Soleus, plantaris and gastrocnemius VEGF mRNA responses to hypoxia and exercise are preserved in aged compared with young female C57BL/6 mice

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Abstract

Aims: In humans, skeletal muscle capillarization and the vascular endot- helial growth factor (VEGF) mRNA response to acute exercise are lower in aged compared with young. The exercise-induced increase in VEGF mRNA has been proposed to involve hypoxic regulation of VEGF and is believed to be fibre type-dependent. We hypothesized that attenuated VEGF mRNA responses to hypoxia and exercise with advanced age would be greatest in oxidative vs. glycolytic muscles.
Methods: 3- and 24-month-old female C57BL/6 mice were exposed to acute hypoxia (FIO2 ¼ 0.06) or performed a single exercise (65% of maximum treadmill running speed) bout. Capillarization and VEGF mRNA wereanalysed in the soleus, plantaris and gastrocnemius muscles.
Results: In each muscle, VEGF mRNA was greater in aged compared with young, while the VEGF mRNA response to acute hypoxia or acute exercise was similar between young and aged. Morphological analysis revealed that type IIA fibre percentage and type IIB capillarization in the plantaris were greater and type IIB fibre cross-sectional area (FCSA) in the gastrocnemius was smaller in aged compared with young.
Conclusions: These findings suggest that ageing does not impair the potential for non-pathological angiogenesis in mice and that acute exercise increases VEGF mRNA in the soleus, plantaris and gastrocnemius muscles, which differ considerably in fibre type percentage.

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Soleus, plantaris and gastrocnemius VEGF mRNA responses to hypoxia and exercise are preserved in aged compared with young female C57BL/6 mice

T. P. Gavin,[1,2,3] L. M. Westerkamp[1,3] and K. A. Zwetsloot[1,3]

1 *Department of Exercise and Sport Science, East Carolina University, Greenville, NC, USA*

2 *Department of Physiology, East Carolina University, Greenville, NC, USA*

3 *Human Performance Laboratory, East Carolina University, Greenville, NC, USA*

Abstract

Aims: In humans, skeletal muscle capillarization and the vascular endothelial growth factor (VEGF) mRNA response to acute exercise are lower in aged compared with young. The exercise-induced increase in VEGF mRNA has been proposed to involve hypoxic regulation of VEGF and is believed to be fibre type-dependent. We hypothesized that attenuated VEGF mRNA responses to hypoxia and exercise with advanced age would be greatest in oxidative vs. glycolytic muscles.

Methods: 3- and 24-month-old female C57BL/6 mice were exposed to acute hypoxia (F_IO_2 ¼ 0.06) or performed a single exercise (65% of maximum treadmill running speed) bout. Capillarization and VEGF mRNA were analysed in the soleus, plantaris and gastrocnemius muscles.

Results: In each muscle, VEGF mRNA was greater in aged compared with young, while the VEGF mRNA response to acute hypoxia or acute exercise was similar between young and aged. Morphological analysis revealed that type IIA fibre percentage and type IIB capillarization in the plantaris were greater and type IIB fibre cross-sectional area (FCSA) in the gastrocnemius was smaller in aged compared with young.

Conclusions: These findings suggest that ageing does not impair the potential for non-pathological angiogenesis in mice and that acute exercise increases VEGF mRNA in the soleus, plantaris and gastrocnemius muscles, which differ considerably in fibre type percentage.

Keywords acute aerobic exercise, acute hypoxia, ageing, vascular endothelial growth factor.

Detrimental changes present in the skeletal muscle of aged individuals include sarcopenia (Coggan *et al.* 1992, Proctor *et al.* 1995), lower oxidative capacity (Coggan *et al.* 1992, Houmard *et al.* 1998), increases in type I and decreases in type IIB muscle fibre percentages (Coggan *et al.* 1992), and fewer capillaries surrounding muscle fibres (Parizkova *et al.* 1971, Coggan *et al.* 1992, Proctor *et al.* 1995, Frontera *et al.* 2000, Croley *et al.* 2005, Ryan *et al.* 2006). These changes likely result both from the ageing process and decreases in physical activity in aged individuals. It is well known that acute exercise increases skeletal muscle vascular endothelial growth factor (VEGF) mRNA in humans (Gustafsson *et al.* 1999, Richardson *et al.* 1999, Gavin *et al.* 2004, 2005, Croley *et al.* 2005, Ryan *et al.* 2006), rats (Breen *et al.* 1996, Gavin *et al.* 2000a, Gavin & Wagner 2001, 2002, Olfert *et al.* 2001a,b) and mice (Choi *et al.* 2005). Hypoxia has been proposed as a potential regulator of exercise-induced increases in VEGF mRNA (Wagner 2001, Birot *et al.* 2003).

Consistent with this, systemic hypoxia increases skeletal muscle VEGF mRNA in rats (Tang *et al.* 1993, 2004, Breen *et al.* 1996).

We have recently observed that the VEGF mRNA response to acute exercise is attenuated in aged vs. young men and women (Croley *et al.* 2005, Ryan *et al.* 2006). In contrast, the exercise-induced increase in gastrocnemius VEGF mRNA is preserved in aged vs. young rats (Rossiter *et al.* 2005). One potential reason for these divergent results may be the fibre type composition of the muscles studied. The rat gastrocnemius is predominantly glycolytic composed of type IIB muscle fibres (Armstrong & Phelps 1984), while the vastus lateralis in humans is predominantly oxidative composed of types I and IIA muscle fibres (Ryan *et al.* 2006). Birot *et al.* (2003) reported that in the plantaris the VEGF mRNA response to acute exercise occurs exclusively in type IIB muscle fibres. In contrast, Brutsaert *et al.* (2002) reported a greater VEGF mRNA response to acute exercise and electrical stimulation in more oxidative compared with glycolytic regions of the gastrocnemius. Type IIB muscle fibres have been proposed to be more susceptible to hypoxia because of their inherently lower muscle capillarization (Birot *et al.* 2003).

The present study was designed to investigate if the discrepancies in VEGF expression between aged humans and animal models of ageing are because of inherent differences in the fibre type composition of the muscles investigated. Young and aged C57BL/6 mice were used instead of aged rates because age-associated differences in skeletal muscle VEGF mRNA expression in response to hindlimb ischaemia exist in this strain of mice (Rivard *et al.* 1999); and while differences in fibre type composition do exist between mice and rats (Talmadge & Roy 1993, Agbulut *et al.* 1996), these differences are small in nature. Thus, young and aged C57BL/6 mice were exposed to acute hypoxia and acute exercise and VEGF mRNA was measured in the soleus (oxidative), plantaris (mixed) and gastrocnemius (glycolytic) muscles. We hypothesized that the VEGF mRNA response to acute hypoxia and acute exercise would be attenuated in the soleus and not the gastrocnemius of aged compared with young C57BL/6 mice.

Materials and methods

This study was approved by the East Carolina University Animal Care and Use Committee. 3- and 24-month-old female C57BL/6 mice were used. Animals were obtained from the National Institute on Aging. The ages of the mice were selected based upon the finding that 24-month-old compared with 3-month-old C57BL/6 mice demonstrate a lower VEGF and angiogenic response to hindlimb ischaemia (Rivard *et al.* 1999). While it is impossible to adequately gauge the relative age of mice with respect to humans, the mortality rate for this age of mouse is approximately 50% (Turturro *et al.* 1999) and is comparable with that of the aged men and women we have recently studied (Croley *et al.* 2005, Ryan *et al.* 2006). Animals were housed in individual cages and allowed standard chow and water *ad libitum* throughout the study.

Exercise protocol

Animals were familiarized with a rodent treadmill and taught to run at 10 m min^{-1}, 10° incline for 5 – 10 min for two consecutive days. On the third day, maximal treadmill speed was assessed by use of an incremental maximal exercise test. The treadmill was placed on a 10° inclination and animals were allowed to warm-up for 5 min at 10 m min^{-1}. After completion of the warm-up, the treadmill speed was increased 3 m min^{-1} every 2 min until the mice were unable to maintain the treadmill speed.

One week following the completion of the maximal exercise test, mice were randomly assigned to groups (rest, 0 h post-exercise, or 1 h post-exercise; $N = 3$ per age per group). Animals either rested or ran on a 10° inclined treadmill at a speed equivalent to 65% of their maximum speed for 1 h. The speeds used for the submaximal exercise bout were 20 m min^{-1} for young and 13 m min^{-1} for aged mice. At the appropriate time, animals were anaesthetized with isoflurane and killed by cervical dislocation. Muscles were removed, weighed and stored at −80 °C until RNA analysis. The gastrocnemius, plantaris and soleus muscles from one hindlimb of the rest animals were 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 ($N = 3$ per age).

Hypoxia protocol

Young and aged animals were exposed to systemic hypoxia ($F_IO_2 = 0.06$) for 2 h ($N = 5$ per age per group). This level of systemic hypoxia was chosen because: (1) 6% O_2 systemic hypoxia increases hypoxia inducible factor-1 (HIF-1) expression in skeletal muscle (Stroka *et al.* 2001); (2) age associated differences in HIF-1 are present in non-skeletal muscle tissue at 7% O_2 systemic hypoxia (Frenkle-Denkberg *et al.* 1999) and (3) age-associated differences in VEGF are because of reduced HIF-1 activation in vascular smooth muscle cells (VSMC; Rivard *et al.* 2000). Immediately following the completion of the 2 h hypoxia exposure, animals were anaesthetized with isoflurane and killed by cervical dislocation. Muscles were removed and stored at −80 °C until RNA analysis. The rest animals from the

exercise protocol served as normoxia controls for the hypoxia protocol.

RNA isolation and real-time PCR

Gastrocnemius muscle was first pulverized under liquid nitrogen. The mRNA from the entire soleus and plantaris muscles and approximately 30 mg of gastrocnemius muscle was isolated by use of an RNeasy fibrous tissue mini kit (Qiagen, Valencia, CA, USA). RNA was quantified fluorometrically using RiboGreen RNA quantitation 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]. RiboGreen RNA quantitation reagent is an ultrasensitive fluorescent nucleic acid stain specific for RNA and is 1000 times more sensitive than absorbance measures. Real-time PCR was conducted in triplicate on 25 ng of cDNA per reaction in 50 lL reaction volumes using TaqMan Universal PCR Master Mix with a commercially available (AB) primer and probe set for mouse VEGF (product no.: Mm00437304_m1) by use of FAM/TAMRA-labelled dye on an AB PRISM 7300 sequence detection system instrument and software (AB). 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.

Muscle morphological analysis

Muscle tissue was sectioned to a thickness of 10 lm on a cryostat, mounted on slides and kept at)20 °C until fixation. Sections were stained for capillaries and fibre type by use of the Rosenblatt technique (Rosenblatt *et al*. 1987).

Muscle sections were viewed under a light microscope (Nikon 400; Nikon, Melville, NY, USA) and a digital image taken of the mid-belly 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 indices were measured (Hepple *et al*. 1997): (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 an artefact-free region within the mid-belly of the respective muscles. Fibre cross-sectional area (FCSA) and perimeter (FP) were measured with the image analysis system and commercial software (SigmaScan; Jandel Scientific, Point Richmond, CA, USA), calibrated to transform the number of pixels (viewed on a computer monitor) into lm from an image of the Rosenblatt stain.

Statistical treatment

For VEGF mRNA in hypoxia and exercise, a two-way anova (age · exercise or age · hypoxia) was used. Following a significant *F*-ratio, a Fisher's LSD *post hoc* test was used. Unpaired Student's *t*-tests were used to compare differences in all other variables between young and aged mice. Significance was established at $P \leq 0.05$ for all statistical sets and data reported are mean ± SD.

Results

Animals

Aged mice were heavier and demonstrated lower maximal treadmill running speeds compared with young mice (Table 1). The mass of the soleus, plantaris and gastrocnemius muscles were greater in aged compared with young when expressed as absolute mass, but lower in aged compared with young when expressed relative to body mass.

C57BL/6 mouse muscle morphology

In the soleus (Table 2), there were no differences in muscle characteristics between young and aged mice. In the plantaris (Table 3), aged mice demonstrated a greater percentage of type IIA fibres and a lower percentage of type IIB fibres. Muscle capillarization (CC, C/F_i, CD and CFPE) of type IIB fibres was greater and C/F_i of type IIA fibres was lower in aged compared with young. In the gastrocnemius (Table 4), FCSA of type IIB fibres was lower in aged compared with young.

VEGF mRNA expression

Soleus, plantaris and gastrocnemius VEGF mRNA from mice exposed to 21% or 6% O_2 are illustrated in Figure 1. There was no age · hypoxia interaction in any muscle (power: 0.050, 0.050, and 0.050 for soleus, plantaris and gastrocnemius, respectively). There was a significant main effect of acute hypoxia to increase VEGF mRNA in each muscle (power: 0.999, 0.873, and 0.999, respectively), but not for age (power: 0.050, 0.050, and 0.050, respectively).

Soleus, plantaris and gastrocnemius VEGF mRNA at rest, 0 h post-exercise and 1 h post-exercise are illustrated in Figure 2. There was no age · exercise interaction in any muscle (power: 0.166, 0.050, and 0.050

Table 1 C57BL/6 mice characteristics (mean ± SD, *N* ¼ 9 for young and aged)

	Young (3 months)	Aged (24 months)	*P*-value
Body mass (g)	20.4 ± 1.0	27.7 ± 2.1	<0.001
Maximum treadmill speed (m min^{-1})	31.0 ± 5.0	20.0 ± 3.0	<0.001
Soleus (mg)	6.3 ± 0.8	7.3 ± 0.8	0.026
Plantaris (mg)	12.3 ± 1.3	13.6 ± 1.0	0.026
Gastrocnemius mass (mg)	93.7 ± 10.0	105.9 ± 5.7	0.006
Soleus/body mass (mg g^{-1})	0.31 ± 0.03	0.26 ± 0.03	0.004
Plantaris/body mass (mg g^{-1})	0.60 ± 0.05	0.49 ± 0.04	<0.001
Gastrocnemius/body mass (mg g^{-1})	4.59 ± 0.34	3.84 ± 0.33	<0.001

Table 2 Skeletal muscle fibre and capillary characteristics in soleus muscle of C57BL/6 mice (mean ± SD, CFPE, capillary-to-fibre perimeter exchange index, *N* ¼ 3 young and aged)

Soleus	Young	Aged	*P*-value
Type I fibre (%)	56.6 ± 6.9	52.6 ± 7.3	0.525
Type IIA fibre (%)	41.9 ± 5.3	45.1 ± 4.9	0.515
Type IIB fibre (%)	1.5 ± 2.6	2.3 ± 2.0	0.705
Fibre cross-sectional area (lm^2)			
Type I	1169 ± 368	1229 ± 238	0.824
Type IIA	923 ± 266	1056 ± 224	0.543
Fibre perimeter (lm)			
Type I	136 ± 22	142 ± 13	0.705
Type IIA	124 ± 17	137 ± 13	0.340
Capillary contacts			
Type I	4.06 ± 0.12	3.71 ± 0.50	0.371
Type IIA	3.85 ± 0.37	3.51 ± 0.74	0.527
Individual capillary-to-fibre ratio			
Type I	1.38 ± 0.04	1.34 ± 0.19	0.709
Type IIA	1.34 ± 0.13	1.26 ± 0.23	0.629
Capillary density (capillaries · mm^{-2})			
Type I	1313 ± 406	1139 ± 255	0.566
Type IIA	1621 ± 584	1304 ± 417	0.487
CFPE (capillaries · 1000lm^{-1})			
Type I	10.28 ± 1.45	9.35 ± 1.37	0.468
Type IIA	11.01 ± 2.45	9.21 ± 2.00	0.404

Table 3 Skeletal muscle fibre and capillary characteristics in plantaris muscle of C57BL/6 mice (mean ± SD, CFPE, capillary-to-fibre perimeter exchange index, *N* ¼ 3 young and aged)

Plantaris	Young	Aged	*P*-value
Type I fibre (%)	27.7 ± 5.2	35.2 ± 8.3	0.258
Type IIA fibre (%)	17.1 ± 7.6	42.2 ± 7.7	0.016
Type IIB fibre (%)	55.2 ± 11.7	22.6 ± 6.1	0.013
Fibre cross-sectional area (lm^2)			
Type I	1444 ± 536	1502 ± 301	0.878
Type IIA	1204 ± 673	932 ± 212	0.540
Type IIB	1723 ± 172	1905 ± 371	0.484
Fibre perimeter (lm)			
Type I	163 ± 31	163 ± 18	0.998
Type IIA	145 ± 42	128 ± 12	0.537
Type IIB	178 ± 7	182 ± 12	0.674
Capillary contacts			
Type I	2.96 ± 0.19	3.35 ± 0.54	0.307
Type IIA	2.72 ± 0.32	2.34 ± 0.05	0.112
Type IIB	2.93 ± 0.15	3.64 ± 0.22	0.010
Individual capillary-to-fibre ratio			
Type I	1.04 ± 0.16	1.18 ± 0.14	0.296
Type IIA	0.99 ± 0.07	0.83 ± 0.05	0.041
Type IIB	1.01 ± 0.04	1.28 ± 0.14	0.036
Capillary density (capillaries · mm^{-2})			
Type I	793 ± 99	922 ± 350	0.572
Type IIA	1109 ± 529	948 ± 180	0.643
Type IIB	640 ± 12	728 ± 37	0.017
CFPE (capillaries · 1000 lm^{-1})			
Type I	6.41 ± 0.22	7.42 ± 1.73	0.373
Type IIA	7.37 ± 2.18	6.46 ± 0.28	0.516
Type IIB	5.76 ± 0.02	7.02 ± 0.32	0.002

for soleus, plantaris and gastrocnemius, respectively). There was a significant main effect of age to increase VEGF mRNA in each muscle (power: 0.748, 0.765, and 0.510, respectively). There was a significant main effect of exercise to increase VEGF mRNA in each muscle (power: 1.000, 0.999, and 1.000, respectively). *Post hoc* analysis found a significant increase in VEGF mRNA at 0 h post-exercise with a significantly greater increase at 1 h post-exercise in each muscle.

Discussion

The principal finding of the present study from C57BL/6 mice is that the VEGF mRNA responses to acute hypoxia and acute aerobic exercise are well maintained in aged compared with young regardless of muscle fibre type composition of the muscle analysed. Our finding of a preserved VEGF mRNA response to acute exercise in aged mice is in contrast to the lower VEGF mRNA response to acute exercise observed in aged compared with young men and women (Croley *et al.* 2005, Ryan *et al.* 2006), but consistent with a recent report in F344 rats (Rossiter *et al.* 2005).

Ageing and VEGF mRNA

Advanced age is associated with impaired ischaemia-induced hypoxic VEGF expression, which results in a

Table 4 Skeletal muscle fibre and capillary characteristics in gastrocnemius muscle of C57BL/6 mice (mean ± SD, CFPE, capillary-to-fibre perimeter exchange index, *N* ¼ 3 young and aged)

Gastrocnemius	Young	Aged	*P*-value
Type IIB fibre (%)	100.0 ± 0.0	100.0 ± 0.0	1.000
Type IIB fibre cross-sectional area (lm^2)	2567 ± 203	2069 ± 219	0.045
Type IIB fibre perimeter (lm)	208 ± 9	192 ± 6	0.068
Type IIB capillary contacts	4.01 ± 0.23	3.56 ± 0.35	0.136
Type IIB individual capillary-to-fibre ratio	1.39 ± 0.10	1.32 ± 0.18	0.625
Type IIB capillary density (capillaries · $mm^{)2}$)	566 ± 33	697 ± 152	0.221
Type IIB CFPE (capillaries · 1000 $lm^{)1}$)	6.42 ± 0.87	6.99 ± 1.02	0.501

reduced angiogenic response (Rivard *et al.* 1999). We had hypothesized that the VEGF mRNA response to acute hypoxia and acute exercise would be lower in the same age (3 months vs. 24 months) and strain (C57BL/6) of mice studied by Rivard *et al.* (1999). In contrast to our hypothesis, the VEGF mRNA response to acute hypoxia and acute exercise were similar between age groups in each muscle analysed.

It is well established that acute aerobic exercise can increase VEGF mRNA in humans (Gustafsson *et al.* 1999, Richardson *et al.* 1999, Gavin *et al.* 2004, 2005, Croley *et al.* 2005, Ryan *et al.* 2006), rats (Breen *et al.* 1996, Gavin *et al.* 2000b, Gavin & Wagner 2001) and mice (Choi *et al.* 2005). We have recently reported that the VEGF mRNA response to acute aerobic exercise at the same relative exercise intensity is lower in aged compared with young men and women (Croley *et al.* 2005, Ryan *et al.* 2006). In contrast, the VEGF mRNA response to the same relative aerobic exercise intensity is preserved in F344 rats (Rossiter *et al.* 2005). Consistent with Rossiter *et al.* (2005), the increase in VEGF mRNA in response to acute aerobic exercise at the same relative intensity was similar in young and aged C57BL/6 mice. It is possible that age-associated discrepancies in the VEGF mRNA response to acute exercise between humans and rodents result from differences in the workload reduction present between young and aged humans and rodents. In our previous studies (Croley *et al.* 2005, Ryan *et al.* 2006), the aged individuals exercised at an absolute workload that was approximately 35% lower than young, while in the current report the aged mice exercised at an absolute workload (treadmill speed · body mass) that was only 13% lower than young. The VEGF mRNA response to exercise is workload-dependent (Breen *et al.* 1996, Gavin & Wagner 2001) and thus the greater loss of maximal workload in aged humans compared with rodents may account for the differences in exercise-induced VEGF mRNA observed between species.

In the current report 6% O_2 systemic hypoxia was used to increase VEGF mRNA because: (1) 6% O_2 systemic hypoxia increases HIF-1 expression in skeletal muscle (Stroka *et al.* 2001); (2) age-associated differences in HIF-1 are present in non-skeletal muscle tissue at 7% O_2 systemic hypoxia (Frenkle-Denkberg *et al.* 1999) and (3) age-associated differences in VEGF are because of reduced HIF-1 activation in VSMC (Rivard *et al.* 2000). In spite of this rationale, there were no age-associated differences in the acute hypoxic VEGF mRNA response in any muscle analysed (Fig. 1). An increase in VEGF mRNA could be due to an increase in transcription, stabilization, or a combination of these two mechanisms. Age-associated differences in hypoxia-induced VEGF expression in VSMC result from differences in HIF-1-regulated VEGF transcription and not mRNA stabilization (Rivard *et al.* 2000). While 6% O_2 does increase skeletal muscle HIF-1, this response was much lower in skeletal muscle compared with other tissues (Stroka *et al.* 2001) and thus may be inadequate to increase VEGF transcription. Increases in skeletal muscle HIF-1 have been observed during submaximal exercise under hypoxic (Tang *et al.* 2004) and reduced blood flow (Ameln *et al.* 2005) conditions.

In human muscle, intracellular PO_2 is 5 – 8 torr at exercise intensities >50% of maximum (Richardson *et al.* 2001). Based on recent findings in humans from Richardson *et al.* (2006) and assuming similar responses between humans and mice, it would be estimated that intracellular PO_2 would be approximately 15 torr during exposure to 6% O_2 systemic hypoxia. If HIF-1 responsiveness is lower in 24-month-old C57BL/6 mice and HIF-1 is involved in the VEGF response to acute exercise, we would have expected a lower VEGF mRNA response in the aged compared with young mice. Thus, either HIF-1 responsiveness in skeletal muscle is not lower in aged compared with young mice or HIF-1 is not involved in the VEGF response to acute exercise. It is not possible to distinguish between these possibilities with the current results.

Interestingly, there was a main effect of age to increase VEGF mRNA in the soleus, plantaris and gastrocnemius muscles in the acute exercise experiments that was not present in the acute hypoxia experiments. These discrepancies likely result from different VEGF regulatory mechanisms between acute systemic hypoxia and acute systemic exercise either due to different transcription factor regulation or differences in mRNA stabilization, which may be augmented in aged

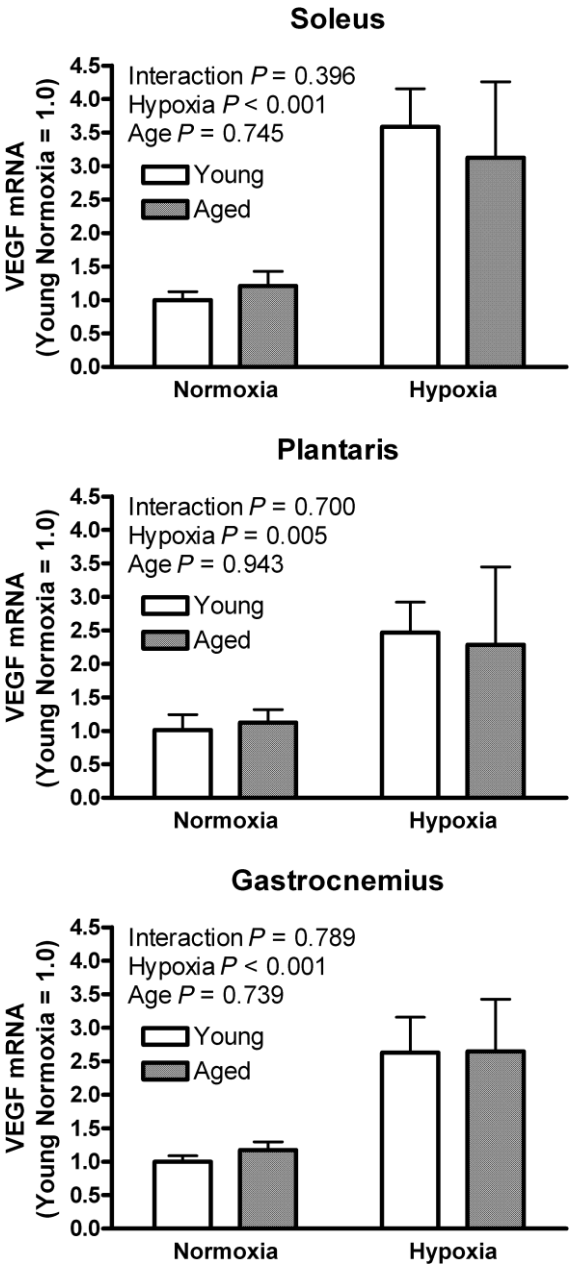

Figure 1 Vascular endothelial growth factor (VEGF) mRNA in soleus (top), plantaris (middle) and gastrocnemius (bottom) at rest and immediately following 2 h of hypoxia (F_IO_2 ¼ 0.06) in young and aged C57BL/6 mice. Hypoxia increased VEGF mRNA in all muscles independent of age (mean ± SD). *N* ¼ 3 per group for young and aged normoxia; *N* ¼ 5 per group for young and aged hypoxia.

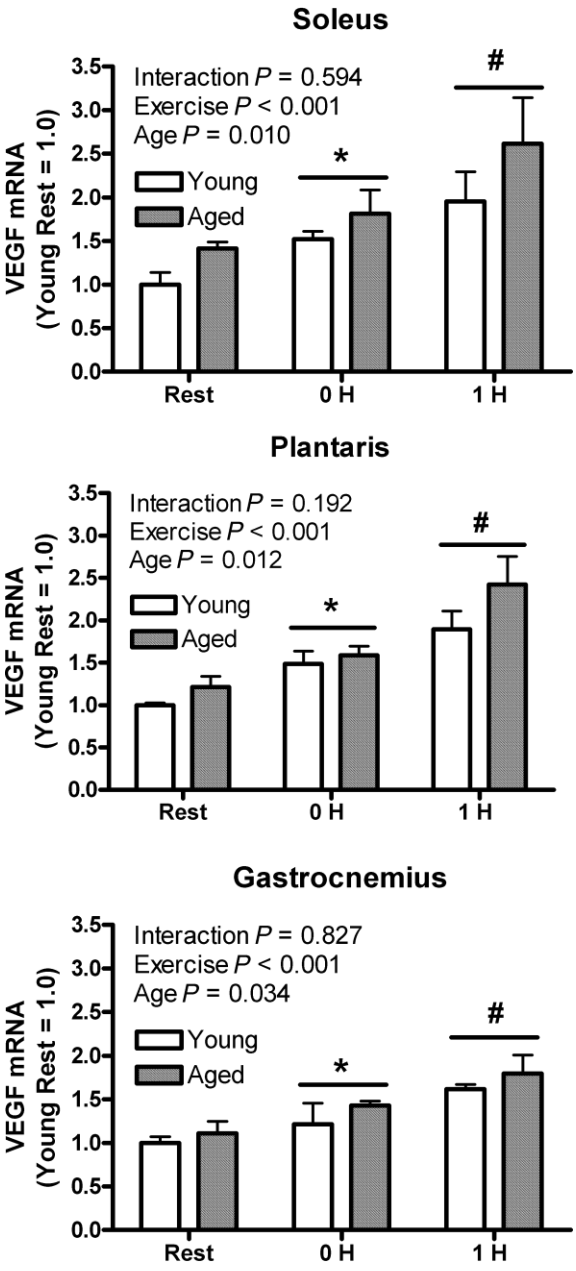

Figure 2 Vascular endothelial growth factor (VEGF) mRNA in soleus (top), plantaris (middle) and gastrocnemius (bottom) at rest, 0 h and 1 h post-exercise in young and aged C57BL/6 mice. Exercise increased VEGF mRNA in all muscles at 0 h with a significantly greater increase at 1 h. Exercise-induced increases in VEGF mRNA were similar in young and aged. VEGF mRNA was greater in aged compared with young in all muscles independent of rest/exercise (mean ± SD). *N* ¼ 3 per group for young and aged; *significantly different than rest; # significantly different than rest and 0 h post-exercise.

compared with young muscle during exercise, but not hypoxia.

We had hypothesized that greater age-associated differences in the VEGF mRNA responses to acute hypoxia and acute exercise would be present in the more oxidative soleus and not in the more glycolytic gastrocnemius. There were no age-associated differences in VEGF mRNA between muscles either in response to acute hypoxia or acute exercise. Birot *et al.* (2003) reported that in the plantaris the VEGF mRNA

response to acute exercise occurs exclusively in type IIB muscle fibres, while Brutsaert *et al.* (2002) reported a greater VEGF mRNA response to acute exercise and electrical stimulation in more oxidative compared with glycolytic regions of the gastrocnemius. Birot *et al.* (2003) suggested that fibre type selective increases in VEGF mRNA may be due to relatively greater muscle hypoxia present in type IIB muscle fibres due to lower capillarization surrounding these fibres. Our data demonstrate an increase in VEGF mRNA in the oxidative soleus as well as the glycolytic gastrocnemius arguing against an increase in VEGF mRNA solely in type IIB muscle fibres.

Ageing and skeletal muscle

Soleus, plantaris and gastrocnemius muscle masses were approximately 12% greater in aged compared with young female mice. When expressed relative to body mass, aged soleus, plantaris and gastrocnemius muscle masses were approximately 20% lower in aged compared with young female mice. Muscle FCSA was 19% lower in aged compared with young gastrocnemius muscle, but not different in soleus or plantaris muscles. This finding is consistent with recent study demonstrating a 47% reduction in gastrocnemius FCSA in 18- vs. 3-month-old male C57BL/6 mice (Rosa *et al.* 2005) and a 28% reduction in gastrocnemius FCSA in 22- vs. 6-month-old female CD1 mice (Wagatsuma 2005). Thus, ageing appears to affect FCSA of glycolytic, but not oxidative muscles and is consistent with several reports in humans where FCSA is lower in type II, but not type I muscle fibres (Coggan *et al.* 1992, Proctor *et al.* 1995, Croley *et al.* 2005).

In the plantaris, the percentage of type IIA fibres was greater and type IIB lower in aged compared with young mice. Similar age-associated shifts in muscle fibre types have been reported in mouse extensor digitorum longus (EDL) muscle (Barton-Davis *et al.* 1998). It must be noted that because of small sample sizes, there is a potential for low power, which would limit our ability to detect small changes in soleus and gastrocnemius phenotype.

Consistent with their use to mimic the human condition, C57BL/6 mice demonstrate several changes in skeletal muscle structure and function including sarcopenia and reduced oxidative capacity (Kwong & Sohal 2000, Rosa *et al.* 2005). The age-associated loss of muscle capillaries present in humans does not occur in rats and in fact may be increased with respect to oxidative capacity in aged compared with young rats (Hepple & Vogell 2004, Mathieu-Costello *et al.* 2005). In C57BL/icrfa (Davidson *et al.* 1999) and CD1 (Wagatsuma 2005) mice, muscle capillarization may be increased in aged compared with young. Our data lend further support that ageing does not systematically lower skeletal muscle capillarization in rodents.

Animal models are attractive in ageing research in that genetic differences between animals are minimized and successful subject recruitment issues are irrelevant. A review of the human ageing literature suggests that muscle capillarization is lower in aged compared with young whether statistical differences were present or not (Parizkova *et al.* 1971, Grimby *et al.* 1982, Jakobsson *et al.* 1990, Coggan *et al.* 1992, Proctor *et al.* 1995, Chilibeck *et al.* 1997, Andersen 2003). Therefore, the vast majority of data in humans suggest that ageing lowers muscle capillarization, which is in contrast to findings in animal models of ageing (Davidson *et al.* 1999, Hepple & Vogell 2004, Mathieu-Costello *et al.* 2005, Wagatsuma 2005). Thus, while animal models of ageing appear well suited to investigating sarcopenia and reduced oxidative capacity, they appear not to be useful in mimicking the effects of ageing on muscle capillarization.

In conclusion, we have demonstrated that skeletal muscle capillarization and the VEGF mRNA responses to acute hypoxia and aerobic exercise are well maintained in aged compared with young C57BL/6 mice. This is in contrast to lower muscle capillarization and a lower VEGF mRNA response to acute aerobic exercise in aged compared with young humans (Croley *et al.* 2005, Ryan *et al.* 2006). Increases in soleus, plantaris and gastrocnemius VEGF mRNA in response to acute exercise suggest that increases in VEGF mRNA are not limited to type IIB muscle fibres. While animals are useful for investigating several structural and functional changes in muscle observed with ageing, animal models do not appear to adequately mimic the non-pathological age-associated changes in muscle capillarization and VEGF expression observed in humans. It is possible though that these discrepancies between mice and humans may be controlled if care is given to adequately match in mice the extent of the decline in function present in humans.

Conflict of interest

There are no conflicts of interest.

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