HIGHLIGHTED TOPIC | Skeletal and Cardiac Muscle Blood Flow

What makes vessels grow with exercise training?

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Prior, Barry M., H. T. Yang, and Ronald L. Terjung. What makes vessels grow with exercise training? J Appl Physiol 97: 1119–1128, 2004; 10.1152/japplphysiol.00035.2004.—Exercise and muscle contractions create a powerful stimulus for structural remodeling of the vasculature. An increase in flow velocity through a vessel increases shear stress, a major stimulus for enlargement of conduit vessels. This leads to an endothelial-dependent, nitric oxide-dependent enlargement of the vessel. Increased flow within muscle, in the absence of contractions, leads to an enhanced capillarity by intussusceptive angiogenesis, a process of capillary splitting by intraluminal longitudinal divide. In contrast, sprouting angiogenesis requires extensive endothelial cell proliferation, with degradation of the extracellular matrix to permit migration and tube formation. This occurs during muscle adaptations to chronic contractions and/or muscle overload. The angiogenic growth factor VEGF appears to be an important element in angiogenesis. Recent advances in research have identified hemodynamic and mechanical stimuli that upregulate angiogenic processes, demonstrated a complexity of potent growth factors and interactions with their corresponding receptors, detected an interaction of cellular signaling events, and identified important tissue reorganization processes that must be coordinated to effect vascular remodeling. It is likely that much of this information is applicable to the vascular remodeling that occurs in response to exercise and/or muscle contractions.

angiogenesis; arteriogenesis; intussusception; sprouting; shear stress; mechanical stretch

BLOOD FLOW TO MUSCLE IS DETERMINED by the balance of central and peripheral influences operating on the existing “plumbing” of the vascular system. The structure of the vascular system is, in turn, designed in a manner to optimize tissue support through the processes of convection and diffusion. Simply described, the large conduit vessels of the systemic circuit permit bulk flow delivery to the relatively small resistance vessels within a muscle at high pressure. The resistance vessels modulate flow distribution to the microvascular network bathing the cells. The capillary network, in turn, functions to permit diffusive exchange (e.g., O2, CO2, glucose, and so forth) between the vascular space and the intracellular volume of the fibers. The focus of this work is to consider what structural changes occur in the vascular system with exercise training and to provide insight as to how these changes develop. In doing so, it is important to distinguish among relevant stimuli that may be introduced by exercise. A number of excellent reviews on these topics are available (11, 12, 16, 21, 34, 43, 48, 77).

THE PROCESSES OF VASCULAR REMODELING

Arteriogenesis

Arteriogenesis is a term applied to the enlargement of existing arterial vessels. This enlargement is not simply distension of the vessel wall due to increased intraluminal pressure or an increase in wall compliance; rather, it is an increase in the caliber (diameter) and wall dimensions, resulting in a larger vessel. For this to occur, there must be remodeling of all three cell types that comprise the arterial vessel: endothelial cells, smooth muscle cells, and fibroblasts. In general, the larger the conduit artery, the thicker and more muscular and fibrous is the vessel wall. A number of excellent reviews summarize the advances in our understanding of arterial vessel enlargement (61, 77, 96).

Angiogenesis

Angiogenesis refers to the formation of new capillaries from existing capillaries. This is different from the process of vasculogenesis, which describes the de novo formation of the vasculature from precursor cells during development. In the absence of pathology (e.g., tumor growth, diabetic retinopa-
there are few physiological circumstances prompting angiogenesis, notably ovarian cycling, placental development, and exercise. Angiogenesis can occur by two primary mechanisms: intussusception and sprouting.

**Capillary intussusception.** Intussusception refers to the process by which a single capillary splits into two capillaries from within, by the formation of a pillarlike structure or longitudinal divide on the luminal side of the capillary (see Fig. 1). Activated endothelial cells extend intraluminally, effectively forming two tubes through which blood can pass. This is thought to be an efficient process of capillary multiplication, requiring less endothelial cell proliferation and offering a greater simplicity in extracellular matrix remodeling, compared with sprouting angiogenesis (21). Recent research has suggested that intussusception may be the primary method of capillary formation during development (21, 51). It is likely that shear forces acting on capillaries may preferentially increase capillarity through intussusception (81). The novel contributions of Hudlicka and colleagues (23) have made significant progress in understanding angiogenesis in muscle challenged by elevated flow, stretch, and contractions. Future work will undoubtedly shed light on the intussusception method of capillary formation, the signaling pathways that lead to endothelial cell pillar formation, and its distinctions from sprouting angiogenesis.

**Sprouting angiogenesis.** Capillary formation by endothelial cell sprouting refers to the process in which activated endothelial cells branch out from an existing capillary, extending through the surrounding matrix to form a cordlike structure (see Fig. 1). The endothelial cell cord is transformed into a tube and attached to the extracellular matrix. Of course, the newly formed tube must reenter the capillary bed by joining with another capillary or venule to become a functional capillary. The newly developed capillary is initially leaky, but it matures to that of the original capillary when pericytes envelop the endothelial cells. Like intussusception, sprouting requires the activation of endothelial cells. In addition, however, the base-

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**Table 1. Factors important in vascular remodeling**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Shear stress</strong></td>
<td>Frictional drag experienced by endothelial cells as blood flow along its surface; initiates signals to enlarge vessel</td>
</tr>
<tr>
<td><strong>Cell stretch</strong></td>
<td>Physical stretch of the vasculature caused by deformation of cells during contractions; initiates signals to stimulate angiogenesis</td>
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<tr>
<td><strong>NO/eNOS</strong></td>
<td>Nitric oxide/endothelial nitric oxide synthase: upregulated by shear stress and exercise training; essential for arteriogenesis induced by VEGF, FGF-2, and exercise</td>
</tr>
<tr>
<td><strong>FGF-2</strong></td>
<td>(Basic) fibroblast growth factor; mitogenic to endothelial cell, smooth muscle cell, and fibroblast; upregulates NO, VEGF; upregulated by shear stress</td>
</tr>
<tr>
<td><strong>VEGF</strong></td>
<td>Vascular endothelial growth factor: binds to its receptors, VEGFR1 (flk-1) and VEGFR2 (flk, KDR); mitogenic to endothelial cells; stimulates endothelial cell migration, promotes chemotaxis; upregulates uPA, tPA, uPA receptor, PA inhibitor (PAI-1), MMP, and NO production; VEGF upregulated by hypoxia, HIF-1 (with or without hypoxia), FGF-2, NO, and therefore vessel shear stress</td>
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<tr>
<td><strong>PIGF</strong></td>
<td>Placental growth factor: binds to VEGFR1 to increase collateral vessel enlargement</td>
</tr>
<tr>
<td><strong>Ang1</strong></td>
<td>Angiopoietin 1: cannot initiate angiogenesis; constitutively expressed throughout body; promotes vascular remodeling, maturation, and stabilization of vessels (via one of its receptors, Tie2)</td>
</tr>
<tr>
<td><strong>Ang2</strong></td>
<td>Angiopoietin 2: cannot initiate angiogenesis; destabilizes vasculature: when VEGF is present, leads to angiogenesis; with VEGF absent, leads to apoptosis and rarefaction</td>
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<tr>
<td><strong>MCP-1</strong></td>
<td>Monocyte chemoattractant protein-1: decreased presence reduces and increased presence enhances collateral vessel enlargement</td>
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<tr>
<td><strong>MMP</strong></td>
<td>Membrane metalloproteinases: instrumental in remodeling extracellular matrix</td>
</tr>
<tr>
<td><strong>uPA, tPA</strong></td>
<td>Urokinase-type and tissue plasminogen activator: initiates extracellular matrix degradation; upregulated by VEGF</td>
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ment membrane and the extracellular matrix must be degraded to permit migration of endothelial cells and expansion of the developing tube. Thus there must be a coordination of multiple processes for sprouting angiogenesis to proceed to completion (23, 42). For example, inhibiting the degradation of the extracellular matrix limits endothelial cell migration and tube formation (38). This could explain why the presence of some apparent angiogenic stimuli by themselves are not sufficient to produce an increase in capillarity.

VASCULAR ADAPTATIONS WITH EXERCISE

Flow Capacity Increases

It is now recognized that the flow capacity of skeletal muscle is far greater than can be supported by cardiac output, if a large mass of muscle would require maximal blood flow during exercise (4). Thus it seems unlikely that significant increases in flow capacity would be a necessary adaptation to support the increased exercise performance observed after training or in elite athletes. Nonetheless, there is general correspondence between the flow capacity of muscle and its biochemical and functional aerobic capacity. When specific muscles are exercise trained, there can be an increase in flow capacity (maximal conductance) (59, 65) and an expected increase in the caliber of the large conduit vessels. Unfortunately, there is little direct evidence, from controlled experiments employing exercise training, showing that major conduit arteries increase in size (86). This is likely due to the modest size increase necessary to appreciably increase flow capacity, since vessel resistance is a function of the radius to the fourth power. More importantly, it is probable that training programs, which are typically set up for experimental purposes, have not optimized the adaptive stimuli necessary to realize exceptional vessel enlargement because the program’s exercise bout intensity and/or duration and overall training program duration may be limited. There is considerable evidence, however, that large flow increases introduced through a major conduit artery result in substantial enlargement of the vessel. Increasing blood flow through the carotid artery, by experimentally introducing an arteriovenous shunt, increased vessel diameter by ~75% (93). Thus it is not surprising that the size of major supply vessels in humans, determined by duplex sonography, scales in relation to the exercise and/or use pattern of the muscle groups supplied by these vessels (47). For example, Hounker and coworkers (47) reported that the subclavian arteries of the dominant arms of elite tennis players and athletes with paraplegia exhibited larger diameters than the respective contralateral and control group arteries. Similarly, increased diameters of the femoral arteries were observed in elite cyclists, whereas decreases were observed with paraplegia, compared with corresponding controls. Thus major conduit vessel size corresponded to activity pattern of the dependent muscle groups, even though the sizes of the great artery (thoracic and abdominal aorta) were not different among all of these groups. Although this evidence of vessel size is not ideal, representing maximal caliber and wall thickness of the vessel in the absence of smooth muscle tone, it is consistent with the known relationship between vessel caliber and vessel flow demand, both apparent within the vascular tree (aorta, iliac, femoral, popliteal, and so forth) and observed experimentally (93). This increase in flow capacity to the major muscle groups of highly trained individuals could contribute to the greater exercise capacity exhibited by these individuals.

Muscle Capillarity Increases

It has long been recognized that exercise training imparts a dramatic adaptation at the other end of the vascular tree (13). As illustrated in Fig. 2, capillarity in the active muscle is meaningfully increased by training, particularly endurance-type training (49). Although high-resistance training programs that result in muscle fiber hypertrophy can actually decrease capillary support, due to the increased fiber area (90), this is not always the case (56), especially if work bouts involve more...
repetitive contractions typically done by bodybuilders. Any increase in muscle capillarity should be important in enhancing blood-tissue exchange properties, since a greater capillary network would 1) increase the surface area for diffusion, 2) shorten the average diffusion path length within the muscle, and 3) increase the length of time for diffusive exchange between blood and tissue. For example, for any given blood flow to the muscle, a greater capillary volume would lengthen the red blood cell transit time and thereby permit a longer time for oxygen exchange. This expectation that trained muscle would exhibit an increased oxygen exchange capacity has been demonstrated in humans (78), dogs (44), and rats (104). In experiments with rat muscle (104), the increase in maximal oxygen consumption (and improvement in muscle performance) was observed when oxygen delivery was controlled to be similar to that of the nontrained muscle (104). Interestingly, although the increase in capillarity is associated with an increase in the maximal oxygen consumption of muscle, there has been no experimental evidence, to our knowledge, demonstrating that the increased capillarity observed in trained muscle is required. Requisite evidence would come from studies in which all muscle adaptations typical of endurance training were induced, except for the increase in muscle capillarity. Furthermore, another factor or other factors appear to be important to this training adaptation. For example, an increase in mitochondrial electron transport capacity was essential to realize the training-induced increase in maximal oxygen consumption (82). It is also unclear just how an increase in capillarity may be functionally important. The value of an enhanced capillary density (i.e., greater capillary network per volume of muscle) was questioned by recent evidence in which oxygen exchange capacity was not improved in dog muscle, despite the same capillary network supplying a much smaller muscle mass as a result of induction of short-term muscle atrophy (44). It has been suggested that the important parameter representing the appropriate match in muscle capillarity is capillary surface area relative to fiber surface area (44), a parameter that increases in muscle after aerobic-type training.

**FACTORS IMPLICATED IN ARTERIOGENESIS**

Enlargement of an arterial vessel occurs in response to elevated internal pressure (55, 85), which increases radial wall stress, and in response to increased blood flow (93), which elevates shear stress on the endothelial surface. Wall mass increases to manage the increased radial wall stress that occurs with high pressure or as vessel diameter increases, according to the Laplace relationship. Large, although short-lived, increases in arterial blood pressure can occur during intense resistance-type exercise (64). It is presently not known whether this type of exercise leads to structural changes in the affected vessels. On the other hand, increases in flow velocity through a given arterial vessel initiates extensive enlargement of the artery in the absence of any increase in luminal pressure. This stimulus is directly related to the increase in shear stress caused by the increased flow velocity (94), as the vessel enlarges to regulate shear stress back to “normal” (93). Conversely, if blood flow is reduced, the diameter of the artery decreases (57). Thus shear stress can be considered the primary hemodynamic stimulus that prompts vessel enlargement.

The entire process of vessel remodeling depends on the presence of the endothelium. In cultured endothelial cells, shear stress upregulates numerous factors implicated in arteriogenesis [e.g., VEGF receptor-2 (VEGFR2), integrin α-β3, angiopoietin receptor Tie2, and endothelial nitric oxide synthase (eNOS) (77)]. Furthermore, increased shear experienced by the endothelium translates to a nitric oxide (NO)-dependent signal necessary for vessel enlargement (84) involving remodeling of the extracellular matrix (92). Experimentally inhibiting NO production with nitro-L-arginine methyl ester eliminates vessel enlargement, even though high flow conditions remain (93), whereas overexpression of eNOS enhances vessel enlargement (1). Thus the competency of endothelial NO production is very important in permitting vascular remodeling through arteriogenesis. Recognition that chronic physical activity upregulates eNOS (9, 60) implies that there is an improved responsiveness for vascular remodeling, compared with sedentary individuals. This may contribute to the general correspondence between an active lifestyle and the lower incidence of cardiovascular disease (14).

**Inflammation.** There is substantial evidence that elements of the inflammatory response are involved in arteriogenesis, at least in collateral vessels after occlusion of a major conduit artery (12). Immediately after occlusion of the vessel, there is a dramatic upregulation of monocyte chemoattractant protein-1, particularly in the tissue where arteriogenesis is active. Invasion of monocytes in the region of the collaterals contributes to the vessel enlargement process, presumably through the release of potent cytokines [e.g., VEGF, fibroblast growth factor (FGF)-2] and/or monocyte-attracting properties, as was the case with placenta growth factor-induced arteriogenesis through VEGF receptor-1 (VEGFR1) (75). Elimination of monocyte presence diminished and exogenous delivery of monocytes stimulated arteriogenesis (52, 75). Furthermore, there is developing evidence that bone marrow-derived endothelial progenitor cells (EPCs) are important in vessel remodeling (6). It is not known whether monocytes or EPCs are involved in any enlargement of conduit vessels induced by exercise, since there is no experimental evidence available. Furthermore, there has been no evaluation of possible monocyte and/or EPC participation in angiogenesis induced within active muscle by endurance-type exercise training, although EPCs have been identified in capillaries within muscle after hindlimb ischemia (6). There may be an expectation that monocyte infiltration could be important in capillary proliferation in active muscle. There is an inflammatory response within muscle at the onset of training, if the exercise task is intense, uncommon, and the muscle is “over used.” However, it seems unlikely that this is critical to the expansion of the capillary network, since muscle soreness and inflammation are quickly resolved and do not occur with continued exercise; on the other hand, angiogenesis continues to completion later in time as the training continues. Interestingly, physical training has been shown to increase circulating EPCs, improve vascular repair after injury, and enhance angiogenesis in mice (58); it has also been shown to increase circulating EPCs and their survival in patients with stable coronary artery disease (58). Whether these findings have a broad application to humans during exercise training and/or are related to the beneficial effects of exercise on cardiovascular disease remains to be explored.
FACTORS IMPLICATED IN ANGIOGENESIS

Growth Factors

VEGF is a potent mitogen of endothelial cells that has been implicated in the angiogenic response to exercise. Although it is reasonable to expect that VEGF and other proangiogenic processes are important in both sprouting and intussusceptive angiogenesis, our present knowledge comes primarily from experiments evaluating sprouting angiogenesis. For example, on stimulation by VEGF, endothelial cells cultured on a collagen gel proliferate, migrate, and form sprouts that eventually appear as tubelike structures (31). In addition, VEGF serves as a chemoattractant (69), which may assist in endothelial cell migration, and has been shown to stimulate smooth muscle cell migration and proliferation (17). VEGF binds to two primary receptors on the endothelial cell: VEGFR1, a fms-like tyrosine kinase (Flt-1) receptor, and VEGFR2, originally termed the fetal liver kinase (Flk/KDR) receptor. VEGF-induced activation of VEGFR2 stimulates endothelial cell proliferation, migration, and differentiation in most cell cultures (8, 30, 99, 101). Activation of VEGFR2 elicits a potent tyrosine kinase signaling cascade, which includes activation of phosphoinositols-3-kinase, phospholipase C-γ, and protein kinase C-ε (101), to stimulate gene expression for a variety of genes, including eNOS (87). In addition, VEGFR2 activation leads to NO production through eNOS via mobilization of intracellular Ca²⁺ stores (18). NO production can be critical to VEGF signaling, since inhibiting NO production diminishes responses to otherwise potent angiogenic stimuli (50, 74). It has been generally accepted that VEGF activation of VEGFR2 elicits the complete angiogenic response (16). Indeed, Milkiewicz et al. (70) observed a high correlation between VEGFR2 protein expression and capillary proliferation induced with muscle activity. Although there is much to be learned about the control of angiogenesis in vivo, VEGF and its binding to receptors must be considered a critical element (2).

Other angiogenic or proangiogenic growth factors can also contribute to angiogenesis. For example, FGF (e.g., FGF-2) is a potent mitogen for endothelial cells, smooth muscle cells, and fibroblasts. Furthermore, FGF-2 can upregulate VEGF (89) and NO production (7). However, FGF-2 does not appear to be upregulated by exercise (35, 79), and its content within trained muscle does not change, even though there was an increased capillarity induced by the training program (20) or with muscle overload (22). Nonetheless, FGF-2 may contribute to the continuation of angiogenesis as it is released from storage sites on degradation of the extracellular matrix (98). The angiopoietins (Ang1 and Ang2) are important cytokines that are not mitogenic to endothelial cells but assist in vascular development and remodeling (24). Ang1 promotes maturation and stabilization of vessels and is expressed widely throughout tissues, whereas Ang2 competes with Ang1 by displacing it from activating the Tie2 receptor and is expressed at sites of vascular remodeling. Thus Ang1 dominance is associated with a stable vasculature, and Ang2 dominance is associated with active angiogenesis. Other cytokines are also implicated in angiogenesis. Activated endothelial cells produce platelet-derived growth factor and transforming growth factor-β, which can recruit pericytes to help complete the newly formed capillary (69). Although a complexity of multiple cytokines and their actions are involved in angiogenesis, VEGF appears to be central in the process of initiating angiogenesis.

Upregulation of VEGF with exercise. Upregulation of VEGF occurs in rat muscle following contractions (2, 5, 40) or with a single bout of moderately intense treadmill running (10). In the latter case, VEGF mRNA was upregulated in the active muscle of rats approximately two- to fourfold by the end of exercise, remained elevated for the next ~4 h, but returned to that of rested muscle within 8 h postexercise. Upregulation of VEGF mRNA also occurs during exercise in humans, in both normal healthy individuals (35, 79) and patients with heart failure (32). Increases in VEGF protein are coincident with these increases in VEGF mRNA after exercise (33). However, a decrease in muscle VEGF protein content has been observed during exercise (25). Muscle VEGF contents may be mobilized as VEGF increases in the interstitium (46) and released by active muscle (45). Thus there is ample reason to expect that mobilization and upregulation of VEGF are important events in the exercise-induced increase in muscle capillarity that occurs with training. Indeed, Armal and coworkers demonstrated that the angiogenesis induced within muscle by chronic stimulation (2) and treadmill running (3) was dependent on the availability of VEGF.

Although experiments to define a clear relationship between the stimulus (exercise dose), VEGF upregulation, and angiogenesis have not been performed, it does appear that VEGF is primarily upregulated in the fibers that undergo angiogenesis. Lloyd et al. (62) observed a relatively modest increase in VEGF mRNA in the fast-twitch red region of the gastrocnemius, compared with the fast-twitch white gastrocnemius region, 2 h after treadmill running with restricted blood flow to the calf muscles. The fast-twitch red region of the gastrocnemius receives approximately fourfold greater blood flow than that measured in the white gastrocnemius section during these exercise conditions (63, 103). Thus the red region has a relative surfeit of blood flow during exercise, compared with the white gastrocnemius region. More importantly, subsequent to the approximately sixfold increase in VEGF mRNA, the white region exhibited a significant increase in muscle capillarity within 10–14 days (62), whereas similar training in a previous study did not produce any change in capillarity in the red gastrocnemius region (105). It should be pointed out, however, that in the basal condition it is the red gastrocnemius region that enjoys a relative rich capillary density, approximately three times that measured in the white gastrocnemius region (37). In fact, basal expression of VEGF mRNA is approximately threefold higher in this high-oxidative red region, compared with the white gastrocnemius region (62). Furthermore, VEGF protein content appears to scale with tissue vascularity among fiber types, as well as after muscle remodeling that increases capillarity (5). Thus the abundance of VEGF mRNA and protein and the upregulation during contractions appear to vary with vascular density and/or active angiogenesis.

Exercise also upregulates other elements that may be important to the angiogenic process. For example, the mRNA of the VEGF receptors VEGFR1 and VEGFR2 are upregulated after muscle activity (26, 28, 62). If this translates into an increase in receptor protein, as observed with electrical stimulation by Milkiewicz et al. (70), there could be an enhanced tissue responsiveness to VEGF and an amplification of the angi-
genic cascade. Exercise has been shown to upregulate eNOS mRNA (62) and protein (97). This could have major implications for angiogenesis because NO production is an important element of VEGF signaling (63, 71). Curiously, inhibiting NO production during training in flow-restricted muscle did not alter the angiogenesis induced by treadmill running (63). Thus there may be other important stimuli, established by active force development and shortening, that interact with the VEGF-mediated angiogenesis pathway (see Mechanical Stretch). Completion of the angiogenic process in active muscle likely involves the angiopoietins. The ratio of Ang2/Ang1 is markedly elevated after an exercise bout and throughout a 3-wk duration of training in muscle undergoing angiogenesis (62). This is expected to favor vascular expansion in the presence of VEGF (66). Although there have been no definitive demonstrations of these events, the evidence developed to date is consistent with this interpretation.

Extracellular Matrix Degradation

Although VEGF appears to be essential for angiogenesis, degradation of the basement membrane surrounding the capillary is necessary for sprouting angiogenesis to proceed. Proteolysis of the basement membrane is achieved by several families of enzymes: matrix metalloproteinases (MMPs), plasminogen, and the urokinase and tissue plasminogen activators (uPA, tPA). This process of extracellular matrix degradation must be highly controlled and coordinated because unguarded dissolution of the extracellular matrix would result in a loss of the integrity, and thereby function, of the microvasculature. Thus a balance of controlling influences must be altered to shift from a situation of stability of the microvasculature to one permitting remodeling of the extracellular matrix and sprouting angiogenesis. Important in this balance are the tissue-localized inhibitors of MMPs (TIMPs). For example, specific ratios of MMPs to TIMPs are needed for efficient activation of some MMPs (39). MMPs are secreted as inactive propeptides and are activated by cleavage of the regulatory peptide sequence. Activation of MMPs is achieved by plasmin and other MMPs. For example, uPA activates plasmin (from plasminogen), which in turn activates MMP-3 (stromelysin) and in turn activates MMP-9 (39). Activation of MMP-2 and activation of MMP-9 appear to be important proteolytic events facilitating angiogenesis. Similarly, uPA is proangiogenic, and PA inhibitor PAI-1 exhibits antiangiogenic effects (16). VEGF-mediated signaling upregulates uPA and tPA activity and mRNA expression (53, 67, 68). Although there is very little information as to how these processes are coordinated to support angiogenesis induced by muscle activity, some interesting studies have confirmed the important of the MMPs. Rivilis et al. (81) observed an increase in MMP-2 activity and an up-regulation of membrane type-1 (MT1)-MMP (i.e., MMP-14) in muscle after overload. MMP-14 has been shown to be upregulated in endothelial cells on exposure to strain in culture (102). This implies that physical stretch developed during contractions could be an important contributor to angiogenesis. In another important study, Haas et al. (38) found that inhibiting the MMPs eliminated the expansion of the capillary network induced by chronic muscle stimulation, although proliferation of endothelial cells continued. It is hoped that future work will add significant new understanding to the remodeling events of the extracellular matrix during angiogenesis induced within active muscle. As described below, the work of Haas, Hudlicka, and coworkers (11, 23, 38, 41, 81, 107, 108) have been instrumental in illuminating our understanding of factors prompting angiogenesis in adult muscle, including the MMPs.

HYPOXIA

One of the most potent stimuli that initiates capillary angiogenesis is hypoxia. Hypoxia exerts its effect primarily through an upregulation of VEGF and the sequelae thereof established. Lowering the PO2 of endothelial cells in culture stimulates cell proliferation, migration, and tube formation, whereas returning the culture medium PO2 to a high value reduces VEGF expression and lessens endothelial cell activation (88). Although it remains unclear how low PO2 is sensed, there is a marked increase in a transcription factor, hypoxia-inducible factor (HIF-1α; both mRNA and protein), which stimulates transcription of the VEGF gene (88). The half-life of HIF-1α is short lived and subject to modulation. HIF-1 also controls other proteins important in angiogenesis (e.g., VEGFR1; Ref. 29) and is engaged in gene regulation of other hypoxia-sensitive responses (e.g., erythropoietin production at altitude). It is important to appreciate that the role of hypoxia should be relegated to angiogenesis in active muscle, where the PO2 can be low, and not to arteriogenesis, where the endothelium of the arterial conduit vessel is perfused with high PO2 blood. The importance of hypoxia as an obvious candidate for the initiation of angiogenesis in active muscle during training was reinforced by data showing that hypoxia enhanced the exercise-induced upregulation of VEGF in rat muscle (10). More recent experiments confirmed the likely involvement of HIF-1α by demonstrating an elevation in HIF-1α mRNA and protein during exercise in humans (36). However, further experimental pursuit of this process in humans led to equivocal results in which exercise in hypoxic/ischemic conditions did not enhance the upregulation of VEGF, compared with exercise in normoxic/hyperemic conditions (35, 79). Thus, although the HIF levels vary within a physiologically relevant range of PO2 (54), no relationship between the PO2 within the active muscle and the VEGF mRNA response was established (79). This conundrum implies that either exercise in normoxia produces a sufficiently low, essentially a “saturated” PO2 response within the active muscle, and that reducing the PO2 further is superfluous or that other factors are involved in modulating the signaling cascade upregulating VEGF.

The upregulation of VEGF in active muscle during exercise is even more intriguing. Wagner and coworkers reported that the large increase in VEGF mRNA initially observed after a bout of exercise was not sustained over subsequent days of training in animals (27) or humans (80). This response pattern was confirmed with ischemic training and shown to occur at a time when angiogenesis was ongoing and became measurable on histochemical evaluation (62). Although adaptations in the cellular environment within the active muscle have likely occurred to ameliorate the VEGF upregulation, it does not seem likely that a significant protection against a low PO2 occurred in this experiment (62), since the animals performed relatively ischemic exercise caused by occlusion of the femoral arteries. Furthermore, Olfert et al. observed that chronic exposure to hypoxia decreased resting mRNA levels of VEGF and...
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VEGF, and the putative development of angiogenesis. It is likely that stimuli other than low \( P_o \) are important in angiogenesis in contracting muscle.

**SHEAR STRESS AND MECHANICAL STRETCH**

Although much evidence shows that mechanical forces, related to shear stress and/or mechanical stretch, can initiate signals that remodel the vasculature, it has been difficult to establish experiments that quantitatively impart and/or control these stimuli and relate them to the angiogenic outcome in the microvasculature of muscle in vivo. This is due to the difficulty in measuring or controlling the stimulus of shear stress and/or mechanical stretch actually experienced by the cells within the muscle in vivo. Nonetheless, there have been some important observations that attest to distinctions in the signaling and angiogenic outcome with different types of mechanical stimuli.

**Shear Stress**

Prolonged delivery of vasodilators to rats increases capillarity of skeletal muscle (91). Chronic administration of an \( \alpha \)-sympathetic blocker (prazosin) increased muscle blood flow approximately threefold (109). This produced a significant increase in red blood cell velocity through the capillaries and an increase in calculated shear stress experienced by the endothelium (19). This work has provided the foundation for the subsequent use of prazosin in evaluating the effects of increased shear stress on capillary and arteriolar remodeling in muscle (23, 76, 95, 108). Most importantly, the nature of this flow-mediated angiogenic response appears to be distinct. The increased capillarity induced by elevating shear stress was achieved primarily by intussusceptive angiogenesis (23, 108), in contrast to that observed with muscle overload (11, 22, 107) or probably exercise training (11). By exhaustive analyses of high-resolution micrographs, Hudlicka and coworkers (23, 108) were able to identify a marked elevation in the frequency of capillaries with longitudinal divides on the luminal side of the capillary. Furthermore, these investigators subsequently reported that activation of MMP-2 and MT1-MMP was not evident with flow-mediated intussusceptive angiogenesis, again in contrast to that observed in sprouting angiogenesis (81). However, in keeping with signaling events induced by shear stress in large arteries, VEGF is implicated in angiogenesis as both VEGF protein accumulation and endothelial cell proliferation were observed in both intussusceptive angiogenesis and sprouting angiogenesis (81). The absence of an up-regulation of VEGF during high flow conditions in quiescent dog muscle (83) does not necessarily dismiss the importance of this growth factor in flow-mediated angiogenesis. In that experiment, blood flows achieved in the high oxidative capacity, high vascular capacity muscle in the absence of contractions may not have produced similar flow profiles within the microvasculature as achieved during contractions. Furthermore, shear stress can activate the VEGFR2 pathway, independent of VEGF (100). Thus an elevated shear stress is likely an important mechanical signal prompting angiogenesis within muscle.

**Mechanical Stretch**

In addition to the intussusceptive angiogenesis associated with increased blood flow, there is evidence for sprouting angiogenesis in skeletal muscle. Muscle overload, by ablation of synergists (11, 22, 107), and muscle contractions, in response to chronic intermittent nerve stimulation (11), lead to sprouting angiogenesis. In both of these experimental treatments, contraction of the muscle leads to mechanical forces acting on the tissue that could initiate angiogenesis, in the former case by performing the work of synergist and in the latter by the unaccustomed frequency of contractions. Stretch of cells in culture upregulates VEGF (mRNA and protein), leads to enhanced endothelial cells migration and tube formation (106), activates MT1-MMP (102), and upregulates Ang2 and Tie2 expression (15). All of these actions would support sprouting angiogenesis if they occurred within active muscle. Although blood flow is expected to be increased during contractions, thereby establishing an increase in shear stress, it is reasonable to expect that mechanical forces developed within active muscle are a stimulus that fosters sprouting angiogenesis.

VEGF is elevated in muscle with treatments of hyperemia, overload, and contractions. Thus the presence of this growth factor does not seem to be a feature in distinguishing sprouting vs. intussusceptive angiogenesis, although there is no clear resolution of its relation to endothelial cell proliferation (11). There is, however, compelling evidence of a distinction in the response of the MMPs. MMP-2 activity and MT1-MMP mRNA were elevated by muscle overload but not with muscle hyperemia (81). Recall that extracellular matrix degradation is an essential element permitting sprouting angiogenesis to proceed. Indeed, Haas et al. (38) demonstrated that inhibition of MMP activity eliminated the angiogenesis typically induced by muscle stimulation. This work provides the first example of how coordinated processes must be orchestrated in order for angiogenesis to continue to completion in active muscle. Furthermore, there is evidence that this angiogenesis prompted by chronic nerve stimulation requires NO production in its signaling pathway. Administration of a NOS inhibitor eliminated the increase in muscle capillarity typically observed (50). Presently, we are only beginning to understand how muscle contractions induce angiogenesis. It is anticipated that future research will help clarify which angiogenic process is initiating by mechanical and/or hemodynamic stimuli in active muscle.

**SUMMARY**

Research in recent years has resulted in an appreciation of the many angiogenic growth factors, critical receptors, signaling pathways, and their coordinated interactions that must be involved in vascular remodeling. In addition, controlled studies have exposed important chemical and mechanical stimuli that initiate and propagate the angiogenic process. This is particularly evident in a shear stress-mediated enlargement of conduit vessels. It is likely that much of this information is applicable to the events within active muscle to increase capillarity and to improve muscle function. However, there is much research needed to understand how this physiological angiogenesis is controlled to accommodate the demands within active muscle.

**ACKNOWLEDGMENTS**

We thank Don Conner for preparing the illustration.
REFERENCES


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