Applied Physiology, Nutrition, and Metabolism
Physiologie appliquée, nutrition et métabolisme

**Regulation of Skeletal Muscle Capillary Growth in Exercise
and Disease**

Journal:	<i>Applied Physiology, Nutrition, and Metabolism</i>
Manuscript ID	apnm-2015-0336.R1
Manuscript Type:	Award Lecture
Date Submitted by the Author:	31-Aug-2015
Complete List of Authors:	Haas, Tara; York University, Nwadozi, Emmanuel; York University,
Keyword:	hypoxia, blood flow, growth factors, peripheral artery disease, diabetes

1   Regulation of Skeletal Muscle Capillary Growth in Exercise and Disease

2   Tara L. Haas* and Emmanuel Nwadozi

3   Angiogenesis Research Group, School of Kinesiology and Health Science,

4   York University, Toronto, ON

5
6   Short Title: Skeletal muscle angiogenesis

7
8   * Address for Correspondence:

9   Tara Haas, PhD
10   Angiogenesis Research Group
11   York University
12   Rm. 341 Farquharson Building
13   4700 Keele St.
14   Toronto, ON
15   M3J 1P3   Canada
16   Phone: (416) 736-2100 x77313
17   Email: thaas@yorku.ca

21 **Abstract:**

22 Capillaries, which are the smallest and most abundant type of blood vessel, form the primary
23 site of gas, nutrient and waste transfer between the vascular and tissue compartments.
24 Skeletal muscle exhibits the capacity to generate new capillaries (angiogenesis) as an
25 adaptation to exercise training, thus ensuring that the heightened metabolic demand of the
26 active muscle is matched by an improved capacity for distribution of gases, nutrients and waste
27 products. This review summarizes the current understanding of the regulation of skeletal
28 muscle capillary growth. The multi-step process of angiogenesis is co-ordinated through the
29 integration of a diverse array of signals associated with hypoxic, metabolic, hemodynamic and
30 mechanical stresses within the active muscle. The contributions of metabolic and mechanical
31 factors to the modulation of key pro- and anti- angiogenic molecules are discussed within the
32 context of responses to a single aerobic exercise bout, short term and long term training.
33 Finally, the paradoxical lack of angiogenesis in peripheral artery disease and diabetes, and the
34 implications for disease progression and muscle health are discussed. Future studies that
35 emphasize an integrated analysis of the mechanisms that control skeletal muscle capillary
36 growth will enable development of targeted exercise programs that effectively promote
37 angiogenesis in healthy individuals and in patient populations.

38 Keywords: Hypoxia; Blood Flow; Growth Factors; Peripheral artery disease; Diabetes

39

40

41 ***Structure and function of capillaries***

42 Capillaries form the smallest unit of the vasculature, with an average diameter of $\sim 5 \mu\text{m}$. It is
43 estimated that capillaries comprise 2-3% of the skeletal muscle mass. For the past century, it
44 has been recognized that a very tight relationship exists between the capillary and the
45 myofibre. Within skeletal muscle, capillaries are oriented longitudinally in parallel with the
46 myofibres, with short interconnecting capillaries that form looping networks (Fig.1). The
47 intimate proximity of capillaries to the myofibres, together with morphological features of the
48 capillary that favour efficient trans-capillary diffusion (endothelial cell thickness of $\sim 0.2 \mu\text{m}$ in
49 non-nuclear regions) establish the capillary as the critical site of nutrient and gas exchange and
50 waste removal (Bruns and Palade 1968; Simionescu et al. 1974). The capillary network also
51 plays a significant role in establishing skeletal muscle insulin sensitivity through controlling the
52 transfer of plasma insulin to the surrounding myocytes (Azizi et al. 2015; Barrett et al. 2009;
53 Lillioja et al. 1987; Stanford and Goodyear 2014). August Krogh first modeled the oxygen
54 exchange properties of the skeletal muscle capillary network. By relating skeletal muscle
55 capillary density to the metabolic rate of a broad variety of animals, he estimated the tissue
56 volume that could receive a sufficient amount of oxygen from an individual capillary (Krogh
57 1919). His work made the seminal contribution in recognizing that the number and distribution
58 of capillaries establishes the oxygenation and, consequently, the metabolic potential of the
59 surrounding skeletal myocytes. Romanul (1964) provided evidence that the number of
60 capillaries surrounding a muscle fiber correlates positively with the fiber's oxidative metabolic
61 potential. Efforts made to map the architecture of the skeletal muscle capillary network have
62 been critical in promoting a greater understanding of how the vasculature meets the unique
63 needs of each muscle fiber type (Al-Shammari et al. 2014; Egginton and Gaffney 2010; Plyley
64 1990; Plyley and Groom 1975).

65 ***Adaptation of the capillary network with muscle activity***

66 Another substantial advancement in this field of research occurred with the recognition that
67 capillary number within muscle is not static, but that it can vary dependent on changes in
68 muscle activity. Angiogenesis is defined as the growth of new capillaries from pre-existing

ones; it ensures that increases in skeletal muscle metabolic capacity are matched with an increased capacity for small molecule exchange. For example, oxygen diffusion is optimized by virtue of an increased surface area for exchange and reduced diffusion distance. Carrow and colleagues (Carrow et al. 1967) reported that a greater number of capillaries were present within the muscle of exercised rats compared to their sedentary counterparts. While the authors concluded that this phenomena was due to an increased number of perfused capillaries following exercise, in retrospect, this study likely represented one of the first observations of the phenomenon known as exercise-induced angiogenesis. Subsequently, the use of rabbit and rat models of electrical stimulation –induced muscle activity, together with standard histological and intra-vital microscopy techniques, provided unequivocal evidence that expansion of the capillary network occurs via addition of new capillaries in glycolytic muscle subjected to a sustained increase in metabolic demand (Brown et al. 1976; Cotter et al. 1973; Myrhaage and Hudlická 1978). Concurrently, researchers made significant progress in demonstrating that these processes were not confined to animal models, but were valid also in human skeletal muscle. In the early 1970s, Hermansen and Wachtlova (1971) reported higher capillary to fiber ratio in trained compared to untrained individuals a finding later confirmed by Andersen (1975). However, Andersen and Henriksson's seminal study (1977), based on histological analysis of muscle biopsies from individuals before and through-out 8 weeks of training, provided the first direct evidence that exercise induced capillary growth occurs in human skeletal muscle. These researchers also demonstrated that maximal oxygen consumption increased with a similar time frame as the increases in capillary density, thus postulating a causal relationship between capillary number and improvements in maximal oxygen consumption.

What stimulates exercise induced angiogenesis?

Exercise is a multi-factorial stimulus, with numerous likely contributors to the initiation of the angiogenic response. These stimuli can be distinguished generally as being metabolic or mechanical in nature. Investigators have attempted to characterize the contributions of these stimuli, employing experimental models that allow independent manipulation of an individual

stimulus. However, it should be emphasized that exercise evokes simultaneous changes in all of these factors.

Hypoxia and Metabolites: Reduced oxygen tension appears to be a strategic stimulus for the purpose of initiating a negative feedback loop that would culminate in capillary growth and enhanced oxygen delivery. However, the contribution of hypoxia to exercise induced angiogenesis remains controversial. Numerous studies have documented a transient reduction in intramuscular pO_2 at the initiation of exercise, but pO_2 is restored rapidly by the concomitant increase in muscle blood flow (Hudlicka 1998; Milkiewicz et al. 2003; Molé et al. 1999; Richardson et al. 1995). However, at high workloads, enhanced perfusion may be insufficient to restore oxygen deficit (Richardson et al. 1995). Thus, the magnitude and duration of a hypoxic challenge during exercise likely depends heavily on muscle workload and the potential for blood flow restriction due to elevated intramuscular pressures. Adenosine, which may be released during, and in proportion to, muscle activity also exerts angiogenic influences (Høier et al. 2010).

Mechanical Stresses: Muscle overload, induced experimentally when a muscle is required to bear the load of a synergistic muscle, increases metabolic demand, alters tensile strain and results in muscle hypertrophy and new capillary growth (Egginton et al. 1998; Holly et al. 1980; James 1981). While metabolic stress associated with muscle overload likely contributes to the induction of an angiogenic response in this model, several studies indicate that tensional forces associated with the stretch of muscle also contribute to the angiogenic process (Egginton 1998; Egginton 2011). Capillaries are tethered through extracellular matrix to the surrounding muscle tissue. Thus, capillary shape is altered and stretched with increases in sarcomere length (Ellis et al. 1990). Cultured endothelial cells subjected to static stretch increase production of pro-angiogenic factors (Milkiewicz et al. 2007b). In accordance with this concept, the interstitial fluid collected from muscle after repeated passive leg movement was shown to increase endothelial cell proliferation (Hellsten et al. 2008; Hoier et al. 2013b). Stretch/overload may activate integrins on the abluminal surface of the endothelium and induce signaling to promote endothelial cell proliferation. Secretion of growth factors from fibroblasts, smooth muscle cells

and pericytes, as well as release of matrix-associated growth factors, can contribute to this angiogenic response.

Shear stress refers to the frictional force generated on the endothelial cell surface as blood flows. In resting muscle, capillary shear stress is estimated to be $5 \text{ dynes}\cdot\text{cm}^{-2}$. During activity, vasodilation of upstream arterioles increases muscle blood flow and results in capillary shear stresses of $15\text{--}20 \text{ dynes}\cdot\text{cm}^{-2}$. Clarke (1918) first proposed the link between blood flow and capillary growth by observing the growth of capillaries in tadpole tails. By observing the same region over the course of weeks, he observed that areas with high velocity formed new vessels while in low velocity areas, vessel number diminished. The administration of vasodilator drugs has been used to isolate the impact of increased blood flow from muscle metabolism in order to investigate its effects on capillarization. Treatment with the α -1 adrenergic receptor antagonist, prazosin, causes a sustained increase in capillary shear stress that is similar to the level achieved during muscle activity (Davey 1982; Ziada et al. 1989), and an increase in capillary to fiber ratio is detectable within 7–14 days (Gee et al. 2010; Rivilis et al. 2002; Zhou et al. 1998).

Sprouting and non-sprouting angiogenesis

The formation of new capillaries has been documented to occur via two distinct morphological processes; abluminal sprouting and luminal splitting/intussusception. The former involves a coordinated sequence of events including proliferation of endothelial cells, spatially restricted proteolytic degradation of the capillary basement membrane, and migration of sprouting tip cells into the interstitial (Fischer et al. 2006; Geudens and Gerhardt 2011). The sprout forms an anastomosis with another capillary and a patent lumen is formed, generating a new flow channel (Geudens and Gerhardt 2011). This type of angiogenesis has been observed with muscle activity, such as that evoked by chronic electrical stimulation (Myrhage and Hudlická 1978).

Intussusception has been described in models of blood flow-induced angiogenesis (Clark and Clark 1940; Patan et al. 1993). In skeletal muscle, this process has been identified as “luminal

splitting". It occurs when capillary endothelial cells forming luminal protrusions that bridge across the lumen and fuse with the opposite wall, which generates 2 parallel flow channels (Zhou et al. 1998). These flow channels then separate into two distinct capillaries, which may occur due to forces exerted by perivascular cells. Unlike sprouting angiogenesis, splitting involves minimal proliferation and is not associated with proteolytic degradation of the basement membrane. Intussusception describes a similar process in which luminal pillars that are comprised both of endothelial cells and surrounding perivascular cells form bridges across the vessel and generate multiple flow pathways (Styp-Rekowska et al. 2011). In each case, remodeling is stimulated by elevated shear stress and it helps the microvascular network to adapt rapidly to local changes in shear stress while maintaining the overall branching hierarchy of the microvascular network.

Sprouting and splitting types of capillary growth result in morphologically distinct capillary networks. While sprouting angiogenesis creates a network that is dominated by many short inter-connecting capillary loops, splitting angiogenesis generates a network comprised of numerous long capillaries in parallel. Aerobic muscle activity does elicit both forms of angiogenesis (Egginton et al. 2001). From a therapeutic point of view, it is attractive to consider that there may be an opportunity to stimulate angiogenesis through manipulation of blood flow and induction of luminal splitting even if sprouting angiogenesis is limited.

Angiogenic Balance: Factors that modulate angiogenesis

Judah Folkman, who pioneered research in the field of tumour angiogenesis, provided evidence that capillary growth is controlled by a specific factor that initially was identified simply as "tumour angiogenesis factor" (Gimbrone et al. 1972; Sherwood et al. 1971). Folkman postulated that inhibition of this factor could be used to control tumour angiogenesis, and hence, tumour growth. Subsequently, his research extended to the identification of multiple endogenous inhibitors of angiogenesis (Folkman 1996). This research provided the framework for understanding the process of angiogenesis in a variety of pathological and physiological conditions. It is now appreciated that angiogenesis within skeletal muscle is tightly controlled by a balance between pro- and anti- angiogenic signals (Gustafsson 2011; Olfert & Birot 2011).

An abundance of pro-angiogenic factors favours the growth of new capillaries while an excess of anti-angiogenic factors restricts new capillary growth and may result in the loss, or rarefaction, of pre-existing capillaries. In the adult, it is appropriate to maintain stable mature capillary networks within most tissues, and this requires a net balance between growth and regression stimuli. Over the past 30 years, dozens of factors that exert pro- or anti-angiogenic functions have been identified and characterized in a wide variety of model systems. This review will be limited to the description of the best characterized factors that been investigated within skeletal muscle.

Pro-Angiogenic Factors:

Vascular Endothelial Growth Factor-A (VEGF-A) is the most widely studied pro-angiogenic molecule and is capable of stimulating all aspects of the angiogenic sprouting process. Multiple isoforms of VEGF-A (typically identified by their amino acid length) are generated as a consequence of alternative splicing of the primary mRNA transcript (Neufeld et al. 1996). These isoforms differ predominantly in their capacity to bind heparin sulphate proteoglycans within the extracellular matrix. VEGF-A₁₆₅ and VEGF-A₁₈₉, both which contain the heparin sulfate binding domain encoded in exon 6, together constitute approximately 70% of the VEGF-A transcript produced by human skeletal muscle, irrespective of fibre type (Gustafsson et al. 2005). Much of this secreted VEGF-A is retained within the matrix surrounding the capillaries. The deposition of matrix-associated VEGF-A may establish a chemotactic gradient that attracts and guides the tip cells of capillary sprouts (Vempati et al. 2014). We found that VEGF-A secreted by C2C12 myoblasts is retained in the cell-free extracellular matrix and that it can in turn activate angiogenic signaling in cultured endothelial cells (Gorman et al. 2014). On the other hand, endothelial cells produce a higher amount of VEGF-A₁₂₁ (Gorman et al. 2014), which lacks exon 6 and thus is freely diffusible within the extracellular fluid. While all isoforms of VEGF-A bind to and activate VEGFR2, only isoforms with exon 7 (in muscle, VEGF-A_{-165,189}) interact with the co-receptor neuropilin-1. It is postulated that this interaction may result in differences in the dynamics of receptor localization and internalization as well as promote unique intracellular signal pathways (Plein et al. 2014). Numerous cells within the skeletal

210 muscle produce and secrete VEGF-A, including myofibres, endothelial cells, satellite cells,
211 fibroblasts and macrophages (Maharaj et al. 2006). Despite these multiple cellular sources of
212 VEGF-A, studies utilizing mice that are deficient in skeletal myofibre VEGF-A have provided clear
213 evidence that myofibre-derived VEGF-A is critical in promoting angiogenesis in response to a
214 metabolic stimulus such as exercise or muscle overload (Gorman et al. 2014; Olfert et al. 2010).

215
216 In muscle, production of VEGF-A is enhanced by hypoxic and metabolic stress (De Bock et al.
217 2013; Hoier and Hellsten 2014). This pattern of regulation strongly suggests that VEGF-A
218 production is linked in tight association with the metabolic needs of the myofibres. Distinct
219 from this established paradigm, we demonstrated recently that nitric oxide (a signaling
220 mediator generated by endothelial cells in response to elevated shear stress) can promote
221 skeletal myocyte production of VEGF-A (Uchida et al. 2015). Furthermore, deficiency in
222 myocyte-derived VEGF-A resulted in a loss of blood flow-induced angiogenesis (Uchida et al.
223 2015). These findings also serve to highlight the significance of bi-directional cross-talk between
224 capillary endothelial cells and the surrounding skeletal myofibres in co-ordinating appropriate
225 adaptive responses within the microvascular network.

226
227 Matrix Metalloproteinases (MMPs) are responsible for proteolysis of the matrix proteins that
228 comprise the capillary basement membrane, allowing for tip cell sprouting during abluminal
229 sprouting angiogenesis (Brew and Nagase 2010; Haas and Madri 1999). MMP activity is
230 essential for activity-induced angiogenesis as the pharmacological inhibition of MMP enzymatic
231 activity prevented basement membrane proteolysis, abluminal sprout formation and new
232 capillary growth in rat EDL muscle in response to prolonged electrical stimulation of muscle
233 activity (Haas et al. 2000). The MMP family is extensive, however, several family members
234 (MMP-2, MMP-9 and membrane type 1 (MT1)-MMP/MMP-14) have been reported to increase
235 in exercising muscle in rats and humans, prior to the detection of increased capillary number
236 (Haas et al. 2000; Rullman et al. 2007, 2009). MMP-9 cleaves heparan sulfate proteoglycans,
237 thus releasing matrix-associated growth factors such as VEGF-A (Lee et al. 2005). This may
238 facilitate rapid activation of pro-angiogenic signaling within the local environment in response

to exercise. In contrast, the process of flow-induced capillary growth does not involve basement membrane degradation and there is no increase in production of MMPs (Rivlis et al. 2002). In fact, cultured endothelial cells exposed to elevated blood flow exhibit reduced levels of MMP-2 and MT1-MMP and increased production of the protease inhibitor TIMP1 (Milkiewicz et al. 2006b, 2008; Yun et al. 2002), which together may ensure stability of the capillary basement membrane.

Angiopoeitins (ANGPT1 and ANGPT2) are ligands for the tyrosine kinase receptor Tie2.

ANGPT1-induced activation of Tie2 is associated with enhancement of pericyte-endothelial cell interactions, strengthening of endothelial-endothelial junctions and the maintenance of a stable capillary network (Augustin et al. 2009). ANGPT1 and ANGPT2 may be produced by satellite cells and skeletal myocytes as well as by endothelial cells. ANGPT2 expression can be stimulated by hypoxia and by VEGF-A (Andersson et al. 1988; Oh et al. 1999; Yuan et al. 2000). ANGPT2 competes for binding to Tie2, but is considered to be a weak receptor agonist that functions as a competitive antagonist of ANGPT1, preventing activation of the receptor and downstream signal pathways (Thurston and Daly 2012). Consequently, elevated levels of ANGPT2 reduce Tie2 activation, and vessel de-stabilization may result. If this occurs with concurrent activation of the VEGF-A/VEGFR2 pathway, ANGPT2 enhances the sprouting angiogenic process. However, in the absence of VEGF-A signaling, elevated levels of ANGPT2 can promote endothelial cell death and vessel regression (Fagiani and Christofori 2013).

Nitric Oxide (NO) is produced in endothelial cells through the enzymatic activity of endothelial nitric oxide synthase (eNOS, or NOS3) (Förstermann and Sessa 2012). eNOS catalyzes the conversion of L-arginine and molecular oxygen to NO and L-citrulline. eNOS exhibits basal constitutive activity, which can be enhanced by elevated intracellular calcium and by phosphorylation of Ser1177 by Akt (Sessa 2009). These intracellular signals are stimulated by endothelial cell exposure to elevated shear stress as well as by numerous growth factor receptors that recruit Akt as part of their intracellular signaling network. Within arterioles, NO diffuses to the surrounding vascular smooth muscle cells, stimulating guanylate cyclase-cGMP-protein kinase G-dependent relaxation. In addition to the critical role that NO plays in the

267 modulation of vascular smooth muscle tone, NO has been shown to exert pro-angiogenic
268 influences (Morbideilli et al. 2003; Papapetropoulos et al. 1997). Indirectly, NO production and
269 arteriolar vasodilation induces angiogenesis as a consequence of increasing blood flow through
270 the downstream capillaries. However, cultured cell experiments demonstrate that elevated
271 NO, in the absence of a flow stimulus, increases endothelial cell proliferation and migration
272 (Morbideilli et al. 1996). Our recent observation that NO stimulates skeletal myocyte
273 production of VEGF-A provides further explanation of a mechanism through which endothelial
274 cells can relay messages to the surrounding tissue to co-ordinate and enhance pro-angiogenic
275 signals (Uchida et al. 2015). Considering these pleiotropic influences of NO, it is not surprising
276 that the capacity of skeletal muscle capillaries to undergo angiogenesis in response to
277 metabolic or to blood flow stimuli is significantly impaired by pharmacological inhibition of
278 eNOS activity or by genetic knock-out of NOS3 (Baum et al. 2004; Hudlická et al. 2000; Williams
279 et al. 2006).

280 Angiotensin II (Ang II) has been shown to play a role in angiogenesis during skeletal muscle
281 regeneration (Bellamy et al. 2010). Ang II treatment of endothelial cells *in vitro* enhanced tube
282 formation and migration (Bellamy et al. 2010). Stimulation with exogenous Ang II was reported
283 to upregulate ANGPT2 mRNA in cultured retinal endothelial cells (Otani et al. 2001) and to
284 increase VEGF-A and MMP-2 mRNA levels in both C2C12 myocytes and microvascular
285 endothelial cells (Gorman et al. 2014). While *in vitro* experiments indicate a pro-angiogenic role
286 of Ang II, evidence that it is a significant contributor in the context of physiological skeletal
287 muscle angiogenesis is mixed. Amaral and colleagues reported that the AT1 receptor inhibitor
288 losartan prevented activity-induced angiogenesis (Amaral et al. 2001a, 2001b). However,
289 losartan did not interfere with VEGF-A production or angiogenesis in response to muscle
290 overload (Gorman et al. 2014). Other studies have reported a pro-angiogenic influence of
291 losartan in tumour growth and within the brain microvasculature (Forder et al. 2005;
292 Munzenmaier and Greene 2006; Walther et al. 2003). A challenge in the interpretation of these
293 findings is that Ang II can activate multiple receptors sub-types and the relative distribution of
294 these receptor sub-types differs between vascular beds. Further, use of losartan was reported
295 to induce compensatory increases in circulating levels of Ang II (Munzenmaier and Greene

2006), which in turn could promote conversion to Ang(1-7) through the actions of angiotensin converting enzyme 2. Consequently, utilization of an AT1 receptor blocker such as losartan may provoke the over-activation of AT2 or Ang(1-7) (mas) receptors, which by themselves have been reported to induce both pro- and anti-angiogenic effects. Considering the known complexity of this signaling axis and the divergent angiogenic influences that have been reported to date, it is apparent that a complete understanding of how Ang II may contribute to the process of skeletal muscle angiogenesis remains to be established.

Anti-angiogenic Factors:

Tissue Inhibitor of Matrix Metalloproteinases (TIMPs) are proteins that restrict the activity of MMPs (Brew and Nagase 2010). They interact with the catalytic domain of the MMPs, preventing MMP access to ligands. They also can interfere with the activation process, which requires cleavage of a pro-domain from the MMP. By restricting MMP activity, TIMPs tend to reduce the remodeling of extracellular matrix proteins and cleavage of cell surface proteins. However, these actions ultimately may inhibit or promote angiogenic signaling. For example, cleavage of extracellular matrix proteins may expose pro-angiogenic domains, but it also can cause the release of anti-angiogenic fragments such as angiostatin (described below) (Suhr et al. 2009). In this case, inhibition of proteolytic activity prevents such anti-angiogenic actions. It also has been proposed that TIMPs exert cellular functions independently of MMP inhibition (Ries 2014). For example, TIMP1 is postulated to exert pro-proliferative and pro-survival cues in cancer cells (Stetler-Stevenson 2008). However, we have not been able to detect this effect in cultured endothelial cells (Mandel et al. 2014), thus the potential relevance of these functions to endothelial cell behaviour remains poorly established. We found that exposure of endothelial cells to elevated shear stress induces the increased production of TIMP1, indicating that TIMP1 may play a protective anti-proteolytic role that assists in maintaining capillary integrity in the face of elevated hemodynamic stress (Milkiewicz et al. 2008; Uchida and Haas 2014).

Endostatin and Angiostatin: Proteolytic cleavage of the C-terminal domain of collagen XVIII releases a 20 kDa fragment that is referred to as endostatin (O'Reilly et al. 1997). MMP-9 is

among the group of MMPs and elastases capable of generating this cleavage product (Ferrerias et al. 2000). Endostatin exerts anti-angiogenic functions by competing with collagen for binding to integrins α_v and α_5 , and thus interfering with migration and cell survival signaling (Faye et al. 2009). Angiostatin is a 38 kDa cleavage fragment of plasminogen, and has been shown to inhibit endothelial cell proliferation and induce apoptosis (O'Reilly et al. 1994). Through interactions with the cell surface receptor angiomin, it may interfere with cell-matrix attachment and cell migration (Trojanovsky et al. 2001). Because of the increased activity of MMPs in response to exercise, it is feasible to expect increased production of these angiostatic cleavage products. However, their contributions to the process of capillary growth in muscle have been difficult to ascertain, in part because it requires accurate detection of the cleavage peptide rather than detection of mRNA or the originating protein. It is notable that elevated levels of endostatin and angiostatin have been found within skeletal and cardiac muscle of diabetic patients and rodents with metabolic syndrome (Dodd et al. 2013; Sodha 2008), suggesting that these factors may contribute to establishing the general balance between pro and anti-angiogenic factors within muscle under basal conditions and have greater relevance under disease conditions.

Thrombospondin-1 (THBS1) is a large matricellular protein that interacts with dozens of secreted and matrix proteins and multiple cell surface receptors (Adams 1997; Resovi et al. 2014; Silverstein et al. 1986). THBS1 also regulates the activation of latent transforming growth factor β (TGF β), implicating it indirectly in promoting the fibrotic influences associated with TGF β signaling (Murphy-Ullrich and Poczatek 2000). Considering the diversity of these interactions, it is not surprising that THBS1 has been reported to exert numerous cell and context-specific biological functions (Chen et al. 2000). THBS1 can inhibit endothelial cell proliferation and migration and induce endothelial cell apoptosis, and thus is considered to be a potent anti-angiogenic factor (Iruela-Arispe et al. 2004). Consistent with this influence, the deletion of THBS1 increases basal capillary number in skeletal and cardiac muscle and enhances exercise capacity in mice (Malek and Olfert 2009).

Intracellular Regulators of Angiogenic Status

Considering that multiple factors exert positive and negative influences on the angiogenic process within the muscle, capillary growth requires some degree of co-ordinated regulation of these factors. It is attractive to consider the involvement of “master regulators”, which could integrate the signals originating from the various exercise-dependent stimuli in order to elicit a program of alterations that enhance angiogenic responsiveness. Transcription factors have the potential to act in this role given their capacity to promote transcription of a set of genes.

Hypoxia Inducible Factors: Low cellular oxygen levels stabilize the transcriptional regulators hypoxia inducible factors (HIF) 1 α and 2 α . This occurs due to reduced targeting of HIF α for degradation as a consequence of inhibition of the oxygen-sensitive prolyl hydroxylase. Either HIF1 α or HIF2 α forms a functional DNA binding protein with HIF1 β and acts to enhance the transcription of numerous genes that contain hypoxia-response elements (HRE) (Semenza 2014). HIF1 α is an established transcriptional regulator of VEGF-A (Pugh and Ratcliffe 2003) and it also increases production of ANGPT2 (Mandriota and Pepper 1998), suggesting that it co-ordinates the expression of these two growth factors to promote an angiogenic response. HIF1 α plays an important role in exercise induced angiogenesis. Destabilization of HIF proteins prevented overload-induced angiogenesis in rats (Milkiewicz et al. 2007a). Furthermore, exercise-induced angiogenesis failed to occur in mice lacking skeletal muscle HIF1 α (Mason et al. 2007). The latter study has been suggested as evidence that hypoxia is an important stimulus of exercise-driven capillary growth. However, HIF1 α transcriptional activity can be induced independently of hypoxia, via stimuli such as mechanical stretch and growth factors, which appear to act through MAPK signaling and generation of reactive oxygen species (Chang et al. 2003; Milkiewicz et al. 2007a; Richard et al. 2000). Interestingly, endurance athletes have elevated levels of enzymes that negatively regulate HIF1 α (prolyl hydroxylases and factor inhibiting HIF), and lower levels of HIF target genes, indicating a reduced reliance on HIF-dependent signal pathways with sustained endurance training (Lindholm et al. 2014a).

Peroxisome-proliferator-activated receptor γ co-activator 1 α (PGC1 α) is a transcriptional co-activator that is active in situations of intracellular energy deficit. In humans, PGC1 α mRNA increases in active muscle following a single exercise bout, and this is augmented under flow-

restricted conditions (Norrbon et al. 2004), consistent with its role as an “energy/metabolism sensor”. PGC1 α , through interaction with estrogen-related receptor (ERR) α , enhances VEGF-A transcription in response to hypoxic or metabolic stress (Arany et al. 2008). PGC1- α /ERR- α also was shown to contribute significantly to the upregulation of VEGF-A, and the induction of capillary growth in response to exercise in mice (Chinsomboon et al. 2009).

Forkhead family Box O1 (FoxO1) is a transcription factor that has been established to exert anti-angiogenic effects through regulating the transcription of downstream targets such as cyclinD1 and p27^{Kip1} (Eijkelenboom and Burgering 2013) as well as eNOS, Sprouty2 (an inhibitor of the MAPK pathway) and THBS1 (Paik et al. 2007; Potente et al. 2005; Roudier et al. 2013b). Overexpression of FoxO1 also leads to a reduction in VEGF-A mRNA (Shikatani et al. 2012). We found that mice deficient in endothelial FoxO proteins exhibited an accelerated capillary growth in response to exercise training (Slopach et al. 2014). Since FoxO proteins can regulate the transcription of multiple genes, including cell cycle regulators and mediators of cell migration, it is probable that FoxO proteins exert their restraining influence through a number of gene targets.

Murine double minute (Mdm2) (or its human counterpart Hdm2) is an E3 ubiquitin ligase with a well-established role as a regulator of the tumour suppressor p53, which it poly-ubiquitinates, thus targeting it for proteasomal degradation. Mdm2 also promotes HIF1 α stabilization, which can in turn facilitate a higher level of VEGF-A transcription (Carroll and Ashcroft 2008). Recent data indicate that Mdm2 is an important regulator of VEGF-A production in skeletal muscle. Mdm2 levels are elevated in muscle of exercise trained mice and humans (Roudier et al. 2012, 2013a). Mice with reduced levels of Mdm2 exhibited lower basal muscle capillarization and a diminished capacity to increase VEGF-A, or to increase capillary number, with exercise (Roudier et al. 2012). These findings illustrate the significant role of Mdm2 in regulating muscle production of VEGF-A. Mdm2 also interacts with FoxO1 in endothelial cells: Mdm2 protein level and phosphorylation status correlates inversely with FoxO1 protein expression, suggesting that Mdm2 is in fact a negative regulator of FoxO1 (Milkiewicz et al. 2011; Roudier et al. 2012).

Integration of Angiogenic Signals in Response to Exercise

To gain a comprehensive understanding of the regulation of exercise-induced angiogenesis, it is crucial to integrate data for the timing and magnitude of changes in the various angiogenic factors and consider the cumulative effects of the modulation of all of these individual factors. Below and in Fig. 2, the data available for alterations in angiogenic and anti-angiogenic factors over the course of exercise training are summarized in 3 distinct stages: 1) a single bout of aerobic exercise; 2) repeated bouts of aerobic exercise; or, 3) sustained (long term) aerobic exercise training. Consideration is given to the cellular/extracellular niche associated with these factors, based on current knowledge.

Stage 1: A single bout of aerobic exercise (Fig. 2A) disrupts homeostasis within the active muscle, simultaneously initiating multiple “stress” signals that are generated by the combined reduction of tissue pO₂, generation of metabolites, elevated capillary shear stress, NO production and tensional stress on myocytes and capillaries. A single bout of exercise induces a significant transient increase in VEGF-A mRNA and protein in muscle (Biro et al. 2003; Breen et al. 1996; Gustafsson et al. 1999; Richardson et al. 1999). VEGF-A production may be driven by altered activity of HIF1 α , as its protein level and DNA binding activity increases with an acute exercise bout (Ameln 2005) or by PGC1 α , which also increases in active human muscle, particularly under flow-restricted conditions (Norrbon et al. 2004). Mdm2 contributes to the acute increase in myocyte VEGF-A (Roudier et al. 2012). An acute bout of exercise also stimulates the exocytosis of VEGF-A from vesicles within the myocytes and the potential release of matrix-associated VEGF-A protein by MMP-9 (Hoier et al. 2013a; Rullman et al. 2007). Together, these alterations are expected to enhance VEGF-A bioavailability, increasing VEGF-A binding to VEGFR2 and the activation of downstream signaling. When exercise ceases, the stimuli return to basal (pre-exercise) levels and, within several hours post-exercise, so do the mRNA and protein levels of VEGF-A. ANGPT2 and Tie2 mRNA also increase in response to an acute exercise bout (Hoier et al. 2012; Lloyd et al. 2003). However, there is no evidence for an increase in ANGPT2 protein with an acute exercise bout (Gustafsson et al. 2007), thus it is unlikely that ANGPT2 plays a major role in the acute exercise response. Increased endothelial

cell proliferation, sprouting or luminal splitting is not detectable after a single exercise bout (Egginton 2009). It is possible that a threshold, in terms of duration and/or magnitude of signal, must be attained in order to accumulate sufficient intracellular changes in protein expression/activation to drive these complex cellular processes.

However, the simultaneous activation of anti-angiogenic signals, which exert opposing intracellular signals, also will repress angiogenic behaviour. In mice, an acute exercise bout has been reported to increase levels of VEGFR1 (Birot et al. 2003), which acts as a competitive antagonist of VEGFR2 signaling. Endostatin levels within muscle homogenates were reported to increase following a single exercise bout (Olenich et al. 2013). THBS1 mRNA and protein levels increase transiently with a single bout of exercise (Hoier et al. 2012; Slopach et al. 2014), driven by increases in FoxO proteins (Slopach et al. 2014).

Stage 2: Adaptations to the acute exercise response (Fig. 2B) begin to be detectable as training continues (i.e. 7 days to 2 weeks). The “basal” level of VEGF-A protein shows a tendency to increase in some studies (Gustafsson et al. 2007; Olenich et al. 2013). This could reflect an accumulation of VEGF-A, for example, if the half-life of VEGF-A protein is long enough that protein levels do not return to basal before the subsequent exercise bout again stimulates new production of VEGF-A. This in turn should extend the duration of VEGFR2 activation on the adjacent capillary endothelial cells, potentially sufficient to trigger the necessary intracellular signals for cell cycle progression and tip cell formation. Interestingly, repeated training appears to reduce HIF1 α mRNA (Lundby et al. 2006), suggesting that it may not play a dominant role in maintaining the continued expression of VEGF-A with repeated training. Increased production of VEGFR2 with exercise training also promotes enhanced VEGF-A dependent signaling (Gustafsson et al. 2007; Lloyd et al. 2003). At the same time, exercise training results in elevated basal levels of ANGPT2 mRNA and protein (Gustafsson et al. 2007), which may facilitate the processes of endothelial cell sprouting. Increased production of MMP-2 and MMP-14, potentially driven by VEGFR2 signaling, is evident after 7-10 days of muscle activity (Haas et al. 2000; Ispanovic and Haas 2006; Rullman et al. 2009). These enzymes will facilitate basement membrane proteolysis and sprout extension into the extracellular matrix.

Interestingly, increased levels of TIMP1 mRNA were detected in muscle after 10 days to 5 weeks of exercise training (Rullman et al. 2007, 2009). Similarly, TIMP1 protein was found to increase post-exercise in exercise trained individuals (Hoier et al. 2012). The role played by TIMP1 in this context is not well established. eNOS mRNA levels also are increased by exercise training in ischemic rat muscles, suggesting that NO production capacity might be increased with training (Lloyd et al. 2003).

While these pro-angiogenic signals accrue, the increase in THBS1 mRNA in response to acute exercise is restrained with training (Slopach et al. 2014). In mice, this correlates closely with a reduction in both FoxO1 and FoxO3a mRNA and protein, which exhibit a lack of increase in response to exercise after 7 days of training (Slopach et al. 2014). Mdm2 may contribute to the restraint of FoxO proteins through inducing their ubiquitination and degradation (Milkiewicz et al. 2011). Thus, the reduced inhibitory influence of FoxO/THBS1 should have the consequence of enhancing endothelial cell responsiveness to VEGF-A and other pro-angiogenic factors. However, the acute exercise response of THBS1 mRNA remained detectable in humans with exercise training (Hoier et al. 2012), thus there may be aspects related to exercise intensity and/or frequency that modulate the effectiveness of FoxO/THBS1 down-regulation.

Stage 3: Attainment of a trained steady-state (Fig. 2C) occurs after 4-8 weeks, when training is repeated without continuous increases in workload. After 4-8 weeks of continual training, many pro-angiogenic (MMPs, HIF α , VEGF-A) and anti-angiogenic (THBS1) factors are no longer different from pre-training levels (Hoier et al. 2012; Lundby et al. 2006; Olenich et al. 2013; Richardson et al. 2000). Basal levels of THBS1 protein were reported to increase after 28 days of training in mice (Olenich et al. 2013); however, no change in basal THBS1 mRNA or protein was detectable in human skeletal muscle after training (Hoier et al. 2012). Alterations in the levels of some factors may facilitate the establishment of a mature and stable capillary network. For example, Tie2 was found to increase following repeated exercise bouts in rats (Lloyd et al. 2003), which may help to stabilize endothelial cell junctions. Microarray analysis of muscle from endurance trained individuals provided evidence for increased mRNA levels of vessel stabilization factors that are known to promote basement membrane deposition and

pericyte coverage (ANGPT1, Tie1, Tie2, TGF β 2 and TGF β R2) (Timmons et al. 2005). Conceptually, this reduced responsiveness is thought to occur because the adaptations in capillary number (together with concomitant adaptations within the skeletal myocytes) that took place during the initial stages of training have contributed in a negative feedback loop to reduce the magnitude of the stimuli that initiated angiogenesis. Alternatively, epigenetic modifications such as DNA methylation have been reported to occur in muscle as a consequence of long term training (Lindholm et al. 2014b), which also could contribute to the shift in expression pattern of angiogenic factors by modulating transcription factor access to the promoter enhancer regions of these genes. Ultimately, the degree to which an individual's capillary network adapts to training is affected by their genetic predisposition to mount an effective adaptive response (Keller et al. 2011). However, the characteristics that define a "responder" require further elucidation.

In summary, the stimulus of exercise initially evokes the production or activation of both pro- and anti-angiogenic factors. At this initial time point, the pro-angiogenic factors are not sufficiently strong or long in duration to enable the initiation of capillary sprouting. However, angiogenic growth factors accumulate with repeated exercise bouts, provoking sustained alterations in intracellular signaling that serve to stimulate endothelial cell proliferation and sprouting as well as to repress the production of anti-angiogenic factors. The precise timing for transition to this state is likely highly dependent on a combination of factors including parameters of the exercise regimen as well as the individual's baseline levels of pro- and anti-angiogenic regulators, as influenced by genetic/epigenetic mechanisms. A new steady state is attained following long term training. If the exercise intensity remains constant, it will no longer provoke the magnitude of intracellular signals required to maintain the angiogenic cascade.

Pathophysiological Influences affecting Skeletal Muscle Capillary Networks

The balance achieved between capillary density and muscle metabolic demand in healthy individuals can be disrupted in various disease states, which can have profound negative effects on muscle performance and whole body metabolic homeostasis. It is relevant to consider both

the underlying influences of these diseases on capillaries within the muscle microenvironment as well as the potential of exercise therapy to enhance capillary number and improve muscle function.

Peripheral Artery Disease (PAD)

PAD is characterized by reduced blood flow (commonly to the lower limb), which causes muscle ischemia, claudication (intermittent leg pain) and reduced exercise tolerance. Ultimately, this condition may deteriorate to the point of critical limb ischemia, which is such an extreme reduction in limb blood flow that it results in tissue necrosis necessitating amputation. Several studies have defined a strong positive correlation between capillary number and indicators of exercise tolerance such as peak oxygen consumption, peak walking time and time until onset of claudication (Askew et al. 2005; Duscha et al. 2011; Robbins et al. 2011). These data indicate that the extent of muscle capillarization is a major factor in determining exercise tolerance, and imply that an increase in capillarization can play a critical role in improving muscle function and, ultimately, the health of PAD patients (see Haas et al. 2012 for a more detailed review).

Although a marked reduction of oxygen tension occurs in the ischemic tissue, which is a key stimulus for induction of hypoxia-responsive genes including VEGF-A, capillary density remains unchanged or even is reduced within the ischemic lower leg muscles of PAD patients (Duscha et al. 2011; Henriksson et al. 1980; Jansson et al. 1988; Robbins et al. 2011). Increased expression of VEGF-A is observed within ischemic muscle, indicating that a lack of VEGF-A is not the underlying problem (Duscha et al. 2011; Couffinhal et al. 1998; Milkiewicz et al. 2006a). The paradoxical lack of angiogenesis in the presence of increased VEGF-A suggests that elevated levels of intrinsic anti-angiogenic signals counter-act the actions of pro-angiogenic molecules. Some studies have observed a reduction in VEGFR2 as well as increased levels of VEGFR1, which may act as a competitive antagonist to VEGF-A/VEGFR2 signaling (Hazarika et al. 2007; Imoukhuede et al. 2013; Milkiewicz et al. 2003). We found that FoxO1 is upregulated within ischemic muscle of PAD patients, correlating with indicators of disease severity. In mice, endothelial FoxO deficiency promoted new capillary growth in ischemic muscle (Roudier et al. 2013b). We and others have detected elevated levels of THBS1 in ischemic muscle, which is not

surprising considering the capacity of FoxO1 to enhance THBS1 expression, (Chu et al. 2015; Favier et al. 2005; Roudier et al. 2013b). Thus, work to date strongly supports the concept that high levels of anti-angiogenic factors within the ischemic muscle impede the capacity for capillaries to respond well to an angiogenic stimulus.

Obesity, Insulin Resistance and Type 2 Diabetes

Animal models have demonstrated that type 2 diabetes results in pronounced microvascular dysfunction at the level of skeletal muscle arterioles, involving increased alpha-adrenergic tone, greater ROS production and NO scavenging, and reduced insulin-dependent production of NO (Barrett et al. 2011; Goodwill et al. 2008; Goodwill and Frisbee 2012; Novielli and Jackson 2014; Xiang et al. 2008), resulting in a pro-inflammatory endothelial phenotype and excessive vasoconstriction. Consequently, the volume of blood delivered to the downstream capillary networks is reduced and also its distribution is more heterogeneous (i.e. a reduced proportion of capillaries receive flow) (Benedict et al. 2011; Butcher et al. 2014; Ellis et al. 2010; Frisbee 2007; Padilla et al. 2006). These alterations impair oxygen delivery even under resting conditions (Ellis et al. 2010), suggesting that these individuals will present with more severe ischemia when coupled with PAD. This is a significant health concern, because diabetic individuals are 2-3 times more likely to develop PAD than non-diabetic individuals (Norgren et al. 2007). Not surprisingly, revascularization interventions also are less successful in diabetic PAD patients, resulting in a greater than 20-fold higher life time risk for major amputation (Jude et al. 2010; Norgren et al. 2007).

While the important link between capillary density, metabolic exchange and insulin sensitivity is recognized, relatively few studies have investigated the impact of obesity on key regulatory mechanisms that control skeletal muscle capillarization. Several studies have reported a lower capillary density in obese compared to healthy subjects (Gavin et al. 2005; Mårin et al. 1994). Notably, rarefaction of microvessels in the skeletal muscle may be more severe when obesity is associated with insulin resistance or diabetes (Pasarica et al. 2009; Solomon et al. 2011).

Diabetes may generate a microenvironment that represses the appropriate angiogenic response to ischemia. Reactive oxygen species, which are elevated within skeletal muscle of

insulin resistant and diabetic animals (Bonnard et al. 2008; Fridlyand and Philipson 2006), appear to exert an anti-angiogenic influence (Ebrahimian et al. 2006). Endothelial cell apoptosis was reported to be elevated within muscle of leptin receptor deficient mice (*db/db*) compared to WT controls and these mice also express higher levels of thrombospondin-1 (Emanueli et al. 2007). However, more research is needed to generate a comprehensive knowledge of the cellular mechanisms that regulate muscle capillarization under diabetic conditions and to understand how these signal pathways will influence the angiogenic responsiveness of skeletal muscle capillaries within ischemic muscle.

Exercise has been shown to stimulate the upregulation of angiogenic factors such as VEGF-A and VEGFR2 in ischemic and diabetic muscle (Kivelä et al. 2008; Lloyd et al. 2003). However, the magnitude of these responses may be smaller than that detected in healthy muscle. From the perspective that angiogenesis is governed by the balance between pro-angiogenic and anti-angiogenic signals, it is likely that increased levels of anti-angiogenic factors within ischemic and/or diabetic microenvironment will impede or delay the initiation of angiogenesis in response to exercise.

Concluding Perspective

Angiogenesis is a long term adaptive process that is important in optimizing the delivery of oxygen and nutrients to meet chronic alterations in the local metabolic needs of skeletal muscle. It is recognized that insufficiencies in angiogenesis contribute to limited improvements in exercise adaptations in some individuals and underpin the skeletal muscle dysfunction in conditions such as peripheral artery disease and diabetes. While significant progress has been made in defining factors that promote capillary growth, it is evident that multiple gaps in knowledge remain related to the integration of angiogenic responses. Research methodologies often exacerbate the challenges in understanding this complex process, as the considerable diversity in exercise regimens and post-exercise collection sample times utilized by researchers limits the consolidation of knowledge across studies. As researchers take advantage of current genomic, proteomic and bioinformatics tools, we will begin to visualize the patterns of gene and protein expression changes that are associated with the angiogenic process, rather than

605 simply describing the regulation of factors individually. This type of approach is necessary to be
606 able to construct a comprehensive framework that delineates the regulatory control of skeletal
607 muscle angiogenesis. Such knowledge then can be applied to identify potential biomarkers that
608 stratify individuals' 'angiogenic capacity', and to optimize exercise modalities that elicit capillary
609 growth within healthy individuals and patient populations.

610
611 ***Acknowledgements:***

612 TLH is the recipient of the 2013 APMN President's Award Lecture (Canadian Society for Exercise
613 Physiology). TLH's research is funded by NSERC, HSF and CIHR. EN received funding from OGS
614 and NSERC PGS-A graduate scholarships.

615
616 ***Conflict of Interest:***

617 Authors have no conflict of interest to report.

References

- Adams, J.C. 1997. Thrombospondin-1. *Int. J. Biochem. Cell Biol.* 29(6): 861–865.
- Amaral, S.L., Linderman, J.R., Morse, M.M., and Greene, A.S. 2001a. Angiogenesis induced by electrical stimulation is mediated by angiotensin II and VEGF. *Microcirculation* 8(1): 57–67.
- Amaral, S.L., Papanek, P.E., and Greene, A.S. 2001b. Angiotensin II and VEGF are involved in angiogenesis induced by short-term exercise training. *Am. J. Physiol. Heart Circ. Physiol.* 281(3): H1163–1169.
- Ameln, H. 2005. Physiological activation of hypoxia inducible factor-1 in human skeletal muscle. *FASEB J.* 19(8):1009-1011. doi: 10.1096/fj.04-2304fje.
- Andersen, P. 1975. Capillary density in skeletal muscle of man. *Acta Physiol. Scand.* 95(2): 203–205. doi: 10.1111/j.1748-1716.1975.tb10043.x.
- Andersen, P., and Henriksson, J. 1977. Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. *J. Physiol.* 270(3): 677–690.
- Andersson, U., Sander, B., Andersson, J., and Möller, G. 1988. Concomitant production of different lymphokines in activated T cells. *Eur. J. Immunol.* 18(12): 2081–2084. doi: 10.1002/eji.1830181232.
- Arany, Z., Foo, S.Y., Ma, Y., Ruas, J.L., Bommi-Reddy, A., Girnun, G., et al. 2008. HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1 α . *Nature* 451(7181): 1008–1012. doi: 10.1038/nature06613.
- Askew, C.D., Green, S., Walker, P.J., Kerr, G.K., Green, A.A., Williams, A.D., et al. 2005. Skeletal muscle phenotype is associated with exercise tolerance in patients with peripheral arterial disease. *J. Vasc. Surg.* 41(5): 802–807. doi: 10.1016/j.jvs.2005.01.037.
- Augustin, H.G., Koh, G.Y., Thurston, G., and Alitalo, K. 2009. Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system. *Nat. Rev. Mol. Cell Biol.* 10(3): 165–177. doi: 10.1038/nrm2639.
- Azizi, P.M., Zyla, R.E., Guan, S., Wang, C., Liu, J., Bolz, S.S., et al. 2015. Clathrin-dependent entry and vesicle-mediated exocytosis define insulin transcytosis across microvascular endothelial cells. *Mol. Biol. Cell* 26(4): 740–750. doi: 10.1091/mbc.E14-08-1307.
- Barrett, E.J., Eggleston, E.M., Inyard, A.C., Wang, H., Li, G., Chai, W., et al. 2009. The vascular actions of insulin control its delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action. *Diabetologia* 52(5): 752–764. doi: 10.1007/s00125-009-1313-z.
- Barrett, E.J., Wang, H., Upchurch, C.T., and Liu, Z. 2011. Insulin regulates its own delivery to skeletal muscle by feed-forward actions on the vasculature. *Am. J. Physiol. Endocrinol. Metab.* 301(2): E252–263. doi: 10.1152/ajpendo.00186.2011.

- 651 Baum, O., Da Silva-Azevedo, L., Willerding, G., Wöckel, A., Planitzer, G., Gossrau, R., et al. 2004.
652 Endothelial NOS is main mediator for shear stress-dependent angiogenesis in skeletal muscle after
653 prazosin administration. *Am. J. Physiol. Heart Circ. Physiol.* 287(5): H2300–2308. doi:
654 10.1152/ajpheart.00065.2004.
- 655 Bellamy, L.M., Johnston, A.P.W., De Lisio, M., and Parise, G. 2010. Skeletal muscle-endothelial cell cross
656 talk through angiotensin II. *Am. J. Physiol. Cell Physiol.* 299(6): C1402–1408. doi:
657 10.1152/ajpcell.00306.2010.
- 658 Benedict, K.F., Coffin, G.S., Barrett, E.J., and Skalak, T.C. 2011. Hemodynamic systems analysis of
659 capillary network remodeling during the progression of type 2 diabetes. *Microcirculation* 18(1): 63–73.
660 doi: 10.1111/j.1549-8719.2010.00069.x.
- 661 Birot, O.J.G., Koulmann, N., Peinnequin, A., and Bigard, X.A. 2003. Exercise-induced expression of
662 vascular endothelial growth factor mRNA in rat skeletal muscle is dependent on fibre type. *J. Physiol.*
663 552(Pt 1): 213–221. doi: 10.1113/jphysiol.2003.043026.
- 664 De Bock, K., Georgiadou, M., and Carmeliet, P. 2013. Role of endothelial cell metabolism in vessel
665 sprouting. *Cell Metab.* 18(5): 634–647. doi: 10.1016/j.cmet.2013.08.001.
- 666 Bonnard, C., Durand, A., Peyrol, S., Chanseane, E., Chauvin, M.A., Morio, B., et al. 2008. Mitochondrial
667 dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. *J.*
668 *Clin. Invest.* 118(2): 789–800. doi: 10.1172/JCI32601.
- 669 Breen, E.C., Johnson, E.C., Wagner, H., Tseng, H.M., Sung, L.A., and Wagner, P.D. 1996. Angiogenic
670 growth factor mRNA responses in muscle to a single bout of exercise. *J. Appl. Physiol.* 81(1): 355–361.
- 671 Brew, K., and Nagase, H. 2010. The tissue inhibitors of metalloproteinases (TIMPs): an ancient family
672 with structural and functional diversity. *Biochim. Biophys. Acta* 1803(1): 55–71. doi:
673 10.1016/j.bbamcr.2010.01.003.
- 674 Brown, M.D., Cotter, M.A., Hudlická, O., and Vrbová, G. 1976. The effects of different patterns of muscle
675 activity on capillary density, mechanical properties and structure of slow and fast rabbit muscles. *Pflüg.*
676 *Arch. Eur. J. Physiol.* 361(3): 241–250.
- 677 Bruns, R.R., and Palade, G.E. 1968. Studies on blood capillaries. I. General organization of blood
678 capillaries in muscle. *J. Cell Biol.* 37(2): 244–276.
- 679 Butcher, J.T., Stanley, S.C., Brooks, S.D., Chantler, P.D., Wu, F., and Frisbee, J.C. 2014. Impact of
680 increased intramuscular perfusion heterogeneity on skeletal muscle microvascular hematocrit in the
681 metabolic syndrome. *Microcirculation.* 21(8): 677–687. doi: 10.1111/micc.12146.
- 682 Carroll, V.A., and Ashcroft, M. 2008. Regulation of angiogenic factors by HDM2 in renal cell carcinoma.
683 *Cancer Res.* 68(2): 545–552. doi: 10.1158/0008-5472.CAN-06-4738.

- 684 Carrow, R.E., Brown, R.E., and Van Huss, W.D. 1967. Fiber sizes and capillary to fiber ratios in skeletal
685 muscle of exercised rats. *Anat. Rec.* 159(1): 33–39. doi: 10.1002/ar.1091590106.
- 686 Chang, H., Shyu, K.G., Wang, B.W., and Kuan, P. 2003. Regulation of hypoxia-inducible factor-1 α by
687 cyclical mechanical stretch in rat vascular smooth muscle cells. *Clin. Sci. Lond. Engl.* 1979 105(4): 447–
688 456. doi: 10.1042/CS20030088.
- 689 Chen, H., Herndon, M.E., and Lawler, J. 2000. The cell biology of thrombospondin-1. *Matrix Biol. J. Int.*
690 *Soc. Matrix Biol.* 19(7): 597–614.
- 691 Cherwek, D.H., Hopkins, M.B., Thompson, M.J., Annex, B.H., and Taylor, D.A. 2000. Fiber type-specific
692 differential expression of angiogenic factors in response to chronic hindlimb ischemia. *Am. J. Physiol.*
693 *Heart Circ. Physiol.* 279(3): H932–938.
- 694 Chinsomboon, J., Ruas, J., Gupta, R.K., Thom, R., Shoag, J., Rowe, G.C., Sawada, N., et al. 2009. The
695 transcriptional coactivator PGC-1 α mediates exercise-induced angiogenesis in skeletal muscle. *Proc.*
696 *Natl. Acad. Sci. U. S. A.* 106(50): 21401–21406. doi: 10.1073/pnas.0909131106.
- 697 Chu, L.H., Vijay, C.G., Annex, B.H., Bader, J.S., and Popel, A.S. 2015. PADPIN: Protein-Protein Interaction
698 Networks of Angiogenesis, Arteriogenesis, and Inflammation in Peripheral Arterial Disease. *Physiol.*
699 *Genomics.* doi: 10.1152/physiolgenomics.00125.2014.
- 700 Clark, E.R. 1918. Studies on the growth of blood-vessels in the tail of the frog larva?by observation and
701 experiment on the living animal. *Am. J. Anat.* 23(1): 37–88. doi: 10.1002/aja.1000230103.
- 702 Clark, E.R., and Clark, E.L. 1940. Microscopic observations on the extra-endothelial cells of living
703 mammalian blood vessels. *Am. J. Anat.* 66(1): 1–49. doi: 10.1002/aja.1000660102.
- 704 Cotter, M., Hudlická, O., Pette, D., Staudte, H., and Vrbová, G. 1973. Changes of capillary density and
705 enzyme pattern in fast rabbit muscles during long-term stimulation. *J. Physiol.* 230(1): 34P–35P.
- 706 Couffignal, T., Silver, M., Zheng, L.P., Kearney, M., Witzenbichler, B., and Isner, J.M. 1998. Mouse model
707 of angiogenesis. *Am. J. Pathol.* 152(6): 1667–1679.
- 708 Dodd, T., Wiggins, L., Hutcheson, R., Smith, E., Musiyenko, A., Hysell, B., et al. 2013. Impaired Coronary
709 Collateral Growth in the Metabolic Syndrome Is in Part Mediated by Matrix Metalloproteinase 12-
710 Dependent Production of Endostatin and Angiostatin. *Arterioscler. Thromb. Vasc. Biol.* 33(6): 1339–
711 1349. doi: 10.1161/ATVBAHA.113.301533.
- 712 Duscha, B.D., Robbins, J.L., Jones, W.S., Kraus, W.E., Lye, R.J., Sanders, J.M., et al. 2011. Angiogenesis in
713 skeletal muscle precede improvements in peak oxygen uptake in peripheral artery disease patients.
714 *Arterioscler. Thromb. Vasc. Biol.* 31(11): 2742–2748. doi: 10.1161/ATVBAHA.111.230441.
- 715 Ebrahimian, T.G., Heymes, C., You, D., Blanc-Brude, O., Mees, B., Waeckel, L., et al. 2006. NADPH
716 oxidase-derived overproduction of reactive oxygen species impairs postischemic neovascularization in
717 mice with type 1 diabetes. *Am. J. Pathol.* 169(2): 719–728. doi: 10.2353/ajpath.2006.060042.

- 718 Egginton, S. 2009. Invited review: activity-induced angiogenesis. *Pflüg. Arch. Eur. J. Physiol.* 457(5): 963–
719 977. doi: 10.1007/s00424-008-0563-9.
- 720 Egginton, S. 2011. Physiological factors influencing capillary growth. *Acta Physiol. Oxf. Engl.* 202(3): 225–
721 239. doi: 10.1111/j.1748-1716.2010.02194.x.
- 722 Egginton, S., and Gaffney, E. 2010. Tissue capillary supply--it's quality not quantity that counts! *Exp.*
723 *Physiol.* 95(10): 971–979. doi: 10.1113/expphysiol.2010.053421.
- 724 Egginton, S., Hudlická, O., Brown, M.D., Walter, H., Weiss, J.B., and Bate, A. 1998. Capillary growth in
725 relation to blood flow and performance in overloaded rat skeletal muscle. *J. Appl. Physiol.* 85(6): 2025–
726 2032.
- 727 Egginton, S., Zhou, A.L., Brown, M.D., and Hudlická, O. 2001. Unorthodox angiogenesis in skeletal
728 muscle. *Cardiovasc. Res.* 49(3): 634–646.
- 729 Eijkelenboom, A., and Burgering, B.M.T. 2013. FOXOs: signalling integrators for homeostasis
730 maintenance. *Nat. Rev. Mol. Cell Biol.* 14(2): 83–97. doi: 10.1038/nrm3507.
- 731 Ellis, C.G., Goldman, D., Hanson, M., Stephenson, A.H., Milkovich, S., Benlamri, A., et al. 2010. Defects in
732 oxygen supply to skeletal muscle of prediabetic ZDF rats. *Am. J. Physiol. Heart Circ. Physiol.* 298(6):
733 H1661–1670. doi: 10.1152/ajpheart.01239.2009.
- 734 Ellis, C.G., Mathieu-Costello, O., Potter, R.F., MacDonald, I.C., and Groom, A.C. 1990. Effect of sarcomere
735 length on total capillary length in skeletal muscle: in vivo evidence for longitudinal stretching of
736 capillaries. *Microvasc. Res.* 40(1): 63–72.
- 737 Emanuelli, C., Caporali, A., Krankel, N., Cristofaro, B., Van Linthout, S., and Madeddu, P. 2007. Type-2
738 diabetic *Lepr(db/db)* mice show a defective microvascular phenotype under basal conditions and an
739 impaired response to angiogenesis gene therapy in the setting of limb ischemia. *Front. Biosci. J. Virtual*
740 *Libr.* 12: 2003–2012.
- 741 Fagiani, E., Christofori, G. 2013. Angiopoietins in angiogenesis. *Cancer Lett.* 328(1): 18–26. doi:
742 10.1016/j.canlet.2012.08.018.
- 743 Favier, J., Germain, S., Emmerich, J., Corvol, P., and Gasc, J.M. 2005. Critical overexpression of
744 thrombospondin 1 in chronic leg ischaemia. *J. Pathol.* 207(3): 358–366. doi: 10.1002/path.1833.
- 745 Faye, C., Moreau, C., Chautard, E., Jetne, R., Fukai, N., Ruggiero, F., et al. 2009. Molecular interplay
746 between endostatin, integrins, and heparan sulfate. *J. Biol. Chem.* 284(33): 22029–22040. doi:
747 10.1074/jbc.M109.002840.
- 748 Ferreras, M., Felbor, U., Lenhard, T., Olsen, B.R., and Delaissé, J. 2000. Generation and degradation of
749 human endostatin proteins by various proteinases. *FEBS Lett.* 486(3): 247–251.

- 750 Fischer, C., Schneider, M., and Carmeliet, P. 2006. Principles and therapeutic implications of
751 angiogenesis, vasculogenesis and arteriogenesis. *Handb. Exp. Pharmacol.* (176 Pt 2): 157–212.
- 752 Folkman, J. 1996. Endogenous inhibitors of angiogenesis. *Harvey Lect.* 92: 65–82.
- 753 Forder, J.P., Munzenmaier, D.H., and Greene, A.S. 2005. Angiogenic protection from focal ischemia with
754 angiotensin II type 1 receptor blockade in the rat. *Am. J. Physiol. Heart Circ. Physiol.* 288(4): H1989–
755 1996. doi: 10.1152/ajpheart.00839.2004.
- 756 Förstermann, U., and Sessa, W.C. 2012. Nitric oxide synthases: regulation and function. *Eur. Heart J.*
757 33(7): 829–837, 837a–837d. doi: 10.1093/eurheartj/ehr304.
- 758 Fridlyand, L.E., and Philipson, L.H. 2006. Reactive species and early manifestation of insulin resistance in
759 type 2 diabetes. *Diabetes Obes. Metab.* 8(2): 136–145. doi: 10.1111/j.1463-1326.2005.00496.x.
- 760 Frisbee, J.C. 2007. Obesity, insulin resistance, and microvessel density. *Microcirculation.* 14(4-5): 289–
761 298. doi: 10.1080/10739680701282945.
- 762 Gavin, T.P., Stallings, H.W., Zwetsloot, K.A., Westerkamp, L.M., Ryan, N.A., Moore, R.A., et al. Lower
763 capillary density but no difference in VEGF expression in obese vs. lean young skeletal muscle in
764 humans. *J. Appl. Physiol.* 98(1): 315–321. doi: 10.1152/jappphysiol.00353.2004.
- 765 Gee, E., Milkiewicz, M., and Haas, T.L. 2010. p38 MAPK activity is stimulated by vascular endothelial
766 growth factor receptor 2 activation and is essential for shear stress-induced angiogenesis. *J. Cell. Physiol.*
767 222(1): 120–126. doi: 10.1002/jcp.21924.
- 768 Geudens, I., and Gerhardt, H. 2011. Coordinating cell behaviour during blood vessel formation.
769 *Development* 138(21): 4569–4583. doi: 10.1242/dev.062323.
- 770 Gimbrone, M.A., Leapman, S.B., Cotran, R.S., and Folkman, J. 1972. Tumor dormancy in vivo by
771 prevention of neovascularization. *J. Exp. Med.* 136(2): 261–276.
- 772 Goodwill, A.G., and Frisbee, J.C. 2012. Oxidant stress and skeletal muscle microvasculopathy in the
773 metabolic syndrome. *Vascul. Pharmacol.* 57(5-6): 150–159. doi: 10.1016/j.vph.2012.07.002.
- 774 Goodwill, A.G., James, M.E., and Frisbee, J.C. 2008. Increased vascular thromboxane generation impairs
775 dilation of skeletal muscle arterioles of obese Zucker rats with reduced oxygen tension. *Am. J. Physiol.*
776 *Heart Circ. Physiol.* 295(4): H1522–1528. doi: 10.1152/ajpheart.00596.2008.
- 777 Gorman, J.L., Liu, S.T.K., Slopach, D., Shariati, K., Hasanee, A., Olenich, S., et al. 2014. Angiotensin II
778 evokes angiogenic signals within skeletal muscle through co-ordinated effects on skeletal myocytes and
779 endothelial cells. *PloS One* 9(1): e85537. doi: 10.1371/journal.pone.0085537.
- 780 Gustafsson, T. 2011. Vascular remodelling in human skeletal muscle. *Biochem. Soc. Trans.* 39(6): 1628–
781 1632. doi: 10.1042/BST20110720.

- 782 Gustafsson, T., Ameln, H., Fischer, H., Sundberg, C.J., Timmons, J.A., and Jansson, E. 2005. VEGF-A splice
783 variants and related receptor expression in human skeletal muscle following submaximal exercise. *J.*
784 *Appl. Physiol.* 98(6): 2137–2146. doi: 10.1152/jappphysiol.01402.2004.
- 785 Gustafsson, T., Puntchart, A., Kaijser, L., Jansson, E., and Sundberg, C.J. 1999. Exercise-induced
786 expression of angiogenesis-related transcription and growth factors in human skeletal muscle. *Am. J.*
787 *Physiol.* 276(2 Pt 2): H679–685.
- 788 Gustafsson, T., Rundqvist, H., Norrbom, J., Rullman, E., Jansson, E., and Sundberg, C.J. 2007. The
789 influence of physical training on the angiopoietin and VEGF-A systems in human skeletal muscle. *J. Appl.*
790 *Physiol.* 103(3): 1012–1020. doi: 10.1152/jappphysiol.01103.2006.
- 791 Haas, T.L., Lloyd, P.G., Yang, H.T., and Terjung, R.L. 2012. Exercise training and peripheral arterial
792 disease. *Compr. Physiol.* 2(4): 2933–3017. doi: 10.1002/cphy.c110065.
- 793 Haas, T.L., and Madri, J.A. 1999. Extracellular matrix-driven matrix metalloproteinase production in
794 endothelial cells: implications for angiogenesis. *Trends Cardiovasc. Med.* 9(3-4): 70–77.
- 795 Haas, T.L., Milkiewicz, M., Davis, S.J., Zhou, A.L., Egginton, S., Brown, M.D., et al. 2000. Matrix
796 metalloproteinase activity is required for activity-induced angiogenesis in rat skeletal muscle. *Am. J.*
797 *Physiol. Heart Circ. Physiol.* 279(4): H1540–H1547.
- 798 Hazarika, S., Dokun, A.O., Li, Y., Popel, A.S., Kontos, C.D., and Annex, B.H. 2007. Impaired angiogenesis
799 after hindlimb ischemia in type 2 diabetes mellitus: differential regulation of vascular endothelial growth
800 factor receptor 1 and soluble vascular endothelial growth factor receptor 1. *Circ. Res.* 101(9): 948–956.
801 doi: 10.1161/CIRCRESAHA.107.160630.
- 802 Hellsten, Y., Rufener, N., Nielsen, J.J., Høier, B., Krstrup, P., and Bangsbo, J. 2008. Passive leg movement
803 enhances interstitial VEGF protein, endothelial cell proliferation, and eNOS mRNA content in human
804 skeletal muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294(3): R975–982. doi:
805 10.1152/ajpregu.00677.2007.
- 806 Henriksson, J., Nygaard, E., Andersson, J., and Eklöf, B. 1980. Enzyme activities, fibre types and
807 capillarization in calf muscles of patients with intermittent claudication. *Scand. J. Clin. Lab. Invest.* 40(4):
808 361–369.
- 809 Hermansen, L., and Wachtlova, M. 1971. Capillary density of skeletal muscle in well-trained and
810 untrained men. *J. Appl. Physiol.* 30(6): 860–863.
- 811 Hoier, B., and Hellsten, Y. 2014. Exercise-induced capillary growth in human skeletal muscle and the
812 dynamics of VEGF. *Microcirculation* 21(4): 301–314. doi: 10.1111/micc.12117.
- 813 Hoier, B., Nordsborg, N., Andersen, S., Jensen, L., Nybo, L., Bangsbo, J., et al. 2012. Pro- and anti-
814 angiogenic factors in human skeletal muscle in response to acute exercise and training. *J. Physiol.* 590(Pt
815 3): 595–606. doi: 10.1113/jphysiol.2011.216135.

- 816 Høier, B., Olsen, K., Nyberg, M., Bangsbo, J., and Hellsten, Y. 2010. Contraction-induced secretion of
817 VEGF from skeletal muscle cells is mediated by adenosine. *Am. J. Physiol. Heart Circ. Physiol.* 299(3):
818 H857–862. doi: 10.1152/ajpheart.00082.2010.
- 819 Hoier, B., Prats, C., Qvortrup, K., Pilegaard, H., Bangsbo, J., and Hellsten, Y. 2013a. Subcellular
820 localization and mechanism of secretion of vascular endothelial growth factor in human skeletal muscle.
821 *FASEB J.* 27(9): 3496–3504. doi: 10.1096/fj.12-224618.
- 822 Hoier, B., Walker, M., Passos, M., Walker, P.J., Green, A., Bangsbo, J., et al. 2013b. Angiogenic response
823 to passive movement and active exercise in individuals with peripheral arterial disease. *J. Appl. Physiol.*
824 115(12): 1777–1787. doi: 10.1152/jappphysiol.00979.2013.
- 825 Holly, R.G., Barnett, J.G., Ashmore, C.R., Taylor, R.G., and Molé, P.A. 1980. Stretch-induced growth in
826 chicken wing muscles: a new model of stretch hypertrophy. *Am. J. Physiol.* 238(1): C62–71.
- 827 Hudlicka, O. 1998. Is physiological angiogenesis in skeletal muscle regulated by changes in
828 microcirculation? *Microcirculation* 1994 5(1): 7–23.
- 829 Hudlická, O., Brown, M.D., and Silgram, H. 2000. Inhibition of capillary growth in chronically stimulated
830 rat muscles by N(G)-nitro-L-arginine, nitric oxide synthase inhibitor. *Microvasc. Res.* 59(1): 45–51. doi:
831 10.1006/mvre.1999.2193.
- 832 Imoukhuede, P.I., Dokun, A.O., Annex, B.H., and Popel, A.S. 2013. Endothelial cell-by-cell profiling
833 reveals the temporal dynamics of VEGFR1 and VEGFR2 membrane localization after murine hindlimb
834 ischemia. *Am. J. Physiol. Heart Circ. Physiol.* 304(8): H1085–1093. doi: 10.1152/ajpheart.00514.2012.
- 835 Iruela-Arispe, M.L., Luque, A., and Lee, N. 2004. Thrombospondin modules and angiogenesis. *Int. J.*
836 *Biochem. Cell Biol.* 36(6): 1070–1078. doi: 10.1016/j.biocel.2004.01.025.
- 837 Ispanovic, E., and Haas, T.L. 2006. JNK and PI3K differentially regulate MMP-2 and MT1-MMP mRNA and
838 protein in response to actin cytoskeleton reorganization in endothelial cells. *Am. J. Physiol. Cell Physiol.*
839 291(4): C579–588. doi: 10.1152/ajpccell.00300.2005.
- 840 James, N.T. 1981. A stereological analysis of capillaries in normal and hypertrophic muscle. *J. Morphol.*
841 168(1): 43–49. doi: 10.1002/jmor.1051680106.
- 842 Jansson, E., Johansson, J., Sylvén, C., and Kaijser, L. 1988. Calf muscle adaptation in intermittent
843 claudication. Side-differences in muscle metabolic characteristics in patients with unilateral arterial
844 disease. *Clin. Physiol. Oxf. Engl.* 8(1): 17–29.
- 845 Jude, E.B., Eleftheriadou, I., and Tentolouris, N. 2010. Peripheral arterial disease in diabetes--a review.
846 *Diabet. Med. J. Br. Diabet. Assoc.* 27(1): 4–14. doi: 10.1111/j.1464-5491.2009.02866.x.
- 847 Keller, P., Volvaard, N.B.J., Gustafsson, T., Gallagher, I.J., Sundberg, C.J., Rankinen, T., et al. A
848 transcriptional map of the impact of endurance exercise training on skeletal muscle phenotype. *J. Appl.*
849 *Physiol.* 110(1): 46–59. doi: 10.1152/jappphysiol.00634.2010.

- 850 Kivelä, R., Silvennoinen, M., Lehti, M., Jalava, S., Vihko, V., and Kainulainen, H. 2008. Exercise-induced
851 expression of angiogenic growth factors in skeletal muscle and in capillaries of healthy and diabetic
852 mice. *Cardiovasc. Diabetol.* 7: 13. doi: 10.1186/1475-2840-7-13.
- 853 Krogh, A. 1919. The number and distribution of capillaries in muscles with calculations of the oxygen
854 pressure head necessary for supplying the tissue. *J. Physiol.* 52(6): 409–415.
- 855 Lee, S., Jilani, S.M., Nikolova, G.V., Carpizo, D., and Iruela-Arispe, M.L. 2005. Processing of VEGF-A by
856 matrix metalloproteinases regulates bioavailability and vascular patterning in tumors. *J. Cell Biol.* 169(4):
857 681–691. doi: 10.1083/jcb.200409115.
- 858 Lillioja, S., Young, A.A., Culter, C.L., Ivy, J.L., Abbott, W.G., Zawadzki, J.K., et al. 1987. Skeletal muscle
859 capillary density and fiber type are possible determinants of in vivo insulin resistance in man. *J. Clin.*
860 *Invest.* 80(2): 415–424. doi: 10.1172/JCI113088.
- 861 Lindholm, M.E., Fischer, H., Poellinger, L., Johnson, R.S., Gustafsson, T., Sundberg, C.J., et al. 2014a.
862 Negative regulation of HIF in skeletal muscle of elite endurance athletes: a tentative mechanism
863 promoting oxidative metabolism. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 307(3): R248–255. doi:
864 10.1152/ajpregu.00036.2013.
- 865 Lindholm, M.E., Marabita, F., Gomez-Cabrero, D., Rundqvist, H., Ekström, T.J., Tegnér, J., et al. 2014b. An
866 integrative analysis reveals coordinated reprogramming of the epigenome and the transcriptome in
867 human skeletal muscle after training. *Epigenetics* 9(12): 1557–1569. doi:
868 10.4161/15592294.2014.982445.
- 869 Lloyd, P.G., Prior, B.M., Yang, H.T., and Terjung, R.L. 2003. Angiogenic growth factor expression in rat
870 skeletal muscle in response to exercise training. *Am. J. Physiol. Heart Circ. Physiol.* 284(5): H1668–1678.
871 doi: 10.1152/ajpheart.00743.2002.
- 872 Lundby, C., Gassmann, M., and Pilegaard, H. 2006. Regular endurance training reduces the exercise
873 induced HIF-1 α and HIF-2 α mRNA expression in human skeletal muscle in normoxic conditions.
874 *Eur. J. Appl. Physiol.* 96(4): 363–369. doi: 10.1007/s00421-005-0085-5.
- 875 Maharaj, A.S.R., Saint-Geniez, M., Maldonado, A.E., and D'Amore, P.A. 2006. Vascular endothelial
876 growth factor localization in the adult. *Am. J. Pathol.* 168(2): 639–648. doi: 10.2353/ajpath.2006.050834.
- 877 Malek, M.H., and Olfert, I.M. 2009. Global deletion of thrombospondin-1 increases cardiac and skeletal
878 muscle capillarity and exercise capacity in mice. *Exp. Physiol.* 94(6): 749–760. doi:
879 10.1113/expphysiol.2008.045989.
- 880 Mandel, E.R., Uchida, C., and Haas, T.L. 2014. Regulation of Proteolysis in Vascular Remodeling. *In* Role
881 of Proteases in Cellular Dysfunction. *Edited by* N.S. Dhalla and S. Chakraborti. Springer New York, New
882 York, NY. pp. 295–319. Available from http://link.springer.com/10.1007/978-1-4614-9099-9_16.

- 883 Mandriota, S.J., and Pepper, M.S. 1998. Regulation of angiopoietin-2 mRNA levels in bovine
884 microvascular endothelial cells by cytokines and hypoxia. *Circ. Res.* 83(8): 852–859.
- 885 Mårin, P., Andersson, B., Krotkiewski, M., and Björntorp, P. 1994. Muscle fiber composition and capillary
886 density in women and men with NIDDM. *Diabetes Care* 17(5): 382–386.
- 887 Mason, S.D., Rundqvist, H., Papandreou, I., Duh, R., McNulty, W.J., Howlett, R.A., et al. 2007. HIF-1alpha
888 in endurance training: suppression of oxidative metabolism. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*
889 293(5): R2059–2069. doi: 10.1152/ajpregu.00335.2007.
- 890 Milkiewicz, M., Doyle, J.L., Fudalewski, T., Ispanovic, E., Aghasi, M., and Haas, T.L. 2007a. HIF-1alpha and
891 HIF-2alpha play a central role in stretch-induced but not shear-stress-induced angiogenesis in rat
892 skeletal muscle. *J. Physiol.* 583(Pt 2): 753–766. doi: 10.1113/jphysiol.2007.136325.
- 893 Milkiewicz, M., Hudlicka, O., Shiner, R., Egginton, S., and Brown, M.D. 2006a. Vascular endothelial
894 growth factor mRNA and protein do not change in parallel during non-inflammatory skeletal muscle
895 ischaemia in rat. *J. Physiol.* 577(Pt 2): 671–678. doi: 10.1113/jphysiol.2006.113357.
- 896 Milkiewicz, M., Hudlicka, O., Verhaeg, J., Egginton, S., and Brown, M.D. 2003. Differential expression of
897 Flk-1 and Flt-1 in rat skeletal muscle in response to chronic ischaemia: favourable effect of muscle
898 activity. *Clin. Sci.* 105(4): 473–482. doi: 10.1042/CS20030035.
- 899 Milkiewicz, M., Kelland, C., Colgan, S., and Haas, T.L. 2006b. Nitric oxide and p38 MAP kinase mediate
900 shear stress-dependent inhibition of MMP-2 production in microvascular endothelial cells. *J. Cell.*
901 *Physiol.* 208(1): 229–237. doi: 10.1002/jcp.20658.
- 902 Milkiewicz, M., Mohammadzadeh, F., Ispanovic, E., Gee, E., and Haas, T.L. 2007b. Static strain stimulates
903 expression of matrix metalloproteinase-2 and VEGF in microvascular endothelium via JNK- and ERK-
904 dependent pathways. *J. Cell. Biochem.* 100(3): 750–761. doi: 10.1002/jcb.21055.
- 905 Milkiewicz, M., Roudier, E., Doyle, J.L., Trifonova, A., Birot, O., and Haas, T.L. 2011. Identification of a
906 mechanism underlying regulation of the anti-angiogenic forkhead transcription factor FoxO1 in cultured
907 endothelial cells and ischemic muscle. *Am. J. Pathol.* 178(2): 935–944. doi:
908 10.1016/j.ajpath.2010.10.042.
- 909 Milkiewicz, M., Uchida, C., Gee, E., Fudalewski, T., and Haas, T.L. 2008. Shear stress-induced Ets-1
910 modulates protease inhibitor expression in microvascular endothelial cells. *J. Cell. Physiol.* 217(2): 502–
911 510. doi: 10.1002/jcp.21526.
- 912 Molé, P.A., Chung, Y., Tran, T.K., Sailasuta, N., Hurd, R., and Jue, T. 1999. Myoglobin desaturation with
913 exercise intensity in human gastrocnemius muscle. *Am. J. Physiol.* 277(1 Pt 2): R173–180.
- 914 Morbidelli, L., Chang, C.H., Douglas, J.G., Granger, H.J., Ledda, F., and Ziche, M. 1996. Nitric oxide
915 mediates mitogenic effect of VEGF on coronary venular endothelium. *Am. J. Physiol.* 270(1 Pt 2): H411–
916 415.

- 917 Morbidelli, L., Donnini, S., and Ziche, M. 2003. Role of nitric oxide in the modulation of angiogenesis.
918 *Curr. Pharm. Des.* 9(7): 521–530.
- 919 Munzenmaier, D.H., and Greene, A.S. 2006. Chronic angiotensin II AT1 receptor blockade increases
920 cerebral cortical microvessel density. *Am. J. Physiol. Heart Circ. Physiol.* 290(2): H512–516. doi:
921 10.1152/ajpheart.01136.2004.
- 922 Murphy-Ullrich, J.E., and Poczatek, M. 2000. Activation of latent TGF-beta by thrombospondin-1:
923 mechanisms and physiology. *Cytokine Growth Factor Rev.* 11(1-2): 59–69.
- 924 Myrhage, R., and Hudlická, O. 1978. Capillary growth in chronically stimulated adult skeletal muscle as
925 studied by intravital microscopy and histological methods in rabbits and rats. *Microvasc. Res.* 16(1): 73–
926 90.
- 927 Neufeld, G., Cohen, T., Gitay-Goren, H., Poltorak, Z., Tessler, S., Sharon, R., et al. 1996. Similarities and
928 differences between the vascular endothelial growth factor (VEGF) splice variants. *Cancer Metastasis*
929 *Rev.* 15(2): 153–158.
- 930 Norgren, L., Hiatt, W.R., Dormandy, J.A., Nehler, M.R., Harris, K.A., Fowkes, F.G.R., et al. 2007. Inter-
931 Society Consensus for the Management of Peripheral Arterial Disease (TASC II). *J. Vasc. Surg.* 45 Suppl S:
932 S5–67. doi: 10.1016/j.jvs.2006.12.037.
- 933 Norrbom, J., Sundberg, C.J., Ameln, H., Kraus, W.E., Jansson, E., and Gustafsson, T. 2004. PGC-1alpha
934 mRNA expression is influenced by metabolic perturbation in exercising human skeletal muscle. *J. Appl.*
935 *Physiol.* 96(1): 189–194. doi: 10.1152/japplphysiol.00765.2003.
- 936 Novielli, N.M., and Jackson, D.N. 2014. Contraction-evoked vasodilation and functional hyperaemia are
937 compromised in branching skeletal muscle arterioles of young pre-diabetic mice. *Acta Physiol. Oxf. Engl.*
938 211(2): 371–384. doi: 10.1111/apha.12297.
- 939 Oh, H., Takagi, H., Suzuma, K., Otani, A., Matsumura, M., and Honda, Y. 1999. Hypoxia and vascular
940 endothelial growth factor selectively up-regulate angiopoietin-2 in bovine microvascular endothelial
941 cells. *J. Biol. Chem.* 274(22): 15732–15739.
- 942 Olenich, S.A., Gutierrez-Reed, N., Audet, G.N., and Olfert, I.M. 2013. Temporal response of positive and
943 negative regulators in response to acute and chronic exercise training in mice. *J. Physiol.* 591(Pt 20):
944 5157–5169. doi: 10.1113/jphysiol.2013.254979.
- 945 Olfert, I.M., and Birot, O. 2011. Importance of Anti-angiogenic Factors in the Regulation of Skeletal
946 Muscle Angiogenesis: Physical Activity and Inhibitors of Angiogenesis. *Microcirculation* 18(4): 316–330.
947 doi: 10.1111/j.1549-8719.2011.00092.x.
- 948 Olfert, I.M., Howlett, R.A., Wagner, P.D., and Breen, E.C. 2010. Myocyte vascular endothelial growth
949 factor is required for exercise-induced skeletal muscle angiogenesis. *Am. J. Physiol. Regul. Integr. Comp.*
950 *Physiol.* 299(4): R1059–1067. doi: 10.1152/ajpregu.00347.2010.

- 951 O'Reilly, M.S., Boehm, T., Shing, Y., Fukai, N., Vasios, G., Lane, W.S., et al. 1997. Endostatin: an
952 endogenous inhibitor of angiogenesis and tumor growth. *Cell* 88(2): 277–285.
- 953 O'Reilly, M.S., Holmgren, L., Shing, Y., Chen, C., Rosenthal, R.A., Moses, M., et al. 1994. Angiostatin: a
954 novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma.
955 *Cell* 79(2): 315–328.
- 956 Otani, A., Takagi, H., Oh, H., Koyama, S., and Honda, Y. 2001. Angiotensin II induces expression of the
957 Tie2 receptor ligand, angiopoietin-2, in bovine retinal endothelial cells. *Diabetes* 50(4): 867–875.
- 958 Padilla, D.J., McDonough, P., Behnke, B.J., Kano, Y., Hageman, K.S., Musch, T.I., et al. 2006. Effects of
959 Type II diabetes on capillary hemodynamics in skeletal muscle. *Am. J. Physiol. Heart Circ. Physiol.* 291(5):
960 H2439–2444. doi: 10.1152/ajpheart.00290.2006.
- 961 Paik, J.H., Kollipara, R., Chu, G., Ji, H., Xiao, Y., Ding, Z., et al. 2007. FoxOs are lineage-restricted
962 redundant tumor suppressors and regulate endothelial cell homeostasis. *Cell* 128(2): 309–323. doi:
963 10.1016/j.cell.2006.12.029.
- 964 Papapetropoulos, A., Desai, K.M., Rudic, R.D., Mayer, B., Zhang, R., Ruiz-Torres, et al. 1997. Nitric oxide
965 synthase inhibitors attenuate transforming-growth-factor-beta 1-stimulated capillary organization in
966 vitro. *Am. J. Pathol.* 150(5): 1835–1844.
- 967 Pasarica, M., Sereda, O.R., Redman, L.M., Albarado, D.C., Hymel, D.T., Roan, L.E., et al. 2009. Reduced
968 Adipose Tissue Oxygenation in Human Obesity Evidence for Rarefaction, Macrophage Chemotaxis, and
969 Inflammation Without an Angiogenic Response. *Diabetes* 58(3): 718–725. doi: 10.2337/db08-1098.
- 970 Patan, S., Haenni, B., and Burri, P.H. 1993. Evidence for intussusceptive capillary growth in the chicken
971 chorio-allantoic membrane (CAM). *Anat. Embryol. (Berl.)* 187(2): 121–130.
- 972 Plein, A., Fantin, A., and Ruhrberg, C. 2014. Neuropilin regulation of angiogenesis, arteriogenesis, and
973 vascular permeability. *Microcirc. N. Y. N* 1994 21(4): 315–323. doi: 10.1111/micc.12124.
- 974 Plyley, M.J. 1990. Quantifying the capillary supply of skeletal muscle. *Can. J. Sport Sci. J. Can. Sci. Sport*
975 15(2): 84–85.
- 976 Plyley, M.J., and Groom, A.C. 1975. Geometrical distribution of capillaries in mammalian striated muscle.
977 *Am. J. Physiol.* 228(5): 1376–1383.
- 978 Potente, M., Urbich, C., Sasaki, K., Hofmann, W.K., Heeschen, C., Aicher, A., et al. 2005. Involvement of
979 Foxo transcription factors in angiogenesis and postnatal neovascularization. *J. Clin. Invest.* 115(9): 2382–
980 2392. doi: 10.1172/JCI23126.
- 981 Pugh, C.W., and Ratcliffe, P.J. 2003. Regulation of angiogenesis by hypoxia: role of the HIF system. *Nat.*
982 *Med.* 9(6): 677–684. doi: 10.1038/nm0603-677.

- 983 Resovi, A., Pinessi, D., Chiorino, G., and Taraboletti, G. 2014. Current understanding of the
984 thrombospondin-1 interactome. *Matrix Biol.* 37: 83–91. doi: 10.1016/j.matbio.2014.01.012.
- 985 Richard, D.E., Berra, E., and Pouyssegur, J. 2000. Nonhypoxic pathway mediates the induction of
986 hypoxia-inducible factor 1 α in vascular smooth muscle cells. *J. Biol. Chem.* 275(35): 26765–26771.
987 doi: 10.1074/jbc.M003325200.
- 988 Richardson, R.S., Noyszewski, E.A., Kendrick, K.F., Leigh, J.S., and Wagner, P.D. 1995. Myoglobin O₂
989 desaturation during exercise. Evidence of limited O₂ transport. *J. Clin. Invest.* 96(4): 1916–1926. doi:
990 10.1172/JCI118237.
- 991 Richardson, R.S., Wagner, H., Mudaliar, S.R., Henry, R., Noyszewski, E.A., and Wagner, P.D. 1999. Human
992 VEGF gene expression in skeletal muscle: effect of acute normoxic and hypoxic exercise. *Am. J. Physiol.*
993 277(6 Pt 2): H2247–2252.
- 994 Richardson, R.S., Wagner, H., Mudaliar, S.R., Saucedo, E., Henry, R., and Wagner, P.D. 2000. Exercise
995 adaptation attenuates VEGF gene expression in human skeletal muscle. *Am. J. Physiol. Heart Circ.*
996 *Physiol.* 279(2): H772–778.
- 997 Ries, C. 2014. Cytokine functions of TIMP-1. *Cell. Mol. Life Sci. CMLS* 71(4): 659–672. doi:
998 10.1007/s00018-013-1457-3.
- 999 Rivilis, I., Milkiewicz, M., Boyd, P., Goldstein, J., Brown, M.D., Egginton, S., et al. 2002. Differential
1000 involvement of MMP-2 and VEGF during muscle stretch- versus shear stress-induced angiogenesis. *Am.*
1001 *J. Physiol. Heart Circ. Physiol.* 283(4): H1430–1438. doi: 10.1152/ajpheart.00082.2002.
- 1002 Robbins, J.L., Jones, W.S., Duscha, B.D., Allen, J.D., Kraus, W.E., Regensteiner, J.G., et al. 2011.
1003 Relationship between leg muscle capillary density and peak hyperemic blood flow with endurance
1004 capacity in peripheral artery disease. *J. Appl. Physiol.* 111(1): 81–86. doi:
1005 10.1152/jappphysiol.00141.2011.
- 1006 Romanul, F.C. 1964. Distribution of capillaries in relation to oxidative metabolism of skeletal muscle
1007 fibres. *Nature* 201: 307–308.
- 1008 Roudier, E., Aiken, J., Slopach, D., Gouzi, F., Mercier, J., Haas, T.L., et al. 2013a. Novel perspective:
1009 exercise training stimulus triggers the expression of the oncoprotein human double minute-2 in human
1010 skeletal muscle. *Physiol. Rep.* 1(2): e00028. doi: 10.1002/phy2.28.
- 1011 Roudier, E., Forn, P., Perry, M.E., and Birot, O. 2012. Murine double minute-2 expression is required for
1012 capillary maintenance and exercise-induced angiogenesis in skeletal muscle. *FASEB J.* 26(11): 4530–
1013 4539. doi: 10.1096/fj.12-212720.
- 1014 Roudier, E., Milkiewicz, M., Birot, O., Slopach, D., Montelius, A., Gustafsson, T., et al. Endothelial FoxO1
1015 is an intrinsic regulator of thrombospondin 1 expression that restrains angiogenesis in ischemic muscle.
1016 *Angiogenesis* 16(4): 759–772. doi: 10.1007/s10456-013-9353-x.

- 1017 Rullman, E., Norrbom, J., Strömberg, A., Wågsäter, D., Rundqvist, H., Haas, T., et al. 2009. Endurance
1018 exercise activates matrix metalloproteinases in human skeletal muscle. *J. Appl. Physiol.* 106(3): 804–812.
1019 doi: 10.1152/jappphysiol.90872.2008.
- 1020 Rullman, E., Rundqvist, H., Wågsäter, D., Fischer, H., Eriksson, P., Sundberg, C.J., et al. 2007. A single
1021 bout of exercise activates matrix metalloproteinase in human skeletal muscle. *J. Appl. Physiol.* 102(6):
1022 2346–2351. doi: 10.1152/jappphysiol.00822.2006.
- 1023 Semenza, G.L. 2014. Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology. *Annu. Rev.*
1024 *Pathol.* 9: 47–71. doi: 10.1146/annurev-pathol-012513-104720.
- 1025 Sessa, W.C. 2009. Molecular control of blood flow and angiogenesis: role of nitric oxide. *J. Thromb.*
1026 *Haemost.* JTH 7 Suppl 1: 35–37. doi: 10.1111/j.1538-7836.2009.03424.x.
- 1027 Al-Shammari, A.A., Gaffney, E.A., and Egginton, S. 2014. Modelling capillary oxygen supply capacity in
1028 mixed muscles: capillary domains revisited. *J. Theor. Biol.* 356: 47–61. doi: 10.1016/j.jtbi.2014.04.016.
- 1029 Sherwood, L.M., Parris, E.E., and Folkman, J. 1971. Tumor Angiogenesis: Therapeutic Implications. *N.*
1030 *Engl. J. Med.* 285(21): 1182–1186. doi: 10.1056/NEJM197111182852108.
- 1031 Shikatani, E.A., Trifonova, A., Mandel, E.R., Liu, S.T.K., Roudier, E., Krylova, A., et al. 2012. Inhibition of
1032 proliferation, migration and proteolysis contribute to corticosterone-mediated inhibition of
1033 angiogenesis. *PloS One* 7(10): e46625. doi: 10.1371/journal.pone.0046625.
- 1034 Silverstein, R.L., Leung, L.L., and Nachman, R.L. 1986. Thrombospondin: a versatile multifunctional
1035 glycoprotein. *Arterioscler. Dallas Tex* 6(3): 245–253.
- 1036 Simionescu, M., Simionescu, N., and Palade, G.E. 1974. Morphometric data on the endothelium of blood
1037 capillaries. *J. Cell Biol.* 60(1): 128–152.
- 1038 Slopack, D., Roudier, E., Liu, S.T.K., Nwadozi, E., Birot, O., and Haas, T.L. 2014. Forkhead BoxO
1039 transcription factors restrain exercise-induced angiogenesis: FoxO proteins delay exercise-driven
1040 angiogenesis. *J. Physiol.* 592(18): 4069–4082. doi: 10.1113/jphysiol.2014.275867.
- 1041 Sodha, N.R. 2008. Increased Antiangiogenic Protein Expression in the Skeletal Muscle of Diabetic Swine
1042 and Patients. *Arch. Surg.* 143(5): 463. doi: 10.1001/archsurg.143.5.463.
- 1043 Solomon, T.P.J., Haus, J.M., Li, Y., and Kirwan, J.P. 2011. Progressive Hyperglycemia across the Glucose
1044 Tolerance Continuum in Older Obese Adults Is Related to Skeletal Muscle Capillarization and Nitric Oxide
1045 Bioavailability. *J. Clin. Endocrinol. Metab.* 96(5): 1377–1384. doi: 10.1210/jc.2010-2069.
- 1046 Stanford, K.I., and Goodyear, L.J. 2014. Exercise and type 2 diabetes: molecular mechanisms regulating
1047 glucose uptake in skeletal muscle. *Adv. Physiol. Educ.* 38(4): 308–314. doi: 10.1152/advan.00080.2014.
- 1048 Stetler-Stevenson, W.G. 2008. Tissue inhibitors of metalloproteinases in cell signaling:
1049 metalloproteinase-independent biological activities. *Sci. Signal.* 1(27): re6. doi: 10.1126/scisignal.127re6.

- 1050 Styp-Rekowska, B., Hlushchuk, R., Pries, A.R., and Djonov, V. 2011. Intussusceptive angiogenesis: pillars
1051 against the blood flow. *Acta Physiol. Oxf. Engl.* 202(3): 213–223. doi: 10.1111/j.1748-1716.2011.02321.x.
- 1052 Suhr, F., Brixius, K., and Bloch, W. 2009. Angiogenic and vascular modulation by extracellular matrix
1053 cleavage products. *Curr. Pharm. Des.* 15(4): 389–410.
- 1054 Thurston, G., and Daly, C. 2012. The complex role of angiopoietin-2 in the angiopoietin-tie signaling
1055 pathway. *Cold Spring Harb. Perspect. Med.* 2(9): a006550. doi: 10.1101/cshperspect.a006650.
- 1056 Timmons, J.A., Jansson, E., Fischer, H., Gustafsson, T., Greenhaff, P.L., Ridden, J., et al. 2005. Modulation
1057 of extracellular matrix genes reflects the magnitude of physiological adaptation to aerobic exercise
1058 training in humans. *BMC Biol.* 3: 19. doi: 10.1186/1741-7007-3-19.
- 1059 Troyanovsky, B., Levchenko, T., Månsson, G., Matvienko, O., and Holmgren, L. 2001. Angiomotin: an
1060 angiostatin binding protein that regulates endothelial cell migration and tube formation. *J. Cell Biol.*
1061 152(6): 1247–1254.
- 1062 Uchida, C., and Haas, T.L. 2014. Endothelial cell TIMP-1 is upregulated by shear stress via Sp-1 and the
1063 TGFβ1 signaling pathways. *Biochem. Cell Biol. Biochim. Biol. Cell.* 92(1): 77–83. doi: 10.1139/bcb-2013-
1064 0086.
- 1065 Uchida, C., Nwadozi, E., Hasane, A., Olenich, S., Olfert, I.M., and Haas, T.L. 2015. Muscle-derived
1066 vascular endothelial growth factor regulates microvascular remodelling in response to increased shear
1067 stress in mice. *Acta Physiol. Oxf. Engl.* 214(3): 349–360. doi: 10.1111/apha.12463.
- 1068 Vempati, P., Popel, A.S., and Mac Gabhann, F. 2014. Extracellular regulation of VEGF: isoforms,
1069 proteolysis, and vascular patterning. *Cytokine Growth Factor Rev.* 25(1): 1–19. doi:
1070 10.1016/j.cytogfr.2013.11.002.
- 1071 Walther, T., Menrad, A., Orzechowski, H.D., Siemeister, G., Paul, M., and Schirner, M. 2003. Differential
1072 regulation of in vivo angiogenesis by angiotensin II receptors. *FASEB J.* 17(14): 2061–2067. doi:
1073 10.1096/fj.03-0129com.
- 1074 Williams, J.L., Cartland, D., Hussain, A., and Egginton, S. 2006. A differential role for nitric oxide in two
1075 forms of physiological angiogenesis in mouse. *J. Physiol.* 570(Pt 3): 445–454. doi:
1076 10.1113/jphysiol.2005.095596.
- 1077 Xiang, L., Dearman, J., Abram, S.R., Carter, C., and Hester, R.L. 2008. Insulin resistance and impaired
1078 functional vasodilation in obese Zucker rats. *Am. J. Physiol. Heart Circ. Physiol.* 294(4): H1658–1666. doi:
1079 10.1152/ajpheart.01206.2007.
- 1080 Yuan, H.T., Yand, S.P., and Woolf, A.S. 2000. Hypoxia up-regulates angiopoietin-2, a Tie-2 ligand, in
1081 mouse mesangial cells. *Kidney Int.* 58(5): 1912–1919. doi:10.1111/j.1523-1755.2000.00363.x

- 1082 Yun, S., Dardik, A., Haga, M., Yamashita, A., Yamaguchi, S., Koh, Y., et al. 2002. Transcription factor Sp1
1083 phosphorylation induced by shear stress inhibits membrane type 1-matrix metalloproteinase expression
1084 in endothelium. *J. Biol. Chem.* 277(38): 34808–34814. doi: 10.1074/jbc.M205417200.
- 1085 Zhou, A., Egginton, S., Hudlická, O., and Brown, M.D. 1998. Internal division of capillaries in rat skeletal
1086 muscle in response to chronic vasodilator treatment with alpha1-antagonist prazosin. *Cell Tissue Res.*
1087 293(2): 293–303.
- 1088
- 1089

Draft

Figure Legends:

Figure 1: Skeletal muscle capillary networks

Skeletal muscle capillaries are oriented predominantly in parallel (arrows) with the long axis of muscle fibers (oriented left to right in this image). Interconnecting lateral branches (asterisks) and capillary loops (arrowheads) ensure thorough perfusion of the adjacent muscle fiber. To obtain this image, a mouse soleus muscle fiber bundle was fixed and stained using *Griffonia simplicifolia*-fluorescein isothiocyanate to detect vascular endothelial cells. The capillary network was visualized on a Zeiss 200M microscope using a 10X objective and captured using a Z series stack, which was subsequently summed and processed by deconvolution (MetaMorph imaging software). The grayscale image was inverted to enhance visualization of the staining. The scale bar represents 50 μm .

Figure 2: Pro- and anti- angiogenic signals in response to aerobic exercise training

Panels illustrate the major stimuli and cell signaling intermediaries associated with A) an acute bout of exercise; B) repeated bouts of exercise; and C) long term training that converge on endothelial cells to promote the induction of angiogenesis or capillary stabilization. Red font indicates activation or upregulation of a stimulus or factor within a time window of 0-4 hours post-exercise; gray font indicates no change or reduction in that factor. Please refer to the main text for details and relevant references. Coll XVIII- collagen XVIII; NO- nitric oxide; PGC1 α - peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; HIF1 α - hypoxia inducible factor 1 alpha; Mdm2- murine double minute 2; MMP-2- matrix metalloproteinase-2; MMP-9 – matrix metalloproteinase-9; MMP-14 – matrix metalloproteinase 14; TIMP-1 – tissue inhibitor of matrix metalloproteinase-1; VEGF-A – vascular endothelial growth factor-A; VEGFR1 –vascular endothelial growth factor receptor 1; VEGFR2-vascular endothelial growth factor receptor 2; ANGPT1 –angiopoietin-1; ANGPT2 –angiopoietin-2; Tie2 –tunica interna endothelial cell kinase 2; FoxO1/3- forkhead box O1/forkhead boxO3; THBS1 –thrombospondin-1.

Fig. 1

Fig. 2