Effect of exercise on HIF-1 and VEGF signaling

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Abstract This review summarizes the literature describing the significance of various conditions, such as hypoxia, oxidative stress, and, above all, physical exercise, in the hypoxia-inducible factor-1 (HIF-1) and vascular endothelial growth factor (VEGF) signaling pathway mainly in skeletal muscle. HIF-1 α acts as a master regulator for the expression of genes involved in the hypoxia response of most mammalian cells. Namely, HIF-1 α initiates transcription of various hypoxia-adaptive genes, such as angiogenesis, glycolysis, and erythropoiesis, after the formation of heterodimer with HIF-1 β . Among them, VEGF is the most potent endothelial specific mitogen, which recruits endothelial cells into hypoxic foci and avascular area and stimulates their proliferation. The study on acute exercise shows that several components of the HIF-1 pathway, involving VEGF and erythropoietin, are activated in response to acute changes in oxygen demand in human skeletal muscle, suggesting that oxygen sensitive pathways could be relevant for adaptation to physical activity by increasing capillary growth. Also, the effects of endurance training on the activity of the HIF pathway in human skeletal muscle under hypoxic conditions appear to be definitely higher than those under normoxic conditions, indicating that combining hypoxia with exercise training appears to improve some aspects of muscle O₂ transport and/or metabolism. On the other hand, increased levels of reactive oxygen species (ROS) due to physical exercise induce the expression of peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC-1 α), which regulates mitochondrial biogenesis in multiple cell types, resulting in increases in VEGF expression and subsequent angiogenesis, strongly suggesting HIF-1 α -independent regulation of VEGF and angiogenesis. Thus, the precise relationship among exercise, the HIF-1 pathway including VEGF, PGC-1 α , and ROS needs further study.

Keywords : exercise, hypoxia, HIF-1, VEGF, PGC-1 α , reactive oxygen species

Introduction

Hypoxia-inducible factor-1 (HIF-1) was discovered in 1992 as the transcription factor of the human *EPO* gene encoding erythropoietin (EPO)¹⁾. HIF-1 is a heterodimeric protein composed of an oxygen-regulated subunit HIF-1 α and a constitutively expressed subunit HIF-1 β . HIF-1 α is hydroxylated by prolyl hydroxylase 1-3 (PHD1-3) on proline residues (Pro402 and Pro564) immediately after *de novo* synthesis²⁾. This modification facilitates the binding of von Hippel-Lindau (VHL) E3 ubiquitin ligase complex with HIF-1 α , and resultant ubiquitination and degradation by 26S proteasome. However, when the intracellular oxygen level is reduced, the enzymatic activities of PHDs are inhibited; and this leads to protein stabilization and nuclear translocation of HIF-1 α , wherein HIF-1 α initiates transcription of various hypoxia-adaptive genes involved

in angiogenesis, glycolysis, erythropoiesis, and catecholamine biosynthesis, after the formation of the active heterodimer with HIF-1 β ³⁾ (Fig. 1)⁴⁾. In contrast, there is growing evidence that HIF-1 α protein stability is regulated by oxygen-independent mechanisms⁵⁾. For example, acute exercise is accompanied by regional and systemic reduced partial pressure of oxygen as well as acidosis, oxidative stress and heat, all of which are stimulatory factors of HIF-1 α ⁶⁾.

To date, more than 100 genes are identified as the transcriptional target of HIF-1⁷⁾. In addition, more than 2% of all human genes are directly or indirectly regulated by HIF-1 in arterial endothelial cells⁸⁾. Among them, vascular endothelial growth factor (VEGF) is the most potent endothelial specific mitogen, which recruits endothelial cells into hypoxic foci and avascular area and stimulates their proliferation⁹⁾. Actually, homozygous deletion of the *Hif1a* gene encoding HIF-1 α in mice results in the arrest of embryonic development at day 8.5 and embryonic

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lethality by day 10.5 with major cardiac malformations, vascular regression, and reduced erythropoiesis¹⁰⁻¹²). Furthermore, homozygous deletion of the *Arnt* gene encoding HIF-1 β also results in embryonic lethality at midgestation with defective vascularization¹³). Thus, HIF and VEGF signaling is essential for the maintenance of the vascular density and oxygen supply in tissue hypoxia.

This review focuses on the significance of various conditions, such as hypoxia, oxidative stress, and, above all, physical exercise, in the HIF-1 α and VEGF signaling pathway mainly in skeletal muscle.

Hypoxia in skeletal muscle

A gradient of oxygen partial pressure (pO_2) exists within the body, due to approximately 160 mmHg in inspired gas, approximately 115 mmHg in arterial blood, and approximately 38 mmHg in capillary blood¹⁴). Within

tissues, the pO_2 gradient depends on the distance of cells from closest O_2 -supplying blood vessel and the metabolic activity and consequent O_2 consumption of the resident cells¹⁵). For instance, intramyocellular pO_2 falls to as low as 3.1 mmHg under normoxia and 2.1 mmHg under hypoxia, respectively¹⁴). Mitochondria house the final biochemical steps in the production of reducing equivalents that react at the terminal oxidases of the respiratory chain with molecular oxygen provided by the respiratory system from the environment. In mitochondria, O_2 finally disappears and oxygen partial pressure (pO_2) goes to zero (Fig. 2)⁴). Mitochondria thus are an effective oxygen sink and this allows organisms to use all of the available oxygen partial pressure (pO_2) of the actual environment to drive the respiratory cascade from lungs through circulation to the mitochondria¹⁶).

HIF-1 α acts as a master regulator for the expression of genes involved in the hypoxia response of most mammalian cells^{1,17}). Nevertheless, it is clearly expressed in various tissues such as the brain, kidney, liver, heart, and skeletal muscle in mice kept in a normoxic condition (21% O_2), whereas it is undetectable in the lungs¹⁸). Further, when mice were kept in extreme hypoxia (6% O_2), the expression levels of HIF-1 α in the brain, kidney, liver, heart, and skeletal muscle were significantly higher, while expression in the lungs was not affected^{18,19}). These findings indicate that there is a clear pO_2 gradient between the lungs and other tissues, and that with the exception of relatively well-oxygenated lungs, low pO_2 and HIF-1 α play physiologically essential roles in homeostasis of various tissues.

Moreover, Mounier et al.²⁰) have shown, in untrained human skeletal muscle under normoxic conditions, higher HIF-1 α protein expression in predominantly oxidative muscles than in predominantly glycolytic muscles. The HIF-1 α mRNA expression pattern, however, was not in agreement with the HIF-1 α protein level. Interestingly, none of the HIF-1 α target genes, like the most studied angiogenic factor involved in muscle angiogenesis, VEGF, exhibited a muscle fibre-specific-related mRNA expression at rest in normoxia. However, soleus presented a significantly higher VEGF protein content than vastus lateralis and triceps muscle. Taken together, such findings indicate that there are muscle-specific differences in HIF-1 α and VEGF expression within human skeletal muscle at rest in normoxic conditions.

HIF-1 α -independent regulation of VEGF and angiogenesis

Peroxisome proliferator-activated receptor (PPAR)- γ coactivator 1 α (PGC-1 α) regulates mitochondrial biogenesis in multiple cell types through coactivation of key transcription factors including nuclear respiratory factors (NRF-1 and NRF-2), estrogen-related receptor- α (ERR α), Gabpa/b, PPARs, and the thyroid hormone receptor²¹) (Fig. 3)²²). PGC-1 α is a mediator of signaling in response

Fig. 1 HIF-1 α -mediated oxygen sensor and hypoxia-inducible gene expression⁴). Oxygen stabilizes hypoxia-inducible factor 1 α (HIF-1 α) through Fe²⁺-dependent proline hydroxylases and asparaginyl hydroxylase(s) which hydroxylate HIF-1 α within its oxygen-dependent degradation domain (ODDD) and C-terminal portion, respectively. Such modification causes recruitment of the von Hippel-Lindau tumour-suppressor protein (pVHL), which targets HIF-1 α for proteosomal degradation thereby silencing HIF-1 α activity. Conversely, hypoxia causes a stabilization of HIF-1 α and activates the MAPK pathway which enhances the transcriptional activity of HIF-1 α through phosphorylation. Both mechanisms contribute to transcriptional activation of downstream angiogenic factor (VEGF), erythropoietin (EPO), glucose transporters (glut 1 and 3) and glycolytic genes (PFK) via HIF-1 α /HIF-1 β dimers (HIF-1). Moreover, lack of oxygen (anoxia) causes HIF-1 α stabilization, possibly by reducing the activity of hydroxylases by depleting their substrate O_2 .

Fig. 2 The pathway of oxygen⁴⁾. Simplified model of the oxygen transport pathway showing the principal structures and the corresponding partial pressure of oxygen (pO₂) in these compartments, during maximal exercise, while breathing normoxic or hypoxic room air. mb, myoglobin. The data are derived from Richardson et al.¹⁴⁾

Fig. 3 PGC-1 α regulates expression of VEGF through coactivation of ERR- α ²²⁾. Speculative model for the role of PGC-1 α in the regulation of angiogenesis during exercise and in response to ischaemia. AMPK, AMP-activated protein kinase; tFAM, mitochondrial transcription factor A.

to deprivation of nutrients and oxygen, and it powerfully regulates VEGF and other angiogenic factors to elicit neovascularization *in vivo*. The regulation of VEGF in response to hypoxia is thought to be mediated primarily through the well-known HIF factors¹⁾. Surprisingly, the novel PGC-1 α /ERR- α pathway (Fig. 3)²²⁾ is apparently independent of the HIF pathway. PGC-1 α ^{-/-} mice are viable, suggesting that PGC-1 α is not essential in embryonic vascularization²²⁾. Angiogenesis in the adult occurs in both physiological and pathological contexts²³⁾. The robust induction of vascularization by PGC-1 α , and its critical function in the response to limb ischaemia, strongly implicate PGC-1 α in the angiogenic response to ischaemia, providing protection against further ischaemic insults²²⁾.

Fig. 4 Schematic representation of HIF target gene expression by PGC-1 α ²¹⁾. PGC-1 α expression results in mitochondrial biogenesis and increased oxygen consumption. The resultant drop in intracellular pO₂ levels leads to decreased HIF hydroxylase activity and HIF stabilization with resultant gene expression that facilitates an increase in oxygen supply. PHD, prolyl hydroxylase.

On the other hand, PGC-1 α is coupled to HIF signaling through the regulation of intracellular oxygen availability, allowing cells and tissues to match increased oxygen demand after mitochondrial biogenesis with increased oxygen supply, because of intracellular hypoxia (Fig. 4)²¹⁾. PGC-1 α -dependent induction of HIF target genes under physiological oxygen concentrations is not through transcriptional coactivation of HIF or up-regulation of HIF-1 α mRNA but through HIF-1 α protein stabilization.

Other candidates have been proposed for the HIF-1 α -independent regulation of VEGF and angiogenesis. Over-expression of suppressor of cytokine signaling 3 (SOCS3), a well-established negative regulator of signal transducer and activator of transcription 3 (STAT3), enhances the mRNA expression of downstream targets of STAT3, c-FOS and VEGF despite inhibition of STAT3 phosphorylation. These increases were correlated with enhanced mRNA expression of genes associated with muscle maturation and hypertrophy (Fig. 5)²⁴⁾. The mechanism by which SOCS3 may mediate these responses, however, is

unknown and will require further investigation. The decline in the regenerative capacity of skeletal muscle with age may be linked to inefficient STAT3/SOCS3 signaling. Actually, a single bout of maximal leg extension exercise (resistance exercise) markedly increased the STAT3 signaling including the SOCS3 gene in human skeletal muscle, accompanied by significant increases in levels of mRNAs for downstream genes of STAT3, c-FOS, JUNB, c-MYC, and VEGF, probably not via HIF signaling²⁵. In addition, the levels of HIF-1 mRNA subunits did not change in response to short-term one-legged exercise training, suggesting no change in HIF-1 mRNA transcript levels in the regulation of training-induced VEGF expression²⁶.

Increased production of reactive oxygen species (ROS), such as H₂O₂, also induces the expression of VEGF A, the prototype VEGF ligand, by a HIF-independent and Sp1-dependent mechanism, in which ligation of VEGF A to VEGF receptor 2 (VEGFR2) results in signal transduction leading to tissue vascularization. Such ligation generates H₂O₂ via an NADPH oxidase-dependent mechanism²⁷. That the function of one mitogen, VEGF, largely depends on the signaling driven by another mitogen, H₂O₂, generates a novel paradigm with major therapeutic implications for a variety of angiogenesis-related disorders²⁸. Furthermore, oxygen-glucose deprivation generates ROS in cerebral endothelial cells; and, in turn, induces VEGF signaling via its receptor, Flk-1 (VEGFR2), and activates extracellular signal-regulated kinase (ERK) 1/2. This suggests that VEGF acts via ERK 1/2 through oxidative-stress-dependent mechanisms, showing that ERK 1/2 can be considered a molecular target for stroke therapy²⁹. Interestingly, high glucose blunts VEGF response to hypoxia in immortalized rat proximal tubular cells. This

effect is mediated by the oxidative stress-regulated HIF-hypoxia-responsible element (HRE) pathway³⁰.

p38 γ MAPK/PGC-1 α signaling regulation by physical exercise and its significance for mitochondrial biogenesis and angiogenesis in skeletal muscle

p38 γ mitogen-activated protein kinase (MAPK), but not p38 α MAPK or p38 β , is required for PGC-1 α upregulation in mitochondrial biogenesis and angiogenesis in response to voluntary wheel running and nerve stimulation in mice. None of the p38 MAPK isoforms were required for endurance exercise-induced IIB-to-IIA fiber-type transformation in these mice³¹. Meanwhile, the calcineurin (CaN)/nuclear factor of activated T-cells (NFAT) regulatory axis controls fiber-type transformation in endurance exercise-induced skeletal muscle adaptation³². Collectively, endurance exercise on one hand induces activation of the Ca²⁺-dependent CaN-NFAT pathway in control of fiber-type transformation and on the other hand activates p38 γ MAPK, which promotes PGC-1 α activity and expression in control of mitochondrial biogenesis and angiogenesis³² (Fig. 6)³¹.

In addition, an acute bout of sprinting exercise that increased ROS production mainly through a nonmitochondrial enzyme, xanthine oxidase (XO), stimulated PGC-1 α expression and other key proteins in the mitochondrial biogenic pathway, such as NRF-1 and transcription factor A, mitochondrial (Tfam). This nuclear-initiated signaling event was accompanied by activation of p38 MAPK and cAMP response element binding protein (CREB) phosphorylation. Inhibition of XO by allopurinol severely attenuated exercise activation of the PGC-1 α signaling pathway, thus providing strong evidence that mitochon-

Fig. 5 SOCS3 increases the expression of STAT3 targets in differentiating myotubes when expression of cFOS (A), JUNB (B) and VEGF (C) mRNA was measured in primary human myoblasts²⁴. Values are arbitrary units normalized to the expression levels of the reference gene cyclophilin representing the mean of 6 replicates $\pm$ SEM. Closed bars (■) represent without SOCS3, open bars (□) represent with SOCS3 overexpression. Significantly different from respective samples without SOCS3: * $p < 0.05$, *** $p < 0.001$.

drial biogenesis in skeletal muscle is controlled at least in part by a redox-sensitive mechanism³³). This view is supported by Holloszy³⁴). Moreover, stretch-stimulated glucose uptake in skeletal muscle is mediated by a ROS- and p38 MAPK-dependent mechanism that appears to be AMPK α 2- and phosphatidylinositol 3-kinase (PI3-K)-independent³⁵). This novel signaling mechanism is distinct from canonical contraction- and insulin-stimulated signaling that increases glucose uptake, probably providing an alternative pathway for the development of novel therapeutic drugs to overcome insulin resistance. MAPK activation is also involved in the contraction-induced secretion of VEGF protein from skeletal muscle, which is partially mediated via adenosine acting on A_{2B} adenosine receptors³⁶).

Double-faced nature of ROS

ROS are widely believed to cause or aggravate several human pathologies such as neurodegenerative diseases, cancer, stroke, and many ailments. Antioxidants are assumed to counteract the harmful effects of ROS and therefore prevent or treat oxidative stress-related diseases³⁷). On the other hand, there is growing evidence that ROS have beneficial effects. This dual nature of ROS means that ROS act as intracellular signaling molecules and as defense mechanisms against micro-organisms³⁸). For instance, the mechanisms by which ROS and cytosolic H₂O₂ levels are involved in the stabilization/activation

and eventual degradation/silencing of HIF-1 α and other redox-sensitive transcription factors, such as MAPK and PI-3K/Akt, are controversially discussed³⁹).

Mitochondria and mitochondria-derived ROS were required for the stabilization of HIF-1 α by MAPK and by exposure of hepatoma cells to 6 h of hypoxia (1.5% O₂)⁴⁰. This hypoxic stabilization of HIF-1 α in hepatoma cells was blocked by the overexpression of catalase, which catalyses dismutation of H₂O₂ into H₂O and O₂, and is an inhibitor of the mitochondria superanion channel. Also, HIF-1 α and VEGF overexpression in pulmonary artery smooth muscle was induced by CoCl₂, which mimics the hypoxic response, via ROS⁴¹). Such overexpression was inhibited by vitamin C and by the overexpression of GPX and catalase. By contrast, the addition of H₂O₂ in the absence of a hypoxic stimulus causes stabilization of HIF-1 α ⁴⁰). Anoxia, on the other hand, may stabilize HIF-1 α essentially through depletion of the co-substrate dioxygen of prolyl hydroxylases, indicating that increases in cytosolic H₂O₂ in consequence of hypoxia-driven ROS production may cause HIF-1 α accumulation in hypoxia through reduction of hydroxylation (Fig. 7)⁴). Actually, during the “acute” increase phase of HIF-1 α (between 0 and 2 h), 8-hydroxy-2'-deoxyguanosine (8-OHdG) was positively correlated with HIF-1 α during sustained hypoxia in humans⁴²). Moreover, ROS-activated signaling events involving MAPK and PI-3K/Akt have been implied in the modulation of the transcriptional activity of HIF-1 α ⁴³). Further, angiotensin II *in vivo* and *in vitro* up-regulates renal VEGF expression by a mechanism that involves HIF-1 activation and oxidative stress⁴⁴).

Thus, it now seems possible that ROS have important roles in the regulation of cell signaling, although these substances are potentially cell damaging. For example, there appears to be a potential feedback loop in which PGC-1 α regulates antioxidant mechanisms via regulation of mitochondrial respiration and ROS. In turn, ROS might regulate antioxidant defense genes through activation of redox-sensitive transcription factors, supporting the view that ROS are important signaling molecules in adaptation to exercise (Fig. 8)⁴⁵). Whereas increasing evidence suggests that mitochondria play a less important role in oxidant production in contracting skeletal muscles than was previously predicted, and the primary site of contraction-induced ROS production in muscle fibers remains unclear^{46,47}), PGC-1 α is transiently induced by acute exercise^{34,48}). This induction has been suggested to occur in response to altered energy demands and because of PGC-1 α interacting with the metabolic enzyme AMPK⁴⁹). PGC-1 α has also been proposed to be a key mediator of long-term adaptation to exercise⁴⁹). Indeed, basal levels of PGC-1 α gene expression increased approximately two-fold after a period of endurance training in humans⁵⁰).

Intriguingly, in conflict with the free radical theory of aging (FRTA)⁵¹), longevity and health-promoting effects of caloric restriction and specifically reduced glucose

Fig. 6 Signaling pathways involved in exercise-induced peroxisome-proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) regulation in skeletal muscle. Current evidence suggests roles for calcineurin (CaN), calmodulin-dependent kinase (CaMK), AMP-activated protein kinase (AMPK), and p38 MAPK in PGC-1 α regulation³¹). Thick arrows depict regulatory events required for exercise-mediated induction of PGC-1 α and subsequent adaptations that have been confirmed by gene deletion studies in animal models. Thin arrows depict regulatory events that have been associated with PGC-1 α regulation, but their requirement for the exercise-dependent induction of PGC-1 α awaits further investigation. ROS, reactive oxygen species.

Fig. 7 Targets of mitochondrial ROS production⁴⁾. Incomplete reduction of O_2 during normal metabolic conversion of pyruvate in mitochondria gives rise to a low level of superoxide anion ($O_2^{\cdot-}$). Exposure of cells to hypoxia increases the aberrant production of $O_2^{\cdot-}$ at the mitochondrial electron chain complex III. Catalyzed or spontaneous dismutation of the unstable superanion and export via anion channels enhances the concentration of cytosolic H_2O_2 and other reactive oxygen species (ROS). Increased ROS may increase the level of oxidatively damaged lipids (lipofuscin) or activate downstream redox-sensitive signal transduction events. For example ROS stabilize HIF-1 α possibly by interfering with the activity of Fe^{2+} -dependent proline hydroxylases. ROS may also activate the MAPK or PI-3K/Akt pathway, which enhances the transcriptional activity of HIF-1 α through its phosphorylation. Lastly, mechanical stress may also increase ROS production that may influence HIF-1 α activation.

Fig. 8 Proposed model of signaling activation by ROS in skeletal muscle in response to exercise⁴⁵⁾. (a) Reactive oxygen species (ROS) are produced from mitochondrial respiration by a leakage of electron from the electron-transport chain to oxygen. This process increases during exercise as a consequence of increased mitochondrial activity. (b) ROS have the potential to regulate cell signaling by affecting redox-sensitive transcription factors (here assigned x, y and z), inducing PGC-1 α , antioxidant defense mechanisms and myokines. (c) PGC-1 α activates gene involved in mitochondrial respiration, enabling increased mitochondrial activity, whereas (d) endogenous antioxidants such as superoxide dismutase (SOD), glutathione peroxidase-1 (GPX1) and catalase buffer the increased amount of ROS. (e) Myokines act in autocrine, paracrine or endocrine fashion to stimulate hypertrophy (IL-6, LIF and IL-15) or angiogenesis (IL-8 and Fstl-1).

metabolism may be due to increased formation of ROS within the mitochondria causing an adaptive response that culminates in subsequently increased stress resistance assumed to ultimately cause a long-term reduction of oxidative stress. This adaptive response is known as mitochondrial hormesis or mitohormesis⁵²). Molecular mediators of endogenous ROS defense (SOD 1 and 2 and GPX) are also induced by exercise, and this effect is blocked by antioxidant supplementation⁵³). Consistent with the concept of mitohormesis, exercise-induced oxidative stress ameliorates insulin resistance and causes an adaptive response promoting endogenous antioxidant defense capacity. As expected or unexpectedly, supplementation with antioxidants may preclude these health-promoting effects of exercise in humans. Taken together, physical exercise induces numerous molecular regulators of insulin sensitivity and antioxidant defense, most of which are almost completely inhibited by antioxidant pretreatment in healthy young men^{52,54}) (Fig. 9)⁵³).

Furthermore, lactate elevation is a ubiquitous characteristic of tumors and wounds. ROS production by Fenton-like reactions from lactate metabolism by stem/progenitor cells (SPCs) accelerates further SPC recruitment and differentiation through thioredoxin 1-mediated elevations in HIF-1 levels and the subsequent synthesis of HIF-1-dependent growth factors, such as VEGF and stromal cell-derived factor⁵⁵).

Effect of exercise on HIF and VEGF signaling

The study on acute exercise by Ameln et al.⁵⁶) is the first

Fig. 9 Mitohormesis links physical exercise and subsequent formation of reactive oxygen species to insulin sensitivity and antioxidant defense⁵³). Physical exercise exerts ameliorating effects on insulin resistance by increasing mitochondrial formation of reactive oxygen species in skeletal muscle to induce expression of PGC-1 α , PGC-1 β , and PPAR γ as inducers of insulin sensitivity, as well as SODs 1 and 2 and glutathione peroxidase 1, key enzymes of ROS defense. Notably, by blocking exercise-dependent formation of ROS due to ingestion of antioxidant supplements, health promoting effects of physical exercise are abolished, and physical exercise fails to promote insulin sensitivity and antioxidant defense in the presence of vitamin C and vitamin E.

to show that several components of the HIF-1 pathway, involving VEGF and EPO, are activated in response to acute changes in oxygen demand in healthy human skeletal muscle. This is due to a concurrent decrease in VHL tumor suppressor protein levels, suggesting that oxygen sensitive pathways could be relevant for adaptation to physical activity by increasing capillary growth in human skeletal muscle. Such findings support the general importance of the HIF pathway (Fig. 10)⁵⁶). VEGF mRNA is further increased when blood flow to the exercising leg is restricted.

Also, several studies have revealed increased levels of the HIF-1 pathway in human skeletal muscle following acute exercise^{57,58}). The positive response of mRNAs for HIF-1 α and HIF-2 α by acute exercise, however, is blunted with training⁵⁸). Moreover, a positive correlation was found between exercise-induced changes in VEGF mRNA on one hand, and changes in HIF-1 α mRNA, HIF-1 β mRNA, and lactate on the other; while no significant differences between the restricted and nonrestricted groups were observed⁵⁷), unlike the study of Ameln et al.⁵⁶). This lack of a further increase in VEGF mRNA when flow restriction was “added” may be explained by the smaller further reduction in oxygen saturation and oxygen tension compared with the rest-to-exercise transition, even if this difference was large enough to induce a greater lactate concentration increase in the restricted condition. Another explanation could be that, even if there was no significant VEGF mRNA difference before exercise between non-restricted and restricted conditions, a strong influence of the preexercise value of VEGF mRNA on the exercise-induced increase makes a difference between the two conditions more difficult to detect.

Exercise induces several pathways that are known to be important for regulating angiogenesis. VEGF is critical for basal and activity-induced regulation of skeletal muscle capillarization; whereas the importance of other growth factor pathways remains to be elucidated. Although several signaling pathways have been identified (Fig. 11)⁵⁹), significant work remains on understanding the intracellular signaling pathways and transcription factors involved in basal and exercise-induced angiogenesis.

For example, in addition to AMPK, exercise activates several signaling pathways that may be the link between metabolism and capillarization including calcium (Ca²⁺)-regulated pathways⁵⁹). Large but transient increases in intracellular Ca²⁺ occur during exercise as a result of neural activation and muscle cell depolarization. Ca²⁺ is intimately involved in actin-myosin cross-bridge formation and is responsible in part for exercise-induced improvements in insulin sensitivity that occur in part through increases in glucose transporter 4. Increasing intracellular Ca²⁺ leads to the activation of several downstream signaling proteins including calmodulin-dependent kinase (CaMK) and CaN, possibly resulting in increased expression of VEGF mRNA in primary human myotubes *in*

vitro, suggesting that calcium may be involved in muscle VEGF regulation⁵⁹); although much more work needs to be done before these studies are finalized. In addition, PPAR- β , which is also known as PPAR δ and is involved in muscle development and metabolism, increases VEGF and promotes muscle angiogenesis through a CaN-dependent pathway⁶⁰.

Moreover, nitric oxide (NO) is involved in muscle VEGF regulation⁶¹ (Fig. 11)⁵⁹. NO is generated by NO synthase (NOS) and is produced in muscle by neuronal NOS (nNOS) and in endothelial cells by endothelial NOS (eNOS). nNOS production of NO regulates, in part, muscle mitochondrial respiration, whereas eNOS production of NO is involved in blood flow regulation. Disrupting normal NO production (via L-NAME) eliminates the increase in collateral blood flow induced by training, but does not disturb the increase in muscle capillarity within the active muscle. Similarly, inhibiting VEGFR kinase activity eliminates the increase in collateral-dependent blood flow, and lessens, but does not eliminate, angiogenesis in the calf muscle, illustrating distinctions between the processes influencing angiogenesis and arteriogenesis⁶¹.

In general, the effects of endurance training on the activity of the HIF pathway in human skeletal muscle under hypoxic conditions appear to be definitely higher than those under normoxic conditions⁶²⁻⁶⁴); although there are several exceptions^{65,66}), indicating that combining hypoxia with exercise training appears to improve some aspects of muscle O₂ transport and/or metabolism⁶⁴). Although there were no significant changes in the levels of mRNAs for HIF-1 α or VEGF in human skeletal muscle after endurance training under normoxic conditions, the changes in HIF-1 α mRNA expression correlated well with those in VEGF mRNA expression (Fig. 12)⁶⁷), suggesting that HIF-1 α influences the training-induced VEGF gene expression or alternatively that HIF-1 α and VEGF expression is co-regulated at the transcriptional level in human skeletal muscle. Taken together, it is envisioned that the cumulative effects of transient changes in transcription during recovery from successive bouts of exercise may represent the underlying kinetic basis for the cellular adaptations associated with endurance training. Furthermore, no plasma VEGF concentrations in overweight men aged 50-60 years were affected by long-term endurance exercise under normoxic conditions⁶⁸). Likewise, low or high intensity resistance exercise did not significantly alter serum VEGF concentrations in humans⁶⁹).

Future directions

As already stated, HIF-1-mediated pathways have multiple functions. Recently, it has been reported that loss of VHL-1 in *C. elegans* significantly increases lifespan and enhances resistance to polyglutamine and amyloid β toxicity⁷⁰). Deletion of HIF-1 is epistatic to VHL-1, indi-

cating that HIF-1 acts downstream of VHL-1 to modulate aging and proteotoxicity. VHL-1 and HIF-1 control longevity by a mechanism distinct from both dietary restriction and insulin/IGF-1-like signaling^{70,71}). Moreover, ROS are increased in respiration mutants of *C. elegans* and mild increases in ROS can stimulate HIF-1 to activate gene expression and promote longevity, suggesting that HIF-1 links respiratory stress in the mitochondria to a nuclear transcriptional response that promotes longevity⁷²). The question whether this is also true for humans, however, cannot be answered at present.

On the other hand, the depression of HIF-1-mediated pathways appears to be a “natural” part of aging. VEGF, VEGFR1, VEGFR2 mRNA and protein levels are reduced in the hearts of aged (23-month-old) sedentary rats compared to young (4-month-old) sedentary rats or aged rats subjected to a swimming regimen for 8 weeks⁷³). Cardiac capillary density mirrored the changes in VEGF expression with age and exercise. In humans, similar studies have shown depressed capillarization and lower VEGF protein in the skeletal muscles of elderly humans compared to young humans^{74,75}). In elderly patients with stable coronary disease or peripheral vascular disease subjected to exercise to an ischaemic threshold, the number of endothelial progenitor cells (EPCs), regulated by HIF-1, and plasma VEGF are increased⁷⁶). Hence, exercise by stimulating HIF-1 signaling, may maintain vascular youth^{77,78}). Moreover, it should be emphasized that a cheap and ready therapy to augment HIF-1-mediated pathways is exercise⁷⁸).

On the contrary, age-associated increases in HIF-1 α and VEGF levels in rat skeletal muscle were observed, possibly due to mitochondrially increased generation of ROS with aging⁷⁹). Unexpectedly, exercise training decreased levels of both HIF-1 α and VEGF in aged rats, which appears to run contradictory to the understanding that HIF-1 α mediates exercise-induced oxidative adaptation to

Fig. 12 Correlation between HIF-1 α and VEGF mRNA expression in human skeletal muscle after endurance training⁶⁷).

skeletal muscle⁸⁰). Thus, the precise relationship of VEGF, exercise, aging, and ROS and the HIF-1 pathway needs further study.

Lastly, the study of the HIF and VEGF signaling (via the ROS production) appears to be shifting from terra incognita to terra firma – ensuring some promising breakthroughs in the near future.

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