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

Authors:
T. P. Gavin, L. M. Westerkamp and K. A. Zwetsloot

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 (FIO2 ¼ 0.06) or performed a single exercise (65% of maximum treadmill running speed) bout. Capillarization and VEGF mRNA were analyzed 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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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.

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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.
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, Bruinsaert 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 rats 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.

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 \(\frac{1}{4} 3\) 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 \(\frac{1}{4} 3\) per age).

Hypoxia protocol
Young and aged animals were exposed to systemic hypoxia (F\(_{2}\)O\(_2\) \(\frac{1}{4} 0.06\)) for 2 h (N \(\frac{1}{4} 5\) 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). mRNA 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) 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 ≤ 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/Fi, CD and CFPE) of type IIB fibres was greater and C/Fi 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% O2 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 respectively).
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

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### Table 2

<table>
<thead>
<tr>
<th>Soleus</th>
<th>Young (3 months)</th>
<th>Aged (24 months)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Type I fibre (%)</td>
<td>56.6 ± 6.9</td>
<td>52.6 ± 7.3</td>
<td>0.525</td>
</tr>
<tr>
<td>Type IIA fibre (%)</td>
<td>41.9 ± 5.3</td>
<td>45.1 ± 4.9</td>
<td>0.515</td>
</tr>
<tr>
<td>Type IIB fibre (%)</td>
<td>1.5 ± 2.6</td>
<td>2.3 ± 2.0</td>
<td>0.705</td>
</tr>
<tr>
<td>Fibre cross-sectional area (µm²)</td>
<td>1169 ± 368</td>
<td>1229 ± 238</td>
<td>0.824</td>
</tr>
<tr>
<td>Fibre perimeter (µm)</td>
<td>923 ± 266</td>
<td>1056 ± 224</td>
<td>0.543</td>
</tr>
<tr>
<td>Capillary contacts</td>
<td>136 ± 22</td>
<td>142 ± 13</td>
<td>0.705</td>
</tr>
<tr>
<td>Type I</td>
<td>1.06 ± 0.12</td>
<td>3.71 ± 0.50</td>
<td>0.371</td>
</tr>
<tr>
<td>Type IIA</td>
<td>3.85 ± 0.37</td>
<td>3.51 ± 0.74</td>
<td>0.527</td>
</tr>
<tr>
<td>Individual capillary-to-fibre ratio</td>
<td>1.38 ± 0.04</td>
<td>1.34 ± 0.19</td>
<td>0.709</td>
</tr>
<tr>
<td>Type I</td>
<td>1.34 ± 0.13</td>
<td>1.26 ± 0.23</td>
<td>0.629</td>
</tr>
<tr>
<td>Type IIA</td>
<td>1313 ± 406</td>
<td>1139 ± 255</td>
<td>0.566</td>
</tr>
<tr>
<td>Type IIB</td>
<td>1631 ± 564</td>
<td>1304 ± 417</td>
<td>0.487</td>
</tr>
<tr>
<td>CFPE (capillaries/1000 µm³)</td>
<td>10.28 ± 1.45</td>
<td>9.35 ± 1.37</td>
<td>0.468</td>
</tr>
<tr>
<td>Type I</td>
<td>11.01 ± 2.45</td>
<td>9.21 ± 2.00</td>
<td>0.404</td>
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</tbody>
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### Table 3

<table>
<thead>
<tr>
<th>Plantaris</th>
<th>Young (3 months)</th>
<th>Aged (24 months)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Type I fibre (%)</td>
<td>27.7 ± 5.2</td>
<td>35.2 ± 8.3</td>
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<tr>
<td>Type IIA fibre (%)</td>
<td>17.1 ± 7.6</td>
<td>42.2 ± 7.7</td>
<td>0.016</td>
</tr>
<tr>
<td>Type IIB fibre (%)</td>
<td>55.2 ± 11.7</td>
<td>22.6 ± 6.1</td>
<td>0.013</td>
</tr>
<tr>
<td>Fibre cross-sectional area (µm²)</td>
<td>1444 ± 536</td>
<td>1502 ± 301</td>
<td>0.878</td>
</tr>
<tr>
<td>Fibre perimeter (µm)</td>
<td>1204 ± 673</td>
<td>932 ± 212</td>
<td>0.540</td>
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<tr>
<td>Capillary contacts</td>
<td>1723 ± 172</td>
<td>1905 ± 371</td>
<td>0.484</td>
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<tr>
<td>Type I</td>
<td>163 ± 31</td>
<td>163 ± 18</td>
<td>0.998</td>
</tr>
<tr>
<td>Type IIA</td>
<td>145 ± 42</td>
<td>128 ± 12</td>
<td>0.537</td>
</tr>
<tr>
<td>Type IIB</td>
<td>178 ± 7</td>
<td>182 ± 12</td>
<td>0.674</td>
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<tr>
<td>Individual capillary-to-fibre ratio</td>
<td>2.96 ± 0.19</td>
<td>3.35 ± 0.54</td>
<td>0.307</td>
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<tr>
<td>Type I</td>
<td>2.72 ± 0.32</td>
<td>2.34 ± 0.05</td>
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</tr>
<tr>
<td>Type IIB</td>
<td>2.93 ± 0.15</td>
<td>3.64 ± 0.22</td>
<td>0.010</td>
</tr>
<tr>
<td>Capillary density (capillaries/mm³)</td>
<td>1.04 ± 0.16</td>
<td>1.18 ± 0.14</td>
<td>0.296</td>
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<tr>
<td>Type I</td>
<td>0.99 ± 0.07</td>
<td>0.83 ± 0.05</td>
<td>0.041</td>
</tr>
<tr>
<td>Type IIB</td>
<td>1.01 ± 0.04</td>
<td>1.28 ± 0.14</td>
<td>0.036</td>
</tr>
<tr>
<td>CFPE (capillaries/1000 µm³)</td>
<td>793 ± 99</td>
<td>922 ± 350</td>
<td>0.572</td>
</tr>
<tr>
<td>Type I</td>
<td>1109 ± 529</td>
<td>948 ± 180</td>
<td>0.643</td>
</tr>
<tr>
<td>Type IIB</td>
<td>640 ± 12</td>
<td>728 ± 37</td>
<td>0.017</td>
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</tbody>
</table>

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### Table 1

<table>
<thead>
<tr>
<th>C57BL/6 mice characteristics</th>
<th>Young (3 months)</th>
<th>Aged (24 months)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (g)</td>
<td>20.4 ± 1.0</td>
<td>27.7 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximum treadmill speed (m min⁻¹)</td>
<td>31.0 ± 5.0</td>
<td>20.0 ± 3.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Soleus (mg)</td>
<td>6.3 ± 0.8</td>
<td>7.3 ± 0.8</td>
<td>0.026</td>
</tr>
<tr>
<td>Plantaris (mg)</td>
<td>12.3 ± 1.3</td>
<td>13.6 ± 1.0</td>
<td>0.026</td>
</tr>
<tr>
<td>Gastrocnemius mass (mg)</td>
<td>93.7 ± 10.0</td>
<td>105.9 ± 5.7</td>
<td>0.006</td>
</tr>
<tr>
<td>Soleus/body mass (mg g⁻¹)</td>
<td>0.31 ± 0.03</td>
<td>0.26 ± 0.03</td>
<td>0.004</td>
</tr>
<tr>
<td>Plantaris/body mass (mg g⁻¹)</td>
<td>0.60 ± 0.05</td>
<td>0.49 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gastrocnemius/body mass (mg g⁻¹)</td>
<td>4.59 ± 0.34</td>
<td>3.84 ± 0.33</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
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 (Rossier et al. 2005). Consistent with Rossier 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 was 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% O₂ systemic hypoxia was used to increase VEGF mRNA because: (1) 6% O₂ 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% O₂ 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% O₂ 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 PO₂ 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 PO₂ would be approximately 15 torr during exposure to 6% O₂ 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

### 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 ≥ 3 young and aged)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Young</th>
<th>Aged</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type IIB fibre (%)</td>
<td>100.0 ± 0.0</td>
<td>100.0 ± 0.0</td>
<td>1.000</td>
</tr>
<tr>
<td>Type IIB fibre cross-sectional area (mm²)</td>
<td>2567 ± 203</td>
<td>2069 ± 219</td>
<td>0.045</td>
</tr>
<tr>
<td>Type IIB fibre perimeter (µm)</td>
<td>208 ± 9</td>
<td>192 ± 6</td>
<td>0.068</td>
</tr>
<tr>
<td>Type IIB capillary contacts</td>
<td>4.01 ± 0.23</td>
<td>3.56 ± 0.35</td>
<td>0.136</td>
</tr>
<tr>
<td>Type IIB individual capillary-to-fibre ratio</td>
<td>1.39 ± 0.10</td>
<td>1.32 ± 0.18</td>
<td>0.625</td>
</tr>
<tr>
<td>Type IIB capillary density (capillaries · mm²)</td>
<td>566 ± 33</td>
<td>697 ± 152</td>
<td>0.221</td>
</tr>
<tr>
<td>Type IIB CFPE (capillaries · 1000 mm³)</td>
<td>6.42 ± 0.87</td>
<td>6.99 ± 1.02</td>
<td>0.501</td>
</tr>
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</table>
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

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**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 (FiO2 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.
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 C57BL6jcrfa (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 response 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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**References**


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