MINI REVIEW

Metabolic regulation of exercise-induced angiogenesis

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Abstract

Skeletal muscle relies on an ingenious network of blood vessels, which ensures optimal oxygen and nutrient supply. An increase in muscle vascularization is an early adaptive event to exercise training, but the cellular and molecular mechanisms underlying exercise-induced blood vessel formation are not completely clear. In this review, we provide a concise overview on how exercise-induced alterations in muscle metabolism can evoke metabolic changes in endothelial cells (ECs) that drive muscle angiogenesis. In skeletal muscle, angiogenesis can occur via sprouting and splitting angiogenesis and is dependent on vascular endothelial growth factor (VEGF) signaling. In the resting muscle, VEGF levels are controlled by the estrogen-related receptor γ (ERR γ). Upon exercise, the transcriptional coactivator peroxisome-proliferator-activated receptor- γ coactivator-1 α (PGC1 α) orchestrates several adaptations to endurance exercise within muscle fibers and simultaneously promotes transcriptional activation of *Vegf* expression and increased muscle capillary density. While ECs are highly glycolytic and change their metabolism during sprouting angiogenesis in development and disease, a similar role for EC metabolism in exercise-induced angiogenesis in skeletal muscle remains to be elucidated. Nonetheless, recent studies have illustrated the importance of endothelial hydrogen sulfide and sirtuin 1 (SIRT1) activity for exercise-induced angiogenesis, suggesting that EC metabolic reprogramming may be fundamental in this process. We hypothesize that the exercise-induced angiogenic response can also be modulated by metabolic crosstalk between muscle and the endothelium. Defining the underlying molecular mechanisms responsible for skeletal muscle angiogenesis in response to exercise will yield valuable insight into metabolic regulation as well as the determinants of exercise performance.

Key Words

- ▶ endothelial metabolism
- ▶ exercise
- ▶ angiogenesis
- ▶ metabolism
- ▶ microvasculature

Introduction

Skeletal muscle is a highly plastic organ which ensures locomotion and is critical for the maintenance of whole body metabolic homeostasis (1, 2). Oxygen, glucose and other nutrients are delivered to the muscle via an ingenious network of blood vessels formed by neatly aligned endothelial cells (ECs). During exercise, the uptake of oxygen and nutrients needs to increase dramatically

(3, 4), and for this reason, blood flow through the vessels surrounding the active muscle fibers increases within seconds (5, 6, 7). In response to repeated exercise bouts, the formation of new capillaries from existing ones, a highly dynamic and tightly controlled process termed angiogenesis, is initiated (8, 9). The increase in muscle vascularization is an early adaptive event to exercise

training, occurring before or simultaneously with the switch in fiber types (10) or the increase in activity of oxidative enzymes in the myofibers (11, 12). Exercise is also the most effective non-surgical therapy to increase muscle angiogenesis and to ameliorate the symptoms of ischemia in peripheral artery disease (13, 14). An increase in muscle capillarity is expected to improve blood–tissue exchange properties by increasing the surface area for oxygen diffusion, nutrient uptake and/or elimination of toxic waste products, and therefore, should determine the metabolic potential of the muscle, a concept that was already recognized by August Krogh in the beginning of the 20th century (15). Nonetheless, despite the intimate link between capillary content and muscle metabolism, the molecular mechanisms underlying muscle angiogenesis in response to exercise are still poorly understood.

Mechanisms of angiogenesis in skeletal muscle

Angiogenesis can occur via distinct mechanisms: vessel sprouting or vessel splitting (also called intussusception or non-sprouting angiogenesis) (16). Vessel sprouting is initiated by the conversion of a quiescent EC into a tip cell, which sends out filopodia and guides a sprout in response to the secretion of pro-angiogenic factors from the hypoxic or metabolically active microenvironment (17). The neighboring cells become stalk cells that proliferate, extend the growing sprout and form a lumen (18). When two sprouts fuse, blood flow reinitiates and the ECs return to quiescence, secrete basement membrane components and form tight junctions (19). In skeletal muscle, only a few electron microscopy studies have observed capillary sprouts following chronic muscle electrostimulation (20, 21, 22, 23) or overload induced by synergist ablation (23, 24) in rodents or upon endurance training in humans (25). Moreover, while the molecular mechanisms that control vessel sprouting during development and certain diseases are widely studied (26, 27, 28), evidence for a conserved program occurring in skeletal muscle in response to exercise training is lacking.

During vessel splitting, capillaries expand via the insertion of pillar-like structures into the vessel lumen (16, 29). A contact zone is formed between opposing capillary walls, followed by the perforation of the contact zone and shaping of a pillar-like structure. Ultimately, expansion of the pillar results in splitting of the primary vessel into two new ones. Vessel splitting may occur in the virtual absence of endothelial proliferation and can allow rapid expansion of a vascular network, which, in contrast to sprouting angiogenesis, does not require the

breakdown of the extracellular matrix (30). Vessel splitting can be induced in the absence of muscle contractions by pharmacologically elevating blood flow (23, 24) or by chronic electrostimulation, in which contractions take place simultaneously to an increase in blood flow (22, 23). This suggests that sprouting and splitting angiogenesis could be complementary or additive phenomena in response to muscle contraction and can be dependent on the mode of contraction. Indeed, differences in the angiogenic response to exercise were reported between exercise programs involving concentric versus eccentric contractions in rats (31). Moreover, isometric and dynamic contractions differentially affect muscle blood flow and could therefore evoke a different angiogenic response (32). The temporal and spatial characteristics of sprouting versus splitting angiogenesis, as well as their relative contribution to the expansion of the vascular network in response to different exercise modes, are not clear and require further investigation.

The growth of functional capillaries following exercise is the result of a tight balance between pro- and anti-angiogenic factors, with the pro-angiogenic vascular endothelial growth factor (VEGF) playing a crucial role. Skeletal muscle VEGF mRNA (33) and protein (34) levels transiently increase after acute exercise bouts and VEGF is essential for exercise-induced angiogenesis (35, 36), though it is not clear whether this is by promoting splitting or sprouting angiogenesis. Even though VEGF is a powerful stimulator of sprouting angiogenesis (17), its overexpression by myoblasts implanted into skeletal muscle increases capillarity essentially via intussusception (29, 37). The VEGF levels in such model, however, are much higher than those observed upon exercise (29). Pharmacological inhibition of VEGF receptor signaling or deletion of *Vegf* in myofibers (35, 36, 38) blunts but does not completely prevent the increase in capillary-to-fiber ratio following exercise training. Of note, since VEGF also promotes contraction-induced hyperemia (39), reduced capillary density following training upon myofiber-specific deletion of *Vegf* could be secondary to reduced blood flow. Moreover, under specific conditions such as muscle damage, other cells within the muscle microenvironment which localize in close proximity to capillaries express pro-angiogenic genes (40), but it remains to be shown whether activation of these cells during exercise can promote angiogenesis.

Exercise-induced changes in metabolism of skeletal muscle cells promote angiogenesis

It is clear that there is an intimate interplay between angiogenesis and metabolism in skeletal muscle.

Indeed, slow-twitch, fatigue-resistant muscles are characterized by high capacity for energy production via oxidative phosphorylation (OXPHOS) and have a dense vascular network, while fast-twitch, fatigue-sensitive muscles display lower OXPHOS, higher capacity for glycolytic energy production and fewer capillaries (11, 41, 42). The estrogen-related receptor γ (ERR γ) is exclusively and abundantly expressed in slow muscle fibers, and activation of ERR γ in fast muscle fibers increases OXPHOS and vascular density, as well as exercise endurance (43). ERR γ controls baseline muscle vascularization by directly upregulating the expression of the *Vegf* and *Fgf1* genes and by activating the energy sensor 5' adenosine monophosphate-activated protein kinase (AMPK) (43), which also promotes *Vegf* transcription (44, 45). Accordingly, mice expressing a dominant negative AMPK in muscle have lower baseline capillarization, but do not show impairments in exercise-induced angiogenesis (45), suggesting that different metabolic regulators control baseline versus exercise-induced vascular density (Fig. 1).

Figure 1

Exercise-induced activation of transcription factors in myofibers stimulates angiogenesis. While ERR γ determines baseline muscle vascularization either via direct binding to the VEGF promoter or via controlling the activity of AMPK, exercise-induced activation of PGC1 α (through recruitment of ERR α to the VEGF promoter), and potentially increased stabilization of HIF1 α , culminates in the increased expression of VEGF and other pro-angiogenic factors. Release of VEGF and other angiogenic factors from the exercising muscle leads to increased muscle vascularization through vessel sprouting or vessel splitting. AMPK, 5' adenosine monophosphate-activated protein kinase; ERR α , estrogen-related receptor α ; ERR γ , estrogen-related receptor γ ; HIF1 α , hypoxia inducible factor 1 α ; PGC1 α , peroxisome-proliferator-activated receptor- γ coactivator-1 α ; VEGF, vascular endothelial growth factor.

The transcriptional coactivator PGC1 α (peroxisome-proliferator-activated receptor- γ coactivator-1 α) is a potent metabolic sensor, which is activated during exercise (46, 47) and orchestrates several adaptations to endurance exercise including mitochondrial biogenesis and increased OXPHOS (48). PGC1 α also controls the expression of *Vegf* and other pro-angiogenic genes by recruiting estrogen-related receptor α (ERR α) to conserved binding sites within their promoters (49, 50). In contrast to *Errr*, loss of *Pgc1a* does not affect baseline muscle capillary density (43, 51), but mice lacking *Pgc1a* or *Erra* in myofibers fail to increase vascular density in response to exercise (50, 52). Even though PGC1 α /ERR α control *Vegf* expression independently of hypoxia signaling via the hypoxia inducible factor (HIF) 1 α (49), hypoxia increases PGC1 α gene expression (49, 53) and the *Pgc1a*-mediated increase in mitochondrial content can indirectly promote cellular hypoxia due to increased oxygen consumption (54). Nonetheless, the role of HIF1 α in regulating muscle angiogenesis is still controversial since muscle-specific loss of *Hif1a* leads to a higher – not lower – capillary-to-fiber ratio (55).

ECs metabolically rewire during angiogenesis

ECs are highly glycolytic and produce 85% of their energy glycolytically (56). However, when they need to sprout into avascular areas, they upregulate glycolysis even further and reducing EC glycolysis by deleting the glycolytic regulators *Pfkfb3* (phosphofructokinase-2/fructose-2,6-bisphosphatase isoform 3) or hexokinase 2 impairs EC migration and proliferation, resulting in impaired angiogenesis during development and under several pathological conditions (56, 57, 58). Beyond ensuring optimal energy provision, glycolytic enzymes (such as the pyruvate kinase muscle isoenzyme PKM2) also prevent cell cycle arrest during angiogenesis (59). ECs do not require mitochondrial ATP production for angiogenesis, but rather use their mitochondria to maintain NAD⁺/NADH balance (60) and as a hub for macromolecule synthesis during proliferation. Indeed, fatty acid oxidation is crucial for the synthesis of nucleotides used in DNA replication, and blocking this process impairs EC proliferation without affecting migration (61). Interestingly, intermediates of lipid synthesis also control proliferation via posttranslational repression of the activity of the mechanistic target of rapamycin complex 1 (mTORC1), which controls cell growth (62). Proliferating ECs also consume high

amounts of glutamine, and glutamine deprivation or deletion of glutaminase 1 decreases vessel sprouting due to decreases in both cell migration and proliferation (63, 64). A detailed overview of the metabolic regulation of EC function during sprouting is provided elsewhere (65, 66), but to the best of our knowledge, there are no studies showing that ECs also metabolically rewire during intussusception.

Do ECs metabolically rewire during exercise-induced muscle angiogenesis?

Only very few studies have addressed the role of skeletal muscle EC metabolism in response to exercise. Exercise-induced angiogenesis requires adequate activation of the NAD⁺-dependent deacetylase sirtuin 1 (SIRT1) nutrient sensor in ECs (67), at least partially because endothelial *Sirt1* is required for VEGF-mediated angiogenesis (67, 68). SIRT1 controls EC function by inhibiting Notch, a potent repressor of sprouting as well as intussusception (69, 70), and via deacetylating and inactivating forkhead box O1 (FOXO1) (69), which ensures endothelial quiescence by limiting EC glycolysis and OXPHOS (71). Activation of SIRT1 by treatment with an NAD⁺ precursor

during exercise training potentiates the increases in capillary density in mice (67). Interestingly, the effect of *Pgc1α* overexpression in myofibers on capillary density also depends on SIRT1 activation in ECs (67). SIRT1 is activated not only by a rise in NAD⁺ during energy depletion, but also by hydrogen sulfide (H₂S). In ECs, the latter is generated by cystathionine γ-lyase (CGL) during dietary restriction of sulfur amino acids (methionine, cysteine) or upon VEGF stimulation. CGL-derived H₂S downregulates endothelial OXPHOS and subsequently increases glucose uptake in an AMPK-dependent but VEGF-independent fashion, leading to an increase in glycolysis-driven EC migration and in EC proliferation through increased biosynthesis of nucleotides via the pentose phosphate pathway (72). These data support a role for glycolytic metabolism in exercise-induced angiogenesis, but future genetic experiments are required to confirm this. Moreover, even though deletion of CGL prevented exercise-induced angiogenesis, these genetic interventions were not restricted to ECs and could therefore be confounded by effects of H₂S in other cells residing in skeletal muscle (72). The proposed model on how EC metabolism changes in response to exercise is illustrated in Fig. 2.

Figure 2

Proposed model for exercise-induced changes in EC metabolism promoting angiogenesis. (Panel A) ECs are highly glycolytic and produce 85% of their energy glycolytically. Mitochondria do not significantly contribute to energy production but rather maintain NAD⁺/NADH balance. (Panel B) During exercise, increased CGL activity leads to H₂S generation. CGL-derived H₂S downregulates endothelial OXPHOS by inhibiting complex IV activity. This leads to increased glucose uptake, glycolysis and pentose phosphate pathway flux in an AMPK-dependent fashion. In addition, exercise-induced angiogenesis requires adequate activation of SIRT1, at least partially because endothelial SIRT1 is required for VEGF-mediated angiogenesis. SIRT1 also controls EC function by inhibiting Notch (NICD indicates active Notch) and via inactivating FOXO1 (forkhead box O1, not shown). 3PG, glyceraldehyde 3-phosphate; AMPK, 5' adenosine monophosphate-activated protein kinase; CGL, cystathionine γ-lyase; G6P, glucose 6-phosphate; NAD, nicotinamide adenine dinucleotide; NICD, Notch intracellular domain; OXPHOS, oxidative phosphorylation; PPP, pentose phosphate pathway; Pyr, pyruvate; ROS, reactive oxygen species; SIRT1, Sirtuin 1; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2.

Outlook: can exercise-induced metabolic crosstalk between ECs and muscle contribute to angiogenesis?

The available evidence thus far indicates that VEGF is driving exercise-induced angiogenesis in skeletal muscle, but the extent of the angiogenic response can be mediated by altering the metabolic ‘fitness’ of ECs. This raises the exciting hypothesis that targeting EC metabolism can promote exercise-induced angiogenesis. Potentially, skeletal muscle cells can modulate EC metabolic fitness as well via metabolic crosstalk during exercise. Indeed, it has already been shown that metabolites that are secreted from myofibers during exercise can either potentiate the effect of VEGF or directly contribute to angiogenesis. For instance, lactate is secreted (mainly) by fast glycolytic fibers during intense exercise. In other settings, lactate renders ECs more responsive to VEGF by promoting an increase in VEGF receptor content due to enhanced HIF1 α stabilization in an α -ketoglutarate- and reactive oxygen species (ROS)-dependent fashion (73, 74). Increasing lactate levels near ischemic muscles also improves their revascularization (75). Exercising muscles also release ATP, which is converted to adenosine in the extracellular space (76). In the retina, adenosine can act through activation of the adenosine A2a receptor, which causes pathological angiogenesis by increasing endothelial glycolysis in a HIF1 α -dependent fashion (77). Whether lactate- and/or adenosine-dependent metabolic rewiring of ECs contributes to exercise-induced angiogenesis remains an open question.

Finally, despite the strong correlation between muscle capillary density and skeletal muscle metabolism (15), as well as the control of *Vegf* (and other angiogenic factors) expression by myofiber metabolic master switches, there is still no conclusive evidence that improving vascular density is *required* for achieving muscle adaptations to training, such as increased oxidative capacity and improved performance. On the other hand, the observation that increased capillary density per se (via the overexpression of SIRT1 in ECs) improves running performance and lowers blood glucose levels in mice (67) indicates that increasing blood vessel density *suffices* to enhance exercise performance and/or affect systemic metabolism. Unraveling the mechanisms underlying these observations will yield valuable insight into metabolic regulation as well as determinants of human performance and will potentially open novel avenues for the treatment of diseases such as peripheral artery disease and insulin resistance.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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