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# Acute resistance exercise increases skeletal muscle angiogenic growth factor expression

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## Abstract

**Aims:** Both aerobic and resistance exercise training promote skeletal muscle angiogenesis. Acute aerobic exercise increases several pro-angiogenic pathways, the best characterized being increases in vascular endothelial growth factor (VEGF). We hypothesized that acute resistance exercise also increases skeletal muscle angiogenic growth factor [VEGF and angiopoietin (Ang)] expression.

**Methods:** Seven young, sedentary individuals had vastus lateralis muscle biopsies and blood drawn prior to and at 0, 2 and 4 h post-resistance exercise for the measurement of VEGF; VEGF receptor [KDR, Flt-1 and neuropilin 1 (Nrp1)]; Ang1 and Ang2; and the angiopoietin receptor – Tie2 expression. Resistance exercise consisted of progressive knee extensor (KE) exercise to determine one repetition maximum (1-RM) followed by three sets of 10 repetitions ($3 \times 10$) of KE exercise at 60–80% of 1-RM.

**Results:** Resistance exercise significantly increased skeletal muscle VEGF mRNA and protein and plasma VEGF protein at 2 and 4 h. Resistance exercise increased KDR mRNA and Tie2 mRNA at 4 h and Nrp1 mRNA at 2 and 4 h. Skeletal muscle Flt-1, Ang1, Ang2 and Ang2/Ang1 ratio mRNA were not altered by resistance exercise.

**Conclusions:** These findings suggest that acute resistance exercise increases skeletal muscle VEGF, VEGF receptor and angiopoietin receptor expression. The increases in muscle angiogenic growth factor expression in response to acute resistance exercise are similar in timing and magnitude with responses to acute aerobic exercise and are consistent with resistance exercise promoting muscle angiogenesis.

*Keywords* angiopoietin, plasma, resistance exercise, skeletal muscle, Tie2, vascular endothelial growth factor, vascular endothelial growth factor receptors.

Several well coordinated steps are required to promote an increase in the number of capillaries, known as angiogenesis. Two growth factor pathways involved in the early stages of angiogenesis are vascular endothelial growth factor (VEGF) and angiopoietin (Papetti & Herman 2002). VEGF increases the proliferation and migration of endothelial cells (ECs) (Connolly *et al.* 1989, Waltenberger *et al.* 1994), while angiopoietin is responsible for vessel stabilization (Papetti & Herman 2002). VEGF is a 45 kDa secretable protein that works through binding to the VEGF receptors, KDR and Flt-1 (Terman *et al.* 1992, de Vries *et al.* 1992, Ferrara 1999). VEGF binding to KDR increases EC mitogenesis and chemotaxis, while VEGF binding to Flt-1 lacks such

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responses (Waltenberger *et al.* 1994). VEGF binding to KDR is facilitated by neuropilin 1 (Nrp1) (Soker *et al.* 1998).

It is well recognized that endurance exercise training promotes many adaptations in skeletal muscle including increases in oxidative enzymes and in the number of capillaries surrounding muscle fibres (Saltin *et al.* 1968, Andersen & Henriksson 1977). In humans, acute aerobic exercise increases the expression of VEGF and the VEGF receptors (KDR, Flt-1 and Nrp1) (Gustafsson *et al.* 1999, 2005, Gavin *et al.* 2004, Ryan *et al.* 2006). VEGF is important for basal skeletal muscle capillarization as well as aerobic exercise-induced angiogenesis (Amaral *et al.* 2001, Tang *et al.* 2004, Wagner *et al.* 2006).

Less well known is that resistance exercise can also promote skeletal muscle angiogenesis (McCall *et al.* 1996, Green *et al.* 1999, Campos *et al.* 2002). VEGF mRNA is increased 2.9-fold above rest 24 h post-resistance exercise in young and aged men as determined by pooled mRNA microarray analysis (Jozsi *et al.* 2000). In response to aerobic exercise, VEGF mRNA is rapidly increased within 30 min post-exercise (Gustafsson *et al.* 1999) and returns to baseline by 20 h post-exercise (Hiscock *et al.* 2003) in humans. Given that VEGF mRNA is elevated at 24 h post-resistance exercise, we questioned if increases in VEGF and VEGF receptor expression in response to resistance exercise are as rapid as previously observed with aerobic exercise in humans.

The angiopoietin system consists of angiopoietin 1 and 2 (Ang1 and Ang2) and their common receptor Tie2. The angiopoietins are not prototypical angiogenic growth factors, but rather permissively allow for the proper interaction between ECs and supporting cells (Gale & Yancopoulos 1999). Capillary stability is promoted when the concentration of Ang1 is greater than Ang2, while capillary instability is promoted when Ang2 is greater than Ang1 thus Ang2 acts as a naturally occurring antagonist to Ang1 (Gale & Yancopoulos 1999). Greater Ang2 would facilitate capillary sprouting at the initiation of angiogenesis, while greater Ang1 would facilitate the stabilization of capillaries prior to and after the completion of angiogenesis. In rodents, acute treadmill exercise with ischaemia (Lloyd *et al.* 2003) and synergistic ablation-induced muscle overload (Williams *et al.* 2006) increase muscle Ang2 expression. Whether resistance exercise increases the angiopoietins is unknown in humans.

Therefore, the primary purpose of the current study was to investigate if acute resistance exercise rapidly (within 4 h) increases skeletal muscle VEGF expression. The secondary purpose was to investigate if acute resistance exercise also increases VEGF receptor and angiopoietin mRNA expression. We hypothesized that acute resistance exercise increases: (i) skeletal muscle VEGF mRNA and protein; (ii) skeletal muscle VEGF receptor (KDR, Flt-1, and Nrp1) mRNA; and (iii) skeletal muscle Ang2 and Tie2 mRNA.

## Materials and methods

### *Subjects*

Seven sedentary, young (range 24–32 years of age) individuals (six males, one female) volunteered to participate in the study after receiving written and verbal explanations of the content and intent of the study in accordance with the University & Medical Center Institutional Review Board. All subjects were healthy non-smokers, with no history of cardiopulmonary disease. Subject characteristics are in Table 1. Sedentary subjects were defined as participating in < 1 h of strenuous physical activity per week.

### *Determination of one repetition maximum and resistance exercise bout*

Subjects reported to the laboratory after a 12 h fast and were allowed water *ad libitum* throughout the study. Two legged knee extensor (KE) one repetition maximum (1-RM) was determined by having subjects lift a progressively greater weight until they were unable to continue. Subjects were given 1 min rest between each attempt and the weight was increased 9 kg before the next attempt. The highest successfully lifted weight was designated as 1-RM.

After a 5-min rest period, subjects performed three sets of 10 repetitions (three set × 10 reps) of two-legged KE at 60–80% of 1-RM with 2 min rest between each set. If a subject was unable to complete a set, they were assisted only enough to allow for the completion of the 10 repetitions. If during the third set subjects were able to successfully complete 10 repetitions unassisted, they were instructed to complete as many repetitions as possible until fatigue. No subject exceeded 10 repetitions on the third set.

### *Muscle biopsies and blood draws*

Prior to the determination of 1-RM and at 0, 2 and 4 h post-exercise, a muscle biopsy from the vastus lateralis

**Table 1** Subject Characteristics

| | |
|---|---|
| Age, years | 26 ± 1 |
| Height, m | 1.75 ± 0.03 |
| Mass, kg | 86.9 ± 10.4 |
| 1-RM, kg | 110 ± 9 |

1-RM, one repetition maximum.
Mean ± SE. $n = 7$.



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and blood from an antecubital vein were obtained. During exercise, skeletal muscle derived VEGF protein is released into the muscle interstitial space (Hoffner *et al.* 2003), enters into the venous circulation (Hiscock *et al.* 2003), and is the likely source for the increase in circulating VEGF in response to aerobic exercise (Nemet *et al.* 2002, Kraus *et al.* 2004). Biopsies were alternated between legs and sites were separated by at least 3 cm as previously described (Gavin *et al.* 2004). Muscle biopsy sampling at sites separated by 3 cm and 2 h does not increase VEGF mRNA expression in resting biopsy samples (TP Gavin, unpublished data). The leg for the resting biopsy samples was alternated between subjects to account for dominant and non-dominant legs. Samples were stored at −80 °C until analysis.

### *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, Valencia, CA, USA). RNA was quantified fluorometrically using RiboGreen RNA Quantitation Reagent and 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). Real time PCR was conducted in duplicate on 25 ng of cDNA per reaction in 50 $\mu$L reaction volumes using TaqMan Universal PCR Master Mix with commercially available (AB) primer and probe sets for human VEGF (product #: Hs00173626_m1), KDR (product #: Hs00176676_m1), Nrp1 (product #: HS00818574_m1), Ang1 (product #: HS00375822_m1), Ang2 (product #: HS00169867_m1) and Tie2 (product #: HS00176096_m1) by use of FAM/TAMRA labelled dye on an AB PRISM 7300 sequence detection system instrument and software. The Flt-1 primer and probe set was designed using Primer Express software (AB) that selects primer and probes optimized for use with AB system products as previously described (Ryan *et al.* 2006). 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 # – 4310893E) multiplexed during the analysis of each specific gene.

### *VEGF protein analysis*

A portion of the muscle biopsy sample was homogenized in RIPA (1 × PBS, 1% Igepal, 0.5% sodium deoxycholate, 0.1% SDS with protease inhibitors) as previously described (Gavin *et al.* 2004). Total protein was measured by BCA (Bio-Rad Laboratories, Hercules, CA, USA). Skeletal muscle VEGF was analyzed from 50 $\mu$g of total protein. Plasma was separated from venous samples obtained in the presence of EDTA. Skeletal muscle and plasma VEGF were determined by ELISA according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA).

### *Statistical treatment*

A one-way repeated measures analysis of variance was used. Following a significant $F$ ratio, a Fisher's LSD *post-hoc* analysis was used. Significance was established at $P \leq 0.05$ for all statistical sets and data reported are Mean ± SE.

## Results

The VEGF response to resistance exercise for muscle mRNA, muscle protein, and plasma protein are in Figure 1. Exercise significantly increased muscle VEGF mRNA and protein and plasma VEGF protein at 2 and 4 h.

The VEGF receptor responses to resistance exercise are in Figure 2. Exercise increased KDR at 4 h and Nrp1 mRNA at 2 and 4 h. There was no effect of exercise on muscle Flt-1 mRNA.

The angiopoietin mRNA responses to resistance exercise are in Figure 3. Exercise increased muscle Tie2 mRNA at 4 h. There was no effect of resistance exercise on Ang1, Ang2, or the ratio of Ang2/Ang1 mRNA.

## Discussion

The principal finding from the current report is that resistance exercise significantly increases skeletal muscle VEGF mRNA and protein, plasma VEGF protein, and skeletal muscle VEGF receptor (KDR and Nrp1) mRNA. Interestingly, while we failed to observe an increase in mRNA for either Ang2 or Ang2/Ang1 ratio, resistance exercise did increase skeletal muscle Tie2 mRNA. These results suggest that early angiogenic signalling for VEGF are increased by acute resistance exercise, consistent with VEGF being important for resistance exercise induced skeletal muscle angiogenesis.

### *VEGF and exercise*

In the current report, acute resistance exercise increased VEGF mRNA threefold and VEGF protein 15%. The magnitude of the VEGF mRNA response in the current report is similar to the magnitude recently reported by Trenerry *et al.* (2007) in response to acute resistance exercise. In response to acute systemic aerobic exercise,

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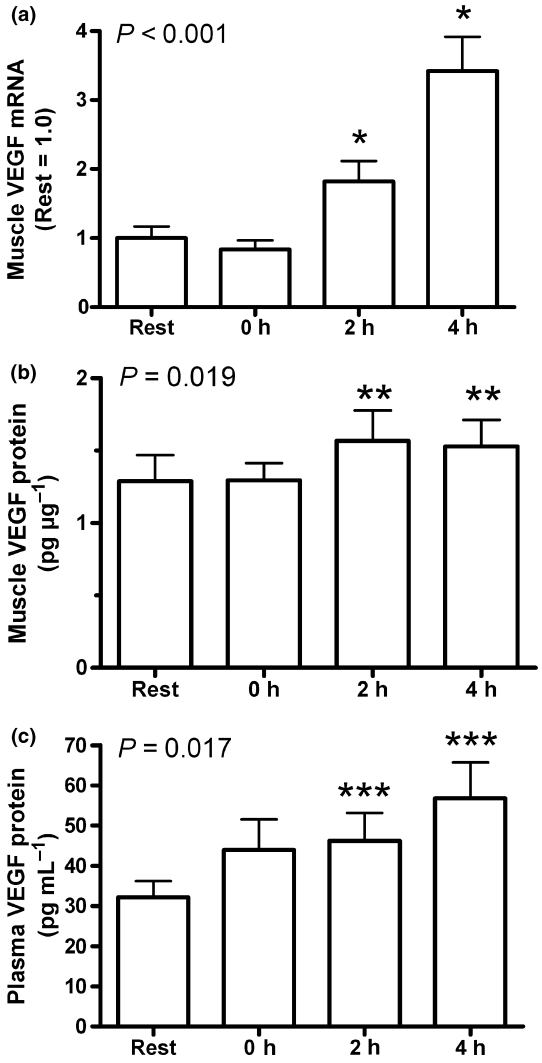


**Figure 1** Skeletal muscle vascular endothelial growth factor (VEGF) mRNA (a) and protein (b) and plasma VEGF protein (c) in response to acute resistance exercise. Skeletal muscle VEGF mRNA and protein and plasma VEGF protein were significantly increased at 2 and 4 h. *Significantly different than all other time points ($P \leq 0.05$). **Significantly different than Rest and 0 h ($P \leq 0.05$). ***Significantly different than Rest ($P \leq 0.05$). Mean ± SE. $n = 7$.

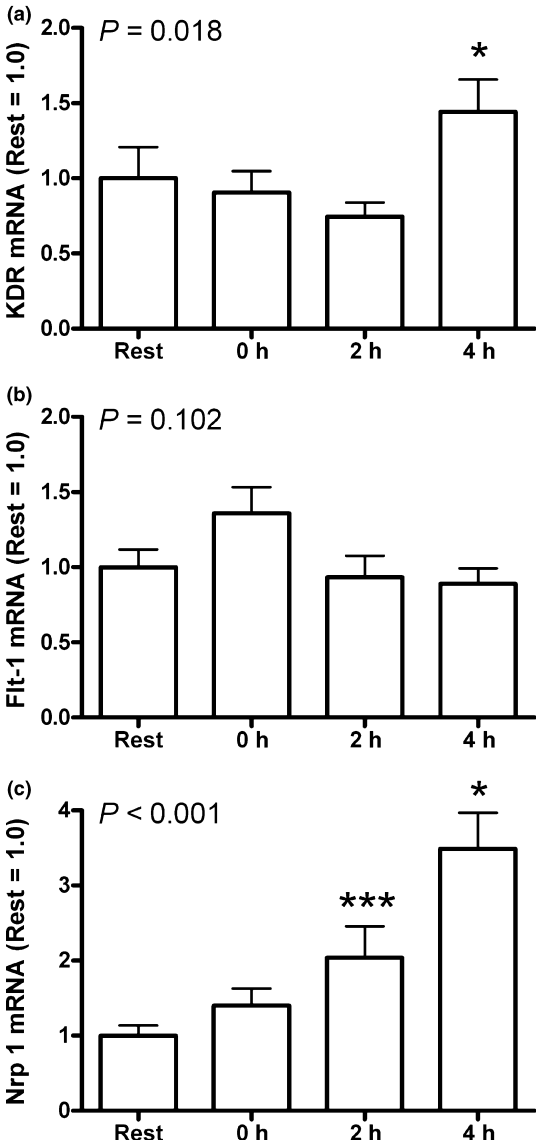


**Figure 2** Skeletal muscle KDR (a), Flt-1 (b), and Nrp1 (c) mRNA in response to acute resistance exercise. KDR was increased at 4 h and neuropilin 1 (Nrp1) mRNA was increased at 2 and 4 h post-resistance exercise. Flt-1 mRNA was not changed by resistance exercise. *Significantly different than all other time points ($P \leq 0.05$). ***Significantly different than Rest ($P \leq 0.05$). Mean ± SE. $n = 7$.

VEGF mRNA is increased three- to fivefold and VEGF protein 15–50% (Ryan *et al.* 2006, Rullman *et al.* 2007). Thus, the magnitude of the VEGF mRNA and protein responses to exercise is similar between acute aerobic and resistance exercise consistent with muscle VEGF mRNA being translated into VEGF protein in response to acute aerobic and resistance exercise.

In the current report, acute resistance exercise increased VEGF mRNA and protein at 2 and 4 h post-exercise. The time course of the increase in VEGF mRNA is similar to that recently reported by Trenerry *et al.* (2007) in response to acute resistance exercise. In response to acute systemic aerobic exercise, VEGF mRNA is not statistically increased at 0 h post-exercise and is significantly increased at 2 and 4 h post-exercise (Gavin *et al.* 2004). VEGF protein is increased by acute systemic aerobic exercise at 2 and 4 h post-exercise (Ryan *et al.* 2006, Rullman *et al.* 2007). Thus, the time courses of the VEGF mRNA and protein responses to exercise are similar between acute aerobic and resistance modes.

Skeletal muscle fibres can secrete VEGF into the interstitial space during aerobic exercise (Hoffner *et al.*



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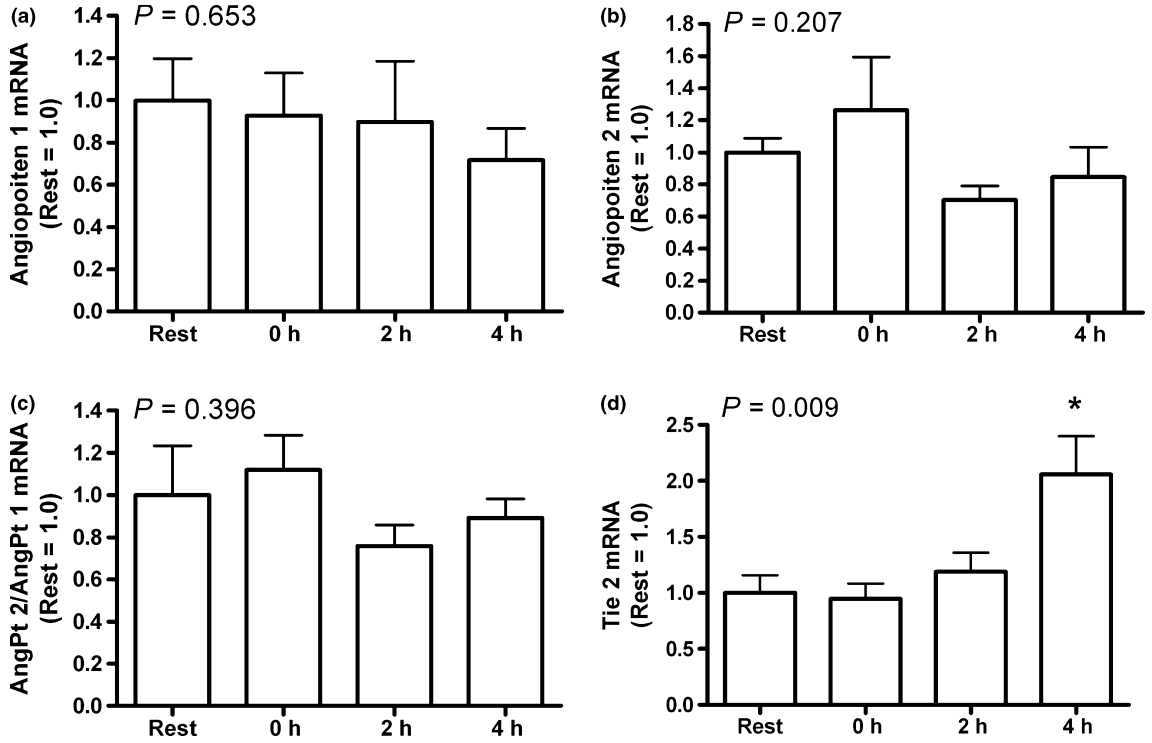


**Figure 3** Skeletal muscle angiopoietin 1 (Ang1) (a), angiopoietin 2 (Ang2) (b), Ang2/Ang1 ratio (c), and Tie2 (d) mRNA in response to acute resistance exercise. Tie2 mRNA was significantly increased at 4 h post-resistance exercise. Exercise had no effect on Ang1, Ang2, or the ratio of Ang2/Ang1 mRNA. *Significantly different than all other time points ($P \leq 0.05$). Mean ± SE. $n = 7$.

2003) and circulating VEGF can be increased in response to acute aerobic exercise (Nemet *et al.* 2002, Kraus *et al.* 2004). It is attractive to hypothesize that acute exercise increases VEGF production and release from muscle such that circulating VEGF is increased. During the preparation of the current manuscript, Rullman *et al.* (2007) demonstrated that muscle VEGF mRNA and protein are increased in response to acute aerobic exercise, that femoral vein plasma VEGF is increased at 17 min post-exercise, but that both femoral vein and artery VEGF are decreased at 2 h post-exercise. One reason for this discrepancy may due to sampling from an antecubital vein [current report and (Nemet *et al.* 2002)] and femoral vein and artery (Rullman *et al.* 2007). Regardless of the discrepancy in the reports on the response to acute aerobic exercise, the current report demonstrates an increase in plasma VEGF protein in response to acute resistance exercise.

The regulation of exercise-induced skeletal muscle VEGF expression is poorly understood. One possible regulator of VEGF transcription is hypoxia-inducible factor 1 (HIF-1), a well-known transcriptional regulator of VEGF gene expression. In humans, acute aerobic exercise activates muscle HIF-1 (Ameln *et al.* 2005), while in animals synergistic ablation, a model of chronic muscle stretch and overload, increases muscle HIF-1 (Williams *et al.* 2006). One limit to the synergistic ablation model is that it is impossible to separate acute and chronic muscle overload effects, thus the results from Williams *et al.* (2006) likely reflect an integration of these two conditions. While aerobic exercise-induced VEGF expression has been proposed to involve hypoxia and HIF-1 (Wagner 2001), HIF-1 can be activated in the absence of hypoxia by factors such as nitric oxide (NO) in glioblastoma and hepatoma cells (Kimura *et al.* 2000) and insulin in Hep-G2 cells (Zelzer *et al.* 1998). Therefore, it is possible that the increase in VEGF mRNA following resistance exercise may involve HIF-1 in spite of the low potential for prolonged muscle hypoxia.

Other potential regulators of VEGF transcription are the signalling pathways including phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) and signal transducer and activator of transcription 3 (STAT3). mTOR is a key regulator of skeletal muscle protein synthesis. In C2C12 cells, overexpression of Akt increases VEGF transcription independent of HIF-1 (Takahashi *et al.* 2002). In response to acute resistance exercise, skeletal muscle VEGF mRNA is increased in a temporal manner consistent with STAT3 activation (Trenerry *et al.* 2007). Thus, multiple pathways may potentially regulate VEGF gene expression in response to resistance exercise.

 

Traditionally, angiogenesis was considered to result exclusively from the proliferation, migration, and remodelling of pre-existing, fully differentiated ECs residing in the parent vessels (Folkman 1971). However, recent evidence suggests that exercise-induced angiogenesis may be modulated by stem cells not resident in skeletal muscle (Li *et al.* 2006). A specific subset of these cells, referred to as endothelial progenitor cells (EPCs), can enhance blood flow recovery and angiogenesis in response to hindlimb ischaemia (Kalka *et al.* 2000) and can differentiate into ECs *in situ* (Asahara *et al.* 1997). Prolonged ischaemia can increase circulating EPCs (Takahashi *et al.* 1999, Shintani *et al.* 2001) due in part to VEGF-mediated mobilization of bone marrow-derived EPCs (Takahashi *et al.* 1999, Adams *et al.* 2004). Physical training has been shown to increase circulating EPCs both in mice and humans (Adams *et al.* 2004, Laufs *et al.* 2004, Rehman *et al.* 2004). Thus, exercise-induced increases in circulating VEGF may play an important role in mobilizing EPCs and future work should investigate the EPC response to acute resistance exercise.

### *VEGF receptors and exercise*

Resistance exercise significantly increased KDR and Nrp1 mRNA, but not Flt-1 mRNA. Acute aerobic exercise increases KDR, Flt-1 and Nrp1 mRNA in humans (Gavin *et al.* 2004, Gustafsson *et al.* 2005). The magnitude and time course of the increases in KDR and Nrp-1 mRNA in response to acute resistance exercise are similar to previous reports in response to acute aerobic exercise. Both mRNA and protein for Flk-1, the murine analogue of KDR, are increased by muscle overload induced by synergistic ablation (Williams *et al.* 2006). VEGF can directly regulate KDR mRNA expression in ECs (Shen *et al.* 1998). Thus, the secretion of VEGF by muscle fibres during exercise might result in increased VEGF receptor expression.

Increases in KDR and Nrp1 mRNA are consistent with a coordinated VEGF specific angiogenic response to resistance exercise, though the increases in Nrp1 expression preceded the expression of KDR mRNA. Decreases in Flk-1 mRNA precede decreases in Nrp-1 mRNA in denervated muscle (Wagatsuma & Osawa 2006), while aging lowers muscle Nrp-1 mRNA without lowering Flk-1 mRNA (Wagatsuma 2006) suggesting that changes in KDR are not always temporally linked to Nrp-1 in skeletal muscle.

Differential regulation of KDR and Flt-1 is likely. Nitric oxide synthase (NOS) inhibition decreases aerobic exercise-induced increases in Flt-1 mRNA, but not Flk-1 mRNA (Gavin & Wagner 2002). In addition, hypoxia increases the expression of Flt-1, but not Flk-1 (Gerber *et al.* 1997). Thus, the lack of an increase in Flt-1 mRNA in response to resistance exercise as opposed to aerobic exercise likely reflects different intracellular signalling mechanisms between the different exercise modalities.

### *Angiopoietin and exercise*

There is limited data on the effects of acute aerobic or resistance exercise on Ang1, Ang2, or Tie2 regulation in humans. In rats, acute treadmill exercise (2 h post-ex) with ischaemia increases Ang2 mRNA and the Ang2/Ang1 mRNA ratio without changing the expression of Ang1 or Tie2 mRNA (Lloyd *et al.* 2003). It is possible that resistance exercise does not alter the Ang2/Ang1 ratio, however Ang2 protein is increased in response synergistic ablation-induced muscle overload (Williams *et al.* 2006). Six weeks of aerobic exercise training can increase resting skeletal muscle Ang1 and Tie2 mRNA (Timmons *et al.* 2005). Given that resistance exercise promotes angiogenesis and the importance of the angiopoietin pathway in angiogenesis it is possible that the time points used in the current study limited the ability to observe a pro-angiogenic increase in Ang2 or the Ang2/Ang1 ratio. It should also be noted that increases in mRNA may not reflect changes in protein expression, thus the lack of a pro-angiogenic change in the angiopoietins must be interpreted with caution. Further work is necessary to determine if and when resistance exercise may increase Ang2 expression.

### *Resistance exercise and angiogenesis*

Aerobic exercise increases both absolute and relative muscle capillarization as reflected by the number of capillaries surrounding a muscle fibre (NCAF) and capillary density (CD) respectively. Resistance exercise increases NCAF, while maintaining or decreasing CD due to the concomitant increase in muscle fibre size. Both aerobic (Adair *et al.* 1990) and resistance (McCall *et al.* 1996, Green *et al.* 1999) exercise promote an approx. 20–25% increase in NCAF. Thus, the similar responses of VEGF, KDR, Nrp1, and Tie2 to both aerobic and resistance exercise are consistent with similar increases in angiogenesis.

In the current report, we have employed a systemic exercise model of skeletal muscle overload in humans that produces significant increases in growth factor gene expression (Figs 1–3). In humans, resistance exercise of similar intensity and volume produces exercise-induced angiogenesis (McCall *et al.* 1996, Green *et al.* 1999). Although our exercise protocol represents only the initial bout of an exercise training program, it would be expected that training of humans at this intensity and volume would produce exercise-induced angiogenesis.



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In summary, we have demonstrated that VEGF and VEGF receptor expression are increased with acute resistance exercise with similar magnitude of increase and time course as previously observed in response to acute systemic aerobic exercise. Interestingly, in the angiopoietin system, only Tie2 expression was increased in response to resistance exercise, which may be a function of the short-time course under investigation in the current work. Thus, acute resistance exercise rapidly increases the expression of several key angiogenic growth factors consistent with resistance exercise promoting angiogenesis.

## Conflicts of interest

There are no conflicts of interest.

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