Vascular remodelling in human skeletal muscle

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Abstract
Exercise-induced angiogenesis in skeletal muscle involves both non-sprouting and sprouting angiogenesis and results from the integrated responses of multiple systems and stimuli. VEGF-A (vascular endothelial growth factor A) levels are increased in exercised muscle and have been demonstrated to be critical for exercise-induced capillary growth. Only limited information is available regarding the role of other angiogenic and angiostatic factors in exercise, but changes in the angiopoietin family following repetitive bouts of exercise occur in a pattern that is favourable for angiogenesis. Results from other angiogenic model systems, indicate that miRNAs (microRNAs) are important factors in the regulation of angiogenesis and thus to explore their role as regulators of exercise induced angiogenesis will be an important avenue of study in the future. ECM (extracellular matrix) remodelling and activation of MMPs (matrix metalloproteinases) are, to some extent, overlooked players in skeletal muscle adaptation. Degradation of ECM proteins liberates angiogenic factors from immobilized matrix stores and make cell migration possible. In fact, it is known that MMPs become activated by a single bout of exercise in humans, rapid interstitial changes occur long before any changes in gene transcription could result in protein synthesis and inhibition of MMP activity completely abolishes sprouting angiogenesis. A growing body of evidence suggests that circulating and resident progenitor cells, in addition to other cell types located in skeletal muscle tissue, participate in skeletal muscle angiogenesis by various mechanisms. However, more studies are needed before these can be confirmed as mechanisms of exercise-induced capillary growth.

Introduction
Postnatal vessel growth occurs naturally in the female reproductive system, in wound healing and in skeletal muscle in response to exercise. Postnatal vessel growth has been assumed to occur as functional modifications of existing arteries, such as the growth of pre-existing vessels to become functional collateral arteries (arteriogenesis) or the formation of new capillaries from an already established capillary network (angiogenesis) [1–3]. Arteriogenesis in response to increased muscle activity has predominantly been reported in animal models. In contrast with the previous belief that vessel growth from undifferentiated progenitor cells (vasculogenesis) was restricted to embryonic cardiovascular development, evidence suggests that it may also occur in the adult organism [1–3].

Exercise-induced skeletal muscle angiogenesis
Reports of exercise-induced vascular growth, at least in humans, have mainly been related to changes in capillaries. In the 1930s, increased capillarity was reported following muscle activity alone and with voluntary endurance exercise training in rat and guinea pig muscles [3a]. More recent studies confirm that electrical muscle stimulation and exercise training induce increased capillarity in skeletal muscle in various animal species [4]. In human skeletal muscle, the reintroduced percutaneous biopsy technique [5] made it possible to analyse changes in capillarity in response to exercise in humans, and demonstrated that increased physical activity stimulated capillary growth [6] and that cessation of exercise training induced a rapid regression in capillarity [7].

Animal preparations have demonstrated that at least two different types of angiogenesis occur in skeletal muscle: true sprouting angiogenesis and non-sprouting angiogenesis [8]. True sprouting angiogenesis, assumed to be the major type of growth, is initiated by the activation of endothelial cells; basement membrane and ECM (extracellular matrix) are degraded, allowing endothelial cells to migrate to sites where new capillaries are needed. Non-sprouting angiogenesis consists of the splitting of pre-existing capillaries. Two different types exist: longitudinal division and intussusceptions. A suggested advantage of non-sprouting angiogenesis is that it permits rapid expansion of the capillary network because it does not require initial proliferation of endothelial cells, but rather rearrangement and plastic remodelling of existing cells.

Exercise-induced angiogenic stimuli
On the basis of available reports, exercise-induced angiogenesis results from the integrated responses to multiple stimuli of multiple systems, and presumably also involves both...
types of angiogenesis. Hudlicka et al. [9] have provided important data about possible stimuli for angiogenesis in skeletal muscle, including exercise-induced increases in blood flow, shear stress and muscle stretching. Other suggested stimuli of angiogenesis in skeletal muscle are exercise-induced lowered oxygen tension and metabolic alterations [10].

One-legged exercise training with reduced oxygen delivery augmented the increase in capillarization [10] compared with the contralateral leg trained at the same absolute workload under normal conditions. The role of oxygen tension in skeletal muscle angiogenesis has been questioned on the basis of observations at high altitude. It should, however, be noted that the decrease in oxygen tension from rest to exercise largely exceeds that which is possible to achieve in resting skeletal muscle by changes in environmental oxygen pressure [11]. Furthermore, a hypoxic environment achieves only minor additive effects in reduced oxygen tension or disturbed metabolic perturbation compared with that observed at high exercise intensity in normoxia.

Animal models have revealed that non-sprouting angiogenesis is associated with increased blood flow and shear stress, in contrast with reduced oxygen tension/metabolic perturbation and mechanical stretch that appear to stimulate sprouting angiogenesis [8]. It is now well documented that the process of angiogenesis is regulated and mediated by diffusible angiogenic factors [1–3]. The first angiogenic mediator identified in exercising skeletal muscle was described in 1990 [12] and, in animals models, it is now known that growth factors are crucial in both sprouting and non-sprouting angiogenic processes [13,14].

Factors regulating angiogenesis in exercised skeletal muscle

Given the complexity of angiogenesis, it is remarkable that VEGF (vascular endothelial growth factor)-A is such a predominant regulator of this process [1–3]. Using various methodological approaches, VEGF-A has also been demonstrated to be crucial in exercise-induced angiogenesis [15–17]. VEGF-A expression increases at the mRNA level in response to a single bout of exercise [18–25], but this is transient and seems to be associated with each bout of exercise, whereas steady-state levels of VEGF-A protein increase throughout a period of training [25]. Knowledge of the cell types in which VEGF-A expression is induced by exercise may provide information regarding possible regulating stimuli. There is substantial support for skeletal muscle fibre cells as one of the sources. For example, VEGF-A has been measured in dissected pooled human single fibres checked for the absence of markers of endothelial cells, and, in mouse skeletal muscle fibres with Cre/loxP-mediated deleted VEGF-A, exercise-induced angiogenesis is markedly depressed [17,24]. However, other cells such as endothelial cells induce expression of VEGF-A in response to exercise-induced stimuli in animal models and immunohistochemistry localizes VEGF to both endothelial and skeletal muscle cells [22]. It is therefore plausible that exercise induces VEGF-A transcription in various cell types in skeletal muscle tissue.

Expression of the splice variants VEGF-A121, VEGF-A165 and VEGF-A189 increases in response to a single bout of exercise [24], but the relative distribution of VEGF-A splice variant expression changes after exercise. Initially, a relative increase occurs in expression of VEGF-A189, followed by a later relative increase in VEGF-A165. Splice variants containing the domain encoded by exon 6, such as VEGF-A190, are bound tightly to cell-surface heparan sulfate proteoglycans, whereas splice variants lacking this domain, such as VEGF-A121 and VEGF-A165, are diffusible [26]. Thus the temporal pattern of these relative changes in the different splice variants after exercise might represent early endothelial activation followed by a later chemoattractant and differentiation role for the VEGF-A system. In addition to the observed change in VEGF-A, expression of the mRNA for the VEGFRs (VEGF receptors) VEGFR1, VEGFR2 and NRP-1 (neuropilin 1) also increases in response to a single bout of exercise [21,24,25], but, following repetitive exercise, a selective increase occurs in VEGFR2 [25], the major receptor mediating the angiogenic effects of VEGF-A [26].

As discussed above, angiogenesis is a multistep process that includes integration of different signalling pathways. Interaction between VEGF-A and the angiopoietin family (Ang-1 and Ang-2) has been reported to influence markedly the angiogenic process [1–3]. Ang-1 and Ang-2 are vascular endothelial cell-specific factors that share and compete for a common receptor, the tyrosine kinase receptor Tie2. Ang-2 acts as an agonist for Tie2, and seems to maintain and stabilize mature vessels by promoting the interaction between endothelial cells and their surrounding cells. Ang-2 is thought to block the Tie2 receptor: in the absence of VEGF-A, this leads to vessel degradation. Conditions that cause increased expression of Ang-2 and high availability of VEGF-A induce favourable effects on the angiogenic process through Ang-2-induced endothelial destabilization. In both rats and humans, exercise induces a higher Ang-2/Ang-1 ratio [25,27], with a presumably permissive effect on angiogenesis. In skeletal muscle adapted to exercise training, the change in the Ang-2/Ang-1 ratio is reversed [28], and the VEGF-A exercise response is markedly attenuated [23,29]. Such changes might relate to vessel maturation and to an upper limitation of exercise-induced capillary growth.

Numerous other participants in angiogenesis have been described, for example FGF-2 (fibroblast growth factor 2), TGFα (transforming growth factor α), PDGF (platelet-derived growth factor-BB) and HGF (hepatocyte growth factor) [1–3]. Only a few of these have been studied in exercised human skeletal muscle or have been described to show altered expression, but findings have been inconsistent. In contrast with experimental models, in animals the expression of FGF-2, the first angiogenic factor described in skeletal muscle, does not change either after a single bout of exercise [18] or after exercise training. Moreover, compared
with other angiogenic conditions, information is largely lacking on the importance of changed expression and activity of antiangiogenic or angiostatic molecules in exercised skeletal muscle [30]. In animal preparations, some support exists for changed expression of thrombospondin 1 and vasoohbin-1, which might influence the local balance between angiogenic and angiostatic signals following an exercise bout [30].

The regulatory mechanisms behind exercise-induced expression of VEGF-A have been investigated extensively. Consistent with the finding of increased numbers of capillaries following 4 weeks of exercise training with restricted blood flow, reduced oxygen delivery and greater metabolic perturbation in the exercised muscle increase the expression of VEGF-A and VEGFRs [18,24,25] and modify the Ang system towards a balance that favours capillary growth [25]. Moreover, several components of the major transcription factor of hypoxic activation of cellular VEGF-A transcription, HIF-1 (hypoxia-inducible factor 1), are activated in response to a single bout of exercise in healthy human skeletal muscle [31]. However, it seems that the exercise-induced increase in VEGF-A is more complex than activation of HIF-1 alone. For example, an exercise-induced increase in VEGF-A expression occurs in the skeletal muscle of HIF-1α-knockout mice, and, in the exercise models demonstrating an augmented VEGF-A response with restricted blood flow, a similar activation of HIF-1 is observed with non-restricted blood flow to the exercising muscle [31]. The link between metabolism and angiogenesis, i.e. metabolic regulators of angiogenesis, has recently been highlighted [32], and other pathways associated with metabolic perturbation, e.g. AMPK (AMP-activated protein kinase), adenosine and PGC-1α (peroxisome-proliferator-activated receptor γ co-activator-1α), have been shown to activate VEGF-A gene expression [33–35]. In mouse skeletal muscle, PGC-1α has evolved as a principal regulator of exercise-induced expression of VEGF through co-activation of the nuclear receptor ERRα (oestrogen-related receptor α) [35–37]. Support for a similar regulating mechanism in humans is provided by the relationship that exists between the exercise-induced increase in venous lactate concentration and VEGF-A mRNA expression, and the observation that PGC-1α increases in a pattern similar to that of VEGF-A following an exercise bout with and without restricted blood to the exercising skeletal muscle [18,38]. In animal models, increased blood flow induces expression of VEGF-A in skeletal muscle endothelial cells, associated with nitric oxide production and the up-regulation of endothelial nitric oxide synthase activity in endothelial cells [39]. Finally, there is increasing evidence for control of angiogenesis by non-coding miRNAs (microRNAs) [3]. miRNAs target mRNAs to repress translation or degrade the transcript. The importance of miRNAs in angiogenesis has been documented [3] and changed expression of miRNAs has also been shown to be crucial in skeletal muscle remodelling [40]. These findings clearly suggest a role of miRNAs in regulation of exercise-induced vascular growth, but this needs to be explored further.

### Extracellular matrix remodelling and progenitor cells

The muscle environment changes towards a pro-angiogenic state already during the initial phase of exercise, long before any changes in gene transcription could result in altered protein balance [41–43]. Microdialysate obtained from skeletal muscle immediately following exercise stimulates endothelial cell proliferation and contains increased levels of VEGF-A, the latter effect shown to be at least partly dependent on adenosine-induced secretion from skeletal muscle fibre [41,42]. A release of VEGF-A from the muscle is also observed in the initial phase of an exercise bout [43]. Cleavage of ECM proteins is, for example, known to liberate angiogenic factors from immobilized matrix stores [1–3], and provides an additional mechanism behind the observation of a release of VEGF-A in the initial phase of an exercise bout. ECM remodelling is, to some extent, an overlooked player in skeletal muscle adaptation. In angiogenesis, it provides a physical link between vascular cells and their surrounding tissues and has been shown to orchestrate angiogenesis in numerous ways [1–3]. In addition to the release of growth factor, degradation of basement membrane makes cell movement possible and is thus essential in sprouting angiogenesis. The best-known and probably most important protease family in ECM remodelling is the MMP (matrix metalloproteinase) family [44]. In rat skeletal muscle, MMP-2 and MMP-14 increase with chronic electrical stimulation, and inhibition of MMP activity completely abolishes sprouting angiogenesis [45]. This protease family is involved in, and, in many cases, is rate-limiting for, many of the key processes in ECM remodelling, such as the release of sequestered growth factors and cell migration in general and probably also in skeletal muscle. In human skeletal muscle, both MMP-2 and MMP-9 are expressed in various cell types, including skeletal muscle fibres, and both are up-regulated in response to exercise [43,46], although, on the basis of the kinetics of activation of the two MMPs, different stimuli and mechanisms are presumably involved. Similar findings have been reported in the rat [47].

Circulating EPCs (endothelial progenitor cells) and inflammatory cells, as well as resident progenitor cells located in skeletal muscle tissue, have recently been recognized as participants in various aspects of skeletal muscle remodelling [48]. For example, it has been suggested that these cells participate in the regulation of angiogenesis through both differentiation and maturation to endothelial cells as well as by orchestrating the process through paracrine signalling [3,49]. Human peripheral blood contains circulating EPCs that contribute to vascular growth in vivo, and the view that blood vessel formation in adults depends solely on growth from pre-existing capillaries has been questioned [1–3]. However, following initial enthusiasm, the evaluation of the function and importance of these EPCs in postnatal vessel growth has been modified. The extent and mechanisms of EPC participation in angiogenesis processes in human skeletal muscle are not known, but the levels...
of EPCs in the circulation increase following exercise [50]. Nonetheless, investigation of the importance of resident and recruited progenitor cells in exercise-induced vascular growth is warranted. This should include information regarding homing and ECM remodelling, interstitial release of substances participating in chemoattraction and cross-talk between various cell types present in skeletal muscle tissue.

Summary and future perspectives

Exercise-induced angiogenesis in skeletal muscle involves both non-sprouting and sprouting angiogenesis and results from the integrated responses of multiple systems and stimuli. VEGF-A levels increase in exercised muscle, which has been demonstrated to be critical for exercise-induced capillary growth. Only limited information is available regarding the role of other angiogenic and angiostatic factors in exercise, but changes in the Ang family following repetitive bouts of exercise occur in a pattern that is favourable for angiogenesis. Results from other angiogenic model systems indicate that miRNAs are important factors in the regulation of angiogenesis, and thus exploring their role as regulators of exercise-induced angiogenesis is an important route of future study. ECM remodelling and activation of MMPs are, to some extent, overlooked players in skeletal muscle adaptation. Degradation of ECM proteins liberate angiogenic factors from immobilized matrix stores and make cell migration possible. In fact, it is known that MMPs become activated by a single bout of exercise in humans and that rapid interstitial changes occur long before any changes in gene transcription could result in altered protein balance. A growing body of evidence suggests that circulating EPCs and inflammatory cells, as well as resident progenitor cell types located in skeletal muscle tissue, are participants in angiogenesis by various mechanisms. More studies are, however, needed to clarify whether and how they participate in exercise-induced capillary growth.

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References


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