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Capillary growth and regression in skeletal muscle

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Abstract A muscle capillary network is composed of capillaries and anastomoses, and can be modulated under varying conditions. Although exercise induces growth in the capillary network in healthy individuals, physical deconditioning and diabetes cause regression in the capillary network of skeletal muscle. Vascular endothelial growth factor (VEGF) is a critical factor in maintaining the capillary network in skeletal muscle. In addition, the angiopoietin system, is a second family of essential growth factors, that has been identified. Angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) are important angiogenic factors that bind to their common receptor Tie-2 and assist in vascular development and remodeling. Recent studies have demonstrated the contribution of anti-angiogenic factors in controlling angiogenesis in skeletal muscle. Thrombospondin-1 (TSP-1) was shown to be an angiogenesis inhibitor. A balance between pro- and anti-angiogenic factors tightly modulates capillary regression or growth. A chronic decrease in loading and activity resulted in a regression in the capillary network. We have recently shown that the levels of Ang-1 were lower, while levels of Ang-2 were unaffected in atrophied skeletal muscle. Accordingly, the Ang-2/Ang-1 ratio was higher. In addition, the VEGF/TSP-1 ratio was lower. Thus, capillary regression and growth are associated with complex pro- and anti-angiogenic factors in skeletal muscle. Meanwhile, exercise prevents capillary regression associated with a balance between pro- and anti-angiogenic factors in impaired skeletal muscle. Our study provided clear evidence of reduced oxidative enzyme activity levels and capillary regression in skeletal muscle of diabetes. Therefore, exercise has high potential for preventing capillary regression in impaired muscle.

Keywords: capillary, angiogenic factors, microvascular disease, exercise

Introduction

A capillary network that is composed of capillaries and anastomoses in skeletal muscle is essential for delivery of nutrients and O2 exchange. In skeletal muscle, capillaries run longitudinally along the muscle fiber and are connected by narrow anastomoses that run orthogonally to the muscle fibers1). It is well known that the capillary network can be modulated under varying conditions, e.g., the capillary network can expand with exercise2) and functional overload3), and regress with denervation4) or physical deconditioning5,6). In addition, structural or functional impairment within the capillary network of skeletal muscle is caused by disease. For example, diabetes affects the capillary network, e.g., a reduction in capillary number, diameter, and capillary volume in skeletal muscle7,8). These responses are controlled by a balance between pro- and anti-angiogenic factors and their receptors9). The regulatory mechanisms behind the exercise-induced expression of pro-angiogenic factors have been investigated extensively. Conversely, anti-angiogenic factors controlling capillary network plasticity of skeletal muscle are much less understood. Therefore, we highlight the growth and regression of the capillary network in skeletal muscle, and review the evidence that has been presented more recently of angiogenesis and the underlying molecular mechanisms including potential pathways regulating angiogenesis and anti-angiogenesis.

Exercise-induced capillary growth

Exercise leads to an increase in the capillary network in skeletal muscle, which is usually preceded by an increase in oxidative capacity10). Capillary growth is greater around slow and fast oxidative than around glycolytic fibers11). This is because of the following reasons: slow oxi-
ative fibers are continually activated during contraction, but fast glycolytic fibers are only activated during supramaximal contraction. The composition of muscle fiber type exerts a powerful influence on the capillary network. In our previous study, the capillary volume in the soleus muscle, being comprised predominantly of slow oxidative fibers, has been greater than that in the superficial region of the lateral head of the gastrocnemius muscle, which is comprised of fast glycolytic fibers (Fig. 1)\(^1\). In regards to this observation, we have also reported that the capillaries have shown tortuosity in both slow and fast muscles, but higher tortuosity has been observed in slow muscle. In addition, both the number of capillaries and anastomoses were greater in slow than in fast muscle. Conversely, capillary diameter was greater in fast than in slow muscle. Furthermore, the higher capillary volume in the soleus, compared with the superficial region of the lateral head of the gastrocnemius muscle, is also consistent with the larger number of adjacent capillaries in slow and fast oxidative fibers compared with fast glycolytic fibers\(^2\).

Krogh’s pioneering work recognizes the importance of the capillary network for \(O_2\) delivery to skeletal muscle\(^3\). The capillary network may represent a limiting factor for \(O_2\) conductance. Nevertheless, exercise training can increase the capillary network of active skeletal muscle and elicit a parallel increase in \(O_2\) extraction capacity\(^4\).

**Factors regulating angiogenesis in exercised skeletal muscle**

Regarding capillary growth, recent studies have provided information on the molecular events of angiogenesis (Fig. 2). Vascular endothelial growth factor (VEGF) is one of the critical factors\(^5\) and is also essential in maintaining the capillary network in skeletal muscle. VEGF increases in skeletal muscle following contractions by electrical stimulation\(^6\) or treadmill running\(^9\). VEGF is produced by endothelial, perivascular and host tissue cells such as myocytes\(^15\), and mainly binds to endothelial cell-specific receptor tyrosine kinases, i.e., VEGFR1 (fms-like tyrosine kinase 1; Flt-1), VEGFR2 (fetal liver kinase: Flk-1/kinase insert domain receptor: KDR) and VEGFR3 (fms-like tyrosine kinase 4: Flt-4). VEGF-induced activa-
Vascular endothelial growth factor (VEGF)
VEGFR1 (fms-like tyrosine kinase-1; Flt-1)
VEGFR2 (fetal liver kinase; Flk-1/kinase insert domain receptor; KDR)
VEGFR3 (fms-like tyrosine kinase-4; Flt-4)
Angiopoietin-1 (Ang-1)
Angiopoietin-2 (Ang-2)
Tie-2

Thrombospondin-1 (TSP-1)
Endostatin
Angiostatin
Vasohibin-1
Ephrins*

Also, a second family of growth factors specific for the vascular endothelium, \textit{i.e.}, angiopoietin system, was identified\cite{27}. The angiopoietins, angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2), are important angiogenic factors that bind to their common receptor Tie-2 and assist in vascular development and remodeling. Angiopoietins allow for the proper interaction between endothelial cells and supporting cells\cite{28}. Ang-1 promotes vessel stability, whereas Ang-2 has the opposite effect\cite{29}. The Ang-2/Ang-1 ratio is thought to determine if the net effect of the angiopoietins will stabilize or destabilize the vasculature, with an increase in the Ang-2/Ang-1 ratio having a destabilizing effect\cite{2}. The Ang-2/Ang-1 ratio was markedly elevated after exercise, and remained elevated for more than 3 weeks in muscle undergoing angiogenesis\cite{30}. Tie-2 expression was also increased after exercise. Holash et al. have reported that Ang-2 expression in the presence of VEGF leads to vessel growth\cite{31}. Therefore, exercise results in capillary growth associated with increased VEGF, Ang-2, and the Ang-2/Ang-1 ratio in skeletal muscle.

**Disuse-induced capillary regression and protective effects of exercise**

Capillary growth or regression is related to the loading and activity levels in skeletal muscle. A chronic decrease in the loading and activity levels, such as hindlimb unloading, results in capillary regression in skeletal muscle\cite{5,31,32}. In fact, histological sections in skeletal muscle with disuse atrophy demonstrate a decrease in capillary/fiber ratio and capillary diameter\cite{31}. We also found that disuse caused decreases in capillary volume and diameter, and tortuosity, particularly in anastomoses, in association with endothelial cell apoptosis (Fig. 3). Thus, a regression in the capillary network caused by a chronic decrease...
in the loading and activity level is an important morphological change because it may result in a decrease in the oxygen requirement of muscle fiber. These reports reveal disuse-induced adaptive remodeling in the capillary network.

Powers et al. have demonstrated that oxidative stress might contribute to muscle atrophy. In fact, oxidative stress accelerated muscle protein degradation in the unloaded diaphragm via the proteasome system. In turn, an increase in oxidative stress levels caused the oxidation of membrane phospholipids, proteins and DNA, and promoted vascular dysfunction. Also, capillary regression induced by disuse was associated with increased oxidative stress. Since earlier studies have suggested that anti-oxidative nutrients can attenuate skeletal capillary regression induced by disuse, it is thought that anti-oxidants may function as adjuvants to protect muscle endothelial cells from disuse atrophy.

Diabetes-induced capillary regression and protective effects of exercise

Diabetes has been associated with microvascular complications and impaired angiogenesis in skeletal muscle (Fig. 5). Skeletal muscle of diabetes has been associated with a reduction in capillary diameter, capillary/fiber ratio, and reduced capillary diffusing capacity in animal models of diabetes. In addition, we demonstrated regression of the capillary network in skeletal muscle with diabetes, e.g., decreased diameter, tortuosity and volume, using a three-dimensional visualizing method. Therefore, regression of the capillary network in skeletal muscle caused by diabetes can negatively impact O2 exchange, and consequently may play a role in diabetic complications.

It is well known that exercise increases angiogenesis, and the lack of exercise has an opposite effect. In this regard, exercise intervention studies would be very useful. Our study provided clear evidence of reduced oxidative enzyme activity levels and capillary regression in the skeletal muscle of diabetic cases. In addition, exercise is effective in maintaining and improving oxidative metabolism in skeletal muscle. Exercise increased blood flow to skeletal muscles, which induced an increase in the capillary/fiber ratio and capillary density in skeletal muscle of healthy rats. Thus, exercise resulted in an adaptive increase in capillary supply in skeletal muscle. Therefore, exercise may be an effective countermeasure to these

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**Fig. 3** Representative images of capillary in the soleus muscles under control conditions (A) and after hindlimb unloading (HU, B). Capillaries run tortuously along the muscle fibers (indicated by arrows) in control and after HU, but the degree of tortuosity is more remarkable in the control group. Intercapillary anastomoses were observed more clearly in the control group. In HU, the capillaries were shrunken with fewer anastomoses and less tortuosity. Immunohistological images (C, D and E) of endothelial cell (anti-von Willebrand factor, weak intensity dots) and terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling (TUNEL, strong intensity dots). Apoptotic nuclei and endothelial cells were stained with TUNEL (green) and (red), respectively. TUNEL-positive endothelial cells are indicated by arrows and were observed mostly in anastomoses. Frequency of TUNEL-positive endothelial cell (F). *, significantly different from the control muscle at $P < 0.05$. (This figure was cited from ref. 6).
Fig. 4 Representative images of the capillary of the soleus muscles in the control (Con, A), control + anti-oxidant supplementation (Con + ASX, B), hindlimb unloading induced atrophied muscle (HU, C) and hindlimb unloading + anti-oxidant supplementation (HU + ASX, D) based on confocal laser scanning microscopy. Note the qualitatively lower density and tortuosity of capillaries in the hindlimb unloading induced atrophied muscle. Capillary volume (E) and diameter (F) of the Con, Con + ASX, HU and HU + ASX determined using confocal laser microscopy. Both mean capillary volume and diameter were lower in the HU than all other groups. Capillary volume, but not diameter, in the HU + ASX group was not different from Con levels. *, † and ‡ denote a significant difference from the Con, Con + ASX and HU groups, respectively, at P < 0.05. (This figure was cited from ref. 32).

Fig. 5 Representative images of the capillary of the soleus muscle in control (A) and diabetes (B). Transverse sections of the soleus muscle in control (C) and diabetes (D). The visualization of the luminal diameters of the capillaries as light spots using a confocal laser scanning microscope (CLSM) revealed that the capillary diameter was smaller in diabetes than in control. This observation is consistent with the mean muscle capillary volume being 47% lower in diabetes than control. (This figure was cited from refs. 8 and 39).
detrimental effects in diabetic muscle. In human subjects with diabetes, exercise has been reported to increase capillary function\(^{32}\).

**Factors regulating anti-angiogenesis**

It is well known that angiogenic factors induce growth of the capillary network in tissue. Besides, several studies reported the existence of endogenous anti-angiogenic factors (Fig. 2)\(^ {43}\). Thrombospondin-1 (TSP-1) was shown to be an angiogenesis inhibitor\(^ {44}\). TSP-1 is widely presented as a powerful anti-angiogenic factor inhibiting cell migration and inducing apoptosis in endothelial cells. TSP-1 inhibits VEGF and suppresses angiogenesis in tissue by interacting directly with VEGF leading to internalization and degradation of VEGF\(^ {45}\). In addition, Malek and Olfert have reported that the capillarity (capillary action) of skeletal muscle is increased in TSP-1 knock-out mice\(^ {46}\). The expression level of TSP-1 has been shown to be lower in the muscles of chronically exercised mice than controls\(^ {47}\). We also recently reported that the VEGF/TSP-1 ratio was lower in atrophied muscle, reflecting a balance in favor of capillary destabilization and regression\(^ {32}\). These reports indicate that TSP-1 plays an important role in maintaining the capillary network in skeletal muscle.

In addition, there are important anti-angiogenic factors similar to TSP-1, e.g., endostatin, angiosstatin, vasohibin-1, ephrins, and tissue inhibitors of metalloproteinases (reviewed by Olfert and Birot\(^ {9}\)). Endostatin is matrix-derived anti-angiogenic factor, and inhibits angiogenesis by blocking VEGF signaling\(^ {48}\). Angiostatin is an internal cleavage product exhibiting activities that oppose the angiogenic response of plasminogen and is composed of the first four-kringle domains\(^ {49}\). Vasohibin-1 is secreted by endothelial cells and is believed to contribute to vessel stabilization and maturation\(^ {10}\). Ephrins inhibit the proliferation and the migration of endothelial cells in response to Ras/mitogen-activated protein kinase (MAPK) signaling cascade activation by VEGF or angiopoietin-1\(^ {51}\). Nevertheless, Ephrins promote angiogenesis\(^ {52,53}\). Tissue inhibitors of metalloproteinases are the endogenous inhibitors that directly regulate matrix metalloproteinase activity\(^ {24}\). Thus, these factors also regulate capillary growth or regression in skeletal muscle.

**Angiogenic factors in disused and diabetic skeletal muscles**

A balance between pro- and anti-angiogenic factors tightly modulates capillary regression or growth (Fig. 6). We have recently shown that the levels of Ang-1 were lower and those of Ang-2 were unaffected by hindlimb unloading, the Ang-2/Ang-1 ratio was higher in atrophied skeletal muscle compared with controls\(^ {32}\). In addition,

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**Fig. 6** Under control conditions (A), pro- and anti-angiogenic factors are in functional balance with each other resulting in a stabilized and quiescent capillary network in skeletal muscle. Exercise (B) leads to sustain activation of pro-angiogenic factors while the responsiveness of anti-angiogenic factor, resulting in a net balance that favors an angiogenic response and the resulting expansion of the capillary network in skeletal muscle. Disease (C) results in a chronic decrease in the loading and activity level and in the capillary regression in skeletal muscle. Capillary regression in disuse and diabetes is associated with the balance between pro- and anti-angiogenic factors. (This figure was cited from ref. 10).
the VEGF level was low in atrophied muscle. The combination of these pro-angiogenic factors may contribute to the observed capillary regression in atrophied muscle. Roudier et al. have showed that the capillary regression in atrophied muscle occurs in association with a decreased VEGF/TSP-1 ratio\(^{39}\). We have also reported that the VEGF/TSP-1 ratio is lower in the soleus muscle of hindlimb unloading\(^{32}\). Interestingly, TSP-1 knockout mice have greater capillarity in skeletal muscle\(^{40}\). Therefore, the maintenance of VEGF alone is not sufficient to prevent capillary regression in skeletal muscle associated with hindlimb unloading.

It is known that skeletal muscle in animal and human subjects with diabetes has impaired angiogenesis and a reduced capillary network\(^{28,37,47,56}\). In addition, several studies have shown that diabetes affects the expression of several genes involved in angiogenesis in skeletal muscle. We have reported that the Ang-2/Ang-1 ratio was higher in diabetic muscle than controls\(^{7}\). The association between elevated TSP-1 and capillary regression in skeletal muscle has been reported in diabetic mice\(^{30}\). These reports suggest that reduced skeletal muscle capillarization in diabetic cases is associated with the balance between complex pro- and anti-angiogenic factors.

**Summary and future perspectives**

We have focused on a handful of pro- and anti-angiogenic factors in skeletal muscle. In fact, capillary regression and growth are associated with complex pro- and anti-angiogenic factors in healthy and diseased skeletal muscle. Although the details of these mechanisms remain unclear, exercise has much potential for preventing capillary regression in impaired muscle and influencing capillary growth in healthy muscle. The content in this review has partially been discussed in our previous articles.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this article.

**References**


