

Chapter 11

Biochemical Mechanisms of Exercise-Induced Angiogenesis

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Abstract Angiogenesis refers to the growth of new capillaries from a pre-existing capillary bed which can occur during normal physiological and pathological conditions by sprouting and non-sprouting processes, which are activated by different stimuli. Various studies have demonstrated that exercise increases the expression of several growth factors for both sprouting and non-sprouting angiogenesis, including vascular endothelial growth factor and other cytokines in skeletal and cardiac muscle, which are associated with an increase in the number of capillaries in the heart and skeletal muscle. Exercise is known to stimulate the release of several pro- and anti-angiogenic proteins and transcription factors and it appears that hypoxia and/or ischemia play a major role in the growth and expansion of new capillaries and has also been suggested that mechanical forces, such as shear stress or muscle overload, stimulate exercise-induced angiogenesis. More importantly, an in-depth understanding of the factors that influence exercise-induced angiogenesis may contribute to the development of potential therapeutic strategies for the treatment of different diseases including hypertension and ischemic heart disease.

Keywords Angiogenesis • Physical activity • Exercise training • Vascular endothelial growth factor • Matrix metalloproteinase • Angiopoietins • Endostatin • Thrombospondin • Tissue inhibitors of matrix metalloproteinases • Endothelial progenitor cells • Chronic disease

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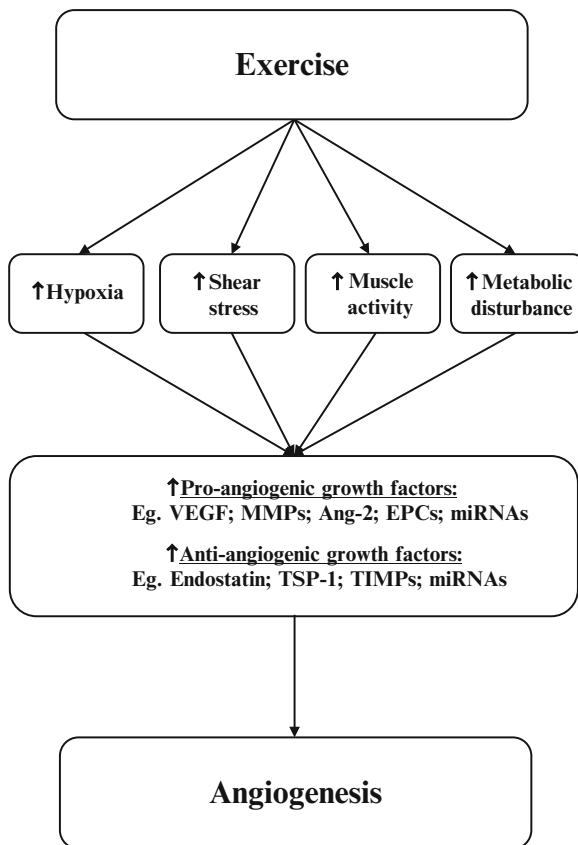
11.1 Introduction

Blood vessels and capillaries play a vital role in supplying oxygen and nutrients to metabolic tissues as well as play a functional role in the endocrine and immune systems [1]. Much of the current understanding of the anatomy and physiology of capillaries stems from the early work of Krogh [2–5], who examined the distribution and number of capillaries in organs and tissues, the structure of the capillary wall, and the exchange of substances through the capillary wall [5]. His work has also elucidated the fact that blood vessels have the capacity to grow (i.e., arteriogenesis) as well as to form new blood vessels from a pre-existing capillary bed, which is known as angiogenesis. The term angiogenesis was first used to describe the formation of new blood vessels during placental growth [6] and was first observed by implanting a transparent chamber into the ear of a rabbit [7]. Angiogenesis is important for normal physiological growth, such as in the female reproductive tract [8], wound healing, and muscle remodeling [9]. Deviations from normal vessel growth and maintenance can lead to several chronic diseases and angiogenesis plays an important role in the healing process after conditions such as stroke [10], myocardial infarction [11], ulcers [12], and neurodegeneration [13]. On the other hand, abnormal blood vessel growth or remodeling is associated with cancer [14], inflammatory disorders [15], pulmonary hypertension [16], and in eye diseases [17].

Physical activity has been shown to be a powerful stimulus of cardiac and skeletal muscle adaptation and physiological remodeling. For example, exercise training plays a major role in the remodeling process to increase capillary density of muscle [18] and mitochondrial enzyme activity after a single bout of exercise [19] as well as after exercise training [20]. These changes may occur because skeletal and cardiac muscle are metabolically active tissues that can increase their metabolic requirements during exercise 30–50-fold compared to basal conditions [21]. Therefore, adequate circulation to supply oxygen and nutrients to these tissues and to remove “metabolic waste” from these tissues is essential for their optimal performance and health. Since its discovery, angiogenesis has been studied extensively and several investigators have attempted to elucidate the mechanisms to explain how exercise stimulates angiogenesis. Hudlicka et al. [22] have provided data on some of the possible stimuli for exercise-induced angiogenesis, including increases in blood flow (hyperemia), shear stress, and muscle stretch. Other suggested stimuli include hypoxia and metabolic disturbance [23] (Fig. 11.1).

Angiogenesis is a highly regulated process and is controlled by several pro- and anti-angiogenic factors which can be turned on when needed and completely shut down [22, 24]. Over 30 years ago, Dvorak et al. [25] discovered a new molecule involved in tumor growth they termed *vascular permeability factor*, which was later renamed as *vascular endothelial growth factor* (VEGF) [26]. VEGF is thought to be the key mediator in the angiogenic process and there is a growing body of evidence indicating that exercise increases the tran-

Fig. 11.1 Simplified pathway of exercise angiogenesis and the suggested mechanisms that exercise affects, including hypoxia, shear stress, muscle activity/stretch, and metabolic disturbance. *VEGF* vascular endothelial growth factor; *MMPs* matrix metalloproteinases; *Ang* angiopoietin; *EPCs* endothelial progenitor cells; *miRNAs* microRNAs; *TSP-1* thrombospondin-1; *TIMPs* tissue inhibitors of matrix metalloproteinases



scription and proteins of VEGF in cardiac and skeletal muscle [27–34]. Two other pro-angiogenic factors that increase with exercise are *matrix metalloproteinases* (MMPs) and *angiopoietins* (Ang-1, Ang-2). The three most investigated anti-angiogenic factors that are upregulated with exercise include endostatin, *thrombospondin-1* (TSP-1), and *tissue inhibitors of matrix metalloproteinases* (TIMPs). Accordingly, evidence for the role of acute and chronic exercise training for upregulating these factors will be reviewed in this article. We will discuss a few other pro- and anti-angiogenic factors to determine if these proteins change in response to acute exercise or chronic exercise training. Since *endothelial progenitor cells* (EPCs) as well as microRNAs (miRNAs) also increase with acute exercise and chronic exercise training, their role in angiogenesis will be reviewed.

11.2 Sprouting and Non-sprouting Angiogenesis Pathways

Animal studies have revealed at least two forms of angiogenesis to occur, at least in skeletal muscle: sprouting angiogenesis and non-sprouting angiogenesis [35]. Irrespective of the cause of angiogenesis, it is assumed that there is a common sequence of events; the activation of endothelial cells followed by the degradation of the basement membrane and the extracellular matrix allowing endothelial cells to migrate to sites where capillaries are needed. Sprouting angiogenesis forms new capillaries via stalk cells and tip cells, whereas non-sprouting angiogenesis occurs when capillaries split into two via intussusception. Non-sprouting angiogenesis is suggested to be a more efficient form of angiogenesis as it permits a more rapid expansion of capillaries and does not require an initial proliferation of endothelial cells [36]. Several reviews have described the details for both sprouting [1, 9, 21, 22, 24, 37–44] and non-sprouting angiogenesis [21, 35, 45–49].

Sprouting angiogenesis is considered to be the major type of angiogenic growth induced by exercise [21] and may be induced by stimuli such as hypoxia, metabolic disturbance, and muscle stretch [35]. It involves the activation of normally quiescent endothelial cells [50], which branch out from an existing capillary and extend through a surrounding matrix to form a cord-like structure. Initiation of sprouting angiogenesis requires the proteolytic degradation of the basement membrane [51], formed by inactive endothelial cells and pericytes. Normally quiescent blood vessels are built with angiogenic sensors, and following the angiogenic signal, pericytes detach from the vessel wall [52] and are removed from the basement membrane [53]. Endothelial cells begin to loosen their junctions from the vessel and the vessel begins to dilate [54]. Permeability of the endothelial cells' layer forces plasma proteins to enter the extracellular matrix and stored angiogenic molecules begin to remodel the extracellular matrix [54]. An endothelial "tip cell" leads the migration of endothelial cells and neighboring stalk cells have filopodia to guide their elongation [55]. In order for the abluminal sprouting capillary to become functional, it must fuse with a neighboring blood vessel and become mature [54]. Endothelial cells return to their inactive state. A myeloid bridge aids the elongation of the stalk as well as the fusion with another blood vessel and allows the initiation of blood flow [54]. Pericytes cover the newly formed capillary and protease inhibitors deposit a basement membrane. Junctions are re-established to ensure optimal blood flow. Regression occurs in the absence of angiogenic stimuli [56, 57].

New capillaries can also form by non-sprouting angiogenesis by intussusception and this was first observed in the rat lung [58]. Opposing capillary walls protrude towards the vessel lumen, and once contact has been established between the capillary walls, the inter-endothelial cell junctions are reorganized and the endothelial bilayer of the capillary becomes centrally perforated [58]. An interstitial pillar-like structure is formed, which is invaded by pericytes and myofibroblasts and these lay down collagen fibrils [59]. The final phase of intussusception is when the pillars increase in girth without any other change in structure [58]. When comparing sprouting vs. non-sprouting angiogenesis, it would appear that non-sprouting angiogenesis is a more efficient way of expanding the capillary network as there is much less endothelial cell proliferation and capillary maturation involved [60].

11.3 Factors That Stimulate Angiogenesis in Response to Exercise

Angiogenesis is a complex, multi-step process and is thought to involve multiple signaling pathways which are coordinated by pro- and anti-angiogenic mediators to generate effective angiogenesis. The following are various pro-angiogenic factors which are considered to be the key mediators in the angiogenic process specifically relevant to exercise-induced angiogenesis.

11.3.1 *Vascular Endothelial Growth Factor in Acute Exercise and Chronic Exercise Training*

VEGF has been shown to play a crucial role in exercise-induced angiogenesis [61–63]. VEGF is a 35–45 kDa peptide growth factor and includes a number of isoforms (VEGF-A to VEGF-D), with the most relevant to exercise-induced angiogenesis being VEGF-A (commonly known as just VEGF). Sources of VEGF include skeletal muscle fibers, endothelial cells, fibroblasts, macrophages, pericytes, as well as mast cells and smooth muscle cells [33, 63]. VEGF plays a major role in endothelial cell stimulation, survival and differentiation, vascular smooth muscle proliferation and migration, and induces capillary permeability and dilation of arterioles [21]. An increase in VEGF transcription is thought to be regulated by several factors. One of these factors includes *hypoxia-inducible factor- α* (HIF-1 α) during hypoxia [64]. VEGF contains a hypoxic-response element [65] and binds to HIF-1 α during hypoxic states. This results in an increase in VEGF transcription when there is reduced oxygen availability and it would seem that HIF-1 α would also regulate VEGF protein during exercise. For example, in response to moderate to heavy exercise (knee extension exercise at 75–100 % maximum work rate), intracellular PO_2 decreases from 30 mm Hg at rest [66] to approximately 3–4 mmHg during exercise in human quadriceps muscle [67]. However, the exercise-induced increase in VEGF is more complex than by activation of HIF-1 α alone. It is pointed out that VEGF expression was still observed in HIF-1 α knockout mice following exercise training [68]. Moreover, there is a reduced VEGF transcription response with restricted blood flow; however, there was still a similar activation in HIF-1 α between the restricted and non-restricted exercising skeletal muscle [69]. Therefore, VEGF must be regulated by some factors other than HIF-1 α .

More recently, metabolic activity has been highlighted as playing a role in VEGF regulation [70]. For example, *AMP-activated protein kinase* (AMPK) has been suggested to play a role in exercise-induced angiogenesis. AMPK is involved in the regulation of the stress response as well as metabolic homeostasis and is associated with increased glucose uptake in muscle [71]. It also leads to the promotion of angiogenesis by increasing the expression of VEGF in skeletal muscle cell culture (i.e., C2C12 cell line) through activation of the AMPK-p38 MAPK-dependant

pathway [72]. Skeletal muscle cell culture has also indicated that adenosine enhances mRNA expression of VEGF and this may be mediated by the A_{2B} receptor [73]. Several studies in animal models have revealed that *peroxisome-proliferator activated receptor γ co-activator-1 α* (PGC-1 α) through the activation of *estrogen-related receptor α* (ERR- α) serves as a principal regulator of increased expression of VEGF through exercise [72–74].

There is a growing body of evidence that VEGF and its receptors VEGFR1, VEGFR2, and *neuropilin 1* (NRP-1), which is also a receptor of VEGF, increase in response to a single bout of exercise [27–34]. Moreover, an acute bout of exercise in untrained subjects releases higher amounts of VEGF mRNA and protein response compared with trained subjects [75]. Several studies have shown that an acute bout of single-leg knee extension exercise lasting for 45 min at approximately 25% maximum workload increases mRNA expression of VEGF by at least twofold and VEGF protein expression ~1.5-fold [27–29]. Splice variants VEGF₁₂₁ and VEGF₁₆₅ increase initially after an acute bout of exercise by 3- and 3.5-fold, respectively, after 45 min of one-leg knee extension exercise at 25% of maximum workload, followed by a 3.5-fold increase in VEGF₁₈₉ [33]. The relative change in expression after exercise may be represented by endothelial activation with VEGF₁₂₁ and VEGF₁₆₅ as they are diffusible [76], whereas VEGF₁₈₉ contains a domain encoded by exon 6 and is bound to cell-surface heparin sulfate proteoglycans which represent a later chemoattractant and differentiation role in the VEGF system [76].

Prolonged moderate intensity exercise training is known to upregulate VEGF mRNA expression and protein content. For example, 60 min of cycling at 50% of maximal aerobic capacity (i.e., VO_{2max}) increases VEGF mRNA expression by approximately 4.5-fold [30], whereas a 60 min bout of cycling at approximately 60% of VO_{2max} increased the concentration of interstitial VEGF by >6-fold compared to resting levels [77]. These data are also supported by Rullman et al. [78] who demonstrated a 1.5-fold increase in VEGF protein content in subjects who exercised for 65 min at ~65 VO_{2max} . The type and intensity of acute endurance exercise may also influence the VEGF response to exercise. In fact, high intensity sprint interval exercise has recently been investigated and appears to be at least as effective as prolonged moderate intensity exercise training for inducing a VEGF response [79]. Specifically, a greater increase in VEGF protein was observed when subjects participated in four 30 s “all out” exercise bouts which were each followed by 5 min of passive rest when compared to subjects who completed 1 h of cycling at 50% of their peak power output. With the high intensity protocol, there was a 90 pg/mL increase in VEGF 4 h after exercise, whereas there was only a 35 pg/mL increase after moderate intensity exercise. Notably, this physiological difference in VEGF content occurred even though the absolute amount of time spent exercising was 97% lower in the high intensity sprint interval group compared to the low intensity group (i.e., 2 min vs. 60 min) [79].

There is also some evidence to suggest that acute resistance exercise induces a VEGF response. In this regard, three sets of ten repetitions at 60–80% of one-repetition maximum (1RM) induce a threefold increase in skeletal muscle VEGF mRNA and 1.5-fold increase in VEGF protein 4 h post-exercise compared to rest [80]. However, this response may be dependent on the type of resistance training

(i.e., isotonic, isometric, or isokinetic). Specifically, three repetitions of either 40% of 1RM or maximal isokinetic knee extension did not increase serum VEGF [81]. High volume resistance training of 3×30 repetitions in combination with a 30% muscle blood flow restriction also induces a VEGF response [82]. Notably, the VEGF response is attenuated 30 min after exercise when muscle blood flow is normal following exercise training (i.e., a 1.5-fold increase), as compared to the same exercise condition when muscle blood flow is restricted (i.e., a 2.5-fold increase). This observation suggests that intermittent hypoxia or ischemia may be a key factor in regulating exercise-induced angiogenesis.

There appears to be an attenuated VEGF response to an acute bout of exercise following chronic exercise training. For example, the arterial and venous plasma levels of VEGF in response to an acute bout of exercise decreased by 12% and 15%, respectively, after seven exercise training sessions over a 10-day period consisting of 45-min of knee extension exercise at 60–70% maximum work rate, as compared to baseline [29]. However, basal VEGF mRNA and protein expression increased twofold after the 7-day training period. The VEGF response after acute exercise appears to be further attenuated after a longer training period. Particularly, 8 weeks of single-leg knee extension exercise three times per week for 60 min at 50% maximum work rate showed an attenuated VEGF response compared to the untrained muscle. This could be explained by the 18% increase in capillary to fiber ratio of the quadriceps and therefore a further angiogenic response may not be required for the given training stimulus [75].

Receptors VEGFR1, VEGFR2, and NRP-1 are known to increase after a single bout of exercise [30, 33, 34, 83], but respond differently after chronic exercise training, which is characterized by a selective increase in NRP-1 and VEGFR2 [34, 83]. Intermittent hypoxia or ischemia may also influence the regulation of VEGF receptors. For example, resting VEGF protein and VEGFR2 protein content increased threefold under restricted blood flow conditions and remained unchanged under the non-restricted blood flow conditions in response to 5 weeks of knee extension exercise training for 45 min per day, four times per week under 20% restricted blood flow on one leg and non-restricted blood flow to the other leg [34]. This is an interesting finding because VEGFR2 is essential for a majority of the angiogenic actions of VEGF [76, 84]. Moreover, subjects who were “high responders” (i.e., an improvement of $+0.71 \pm 0.1$ L/min of O_2 improvement) after 6 weeks of cycling exercise at 75% of their peak VO_2 for 45 min four times per week had an eightfold increase in NRP-1 and a threefold increase in VEGFR-2, while “non-responders” (i.e., $+0.17 \pm 0.1$ L/min of O_2 improvement) had no change [83].

11.3.2 Matrix Metalloproteinases in Acute Exercise and Chronic Exercise Training

It is now well known that MMPs play an important role in the formation, remodeling, and degradation of the extracellular matrix [85]. They are members of the protease family and 25 enzymes of the MMP family have been identified and are

expressed in most tissues [86–88], including skeletal muscle. Specifically, MMP-2 and MMP-9 have been shown to be upregulated in response to exercise [77, 78, 89–93]. They are both collagenases which degrade collagen type IV, which is the most prevalent protein in skeletal muscle basal lamina [89]. It has been suggested that MMP-2 plays a role in the cleavage of the basal lamina and extracellular matrix during angiogenesis, making a path for the migration of endothelial sprouts and the formation of new capillaries [88, 94]. The promoter region of MMP-9 contains a variety of response elements sensitive to a variety of growth factors and cytokines [95] and may also be a regulator of growth factor bioavailability via proteolytic release. There is also evidence indicating that MMP-1, -3, and -14 also increase in response to chronic exercise training [78, 89, 93].

Evidence indicates that the MMP system is activated during an acute bout of exercise as well as following exercise in skeletal muscle [77, 78]. The same exercise protocol used by Hoier et al. [77] discussed in the VEGF section increased MMP-9 mRNA by three and fourfold at 1 and 3 h post-exercise, respectively. However, there were no changes in MMP-2 mRNA. Furthermore, 65 min of cycle ergometer exercise, consisting of 20 min at 50% of VO_{2max} , 40 min at 65% of VO_{2max} , and 5 min at the subject's maximal tolerable work rate elevated vastus lateralis MMP-9 protein content by ~2.5-fold during exercise and MMP-9 mRNA was elevated ~2.5-fold 2 h after the exercise bout [78]. These data indicate that MMP activity occurs before any measurable changes in MMP-9 transcription, suggesting a post-transcriptional mechanism. As observed with VEGF, the intensity of exercise may influence the MMP response to an acute bout of exercise. Moreover, it has been hypothesized that shear stress is increased during body vibration (as reviewed by Mester et al. [96]) and has been hypothesized that the utilization of vibration training in conjunction with hypoxia and cycling exercise will induce an angiogenic response [92]. Specifically, subjects performed 90 min of cycling; 10 min warm up at 50% VO_{2max} followed by ten intervals of 3 min at a workload of 85% VO_{2max} and 5 min recovery at 60% VO_{2max} . This protocol was carried under four different conditions: normal conditions without vibration; normal conditions with vibration; hypoxic conditions without vibration; and hypoxic conditions with vibration. With the exception of normal conditions with vibration, circulating MMP-2 was increased by approximately 10% immediately after an acute bout of exercise. On the other hand, circulating MMP-9 was shown to increase by approximately twofold in all training conditions 4 h post-exercise, therefore indicating a different activating mechanism for these proteins.

MMP-2 and MMP-9 mRNA and protein levels are known to be regulated by chronic exercise training. For example, 10 days of single-leg exercise training (which consisted of 45 min at the subjects' maximum tolerable workload four times per week) increased MMP-2 mRNA and protein content by 3.5- and 5.5-fold, respectively [89]. However, partial leg ischemia (i.e., 20%) did not exert an additional effect above that of exercise training alone [89]. Even so, basal MMP-2 levels remained elevated after 5 weeks of training in both exercise training protocols. Rullman et al. [89] also found a ~3–7-fold increase in MMP-9 in the normal and restricted blood flow conditions, respectively, after an acute bout of exercise, but the

group changes were not different. Moreover, basal MMP-9 mRNA after 10 days of training increased ~5- and ~13-fold after 5 weeks of training in both groups, with no change in protein. Interestingly, basal MMP-14, which activates MMP-2 [94], mRNA, was increased ~5-fold after 10 days of training in both groups and was maintained after 5 weeks.

Chronic exercise training consisting of aerobic and sprint training in conjunction with either calisthenic or resistance training has been shown to increase circulating MMPs [93]. Calisthenic exercises consist of body weight exercises such as push-ups and pull-ups and squats and lunges. Both the calisthenic and resistance exercise groups exercised 5 days per week for 8 weeks. Exercises were performed until exhaustion in the calisthenic group; whereas the resistance exercise group performed three sets of eight repetitions at 80% of the subject's 1RM followed by 30 s of rest between sets. Immediately after an acute resistance exercise test, MMP-1 concentration increased ~1.2-fold and MMP-2 remained unchanged in both groups compared to baseline. Interestingly, MMP-3 concentration decreased ~1.5-fold and MMP-9 concentration increased ~4-fold in response to an acute resistance exercise test after exercise training in the calisthenic exercise group, but no changes were observed in the resistance training group. However, the resistance training group increased basal MMP-1 ~1.2-fold and MMP-2 increased twofold after chronic exercise training, whereas the calisthenic exercise-trained group demonstrated a ~1.4-fold decrease in basal MMP-2, a 1.4-fold decrease in basal MMP-3, and a 1.3-fold increase in MMP-9. These data suggest that the mode of exercise elicits a specific training adaptation of MMPs following chronic exercise training.

11.3.3 Angiotensins in Acute Exercise and Chronic Exercise Training

The angiotensin family (Ang-1 and Ang-2) has been suggested to play a role in the angiogenic process. Angiotensins are vascular endothelial-specific factors that are activated via RTK pathways and modulate vessel development and remodeling [97]. Ang-1 is expressed in many tissues including the myocardium and perivascular cells and plays a role in the maturation of new blood vessels and vascular stabilization [97]. Ang-2 promotes vascular remodeling by either facilitating growth factor actions or vascular regression when no growth signal is present [98] and is thought to lead to vessel degradation in the absence of VEGF. Ang-1 and Ang-2 compete for the tyrosine kinase receptor Tie2, where Ang-1 acts as an agonist for Tie2 [99] and Ang-2 acts as an antagonist [98]. An increase in the Ang-2/Ang-1 ratio is suggested to have a permissive effect on angiogenesis [100].

Evidence indicates that an acute aerobic exercise bout induces a higher Ang-2/Ang-1 ratio in skeletal muscle [77]. The protocol utilized by Hoier et al. [77] found that Ang-2 mRNA was increased ~2-fold 3 h post-exercise while Ang-1 remained unchanged. As a result, the Ang-2/Ang-1 ratio was increased by ~3.5-fold. Moreover, Tie-2 mRNA was increased ~2-fold 3 h post-exercise. However, these

data contradict previous findings by Gustafsson et al. [34], who reported no changes in Ang-1 or Ang-2 mRNA following exercise. These different observations could be explained by differences in exercise mode and intensity utilized in each of the two studies (i.e., cycling at 60% VO_{2max} utilized by Hoier et al. [77] vs. one-legged knee extension at the highest tolerable workload utilized by Gustafsson et al. [34]).

Endurance-trained athletes may have a different angiopoietin response to an acute bout of exercise. Moreover, the response to a lower-intensity, longer duration (e.g., marathon running) vs. a high-intensity, short bout (e.g., 1,500 m sprint) may induce different Ang-1 and Ang-2 responses. Specifically, circulating Ang-1 increased ~2.2-fold immediately after a marathon and remained unchanged after a 1,500 m field test [101]. However, immediately after the marathon and 1,500 m field test, Ang-2 increased 2- and 1.3-fold, respectively. Even so, the Ang-2/Ang-1 ratio remained unchanged after both events. Receptor Tie2 expression may also be altered by resistance exercise. The protocol utilized by Gavin et al. [80] found that vastus lateralis Tie2 mRNA increased twofold 4 h after the acute resistance exercise test. However, Ang-1, Ang-2, and the Ang-2/Ang-1 ratio remained unchanged.

Chronic exercise training appears to alter the response of Ang-1 and Ang-2 to an acute exercise bout and elicits a change in resting levels. Forty-five minutes of cycling four times per week for 6 weeks at 75% VO_{2max} increased vastus lateralis Ang-1 mRNA approximately 2.5-fold in subjects identified as “high responders” by Timmons et al. [83], while “low responders” had no change. In contrast, Ang-2 remained unchanged in both groups and therefore indicating a reversal in the Ang-2/Ang-1 ratio after an acute exercise bout prior to exercise training. However, Hoier et al. [77] demonstrated that Ang-2 mRNA after 4 weeks of aerobic training increased ~1.8-fold 3 h after an acute bout of exercise compared to rest. These data suggest that angiopoietins Ang-1 and Ang-2 tend to change in a time-dependent manner in response to an acute bout of exercise after chronic exercise training. It should also be pointed out that single-leg knee extension under non-restricted and restricted blood flow conditions for 45 min four times per week for 5 weeks did not alter vastus lateralis Ang-1, Ang-2 mRNA, or the Ang-2/Ang-1 ratio, while basal vastus lateralis Ang-1 mRNA was reduced 10 days after training and returned to baseline after 5 weeks [34]. Moreover, basal Ang-2 mRNA and protein increased ~1.5-fold after 10 days of training. Although Ang-2 mRNA remained elevated, Ang-2 protein returned to baseline levels after 5 weeks of training. Even so, basal Ang-2/Ang-1 ratio was elevated ~1.5-fold after 10 days of training and remained elevated after 5 weeks of training.

11.3.4 Other Factors That Stimulate Angiogenesis

Endothelial cells and mural cells produce and activate *transforming growth factor-β* (TGF-β) which plays a critical role in the maturation of blood vessels by covering the vessel with mural cells (see review by Jain [40]). Mice lacking the TGF-β gene die from defects of the vasculature in utero, suggesting an important

role in angiogenesis prior to birth [102]. One hour of one-leg kicking exercise at 67% maximum workload increased TGF- β in the connective tissue of the vastus lateralis approximately twofold [103]. A maximal graded exercise test has also shown to increase serum TGF- β 2.7-fold immediately after exercise and 1.9-fold 2 h after exercise compared to baseline [104]. To our knowledge, TGF- β in response to chronic exercise training has not been investigated. Although *basic fibroblast growth factor* (bFGF or FGF-2), a 18–23 kDa cationic mitogen, has been shown to upregulate VEGF [105] as well as NO production [106], some studies have failed to demonstrate its upregulation in response to acute or chronic exercise training in humans [32, 79, 107].

11.4 Factors That Inhibit Angiogenesis in Response to Exercise

In addition to several pro-angiogenic molecules, angiogenesis is also regulated by several key anti-angiogenic molecules; however, their response to acute exercise and chronic exercise has been investigated in less depth than the pro-angiogenic factors. Some of these anti-angiogenic factors are described below.

11.4.1 Endostatin in Acute and Chronic Exercise Training

Endostatin, a 20-kDa fragment released from collagen XVIII C-terminal fragment, blocks angiogenesis by preventing the release of VEGF and VEGF receptor signaling [108, 109] as well as MMP-2 activity [110]. It can thus be seen to restrict endothelial cell proliferation and migration. Endostatin also plays a role in relaxation of the vessel walls through *endothelial nitric oxide synthase* (eNOS) phosphorylation and prostacyclin release [111, 112]. A 65 min bout of cycling at 65% VO_{2max} utilized by Rullman et al. [78] increased plasma arterial endostatin ~1.7-fold 17 min into the exercise protocol, which was higher than plasma venous endostatin, which increased ~1.6-fold but then returned to resting values at 57 min into exercise and remained at basal levels 120 min post-exercise. Interestingly, plasma venous endostatin continued to increase at 57 min by ~2-fold and was significantly higher compared to plasma arterial endostatin, suggesting an uptake from the circulation into the muscle. Cycling under hypoxic conditions in conjunction with vibration training, utilized by Suhr et al. [92], increased circulating endostatin by 1.1–1.25-fold immediately after exercise, with the greatest increase found under normal conditions and the lowest increase under hypoxic conditions with vibration. Circulating endostatin remained elevated 30 and 60 min post-exercise only in the normal conditions protocol. A maximal exercise test may induce a greater endostatin response. Specifically, plasma endostatin concentrations increased at 30, 120, and 360 min after exercise by 43%, 73%, and 33%, respectively, compared to rest [107]. These data indicate that endostatin increases to a greater extent following heavy intensity exercise, as compared to moderate intensity exercise. On the other hand, chronic

exercise training may reduce resting plasma endostatin, which would thereby promote angiogenesis. Participants who either ran for 60 min or cycled for 90 min three times per week at moderate intensity for 6 months reduced basal circulating endostatin levels by 14% in both groups [113]. Furthermore, participants in both exercise groups significantly reduced basal endostatin levels when compared to a sedentary control group. Nonetheless, further research is needed to more clearly demonstrate the influence of chronic exercise training on the regulation of endostatin mRNA and protein content in humans.

11.4.2 Thrombospondin-1 in Acute and Chronic Exercise Training

Another endogenous inhibitor of angiogenesis is thrombospondin-1 (TSP-1), which is a large 450 kDa matrix glycoprotein produced by a number of cells, including platelet α -granules, smooth muscle cells, endothelial cells, fibroblasts, neutrophils, and macrophages and located in the extracellular compartment of several organs and tissues, including skeletal muscle, bone, skin, lung, and connective tissue [114, 115]. Its primary anti-angiogenic role is to inhibit cell migration and to induce apoptosis in endothelial cells [115–117]. Few data exist on the response of TSP-1 to acute exercise in humans. A 60 min bout of treadmill running at 20 m/min at 10° incline increased rat gastrocnemius TSP-1 mRNA 3.5-fold immediately after exercise and was further increased 6.2-fold 1 h after exercise, compared to resting levels [118]. More recently, 60 min of cycling at 60% VO_{2max} was shown to increase human vastus lateralis TSP-1 mRNA ~2.75-fold at 1 h after exercise and remained elevated for up to 3 h after exercise [77]. Exercise-trained rats, under both normoxic and hypoxic conditions for 60 min a day, 5 days per week for 8 weeks at 18 m/min and 10° incline, were shown to upregulate gastrocnemius TSP-1 mRNA 3.4 and 3.3-fold, respectively [118]. However, this response was not altered by hypoxia. Humans who completed 4 weeks of cycling for 60 min at 60% VO_{2max} also showed a ~2.5-fold increase in TSP-1 mRNA in the vastus lateralis when sampled 1 h after an acute bout of exercise; however, this acute effect was not different from the pre-training response, suggesting that TSP-1 may not be affected by chronic exercise training [77].

11.4.3 Tissue Inhibitors of Matrix Metalloproteinases in Acute Exercise and Chronic Exercise Training

TIMPs are polypeptides containing 184–194 amino acids and are shown to regulate MMP activity by suppressing extracellular matrix turnover and activation of proteins on the cell surface [119]. TIMPs also have other anti-angiogenic properties without inhibiting MMPs through TIMP-2 integrin-mediated binding to endothelial cells (see review by Stetler-Stevenson et al. [120]). Human studies indicate that TIMPs are not upregulated in the circulation [121, 122] or skeletal muscle [77, 78, 89]

in response to acute exercise. This is in contrast to the evidence from an animal study indicating that rat soleus TIMP-1 and -2 mRNA increased twofold 6 h after a bout of eccentric exercise consisting of 17 m/min for 130 min at 13.5° decline, which was sustained for 2 days after running and soleus TIMP-1 protein increased 1.5-fold [123]. The lack of research examining the influence of exercise on the regulation of TIMPs suggests that this area may be investigated more fully in the future. Chronic exercise training appears to increase TIMP concentration in human skeletal muscle. Rullman et al. [89] found that 10 days of single-leg knee extension training increased vastus lateralis TIMP-1 mRNA ~4- and 6-fold 24 h after an acute bout of exercise in the non-restricted and restricted blood flow conditions, respectively, compared to pre-training levels. Moreover, Hoier et al. [77] found that 4 weeks of cycle ergometer training increased vastus lateralis TIMP-1 mRNA ~2-fold 1 and 3 h after an acute bout of exercise. These data indicate that chronic exercise training increases the expression of TIMP-1 mRNA after an acute bout of exercise compared to pre-training states, suggesting that the inhibition of extracellular remodeling is greater after training when there is sufficient capillary growth, which was demonstrated by Hoier et al. [77].

11.4.4 Other Factors That Inhibit Angiogenesis

Angiostatin was the first discovered anti-angiogenic factor found to block tumor neovascularization and growth of metastases [124]. Angiostatin is a 38-kDa proteolytic fragment derived from degradation of MMP. It is plausible that angiostatin is upregulated by exercise because MMP is known to increase in response to acute exercise and chronic exercise training, but this possibility remains to be investigated. Another factor, namely *vasohibin-1* (VASH-1), is secreted by endothelial cells and contributes to vessel stabilization and maturation [125] and is more highly expressed in less vascularized muscle compared to more vascularized muscle [126]. Rats that completed a maximal running treadmill test that lasted 60–90 min increased VASH-1 by ~1.5-fold immediately after exercise in the hindlimb but returned to baseline 4 h after exercise [126]. In contrast, myocardial VASH-1 did not increase immediately after exercise but increased 4 h after the acute exercise bout. The influence that chronic exercise training has on VASH-1 remains to be fully elucidated because this study indicated that 5 days of training for 60 min at 25–30 m/min at 12° incline did not increase VASH-1 levels in rat hindlimb or heart, as compared to baseline.

11.5 Endothelial Progenitor Cells in Acute Exercise and Chronic Exercise Training

Bone marrow contains EPCs which have the ability to multiply and differentiate into endothelial cells (see review by Ribatti [127]) and were first discovered in adult peripheral blood [128]. EPCs have also been shown to reside in interstitial tissue

surrounding distressed tissues and organs in need of vascular regeneration where the EPCs produce a variety of pro-angiogenic cytokines and growth factors, including VEGF, *hepatic growth factor* (HGF), Ang-1, *stroma-derived growth factor* (SDF)-1 α , *insulin-like growth factor* (IGF)-1, and eNOS [129]. They have also been suggested to play an important role in the regulation of angiogenesis through the differentiation and maturation to endothelial cells and by coordination of paracrine signaling (see review by Carmeliet and Jain [37]). Moreover, endothelial progenitors may play a role in exercise-induced angiogenesis; however, the mechanism of their migration from bone marrow is not fully understood.

Several markers of EPCs have been identified to increase after an acute bout of exercise including CD34⁺VEGFR-2⁺ cells, CD34⁺CD133⁺ cells, CD133⁺VE-cadherin⁺ cells, and monocyte/macrophage-derived angiogenic cells (CACs) in healthy subjects [101, 130–132] and appear to be associated with intensity of exercise. Specifically, immediately after a marathon run circulating CD34⁺VEGFR-2⁺, CD133⁺VE-cadherin⁺, and CAC cell count was found to increase ~1.8-, 3.5-, and 4-fold, respectively, compared to rest, and returned to baseline 20 h after the race [101]. After completion of a 1,500 m field test, CD34⁺VEGFR-2⁺, CD133⁺VE-cadherin⁺, and CAC increased ~2.25-, 5-, and 4-fold, respectively, compared to rest. Moreover, group differences were observed, where CD34⁺VEGFR-2⁺ was ~1.8-fold higher in the 1,500 m group compared to the marathon group and CAC cell count was ~2.3-fold higher in the marathon group compared to the 1,500 m group [101]. After a maximal graded exercise test on a cycle ergometer, CD34⁺VEGFR-2⁺ increased by ~1.7-fold, compared to rest [131]. Thirty minutes of running at anaerobic threshold and 80% of anaerobic threshold increased circulating endothelial progenitors by 235% and 263%, respectively [133]. However, no changes in circulating EPCs were observed after 10 min of running at 80% of anaerobic threshold. Therefore, these data suggest that EPCs may be mobilized differently from the bone marrow in response to different intensities of exercise. Moreover, duration of exercise may play an important role in the release of endothelial progenitors from the bone marrow. To our knowledge, there is no data that exists to determine if chronic exercise training increases circulating EPCs in healthy subjects. However, several studies have demonstrated that endothelial progenitors increase after chronic exercise training in patients with cardiovascular disease [132, 134–136].

11.6 miRNAs in Acute Exercise and Chronic Exercise Training

miRNAs are highly conserved 20–30 nucleotide RNAs that regulate the translation of proteins and enhance messenger RNA (mRNA) degradation (see review by Bartel [137]). miRNAs are formed by two major enzymes; Dicer and Drosha. Drosha regulates nuclear processing of the primary miRNAs into precursors, or pre miRNAs [138], and Dicer cleaves pre-miRNA into the mature miRNA [139]. A number of miRNAs have been identified within cardiac and skeletal muscle which may be involved in

cardiogenesis during embryonic development [140] and skeletal muscle myogenesis [141] and remodeling [142]. Several miRNAs including miR-20a [143], miR-210 [144], miR-221/miR-222 [145], and miR-328 [146] have also been identified which have anti-angiogenic effects. Specifically, miR-20a expression is increased by VEGF but has an inhibitory effect on endothelial cell migration and overall capillary formation [147]. Increased miR-328 expression is found to reduce cell adhesion, aggregation, and migration and also regulates the formation of the capillary structure [146]. miR-210 upregulation under normoxic conditions increases endothelial cell tubulogenesis and migration, but under hypoxic conditions, miR-210 decreases capillary formation [144]. It has also been shown that supplementation of miR-210 to the myocardium to the pre-infarct zone induces angiogenesis in a mouse model [148]. miR-221/miR-222 mediate the angiogenic activity of *stem-cell factor* (SCF) [145]. These miRNAs have only more recently been investigated in response to an acute bout of exercise and chronic exercise training. The response of miRNAs to an acute bout of exercise has been investigated in competitive rowers [149]. Specifically, it has been shown that circulating miR-221 and miR-222 are increased ~3.6- and 2.5-fold immediately after a maximal, incremental cycling exercise test, respectively. Further investigations may be warranted to determine if acute exercise has similar effects on miRNA regulation in non-athlete populations.

Baggish et al. [149] investigated the effects of chronic exercise training in rowing athletes. After 90 days of training for an average 13.1 h per week, miR-221 and miR-222 increased ~5.8- and 2.4-fold compared to baseline resting levels, respectively. After the chronic exercise training period, miR-20a was 3.1-fold higher after an acute bout of exercise compared to baseline resting levels. An additional increase in circulating miR-222 was found (fourfold increase) after an acute bout of exercise after training but not in miR-221. These data indicate a different pattern in regulation between several miRNAs before and after chronic exercise training and further investigations are needed among different populations.

11.7 Exercise-Induced Angiogenesis in Chronic Disease

From the foregoing discussion, it is evident that chronic exercise training in healthy individuals may promote angiogenesis by affecting the expression of several angiogenic factors (Table 11.1). The role of chronic exercise training in the primary and secondary prevention of different diseases has also been investigated extensively. Interestingly, regular exercise may reduce the risk of developing over 25 chronic conditions [150] including coronary heart disease, stroke, hypertension, breast and colon cancer, type 2 diabetes, and osteoporosis. It is likely that research examining the biological mechanisms that regulate exercise-induced angiogenesis may guide the identification of novel therapeutic targets for the treatment of chronic diseases. For example, patients with cardiovascular disease who exercise train to a level

Table 11.1 Angiogenic factors that increase with chronic exercise training

Authors	Training conditions		Exercise protocol				Type	Factors
	Special conditions	Frequency	Intensity	Time				
Gustafsson et al. [34]	Normal	4×/week; 5 weeks	Highest tolerable WL	45 min	Single-leg knee extension	↑Ang-2; ↑Tie-1		
Richardson et al. [75]	Restricted blood	3×/week; 8 weeks	50% max WR	60 min	Single-leg knee extension	↑VEGF; ↑VEGFR-1; ↑VEGF		
Hoier et al. [77]	Normal	3×/week; 4 weeks	68% VO_{2max}	60 min	Cycle ergometer	↑MMP-9; ↑TIMP-1; ↑Ang-2; ↑Tie-2		
Timmons et al. [83]	Normal	4×/week; 6 weeks	75% VO_{2max}	45 min	Cycle ergometer	High responders: ↑VEGFR-1; ↑VEGFR-2; ↑NRP-1 ↑Tie-1		
Rullman et al. [89]	Normal	4×/week; 5 weeks	Highest tolerable WL	45 min	Single-leg knee extension	Low responders: none		
	Restricted blood flow					↑MMP-2; ↑MMP-9; ↑MMP-14; ↑TIMP-1		
Urso et al. [93]	Calisthenics RT	5×/week; 8 weeks	To fatigue 80% 1RM	Not indicated	Calisthenics and AT	↑MMP-2; ↑MMP-9; ↑MMP-14; ↑TIMP-1		
Brixius et al. [113]	Running Cycling	3×/week; 6 month	Pulse 2–4 mmol/L lactate	60 min 90 min	RT and AT Running Cycling	None		
Baggish et al. [149]	Elite rowing athlete training	90 days	Not indicated	5.9 h/week	Rowing	↓Endostatin ↓Endostatin ↑miR-146a; ↑miR-222; ↑miR-21; ↑miR-221; ↑miR-20a		

VO_{2max} maximum oxygen consumption; VEGF vascular endothelial growth factor; VEGFR vascular endothelial growth factor receptor; WL workload; Ang angiotensin; Tie angiotensin receptor; WR work rate; NRP neuropilin; eNOS endothelial nitric oxide synthase; MMP matrix metalloproteinase; TIMP tissue inhibitors of matrix metalloproteinases; RM repetition maximum; miR microRNA

sufficient to stimulate angiogenesis and the formation of collateral vessels in ischemic areas of the heart and skeletal muscle are known to decrease the severity of ischemic events [151–153]. The potential of chronic exercise training to upregulate and normalize circulating pro-angiogenic growth factors and EPCs in patients with cardiovascular disease has been documented previously [132, 135, 154–162]; it also has the potential to improve collateral flow in the heart [163] and increase capillary density in skeletal muscle [160]. Various studies examining the role of chronic exercise training and its ability to upregulate several pro- and anti-angiogenic growth factors as well as EPCs are listed in Table 11.2. Similar health benefits may also be attained by peripheral arterial disease patients, which is a population characterized with ischemia of the limbs that often leads to tissue necrosis requiring amputation [164]. Notably, chronic exercise training consisting of 50 min of walking exercise at an intensity below that which induces claudication symptoms twice a week for 6 months significantly elevated EPCs and VEGF in patients diagnosed with peripheral artery disease [135]. Moreover, exercise-induced angiogenesis may have an important therapeutic role in heart failure. It may be noted that patients with heart failure often experience dyspnea and exercise intolerance, which is not only due to insufficient cardiac output and impaired lung function but also due to peripheral pathophysiological maladaptations such as endothelial dysfunction [165], abnormal skeletal morphology and metabolism [166] and ventilatory control [167]. Chronic exercise training consisting of 55 min of upright cycling three times a week for 8 weeks at an intensity that elevated blood lactate to 2–2.5 mmol/L markedly increased circulating EPCs and pro-angiogenic factors, including VEGF amongst a cohort of heart failure patients. This beneficial effect of exercise training washed out 8 weeks after the heart failure patients stopped exercise training [136].

One goal of cancer therapy is to prevent the blood supply of a tumor using anti-angiogenic drugs [168]. Interestingly, even though chronic exercise training induces a positive angiogenic response in the heart and skeletal muscle, it may have an anti-angiogenic effect on tumors. To our knowledge there are currently no human models for investigating the effects of chronic exercise training and tumor blood vessel supply. Furthermore, the potential mechanisms to explain how exercise training exerts an anti-angiogenic effect on tumors still remain to be elucidated. Nonetheless, the effects of chronic exercise training have been examined using a Dalton's lymphoma mouse model [169]. Data from that study [169] indicate that blood supply to the peritoneal cavity was significantly reduced in mice that exercised 30 min per day three times per week at approximately 80% VO_{2max} [169]. Moreover, mice that exercised 60 min a day had a further decrease in blood supply to the peritoneal cavity compared to the 30 min group. One of the potential mechanisms for the reduced blood supply was that VEGF was significantly reduced in the exercise-trained mice vs. the sedentary controls. However, this may be specific to the type of cancer, as it was found that 44 days of voluntary wheel running did not reduce blood supply or tumor growth in a mouse model of human breast cancer [170]. Further studies should continue to examine the influence that chronic exercise training exerts on tumor growth and angiogenesis.

Table 11.2 Chronic exercise training studies in different disease populations

Disease	References	Exercise protocol				Type	Factors
		Frequency	Intensity	Time	Time		
Coronary artery disease	Laufs et al. [133]	4 weeks	60–80% VO_{2max}	25 min	Cycle ergometer; walking	↑EPC	
	Steiner et al. [134]	5x/week; 12 weeks	Not indicated	30–60 min	Cycle ergometer; running	↑EPC; ↑FMD; ↑ NO_x	
	Lee et al. [161]	3x/week; 12 weeks	55–70% VO_{2max}	30 min	Cycle ergometer	↑MBF; ↓VEGF; ↓SDF-1	
Peripheral arterial disease	Schlager et al. [135]	2x/week; 6 months	At symptoms of claudication	50 min	Walking	↑EPC; ↓ADMA	
	Duscha et al. [160]	3x/week; 12–16 weeks	Not indicated	30–40 min	Walking	↑CD	
Hypertension	Sandri et al. [162]	5 days/week; 4 weeks	At symptoms of claudication	Not indicated	Walking	↑VEGF; ↑EPC	
	Hansen et al. [159]	3x/week; 16 weeks	Not indicated	60 min	Endurance and strength exercise	↑C/F ratio; ↑VEGF	
Heart failure	Sarto et al. [136]	3x/week; 8 weeks	60% HRR	55 min	Cycle ergometer	↑EPC; ↑VEGF/SDF-1 ratio	
	Gustafsson et al. [156]	3x/week; 8 weeks	50% peak work rate	~40 min	Single-leg knee extension	↑Citrate synthase; ↑VEGF	
	Gatta et al. [158]	6x/week; 3 weeks	85% max HR	~30 min	Calisthenics; cycle ergometer	↑EPC; ↓MMP-1; ↓TIMP-1; ↑MMP-2/TIMP-1 ratio; ↑MMP-9/TIMP-1 ratio	

*EPC*s endothelial progenitor cells; *FMD* flow-mediated dilation; *NO_x* nitrites/nitrites; *VEGF* vascular endothelial growth factor; *SDF-1* stroma-derived factor 1; *MBF* myocardial blood flow; *ADMA* asymmetric dimethylarginine; *CD* capillary density; *C/F* capillary-to-fiber; *HRR* heart-rate reserve; *MMP* matrix metalloproteinase; *TIMP-1* tissue inhibitors of matrix metalloproteinases-1

11.8 Conclusions

Angiogenesis is a process regulated by pro- and anti-angiogenic factors and plays an important role for normal growth and physiological function. Several disease states are also characterized by deficient or dysregulated angiogenesis. In general, the literature indicates acute and chronic exercise training stimulates angiogenesis by upregulating pro- and anti-angiogenic factors that ultimately increase capillary density in both heart and skeletal muscle. Chronic exercise training may also have an important therapeutic role for regulating angiogenesis healthy as well as clinical populations.

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