1.51 ± 0.13	1.88 ± 0.15*	1.23 ± 0.15#	.1.48 ± 0.16*,#
Type IIB	1.35 ± 0.12	1.63 ± 0.10	1.24 ± 0.14#	1.08 ± 0.17#
Sharing factor				
Type I	2.81 ± 0.04	2.72 ± 0.05	2.77 ± 0.04	2.77 ± 0.04
Type IIA	2.78 ± 0.05	2.74 ± 0.03	2.79 ± 0.07	2.75 ± 0.06
Type IIB	2.93 ± 0.07	2.63 ± 0.05**	2.72 ± 0.08	2.81 ± 0.06
Capillary density (capillaries × mm^{-2})				
Type I	264 ± 16	341 ± 29	303 ± 28	350 ± 63
Type IIA	216 ± 8	292 ± 18*	249 ± 26	298 ± 42*
Type IIB	246 ± 20	278 ± 24	345 ± 53	277 ± 22
CFPE (capillaries × 1000 μm^{-1})				
Type I	4.50 ± 0.17	5.98 ± 0.54*	4.65 ± 0.44	5.50 ± 0.43*
Type IIA	4.20 ± 0.15	5.52 ± 0.44*	4.06 ± 0.40	4.81 ± 0.38*
Type IIB	4.16 ± 0.23	4.82 ± 0.38	4.64 ± 0.49	3.73 ± 0.36

There was significant interaction (age × training status) for Type IIB sharing factor ($P < 0.05$). There were no significant interactions identified between age and training status (age × training status) for any other measured outcome variable. CFPE, capillary-to-fibre perimeter exchange index. *Main effect of exercise. #Main effect of age. **Significantly different from young Pre. Mean ± S.E.M. $N = 6$ for young and 8 for aged.

age and activity level (age × activity level; $P < 0.001$). Acute exercise increased interstitial VEGF protein (Ex) in both YM and AM, though the increase with exercise was greater in YM than AM independent of training status (Pre, 1 week or 8 weeks). There was no difference in Rest interstitial VEGF protein between YM and AM.

Linear regression was performed to identify if interstitial VEGF protein may be an important determinant of muscle capillarization and angiogenesis. There were significant associations between Pre Ex interstitial VEGF protein and Pre type I, IIA and IIB CC (Fig. 5). There were no associations between Pre Rest interstitial VEGF protein

and fibre-type-specific CC or between Rest or Ex interstitial VEGF protein at any time point and the change in fibre-type-specific CC (data not shown).

There was a main effect of exercise to increase VEGF mRNA (Fig. 6). *Post hoc* analysis revealed that VEGF mRNA was increased at 1 week and 8 weeks similarly in YM and AM. There was no effect of age on VEGF mRNA.

Discussion

The principal finding from the current report is that skeletal muscle angiogenesis in response to 8 weeks of aerobic exercise training is similar in young and aged men. These findings suggest that while ageing lowers basal capillarization (Parizkova *et al.* 1971; Coggan *et al.* 1992*b*; Proctor *et al.* 1995; Ryan *et al.* 2006) and impairs the angiogenic (pathological) response to limb ischaemia (Rivard *et al.* 1999; Shimada *et al.* 2004; Yu *et al.* 2006); the angiogenic (physiological) response to exercise training is not inherently impaired in healthy aged men.

It is generally believed that ageing reduces angiogenic potential. Exercise is well known to increase skeletal muscle capillarization in young (Andersen & Henriksson, 1977; Brodal *et al.* 1977; Ingjer, 1979) and aged individuals (Coggan *et al.* 1992*a*; Hepple *et al.* 1997). In the current investigation it was hypothesized that the angiogenic response to the same exercise training regiment would be lower in aged compared with young individuals. In contrast to this hypothesis, increases in CC, C/F_i, and CFPE of type I and IIA muscle fibres were similar in young and aged men. This is consistent with a recent report demonstrating a similar skeletal muscle angiogenic response to exercise training in young and aged Fisher 344 rats (Rossiter *et al.* 2005). In contrast, the angiogenic response to hindlimb ischaemia is impaired in aged compared with young mice (Rivard *et al.* 1999; Shimada

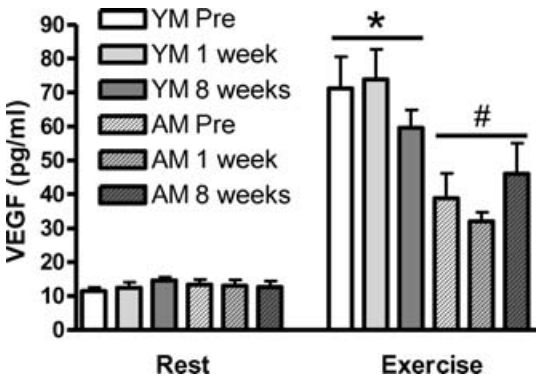

Figure 4. Dialysate VEGF protein prior to (Pre; open bars), after 1 week (light grey bars) and after 8 weeks (dark grey bars) of aerobic exercise training at rest and in response to acute aerobic exercise in young (YM; non-hatched bars) and aged (AM; hatched bars) men
There was no interaction between age, training status (Pre, 1 week, 8 weeks), and activity level (rest/exercise) (age × training status × activity level; $P = 0.301$). Acute exercise increased dialysate VEGF protein in both young and aged men; however, the increase with exercise was greater in young than aged (age × activity level interaction; $P < 0.001$). There were no interactions between age and training status (age × training status interaction; $P = 0.518$) or between training status and activity level (training status × activity level interaction; $P = 0.847$). *Young + exercise significantly different from young + rest, aged + rest, and aged + exercise. #Aged + exercise significantly different from young + rest, aged + rest, and young + exercise. Mean ± S.E.M. $N = 6$ for young and 8 for aged.

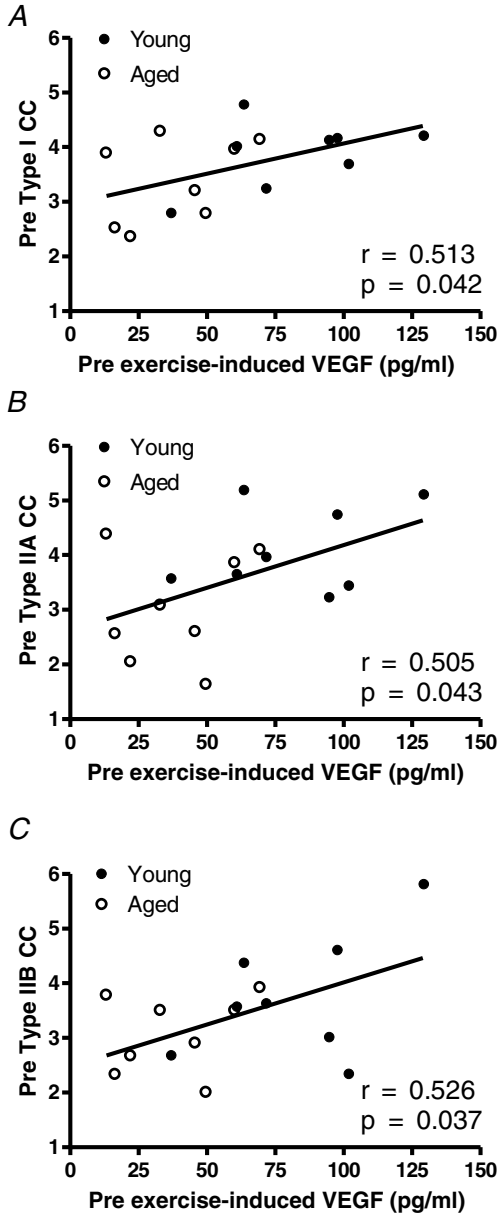

Figure 5. Linear regression between exercise-induced interstitial VEGF protein and skeletal muscle capillary contacts (CC) prior to (*A*, *B* and *C*) in untrained individuals (Pre)
There were significant associations between exercise – induced VEGF protein and CC of type I, IIA and IIB fibres.

et al. 2004; Yu *et al.* 2006). This disparity between physiological (exercise) and pathological (ischaemia) angiogenesis probably reflects differences in the skeletal muscle angiogenic response to the unique stimulus of each condition.

In untrained men, total skeletal muscle VEGF mRNA and protein are lower in aged compared with young following an acute exercise training bout (Ryan *et al.* 2006). We therefore had hypothesized that lower skeletal muscle exercise-induced angiogenesis in aged compared with young men would be due to lower interstitial VEGF protein. While exercise-induced interstitial VEGF protein was lower in aged compared with young independent of exercise training status (Pre, 1 week or 8 weeks), skeletal muscle exercise-induced angiogenesis was not different between young and aged. One possible explanation for this finding is that VEGF is not important for exercise-induced angiogenesis. However, this would be in contrast to findings in transgenic mice that inhibition of muscle-specific VEGF expression inhibits exercise-induced angiogenesis (Wagner *et al.* 2006). A more likely possibility is that a certain minimum increase in interstitial VEGF is required during each acute exercise bout to promote exercise-induced angiogenesis and that greater increases in interstitial VEGF protein do not result in greater angiogenesis. Angiogenesis requires the co-ordinated effort of many systems including vessel destabilization and hyperpermeability (VEGF-dependent step), endothelial cell proliferation and migration (VEGF-dependent step), cell-to-cell interaction and tube formation, attraction of cells to the newly forming vessel, and vessel stabilization. These processes are controlled by many different factors and thus investigation of VEGF expression is one of the many steps where abnormal function may occur.

While the angiogenic response to exercise was not different, basal skeletal muscle capillarization was lower in aged compared with young consistent with several previous reports (Parizkova *et al.* 1971; Coggan *et al.* 1992*b*; Proctor *et al.* 1995; Ryan *et al.* 2006). Interestingly, we observed that greater exercise-induced, but not resting, interstitial VEGF protein was associated with greater CC of type I, IIA and IIB fibres. This finding suggests that increases in interstitial VEGF in response to daily muscular activity may be important for the maintenance of basal skeletal muscle capillarization. Consistent with this, muscular inactivity reduces VEGF expression and promotes the loss of muscle capillaries (Wagatsuma *et al.* 2005; Wagatsuma & Osawa, 2006), while exercise training increases total skeletal muscle VEGF protein in humans (Gustafsson *et al.* 2001; Gustafsson *et al.* 2002). Skeletal muscle capillarization is preserved in aged compared with young master's athletes (Coggan *et al.* 1990). Given that physical activity levels decrease with advanced age, promoting greater physical activity in aged individuals may be important to ameliorate the reduction in muscle capillarization.

It is not possible from the current data to determine why type I CC is similar, while type II fibre capillarization is lower in aged compared with young. Several possibilities may explain this differential response. It may be that the maintenance of type I skeletal muscle capillaries is due to preferentially greater production or localization of VEGF protein surrounding type I fibres compared with type II fibres with ageing. In young rats, exercise-induced increases in VEGF mRNA are greater in more oxidative compared with glycolytic regions of muscle (Brutsaert *et al.* 2002), which is consistent with greater exercise-induced angiogenesis of oxidative compared with glycolytic muscle fibres. It is also possible that preferential maintenance of VEGF receptor expression occurs in capillaries surrounding type I compared with type II muscle fibres with ageing. To our knowledge there are no studies investigating VEGF receptor expression relative to muscle fibre type. It is also possible that greater expression of other angiogenic growth factors may be present in type I fibres in aged compared with young. Future work is necessary to understand these fibre type differences with advanced age.

In the current study, VEGF mRNA was measured to identify if differences in interstitial VEGF protein could be explained by differences in VEGF mRNA. In men, resting and exercise-induced increases in skeletal muscle VEGF mRNA are lower in aged compared with young (Ryan *et al.* 2006). Therefore, we had hypothesized that exercise-training-induced increases in VEGF mRNA would be lower in aged compared with young men and that lower VEGF mRNA might explain potential differences in muscle interstitial VEGF protein. As anticipated, exercise training increased skeletal muscle VEGF mRNA; however, there was no difference in VEGF mRNA between young and aged at any time point during the training program suggesting that differences in VEGF mRNA do not account

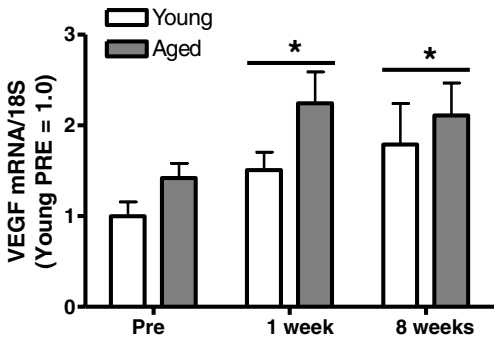

Figure 6. Skeletal muscle VEGF mRNA prior to (Pre) and after 1 week and 8 weeks of aerobic exercise training in young and aged men
Exercise training increased VEGF mRNA at 1 week and 8 weeks similarly in YM and AM. *Time point greater than Pre. Mean ± S.E.M. $N =$ 6 for young and 8 for aged.

for differences in interstitial VEGF protein. It is possible though that VEGF mRNA measured 18 h after exercise may be less important than the transient increase in VEGF mRNA that occurs in response to each individual exercise bout. It should be noted as well that exercise-induced angiogenesis was not different in young and aged and thus any possible differences in angiogenic growth factor responses to exercise would be viewed as physiologically inconsequential.

Advanced age is the leading risk factor for the development of cardiovascular disease and worsens prognosis in patients with cardiovascular disease. One potential mechanism for poor outcomes in aged patients is reduced neovascular potential (Weinsaft & Edelberg, 2001). In the current study, angiogenic potential was not impaired in aged men. Aged individuals like those in the current study are the healthiest among their contemporaries. For example, by age 60 years it is estimated that 50–60% of all individuals are hypertensive (Lakatta & Levy, 2003). The men in the current study were all free of overt cardiovascular disease. Our findings of maintenance of angiogenic potential with ageing may be influenced by the excellent health of our aged subjects. Understanding how the skeletal muscle angiogenic response to exercise can be maintained across the life span in very healthy aged individuals may lead to improvements in the clinical treatment of aged patients.

In summary, we have demonstrated that the angiogenic response to 8 weeks of endurance exercise training is preserved in aged compared with young men and therefore to assume that angiogenic potential is inherently impaired in aged humans appears to be unwarranted. Interestingly, greater exercise-induced interstitial VEGF is associated with greater capillarization of type I, IIA and IIB fibres in untrained men suggesting that VEGF regulation of basal muscle capillarization occurs in response to physical activity. Understanding how exercise training promotes skeletal muscle angiogenesis in populations with known impairments in ischaemia-induced angiogenesis may lead to improvements in the treatment of these populations.

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