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Review

Redox signaling and skeletal muscle adaptation during aerobic exercise

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SUMMARY

Redox regulation is a fundamental physiological phenomenon related to oxygen-dependent metabolism, and skeletal muscle is mainly regarded as a primary site for oxidative phosphorylation. Several studies have revealed the importance of reactive oxygen and nitrogen species (RONS) in the signaling process relating to muscle adaptation during exercise. To date, improving knowledge of redox signaling in modulating exercise adaptation has been the subject of comprehensive work and scientific inquiry. The primary aim of this review is to elucidate the molecular and biochemical pathways aligned to RONS as activators of skeletal muscle adaptation and to further identify the interconnecting mechanisms controlling redox balance. We also discuss the RONS-mediated pathways during the muscle adaptive process, including mitochondrial biogenesis, muscle remodeling, vascular angiogenesis, neuron regeneration, and the role of exogenous antioxidants.

INTRODUCTION

Humans and animals co-exist in an oxygen-dependent environment, where respiration (including inhalation of oxygen and exhalation of carbon dioxide) is essential for producing energy to sustain life. Human and animal species possess internal mechanisms to adapt to a high concentration of oxygen,[1] and it is now well-known that high-intensity exercise can rapidly increase oxygen consumption in skeletal muscle by up to 200-fold, further augmenting the generation of reactive oxygen and nitrogen species (RONS).[2] To avoid oxidative damage by RONS, animals have evolved a complicated redox system, inclusive of an array of antioxidant enzymes and chemicals that synergistically eliminate RONS to maintain redox balance *in vivo*.

Previously, RONS were regarded as detrimental chemical agents that negatively impacted health. RONS is classified as a group of highly reactive oxidants that can cause oxidation and damage to various biological substances in specific cellular compartments.[3] However, with the depth and extension of research on redox regulation, scientists gradually discovered that RONS was not only a group of chemicals harmful to essential cell structures but that they also provide the modulatory stimulus for cell adaption.[4] Maintaining a balanced equilibrium between oxidative stress and antioxidant status is crucial for the normal functioning of an organism.[5,6] Exercise-induced oxidative stress can disrupt this homeostasis and trigger the production of active antioxidant enzymes to counteract oxidative stress and restore equilibrium.

Several studies have endeavored to explore the interplay between RONS and redox signaling in exercise-induced alterations,[4,7] and investigations into exercise-induced redox signaling with regard to muscle adaptation are constantly advancing and evolving. To this end, this review focuses mainly on redox signaling involved in exercise-induced muscular adaptations. The primary narrative will encompass and exclusively detail the source of reactive oxygen species (ROS) and reactive nitrogen species (RNS) during aerobic exercise training, subsequent antioxidant signaling in maintaining redox homeostasis, mitochondrial biogenesis, muscle fiber type switching, vascular angiogenesis, neurogenesis, glucose utilization before finally reviewing the impact of exogenous antioxidant supplementation on aerobic exercise training.

THE PRIMARY SOURCE OF OXYGEN- OR NITROGEN-REACTIVE SPECIES IN AEROBIC EXERCISE

A greater oxygen concentration is consumed during exercise to aid the oxidation of substrates such as glucose and fatty acids in mitochondria while generating ATP to support muscle contraction. Meanwhile, ROS are simultaneously produced from mitochondrial and nonmitochondrial sources, such as the mitochondrial electron transport chain (ETC),[8] NADPH oxidases (NOXs/DUOXs),[9] xanthine oxidase (XO),[10] and others.[11,12] Figure 1 briefly summarizes the RONS sources in myocytes. The mitochondrial ETC and transmembrane NOXs are the primary enzymatic sources for $O_2^{\cdot-}$ and H_2O_2.[13,14] A recent study by Henríquez-Olguin et al. revealed that ROS levels can increase by approximately 86% in human skeletal muscle during moderate-intensity cycling compared to the rest group.[15]

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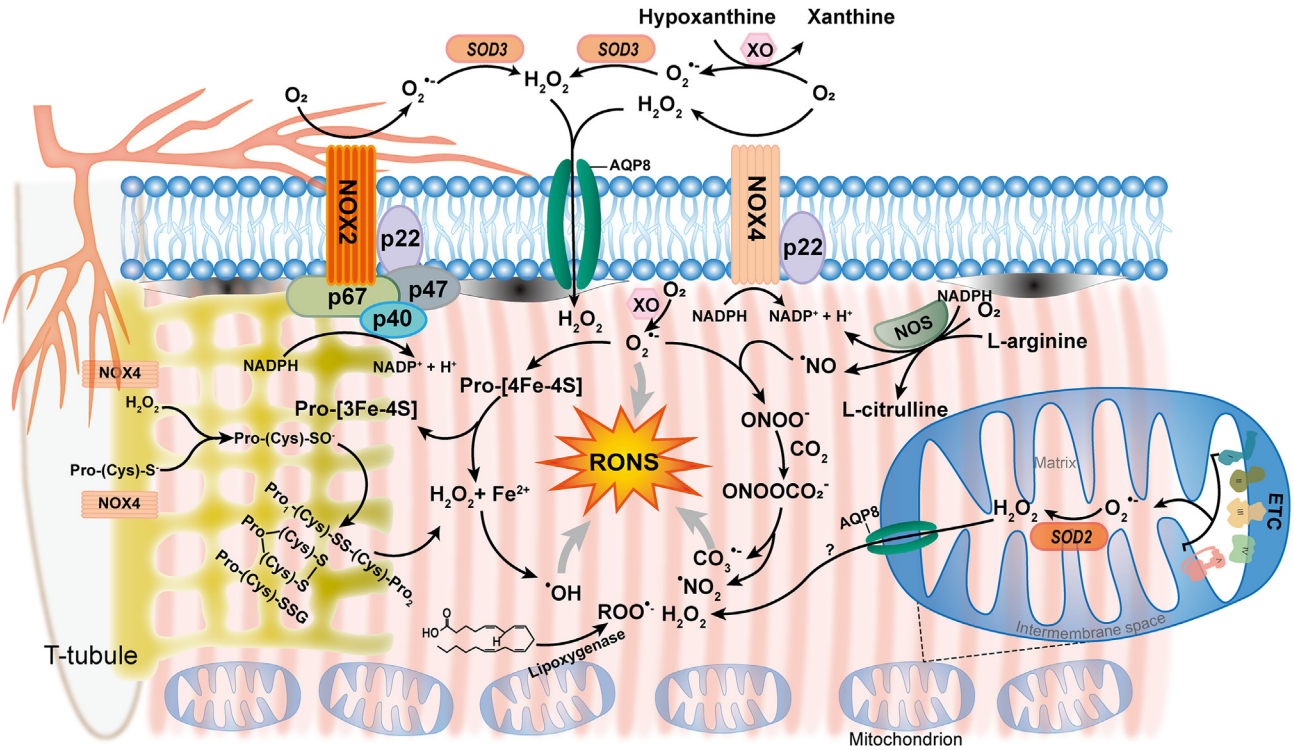


Figure 1. The primary generation of reactive oxygen and reactive nitrogen species in skeletal muscle

The main pathway of reactive oxygen and nitrogen species (RONS) in extracellular and intracellular space is exhibited. Extracellular reactive oxygen species (ROS), including $O_2^{\cdot-}$ and H_2O_2, are produced by xanthine oxidase (XO), NADPH oxidase 2/4 (NOX2/4), and superoxide dismutase 2/3 (SOD2/3). Intracellular RONS consisting of $O_2^{\cdot-}$, H_2O_2, $^{\cdot}OH$, $ROO^{\cdot}$, $^{\cdot}NO$, $ONOO^-$ are generated in the different pathways, such as ETC, NOX2/4, SOD2/3, lipoxygenase (LOX), NOS, as well as their secondary metabolic reactions.

The mitochondria are energy factories in which adenosine triphosphate (ATP) is synthesized by the oxidation of fatty acids and carbohydrates, the primary energy substrates for exercise (Figure 2). At intensities (60–65% of VO_{2max}) that elicit peak fat oxidation, the energetic contribution of plasma free fatty acids (FFAs) and intermyofibrillar lipids (IMFLP) (~1:1) is approximately equal to total carbohydrate utilization.[16,17] However, an increase in intensity at 75% or higher leads to a significant reduction in overall fat oxidation and an elevation in the utilization of glycogen and plasma glucose.[16,17] Meanwhile, with increasing intensity, glycolysis becomes more prominent, leading to elevated lactate and a decreased cytosolic NAD^+/NADH ratio since ATP synthesized from oxidative phosphorylation (OXPHOS) cannot satisfy the demands of exercise.[18] Furthermore, the glycerol-3-phosphate and malate-aspartate shuttles are involved in electron transport to the ETC, which facilitates the regeneration of NAD^+ for glycolysis.[18]

Fatty acids and/or glucose are decomposed and oxidized through a series of enzymatic reactions, generating NADH or $FADH_2$ through β-oxidation and/or the citric acid cycle. Electrons from NADH or $FADH_2$ transmit through the complexes I-IV to molecular oxygen (O_2) and subsequently generate NAD^+, FAD, and H^+. The latter proceeds with proton flux through ATP synthase (complex V), forming ATP and H_2O.[19] During this process, mitochondrial superoxide anion ($O_2^{\cdot-}$) is generated as a result of electron leakage from ETC to O_2, specifically originating from complexes I–III, as evidenced by the inhibition of key electron transfer points.[20–22] Additionally, insufficient electron transporting to O_2 is also an underlying cause of $O_2^{\cdot-}$ formation (Figure 2). An early report indicates that approximately 2–5% of all oxygen consumed by the mitochondria is used to form $O_2^{\cdot-}$.[23] Based on this report, mitochondria were initially hypothesized to serve as the primary source of ROS production in contracting muscle. Nevertheless, most recent reports indicate that approximately 0.15% of oxygen within mitochondria is converted into $O_2^{\cdot-}$.[24,25] During exercise, OXPHOS becomes more efficient, resulting in a decrease in $O_2^{\cdot-}$ production.[26] A previous study demonstrated that the generation of H_2O_2 in isolated muscle fibers is significantly lower under tissue culture conditions mimicking high-intensity exercise compared to mild-intensity exercise or rest.[8] Moreover, the culture conditions that simulate high-intensity exercise seem to promote greater availability of substrates that facilitate myofibers to produce an excess of NADH and $FADH_2$, compared to culture conditions at rest or mild-intensity exercise (these myofibers were exposed to an equivalent level of O_2 partial pressure (PO_2)).[8] A further study demonstrated that mitochondria isolated from cardiomyocytes exposed to high PO_2 produces more $O_2^{\cdot-}$ than those exposed to low PO_2.[27] Moreover, a recent study observed a significant decline in intramuscular PO_2 due to blood flow restriction during exercise, resulting in a reduction of mitochondrial ROS (mtROS) levels.[28] High-intensity aerobic exercise also leads to decreased PO_2 levels,[29] thereby supporting the prevailing notion that mtROS production is attenuated during physical exertion. Collectively, these findings indicate that mitochondrion is not the primary site for the formation of ROS during exercise.[5,7]

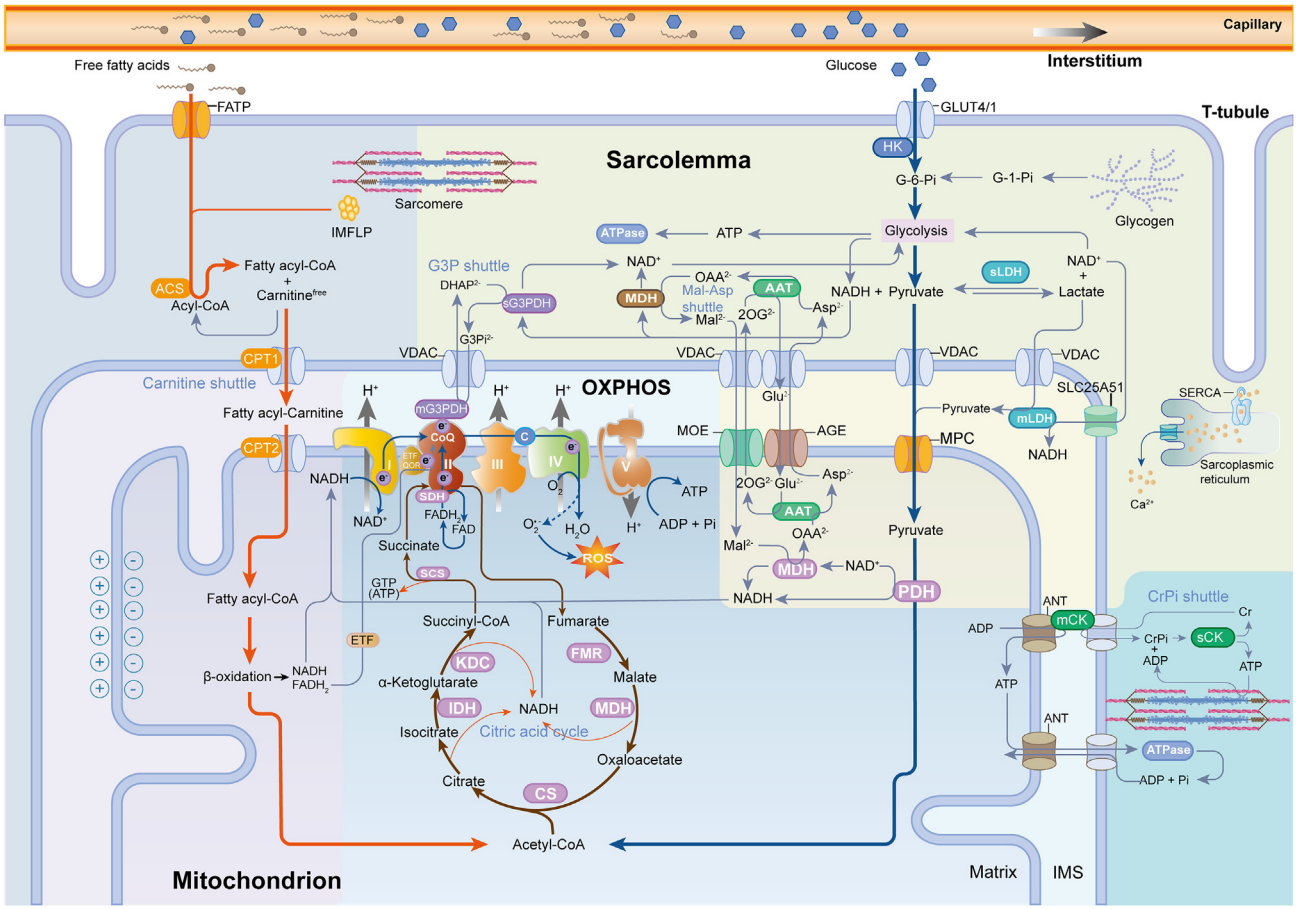


Figure 2. Skeletal muscle metabolism during aerobic exercise

During aerobic exercise, glucose is initially metabolized via glycolysis to produce pyruvate. Subsequently, pyruvate is transported into mitochondria through a voltage-dependent anion channel (VDAC) and a mitochondrial pyruvate carrier (MPC), where it undergoes conversion into acetyl-CoA by pyruvate dehydrogenase (PDH) (represented by the blue line). Finally, acetyl-CoA is oxidized in the citric acid cycle, leading to the generation of NADH and $FADH_2$. Meanwhile, circulating fatty acids, or those that are decomposed from the intermyofibrillar lipid droplet (IMFLP), are transported into mitochondria through a carnitine shuttle. Once inside mitochondria, they undergo β-oxidation and are further oxidized through the citric acid cycle (indicated by the red line) to generate NADH and $FADH_2$. The generated NADH and $FADH_2$ transfer electrons to O_2 via mitochondrial complexes I–IV and produce NAD^+, FAD, and H^+. During these processes, $O_2^{\cdot-}$ is produced due to insufficient electron transfer; meanwhile, the H^+ is transported to the intermembrane space (IMS), and the accumulated H^+ subsequently flows to the matrix via ATP synthase (complex V) to generate ATP. During high-intensity exercise, glycolysis emerges as the predominant pathway for ATP production. Lactate serves as a crucial electron acceptor in regenerating NAD^+, which plays a pivotal role as an electron acceptor in glycolysis. During glycolysis, the glycerol-3-phosphate ($G3Pi^{2-}$) shuttle (G3P shuttle) facilitates electron transfer to coenzyme Q (CoQ) in complex II via sarcomere and mitochondrial glycerol-3-phosphate dehydrogenase (s/mG3PDH). Meanwhile, the electrogenic transport of glutamate (Glu^{2-}) across the inner mitochondrial membrane via the aspartate-glutamate exchanger (AGE) and malate (Mal^{2-}) through the malate/2-oxoglutarate exchanger (MOE) plays a crucial role in regulating mitochondrial lactate oxidation in relation to aerobic glycolysis and the malate-aspartate shuttle. At the same time, the creatine-phosphate (CrPi) shuttle supplies ATP for short-term muscle contraction via adenine nucleotide translocator (ANT) and mitochondrial creatine kinase (s/mCK). ACS, acyl-CoA synthetase; FATP, fatty acid transport protein; GLUT4/1; glucose transporters 4 and 1; HK, hexokinase; sLDH, sarcoplasmic lactate dehydrogenase; mLDH, mitochondrial LDH; $DHAP^{2-}$, dihydroxyacetone phosphate; SLC25A51, solute carrier family 25 A51; G-6-Pi, glucose-6-phosphate; G-1-Pi, glucose-1-phosphate; $2OG^{2-}$, 2-oxoglutarate; OAA^{2-}, oxaloacetate; Asp^{2-}, aspartate; C, cytochrome *c*; CoA, coenzyme A; $DHAP^{2-}$, dihydroxyacetone phosphate; MDH, malate dehydrogenase; AAT, aspartate aminotransferase; OAA^{2-}, oxaloacetate; CS, citrate synthase; IDH, isocitrate dehydrogenase; KDC, α-ketoglutarate dehydrogenase complex; SCS, succinyl-CoA synthetase; SDH, succinate dehydrogenase; FMR, fumarase; ETF, electron-transferring flavoprotein; ETF:QOR, ETF:ubiquinone oxidoreductase. SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase.

A further ROS generation pathway emanates from NOXs, but the unique function of NOXs is producing $O_2^{\cdot-}$ or H_2O_2. NOXs are distributed in various cellular locations, contributing to localized ROS production.[30] The NOX family comprises seven isoforms (NOX1–5, DUOX1, and DUOX2) based on their distinct core catalytic subunit.[31,32] Among the seven members, NOX1, NOX2, and NOX4 are expressed in skeletal muscle cells.[33] Moreover, NOX2 and NOX4 have been extensively investigated in skeletal muscle. NOX2, NOX4, and the subunit $P22^{phox}$ are localized in the sarcolemma and transverse tubules (T-tubules); NOX4 is expressed in the mitochondria.[9] The activation of NOX2 occurs

through the recruitment of active Rac GTPase accompanied by regulatory factors such as $P67^{phox}$, $P47^{phox}$, and $P40^{phox}$, which are essential for generating $O_2^{\cdot-}$.[31] In skeletal muscle, NOX2 and $P22^{phox}$ are located in the membrane and T-tubules, and the subunits $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$ are enriched in the cytosol and translocated to the cell membrane during NOX2 activation.[34,35] Experiments have demonstrated that $p47^{phox}$ can be detected in membrane-enriched areas but not in the cytosolic fraction of the diaphragm. In addition, immunohistochemistry experiments also support that endogenous $p47^{phox}$ is distributed near the sarcolemma and T-tubules in limb muscles.[34] Unlike other members of the NOX subunits, NOX4 is constitutively active, predominantly generating H_2O_2.[36] NOX4 is present in cardiac and limb muscle mitochondria,[9,37] while NOX4 is also found in muscle sarcoplasmic reticulum.[38] A recent study shows that an acute bout of exercise upregulated NOX2 or NOX4 expression in distinct muscular tissues.[39] Exercise-induced expression of NOX4 is an essential source of muscle ROS, which further agitates mitochondrial metabolism.[40] Knockout of the NOX4 gene significantly decreases mice running distance compared to wild-type mice.[40] Tiganis's group reported that NOX4-generated H_2O_2 is essential to activate Nrf2-mediated antioxidant enzyme expression (i.e., GCLC, GCLM, NQO1, Prx1/2/3, and Trx1) and others (i.e., SOD2) in skeletal muscle.[41] Deleting the NOX4 gene in skeletal muscle significantly decreased exercise capacity in mice and deteriorated the expression of several antioxidant enzymes. This process, as mentioned previously, subsequently impacts insulin-mediated signal transduction, leading to the development of insulin resistance or deterioration in glucose metabolism.[41]

Many transcriptional factors such as Nrf2,[42] nuclear factor-kappa B (NF-κB),[43] HIF-1α,[44] STAT1/3,[45] E2F,[46] c-Jun,[47] AP-1,[48] and SMAD3[48] are involved in the regulation of NOX4 expression in various cell types. However, there is a paucity of research on the modulation of NOX2 or NOX4 expression in exercise-induced skeletal muscle adaptation. Thus, the regulation of NOX2 or NOX4 in skeletal muscle remains unclear. In addition, the modulation of other NOXs, such as NOX1, NOX3, NOX5, and DUOX1/2, also requires investigation during exercise.

In previous work, it has been determined that both NADPH and NADH are the substrates of NOXs for the production of $O_2^{\cdot-}$ and H_2O_2.[33] Moreover, NOXs preferentially use NADPH over NADH in non-muscle cells,[31] whereas NADH elicits several folds higher NOX activity than NADPH in skeletal muscle.[34] Since NADH serves as a substrate for NOX2 or NOX4 generating H_2O_2 and NAD^+, whereas NAD^+ is an oxidant involved in the glycolysis and citric acid cycle,[38] these processes enhance the cyclic utility of the NAD^+/NADH. Recent data have demonstrated that ATP levels affect NOX4 activity since it contains an ATP-binding site.[49] ATP can directly bind and negatively regulate NOX4 activity, suggesting that NOX4 is an energy sensor that becomes activated with decreased mitochondrial ATP.[49] MicroRNAs also regulate the expression of the $P47^{phox}$ subunit of NOX through Dicer (ribonuclease III), a key enzyme of microRNA biogenesis.[50,51] Lack of Dicer activity will reduce basal superoxide production through reduced expression of $p47^{phox}$.[50] However, the microRNAs that regulate the expression of $P47^{phox}$ remain unclear.

The XO, distributed on both the extracellular and intracellular surface of myocytes, is widely recognized as a key enzyme in purine metabolism, and its function includes catalyzing the oxidation of hypoxanthine to xanthine and subsequently oxidizing xanthine to uric acid.[52] Additionally, this oxidative process is accompanied by the generation of superoxide.[52] Meanwhile, XO can also reduce nitrate or nitrite to nitric oxide (NO) when endothelial nitric oxide synthase (NOS) activity is diminished.[53] Moreover, recent studies have confirmed that XO and NOXs are primary extracellular and cytoplasmic $O_2^{\cdot-}$/H_2O_2 production sites during exercise.[9,10]

The lipid peroxidation process generates several lipid peroxides, and an optimum concentration can stimulate skeletal muscle adaptation.[54] However, higher lipid peroxidation levels lead to pathological outcomes that may impede or compromise skeletal muscle adaptation during exercise training.[55] Several oxidases, such as cyclooxygenases (COXs), cytochrome p450s (CYPs), lipoxygenases (LOXs), and phospholipase A_2 (PLA_2), participate in the process of lipid peroxidation, which creates lipid-peroxyl and alkyl radicals ($ROO^{\cdot}$, $RO^{\cdot}$), lipid peroxides (ROOH), $O_2^{\cdot-}$, and H_2O_2.[56] The COXs synthesize lipid peroxides and are partially responsible for the peroxidation of linoleic acid, whereas CYPs synthesize epoxyeicosatrienoic acids (EETs), and LOXs are major contributors to the generation of lipid hydroperoxides.[56] Meanwhile, the COXs and the LOXs catalyze arachidonic acid to produce eicosanoid inflammatory factors such as prostaglandin, leukotriene, and thromboxane;[57] Simultaneously, the production of superoxide can also occur during these processes.[58] The PLA_2 family facilitates the hydrolysis of membrane glycerophospholipids, forming arachidonic acid, which serves as a substrate for COXs and LOXs.[59] The Ca^{2+}-dependent PLA_2 ($cPLA_2$) has been detected along the sarcolemma and within the mitochondria, while the Ca^{2+}-independent $iPLA_2$ isoform resides within the cytosol. The inhibition of cPLA2 significantly reduces ROS in contractile muscle compared with untreated control samples.[60] Furthermore, it has been postulated that arachidonic acid interacts with the mitochondrial ETC in the diaphragm, likely at complex I, to generate H_2O_2.[61] Meanwhile, PLA2 can also stimulate NOXs to generate ROS.[62] A recent study showed that $cPLA_2$ could be activated by phosphorylation of c-Jun N-terminal kinase (JNK) by activating the κ-opioid receptor.[63] In addition to the previously mentioned enzymes involved in ROS production, many other enzymes can also produce ROS.[5] These enzymes comprise aldehyde oxidase, amine oxidase, hydroxy acid oxidase, cytochrome *c* oxidase, endoplasmic reticulum oxidoreductase 1 α/β, and other related enzymes. Table 1 summarizes the several oxidases involved in ROS production.

The original $O_2^{\cdot-}$ can be catalyzed by SODs to form H_2O_2,[64] which serves as an additional source of H_2O_2 (Figure 1). H_2O_2 is transported through cell membranes via aquaporins (AQPs) (especially AQP8).[65] There are five members of the AQP family, including AQP3, AQP5, AQP8, AQP9, and AQP11.[66] To date, there is no direct evidence indicating the release of H_2O_2 from mitochondria to the cytosol. In addition, NO, generated from NOS, can react with $O_2^{\cdot-}$ to form harmful peroxynitrite ($ONOO^-$). It can further react with CO_2 to form nitrosoperoxycarbonate ($ONOOCO_2^-$), which then homolyzes to generate carbonate ($CO_3^{\cdot-}$) and nitrogen dioxide radicals ($^{\cdot}NO_2$).[67] In addition, $O_2^{\cdot-}$ and H_2O_2 can further react with lipids to form ROOH or $ROO^{\cdot-}$ (Figure 1). As previously discussed, those new-formed free radicals are byproducts that arise during physiological or pathological processes and have the potential to cause oxidation to lipids, proteins, and nucleotides.[6] Thus, they appear to be detrimental to physiological adaptation, including exercise. Under these conditions, exogenous antioxidants are necessary to neutralize them.

Table 1. Primary oxidases for ROS generation in skeletal muscle

Name	Abbreviation	Location	Product
Xanthine dehydrogenase/oxidase	XO	EC, C	$O_2^{\cdot-}$
NADPH oxidase 1	NOX1	PM	$O_2^{\cdot-}$
NADPH oxidase 2	NOX2	PM	$O_2^{\cdot-}$
NADPH oxidase 3	NOX3	PM	$O_2^{\cdot-}$
NADPH oxidase 4	NOX4	ER, PM, N	$O_2^{\cdot-}/H_2O_2$
NADPH oxidase 5	NOX5	ER	$O_2^{\cdot-}$
Dual oxidase 1	DUOX1	PM	H_2O_2
Dual oxidase 2	DUOX2	PM	H_2O_2
Cytochrome p450s	CP450	ER, M	$O_2^{\cdot-}/H_2O_2$
Cyclooxygenase1	COX1	C	$H_2O_2/ROO^{\cdot-}/ROOH$
Cyclooxygenase2	COX2	C	$H_2O_2/ROO^{\cdot-}/ROOH$
Lipoxygenases	LOX	C	$H_2O_2/ROO^{\cdot-}/ROOH$
D-Amino acid oxidase	DAO	Ps	H_2O_2
L-Amino acid oxidase	LAO	L	H_2O_2
D-Aspartate oxidase	DDO	Ps	H_2O_2
Sulfite oxidase	SUOX	M	H_2O_2
Spermine oxidase	SMOX	C, N	H_2O_2
Sulfhydryl oxidase 1	QSOX1	G	H_2O_2
Sulfhydryl oxidase 2	QSOX2	N, PM, S	H_2O_2
FAD-linked sulfhydryl oxidase	FSO	C, M	H_2O_2
Endoplasmic reticulum oxidoreductase 1 α/β	ERO1A/B	ER	H_2O_2

Note: C, cytoplasm; EC, extracellular; ER, endoplasmic reticulum; G, Golgi apparatus; M, mitochondria; N, nucleus; PM, plasma membrane; Ps, peroxisome; L, lysosome.

CYSTEINE SITE OXIDATION AND EXERCISE-INDUCED ADAPTATION

ROS, especially H_2O_2, plays an essential role in redox signaling, which affects physiological processes, leading to changes in signaling outputs, enzyme activity, gene expression, and membrane and genome integrity.[5] Sulfhydryl groups on Cys are the primary targets for the oxidation or formation of disulfide bonds in several proteins.[68] Figure 3 presents the primary redox reaction of the sulfhydryl group on Cys sites.

It is estimated that about 10–20% of the 214,000 thiols in the cellular cysteine proteome are readily oxidized under aerobic conditions.[69] Xiao and colleagues have screened out more than 30,000 unique cysteine sites among 9,400 individual proteins, and they subsequently developed a comprehensive and quantitative map of the mouse cysteine redox proteome *in vivo*.[70] These cysteine sites are distributed in enzymes, transporters, receptors, and transcription factor regulatory sites, as well as allosteric and macromolecular interaction sites, which can be the initial triggering of redox signals in distinct tissue.[71] There is still limited literature concerning ROS-induced thiol modification for triggering adaption signals during exercise training. However, oxidative modification of the cysteine site of proteins should be one of the principal initial adaptational signaling modes, such as Cys-299 and Cys-304 on AMP-activated protein kinase (AMPK) α subunit (AMPKα)[72] and Cys-185 and Cys-277 on Src kinase.[73] Thus, redox signaling based on thiol sites is intricate, and the precise modulations in physiological signaling transductions and function changes, still need further investigation.

Currently, there is no precise data available that accurately describes the concentration of H_2O_2 either inside or outside cells. It's worth noting that the concentration of physiological H_2O_2 varies across different tissues and cell compartments. Sies et al. estimated that intracellular H_2O_2 concentration in most cells is maintained in the low nanomolar range, typically between 1 and 10 nM, with a several-fold increase observed under stress conditions.[5] Meanwhile, the available data on extracellular H_2O_2 are far from unequivocal, and the probable normal range for plasma H_2O_2 concentrations is approximately 1–5 μM.[74] Thus, there is a gradient of H_2O_2 concentration between extracellular and intracellular spaces. However, few studies have evaluated possible H_2O_2 concentrations specifically in skeletal muscle fibers at rest and/or during exercise. Palomero et al.[75] observed that 15 min contractile activity in isolated muscle fibers increased H_2O_2 levels, which were detected using 5- (and 6-) chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-DCFDA); However, the generated H_2O_2 was lower than 1 μM or potentially as low as 0.1 μM.

During protein synthesis, individual disulfide bonds are formed by protein disulfide isomerases, and one hydrogen peroxide is generated through the reoxidation of protein disulfide isomerases catalyzed by endoplasmic oxidoreduction 1 (ERO1).[76] Hence, H_2O_2 is a by-product of protein folding, and it has been estimated that about 700 nM of H_2O_2 surrounds the endoplasmic reticulum (ER).[77] Overall intracellular H_2O_2 was estimated to be in a range of 1–10 nM, cytosolic H_2O_2 was estimated to be 80 pM, and the amount of H_2O_2 contained in the mitochondrial

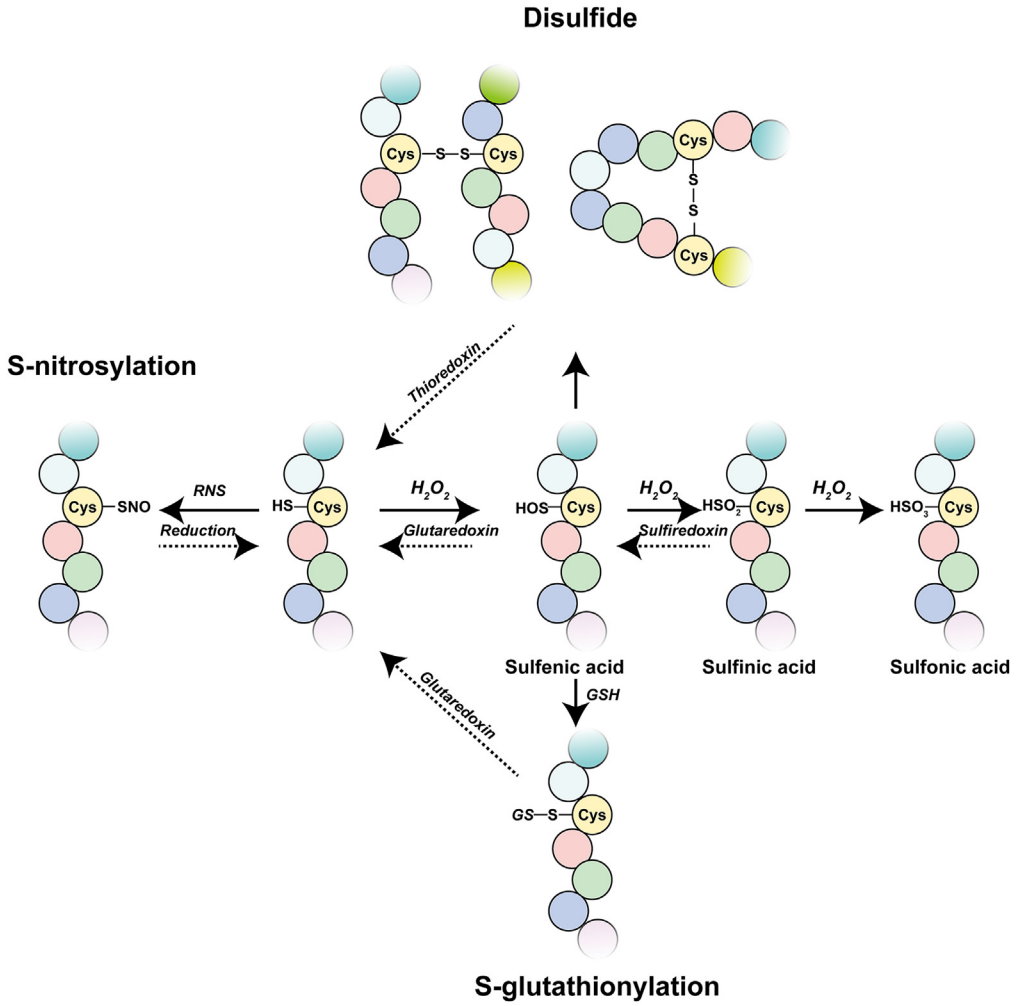


Figure 3. The modification of redox-sensitive cysteine residues

Thiol groups on the modifiable cysteine residues can be oxidized to generate groups of sulfenic acid, sulfinic acid, and sulfonic acid by H_2O_2. The sulfenic and sulfinic acid groups can be reduced to the thiol group through sulfiredoxin and glutaredoxin. The sulfenic acid group on the cysteine residues facilitates the formation of disulfide bonds, which can also be reduced to the thiol group through thioredoxin. The thiol group is nitrosylated as it touches reactive nitrogen species (RNS). However, the oxidized sulfonic acid group is irreversible.

matrix was estimated to be 5–20 nM. Furthermore, a recent estimate showed that approximately 45% of net myoblast H_2O_2 production was from the ETC, and around 45% from NOXs, with the remainder being provided from other enzymatic sources.[78] Thus, mitochondria and NOXs are the primary ROS producers. However, the amount of ROS generation in skeletal muscle in different stages (timing) of exercise is still unclear. In addition, it can be postulated that the generation of $O_2^{\cdot-}/H_2O_2$ during exercise may contribute to the activation of energy metabolism, while the sustained presence of $O_2^{\cdot-}/H_2O_2$ post-exercise could potentially be linked to physiological adaptative processes. The optimum amount of ROS leading to exercise-induced adaptation is termed oxidative eustress; however, an excessive accumulation of ROS can lead to physiological damage or a pathological state, which is referred to as oxidative distress. Sies et al.[5] estimated the physiological response to ranges of H_2O_2, which may also be applicable to exercise-induced adaptation. Figure 4 shows estimated ranges of H_2O_2 concentration which is associated with different physiological states. Quiescent physiological metabolisms, such as proliferation, differentiation, migration, and angiogenesis, require only minimal H_2O_2. Subsequently, elevated H_2O_2 induces adaptational responses to exercise, in which stress sensors such as AMPK, sirtuins (SIRTs), and PGC1α are activated to initiate the transcriptional gene process. Further, higher levels of H_2O_2 generated during exercise can lead to DNA damage, inflammation, neuronal degeneration, and muscle damage. Moreover, higher levels of H_2O_2 exceeding physiological normality may lead to cell death.

PRIMARY MECHANISMS INVOLVED IN MAINTAINING REDOX BALANCE

Reaching and maintaining a redox balance for humans or animals is essential during exercise, and a series of antioxidant enzymes and substances are involved in maintaining redox balance. Table 2 lists the primary antioxidant enzymes and their distribution in the distinct cellular

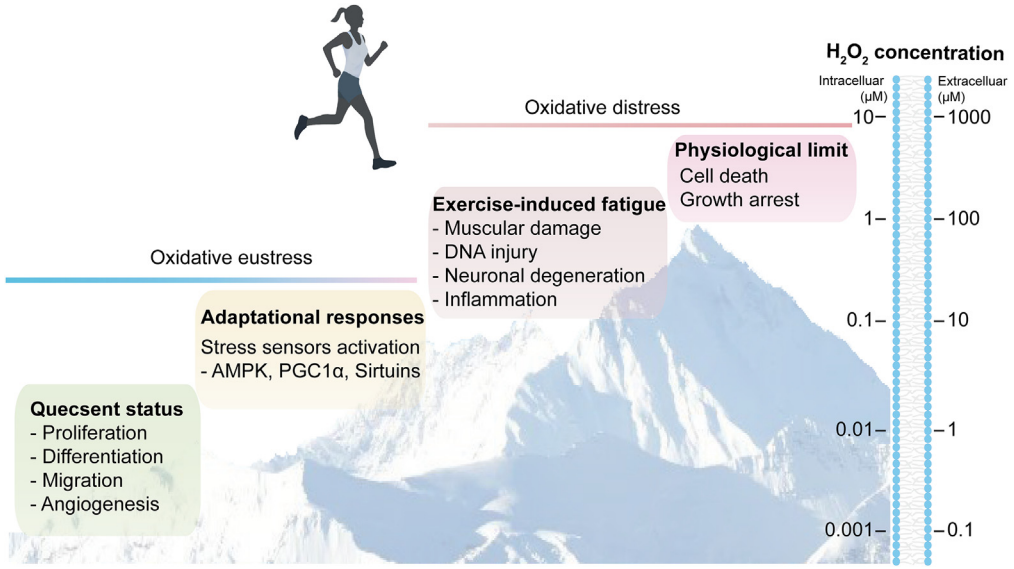


Figure 4. Estimated range of H_2O_2 concentration in exercise-induced adaptation

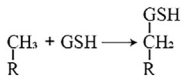
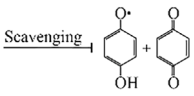
A minimum amount of H_2O_2 (0.01–0.1 μM) is essential for sustaining normal physiological metabolism. A higher concentration of H_2O_2 (0.1 ~ >1 μM) can cause a state of eu/distress. A higher exposure results in inflammation, muscular injury, cell death, and growth arrest. Since there is no precise data available for H_2O_2 concentration during exercise, the elevated H_2O_2 level was estimated based on published studies.[5,75] An estimated 100-fold concentration gradient from extracellular to intracellular is provided for rough orientation. This gradient increases up to 500-fold when considering 5 μM H_2O_2 concentration in blood plasma.[74] The gradient will vary depending on the cell type, intracellular location, and enzymatic sink activity.

compartments and tissues. Modulation of redox balance is performed via a group of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), thioredoxin (Trx), thioredoxin reductase (TrxR), and peroxiredoxin (Prx).[62,68] There are three isoforms of SOD, including SOD1, SOD2, and SOD3, all of which have redox-active transition metals on the active site. SOD1 resides in the cytosol and the mitochondrial intermembrane space, SOD2 in the mitochondria, and SOD3 in the vascular space. The function of SOD is to dismutate $O_2^{\cdot-}$ to form H_2O_2. In addition, CAT is a homotetramer molecule that consists of four subunits, mainly localized in peroxisomes. The role of CAT is to catalyze the dismutation of H_2O_2, resulting in the formation of H_2O and O_2. In addition, mammals have eight isoforms of GPx (GPx1–8), among which only GPx1–4 is involved in muscular metabolism.[79] GPx1 is distributed in the cytosol, nucleus, and mitochondria; GPx2 accumulates in the cytosol and nucleus; GPx3 is a secreted protein found in the cytosol, whereas GPx4 exists in the nucleus, cytosol, and mitochondria and is bound to membranes.[80] GPx5 lacking selenocysteine in the active site, is secreted in male epididymis,[79] GPx6 is found in the epithelium,[80] while GPx7 and GPx8 are involved in protein folding and reside in the ER.[76,81] These enzymes utilize glutathione (GSH) to neutralize ROS, generating oxidized glutathione GSSG. GSSG can be reduced back to GSH by glutathione reductase 1 (GR1).[82,83] Although eight isoforms of GPx were found in the distinct cellular compartments or tissues, the precise regulation of these GPx isoforms under different conditions, especially in exercise adaption, is still unclear.

The thioredoxin system, consisting of NADPH, thioredoxin (Trx), and TrxR, is a crucial disulfide reductase system, critical for protein synthesis and defense against oxidative stress.[84] These enzymes primarily reduce oxidized thiol groups on the cysteine residue of the protein through its disulfide reductase activity, regulating protein dithiol/disulfide balance, which is involved in signal transduction and activation of many redox-sensitive transcription factors.[84] There are two isoforms of Trx and TrxR. The Trx1-TrxR1 isoforms are expressed in the cytosol, and Trx1 can be translocated into the nucleus to change the redox state of transcription factors. Trx2 and TrxR2 play a redox-modulating role in the mitochondria. Glutaredoxin (GRx) is a member of the thioredoxin family, which has the same physiological function as Trx1 and Trx2 and is involved in redox signaling stimulation and sustaining the status of Fe/S protein.[85] Moreover, the GSH and GRx are considered a backup of TrxR to transmit electrons to oxidized Trx.[86] On the other hand, the thioredoxin system can also reduce GSSG back to GSH.[87] In addition, thioredoxin-interacting protein (Txnip) is an inhibitor of Trx, in which Cys-63 and Cys-247 residues can form a stable disulfide-linked bond with Trx active site thiols, to suppress the activity of Trx leading to a state of oxidative stress.[88] In mitochondria, Txnip is a critical regulator of glucose metabolism as Txnip-KO reprograms glucose metabolism to glycolysis and releases more Trx2 to scavenge ROS.[89]

The peroxiredoxin system, composed of six isoforms (Prx I–VI), is also an essential contributor to the maintenance of redox balance.[90,91] Prx I, II, and VI are distributed in the cytosol. Prx III is located in mitochondria, Prx IV is situated predominantly in the ER, and Prx V is located within the cytosol, mitochondria, and peroxisomes.[91] Prx can reduce H_2O_2 and organic hydroperoxides as well as peroxynitrite. The thiol groups on cysteine residues of Prx play a critical role in scavenging ROS, but the oxidized Prx requires Trx-TrxR assistance to be reduced to the original Prx.[91,92] Electron transmission among these antioxidant enzymes is illustrated in Figure 5.

Table 2. Antioxidant enzymes, distribution, and function

Name	Abbreviation	Location	Function
Superoxide dismutase			
Superoxide dismutase 1	SOD1	C, N, M	
Superoxide dismutase 2	SOD2	M	$O_2^{\cdot-} \rightarrow H_2O_2$
Superoxide dismutase 3	SOD3	EC	
Glutathione system			
Glutathione peroxidase 1	GPx1	C, N, M	
Glutathione peroxidase 2	GPx2	C, N	
Glutathione peroxidase 3	GPx3	EC	$H_2O_2 \rightarrow H_2O$
Glutathione peroxidase 4	GPx4	C, N, M	ROOH → ROH
Glutathione peroxidase 5	GPx5	C	$ONOO^- \rightarrow NO_2^-$
Glutathione peroxidase 6	GPx6	M	
Glutathione peroxidase 7	GPx7	ER	
Glutathione peroxidase 8	GPx8	ER	
Glutathione reductase	GR	C, N, M	GSSG → GSH
Glutathione S-transferases	GST	C, N, M, ER	
Thioredoxin family			
Thioredoxin 1	Trx1	C, N, M	
Thioredoxin 2	Trx2	M	$H_2O_2 \rightarrow H_2O$
Glutaredoxins	GRx	C, N, M	R-S-S-R → 2R-SH
Thioredoxin reductase 1	TrxR1	C, N, M	
Thioredoxin reductase 2	TrxR2	M	
Peroxiredoxin family			
Peroxiredoxin 1	Prx1	C, N	
Peroxiredoxin 2	Prx2	C, M	$H_2O_2 \rightarrow H_2O$
Peroxiredoxin 3	Prx3	M	$ONOO^- \rightarrow NO_2^-$
Peroxiredoxin 4	Prx4	ER	ROOH → ROH
Peroxiredoxin 5	Prx5	M, Ps	
Peroxiredoxin 6	Prx6	C, N, EC	
Other antioxidant enzymes			
Catalase	CAT	Ps	$H_2O_2 \rightarrow O_2 + H_2O$
Ferritin heavy chain 1	FHC1	C	$H_2O_2 \rightarrow H_2O$
Metallothionein-3	MT3	C	$H_2O_2 \rightarrow H_2O$ $O_2^{\cdot-} \rightarrow H_2O$ Metal detoxification
NADPH quinone oxidoreductase 1	NQO1	C, M	 $O_2^{\cdot-} \rightarrow H_2O$
Heme oxygenase 1	HO-1	C	$H_2O_2 \rightarrow H_2O$

Note: C, cytoplasm; EC, extra cell; ER, endoplasmic reticulum; M, mitochondria; N, nucleus; Ps, peroxisome.

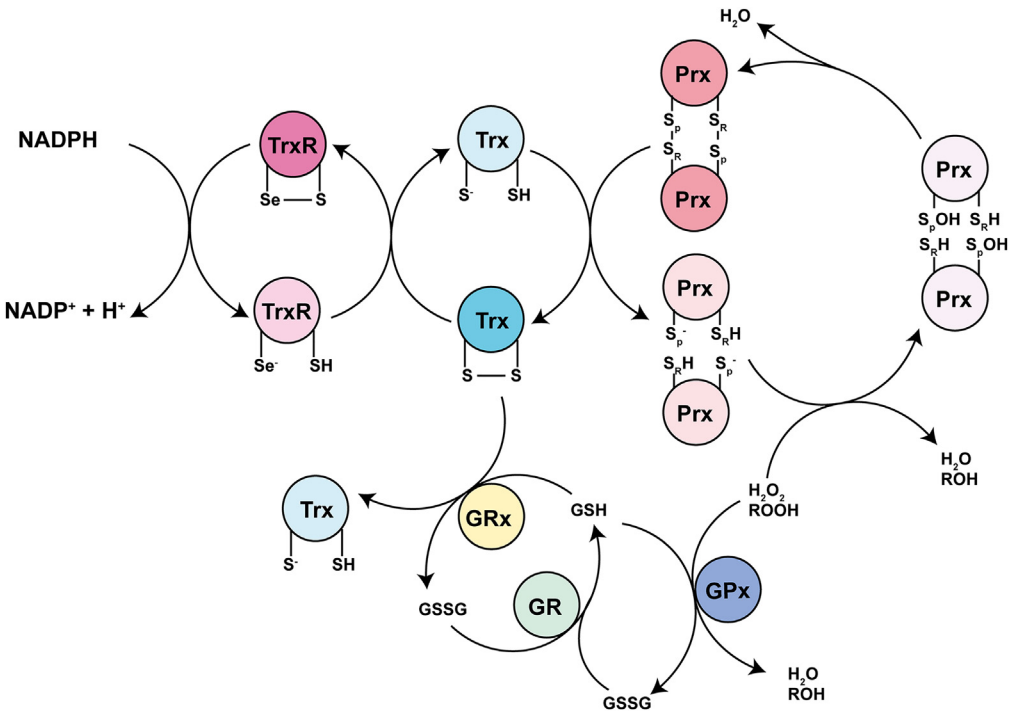


Figure 5. Scheme of the reaction mechanism of typical 2-Cys peroxidase

Under neutral pH conditions, deprotonated CysP reacts with H_2O_2 or ROOH via a nucleophilic attack to form CysP sulfenic acid with their lease of water or ROH. Then, the C-terminal resolving CysR in the other subunit will react with CysP sulfenic acid and form an intermolecular disulfide bond. The Trx and glutathione systems (GSH, GPx, GR, and GRx) participate in the reduction of disulfide bonds to restore their active form.

Meanwhile, the efficacies of Prx (10^7-10^8 $M^{-1}s^{-1}$)[93] in scavenging H_2O_2 are similar to those of well-known antioxidant enzymes such as GPx (10^8 $M^{-1}s^{-1}$)[94] and CAT (10^7 $M^{-1}s^{-1}$).[95] However, the specific protein levels of these three cysteinyl antioxidant enzymes (Trx/Prx/GPx) in skeletal muscle cells (quiescent or exercise) are still unknown. Prxs may play a role in precisely adjusting H_2O_2 levels to meet physiological needs/demands and regulate signal transduction.[96] The growing evidence indicates that Prxs play a critical role in regulating H_2O_2 as a second messenger in receptor-mediated signaling.[97] Methionine sulfoxide reductase (MSR) is another antioxidant enzyme that obtains electrons from the Trx system.[98,99] Free methionine and the methionine residue of a protein can be oxidized to methionine sulfoxide under oxidative stress, and this oxidation can change the protein function. MSR can catalyze the free and protein-bound S- and R-methionine sulfoxide back to methionine.[100,101]

Secondary antioxidant proteins such as ferritin heavy chain (FHC), glutathione S-transferase (GST), and metallothionein-3 (MT3) are also salient for ROS regulation. FHC does not directly scavenge ROS but protects the cell from oxidative damage by preventing the iron-mediated generation of highly reactive $^{\cdot}OH$ from H_2O_2 (Fenton reaction).[102] In addition, glutathione S-transferases (GSTs) contribute to repairing the damage from oxidative stress by catalyzing the GSH thiolate to toxic electrophilic compounds, thus allowing the scavenging of highly reactive carcinogens or radicals.[103] MT3 is involved in regulating metal toxicity and scavenging $O_2^{\cdot-}$ and H_2O_2.[104] Antioxidant enzymes are distributed in the plasma or different cellular compartments, preventing oxidative damage or modulating redox balance. They may also play differential roles in maintaining physiological function. We summarize the main antioxidant enzymes and their functions in Table 2.

MAIN PATHWAYS IN THE REGULATION OF REDOX BALANCE

Humans and animals usually store enough endogenous antioxidants to deal with free radical-mediated damage. Nevertheless, an increased exercise intensity or volume will generate additional free radicals; thus, the body needs to synthesize more antioxidant enzymes and chemicals to neutralize oxidative stress (Figure 6). Nuclear factor erythroid 2-related factor 2 (Nrf2) is a master antioxidant regulator, exerting the transcription of many antioxidant enzymes in response to oxidative stress. Many genes possessing the antioxidant response element (ARE, 5′-TGACNNNGC- 3′) are downstream targets of Nrf2.[105] During exercise, the organism will generate more ROS, which will directly oxidize the cysteines located on the surface of Kelch-like ECH-associated protein 1 (Keap1) and then trigger the Nrf2 pathway to synthesize more antioxidant materials with neutralizing effects.[106] Frequent exercise-induced ROS stimulation promotes increased antioxidant capacity and reaches a different state of homeostasis. Many genes regulated by Nrf2 participate in exercise adaption, such as GST, NAD(P)H quinone oxidoreductase-1 (NQO1), GPx2, and heme oxygenase-1 (HO-1).[107] Moreover, activated Nrf2 is related to GSH synthesis by modulating the expression of glutamate-cysteine ligase complex (GCL), which consists of the catalytic subunit (GCLC) and the modifier subunit

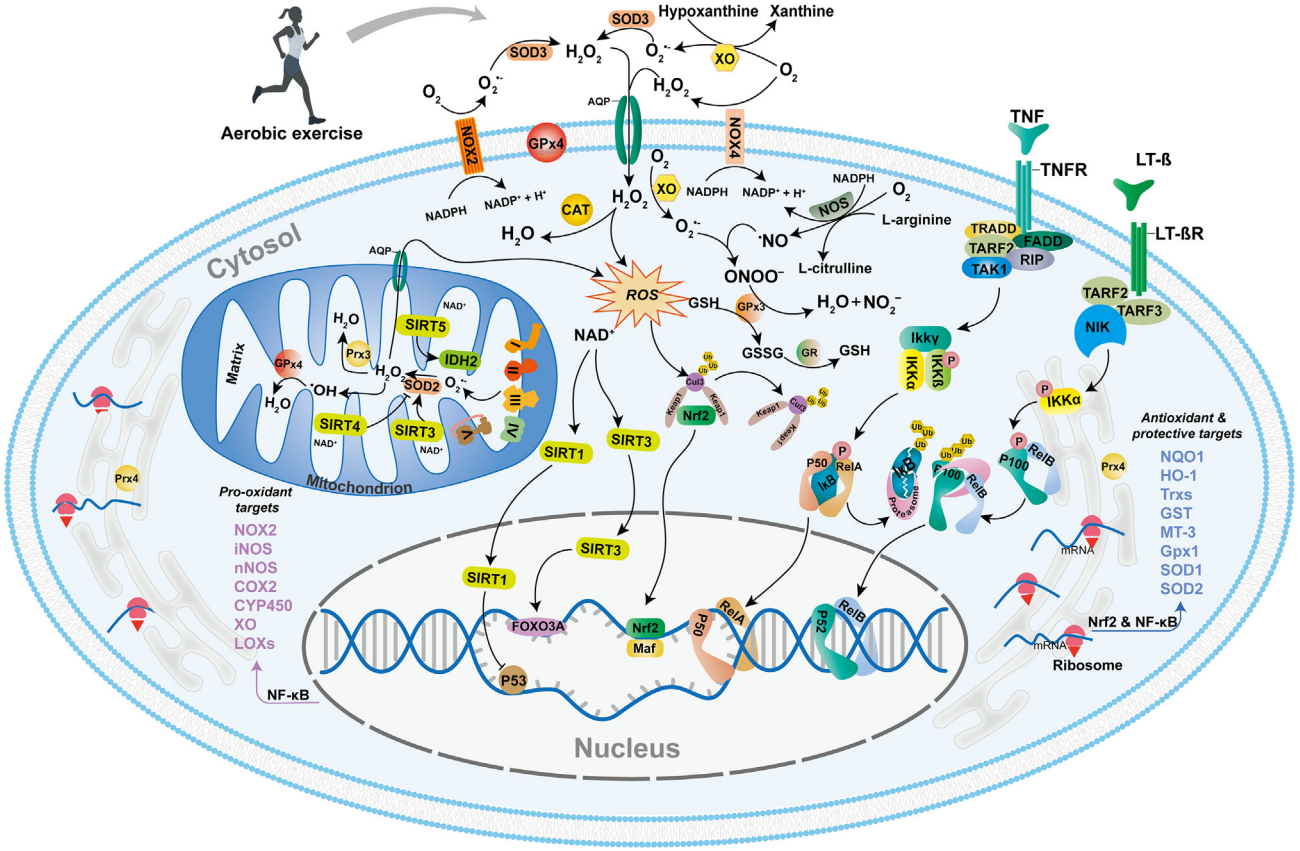


Figure 6. Modulation of ROS through Nrf2 and NF-κB during aerobic exercise

ROS are generated through oxidases such as NOX2, NOX4, XO, or ETC. In homeostasis, Nrf2 is anchored by Kelch-like ECH-associated protein 1 (Keap1), a substrate adaptor for the cullin 3-RING-box protein 1 ubiquitin ligase (Cul3) that induces Nrf2 to degradation by ubiquitination. The keap1 is a cysteine-rich protein, which can be oxidized by ROS, that changes its conformation and detaches Nrf2.[111–113] The released Nrf2 heterodimerizes with musculoaponeurotic fibrosarcoma proteins (Maf proteins). They subsequently bind to the antioxidant response element (ARE) and finally activate the transcription of many antioxidants and detoxifying enzymes.[113,114] NF-κB is activated primarily by two pathways. The canonical NF-κB pathway is triggered by stimulating pro-inflammatory receptors, such as the TNF receptor family, toll-like receptor family, and cytokine receptors for interleukins. Activating TNF receptors (TNFR) leads to the binding of TNFR1-associated death domain protein (TRADD), which will further recruit the Fas-associated death domain (FADD) and TNF receptor-associated factor 2 (TRAF2). TRAF2 forms a complex with receptor-interacting proteins (RIP1), leading to the activation of the NF-κB inhibitor (IκB) kinase (IKK) complex, which comprises two catalytic kinases, IKKα and IKKβ, and a regulatory subunit, IKKγ. p50 is initially inactive as a heterodimer with RelA by interacting with an inhibitory IκB protein. Phosphorylation of IκB on serines 32 and 36 by the activated IKK (primary IKKβ) targets it for ubiquitination. The ubiquitinated IκB is further degraded by proteosomes and disassociates the p50/RelA heterodimer, further translocating into the nucleus and binding to κB sites for specific gene transcription.[115,116] In the noncanonical pathway, the TNF family cytokines, such as lymphotoxin-β (LTβ), activates the LTβ receptor (LTβR), which subsequently recruits TRAF2 and TRAF3, resulting in NF-κB-inducing kinase (NIK) activation. NIK subsequently activates IKKα, which leads to the phosphorylation of p100, resulting in its heterodimerization with RelB. The phosphorylated P100 facilitates its ubiquitination and is processed into a P52-RelB heterodimer, translocating to the nucleus for gene transcription.[116]

(GCLM).[108,109] Nrf2 also activates the expression of many enzymes for NADPH regeneration.[110] In addition, both Trx1 and TrxR1 genes that possess ARE are transcriptionally regulated by Nrf2.[84]

Additionally, the Nrf2-keap1 system is regulated by the TrxR1 and Sirtuin (SIRT) family of NAD^+-dependent deacetylase. A recent study showed that SIRT1 deacetylates Nrf2, leading to its nucleus export and decreased binding of Nrf2 to ARE element,[117] suggesting that SIRT1 is a negative modulator to Nrf2, inhibiting Nrf2-dependent gene transcription. On the contrary, acetylation of Nrf2 by cAMP response element-binding protein (CREB)-binding protein (CBP) promotes Nrf2 nucleus retention.[118] Additionally, the activation of PGC1α by SIRT1 leads to the upregulation of Nrf2 transcription.[119] Meanwhile, Bach1 is an association of small Maf proteins that act as a competitive transcriptional repressor for Nrf2.[120] Some reports have demonstrated that microRNAs can downregulate Bach1 transcription.[121–123] The microRNAs let-7b, let-7c, and miR-98 have been shown to decrease Bach1 expression in the human hepatoma Huh-7 cell line. However, these microRNAs have not been studied in skeletal muscle cells; thus, verifying this assumption in future work is required.

Moreover, redox-sensitive microRNAs are involved in regulating Nrf2.[124] Narasimhan et al. demonstrated that ectopic expression of each of the four microRNAs miR-144, miR-153, miR-27a, and miR-142-5p directly downregulates Nrf2 in neuronal cells, resulting in a reduction in GSH synthesis.[125] A further study showed that microRNA miR200a inhibits the expression of Keap1 in human breast cancer cells, leading to enhanced Nrf2 activation and ARE-mediated antioxidant gene expression.[126] However, there are still a limited number of studies investigating the regulatory role of these microRNAs in Nrf2 in skeletal muscle, and many biochemical puzzles remain that require solutions.

Another essential antioxidant regulator is NF-κB, a critical component in modulating hundreds of genes implicated in cell growth, differentiation, development, and apoptosis.[116] NF-κB has five family members that bind as a homodimer or heterodimer to 10-base pair κB sites. These members contain the Rel-homology domain essential for DNA binding and dimerization.[115] The three Rel members, RelA (p65), RelB, and c-Rel, have a C-terminal transcriptional activation domain (TAD) that positively regulates gene expression. Two precursors, P105 and P100, can be processed to the active forms P50 and P52, respectively (Figure 6). These two proteins lack a TAD, so their activation needs to heterodimerize with one of the Rel proteins.[115,116] The diverse combinations of homo- or hetero-dimer of NF-κB confer their ability to varying affinities to bind κB sites in distinct DNA sequences. Although, with a few exceptions, NF-κB stimulates cell death, in most cases, the expression of NF-κB target genes promotes cell survival.[127]

NF-κB has two regulatory pathways that are referred to as canonical and noncanonical pathways (Figure 6). NF-κB plays a dual role in regulating ROS levels by stimulating the expression of distinct enzymes, which has been comprehensively reviewed by Morgan et al.[115] We briefly introduced the two-sided regulatory pathways of NF-κB. The activation of NF-κB can promote ROS generation by expressing specific oxidases, such as NOX2, inducible or neuronal NO synthase (i/nNOS), cyclooxygenase-2 (COX2), XO, and cytochrome p450 (CYP450), etc. In addition, there is evidence of controlling ROS generation by the NF-κB pathway through modulating c-Jun kinase (JNK), which leads to the inhibition or promotion of ROS production.[128] The activation of NF-κB can also decrease intracellular ROS levels by promoting the expression of many antioxidant enzymes, including SOD1/2, Trxs, metallothionein-3 (MT3), NQO1, HO-1, GPx1, and GST.[115] However, it is unclear what biochemical requirements are needed for distinct pathway activation during aerobic exercise.

ROS are also crucial for NF-κB signaling downstream of the tumor necrosis factor (TNF) pathway. Micromolar concentrations of H_2O_2 can activate NF-κB. However, it has been recognized that H_2O_2 may, in fact, not directly act as an inducer but more of a modulator involved in the NF-κB pathway.[129] Research indicates that mtROS can promote TNF-mediated NF-κB activation.[115,128] Inhibiting mtROS in monocytes and T cells using the mitochondria-specific antioxidant MitoVit E reduces NF-kB activation.[130] It has been suggested that mtROS are essential for NF-kB activation. Meanwhile, Trx1 is involved in regulating NF-κB by controlling oxidative stress. A previous study indicated that Trx1 blocks NF-κB inhibitor (IκB) degradation while, in the nucleus, it enhances NF-kB activity by increasing its affinity to DNA.[131]

Additionally, the dynein light chain (LC8), a component of the dynein motor complex, binds to IκBα (one of the IκB isoforms) in a redox-dependent manner. Oxidization of LC8 by exposure to ROS releases it from IkBα, leading to the activation of NF-kB.[132] Further studies have demonstrated that phosphorylation of Ser-276 on RelA is mediated by a ROS-dependent protein kinase A (PKA) pathway.[133] In the noncanonical pathway, NF-κB-inducing kinase (NIK) is activated by H_2O_2 following IL-1β treatment resulting in subsequent NIK-mediated phosphorylation of IκB kinase α (IKKα) and increased NF-κB activity.[134] ROS can also play a negative role in regulating the NF-κB pathway. A cysteine residue (Cys-62) in the Rel-homology domain of the p50 subunit is subject to oxidation, which decreases its binding affinity to DNA.[135] In addition, sustained oxidative stress may result in the inactivation of the proteasome and subsequently inhibit NF-κB activation by hampering the degradation of IκB.[136] A study by Korn et al. showed that H_2O_2 markedly decreased TNF-induced IKK activity, preventing IκB degradation and NF-κB activation.[137] Similar inhibition of IKK was observed with NO, which targeted the cysteine 179 residue on IKKβ.[138]

Insights into the transcriptional regulation between Nrf2-keap1 and NF-κB pathways have concluded that some of the antioxidant genes are modulated in an intersected way (Figure 6). For instance, SOD1, HO-1, NQO1, and GST are transcriptionally regulated by Nrf2 and NF-κB pathways. However, how these enzymes are transcribed under specific conditions is still unclear. During exercise conditions, the Nrf2-keap1 may be best positioned to activate the transcription of antioxidant genes for sustaining redox homeostasis. At the same time, NF-κB pathways are activated by TNF or other cytokines under distinct inflammatory conditions. However, the mechanism used is not entirely elucidated at this time.

REDOX MODULATIONS BY NAD^+-DEPENDENT SIRTUINS

The modulation of mitochondrial function is a highly complex process that consists of an array of networks. The NAD^+-dependent deacetylases, SIRTs, play an essential role in modulating biological processes.[139] The SIRT family has seven members (SIRT1–7), all having distinct roles in modulating cellular metabolism, such as oxidative stress response, cellular metabolism, glucose homeostasis, and insulin secretion.[140]

SIRT1 and SIRT3 are the most widely studied due to their critical function in redox modulation in mitochondria. SIRT1 seems essential for maintaining Nrf2 levels, as the knockout of SIRT1 inhibited the expression of Nrf2 and HO-1 in neuron cells;[141] however, this has not been verified in muscle cells. SIRT3 is one of the crucial regulators controlling mitochondria redox status. Deacetylation by SIRT3 promotes SOD2 to dismutate $O_2^{\cdot-}$ to H_2O_2.[142] Lee et al. showed that overexpression of SIRT3 mitigated oxidative stress-induced cell death and mitochondrial dysfunction in dopaminergic neurons and astrocytes.[143] In addition, SIRT3 modulates ETC by deacetylating all ETC complexes (including ATP synthase) to improve efficient electron transport, reducing ROS production and maximizing ATP production.[144] SIRT3 deacetylates mitochondrial uncoupling protein 1 (UCP1), promoting the dissipation of proton gradient across the inner mitochondrial membrane, which leads to the generation of heat instead of ATP.[145,146] Additionally, ROS can induce post-translational modifications to promote the activation of UCPs and subsequently trigger mitochondrial uncoupling.[147] Moreover, SIRT3 contributes to glutathione production by activating isocitrate dehydrogenase (IDH2), which catalyzes the oxidation of isocitrate to generate α-ketoglutarate and produce NAD(P)H.[148]

Further, NADPH is crucial in reducing GSSG to GSH[149] and serves as an electronic transmitter for NOS or NOXs to generate NO or $O_2^{\cdot-}$/H_2O_2.[150] SIRT3 was observed to be essential in promoting fatty acid oxidation by deacetylating long-chain acyl-coenzyme A dehydrogenase.[151] SIRT3 controls ROS by activating Forkhead box O3a (FOXO3a), inducing transcription of nuclear and mitochondrial genes involved in the antioxidant system, ETC function. Additionally, SIRT1 modulates FOXO3a to induce cell-cycle arrest and resist oxidative stress and cell death.[152,153] SIRT2 also deacetylates FOXO3a in response to oxidative stress and caloric restriction and subsequently elevates the expression of FOXO3a-targeted genes, such as $p27^{Kip1}$, SOD2, and pro-apoptotic factor Bim.[154] Another essential transcriptional factor in modulating antioxidant enzyme expression is p53, a well-known tumor suppressor negatively regulated by SIRT1.[155] Previous studies have investigated antioxidant pathways regulated by p53, including SOD2, GPx1, and the sestrins (Hi95 and PA26).[156,157] p53 upregulates antioxidant sestrins, which are essential for reducing oxidized peroxiredoxins.[158] Additionally, the downregulation of p53 promotes intracellular ROS and increases DNA oxidation and mutagenesis.[159] SIRT1 and SIRT2 inhibit NF-κB by deacetylating a subunit of NF-κB p65 to alleviate the inflammatory response, oxidative stress, and neurotoxicity.[160–162] Under oxidative stress conditions, SIRT2 has been shown to deacetylate and activate glucose-6-phosphate dehydrogenase, a key enzyme in the pentose phosphate pathway that produces NADPH in the cytosol. SIRT4, located in mitochondria, is involved in the regulation of ROS production. It was found that overexpression or knockdown of SIRT4 increased or decreased ROS levels, respectively, in mitochondria.[163] The authors found that SIRT4 inhibited the binding of SOD2 to SIRT3, resulting in increased acetylation and thereby reducing the activity of SOD2.[163] SIRT5 is expressed in the mitochondria, where it exerts an efficient protein lysine deacetylase, desuccinylase, and demalonylase.[164] It has been shown to play essential roles in fatty acid oxidation, urea cycle, oxidative stress, detoxification, and apoptosis.[165] SIRT5 plays a significant role in enhancing the activity of SOD1 by desuccinylation,[166] as well as deacetylating cytochrome *c*, promoting the efficiency of ETC.[167] Furthermore, the overexpression of SIRT5 in cells resulted in decreased levels of ROS, indicating that it is responsible for regulating antioxidant components within the cells.[168] SIRT6 is distributed in the nucleus of cells and has been shown to function as deacetylation of lysine 9 and 56 on histone 3 (H3K9 and H3K56), which is associated with the transcription of NF-κB dependent genes.[169] By increasing AMP/ATP, SIRT6 indirectly regulates the AMPK-FOXO3a axis, resulting in the expression of SOD2 and CAT.[170] SIRT6 also serves as a coactivator for Nrf2 via connection to the RNA polymerase II complex, promoting the expression of antioxidant or detoxifying enzymes.[171] SIRT7 is also distributed in the nucleus, and tissue expression was observed to be enhanced with higher metabolic activities, particularly in cardiomyocytes and skeletal muscle; however, decreased expression was found to be associated with age.[172] In addition, SIRT7 binds to histones, leading to the transcription of ribosomal DNA.[173] It also acts as a regulator in activating serine-threonine kinase receptor-associated protein (STRAP), a protein that helps sustain TGF-1β and p53 activities, promoting cell proliferation and maintaining redox homeostasis.[174] Figure 7 briefly outlines SIRT (1–7) involved in physiological modulation during aerobic exercise.

EXERCISE-INDUCED ADAPTIVE SIGNALING IN MUSCULAR REMODELING

Mitochondrial biogenesis and muscle fibril transition

Mitochondria are the leading power sources for generating ATP that supplies skeletal muscle, the nervous system, and basal cell metabolism.[175] Slow-twitch muscle fibers are rich in mitochondria, which enables them to sustain endurance activities by efficiently generating ATP through oxidative metabolism. Hence, mitochondrial biogenesis is intricately linked to the transition of muscle fibrils toward the slow-twitch phenotype during aerobic exercise. In this section, we discuss how aerobic exercise promotes mitochondrial biogenesis and muscle fibril transition. Figure 8 briefly summarizes the underlying processes of mitochondrial biogenesis and muscle fibril transition during aerobic exercise.

Numerous studies have demonstrated that aerobic exercise training enhances mitochondrial biogenesis.[176] Peroxisome proliferator-activated receptor gamma coactivator-1α (PGC1α) can promote the activation of nuclear respiratory factor 1 or 2 (NRF1/2), which subsequently stimulate the expression of mitochondrial transcription factor A (mTFA), leading to the transcript of many mitochondrial genes, such as cytochrome *c* oxidase (COX) II, COX IV, and ATP synthase, enzymes involved in electron transport and ATP synthesis.[177–179] Prior studies have shown that endurance training can promote PGC1α[180–182] and NRF1[183] expression and increase mTFA.[183–185] Previous findings have demonstrated that the contraction-induced generation of H_2O_2 in skeletal muscle leads to an upregulation of PGC1α.[186] Furthermore, Irrcher et al. observed that skeletal muscle cells treated with H_2O_2 decreased ATP levels, activated AMPK, and increased PGC1α mRNA, collectively suggesting that H_2O_2 improves PGC1α transcription through AMPK.[187] Concurrently, an increase in cellular lactate, a by-product of glycolysis, results in elevating H_2O_2, which can subsequently lead to upregulating PGC1α.[188] Furthermore, Henriquez-Olguin et al. demonstrated an immediate upregulation of NOX2 levels within the skeletal muscle of mice following a 60-min swimming exercise, accompanied by elevated mRNA level of mTFA and citrate synthase.[189] Brendel et al. found that NOX4-knockout mice reduce exercise-induced mitochondrial-related gene expression, including PGC1α, NADH dehydrogenases, and citrate synthase.[190] According to a recent report, NOX4 is essential for regulating PGC1α expression by promoting Nrf2-mediated redox balance.[41] Thus, optimum ROS have been suggested to be a vital factor for aerobic exercise-responsive PGC1α expression and metabolic adaption in skeletal muscle.

Many studies demonstrate that AMPK is a sensor of metabolic stress and nutrient deprivation and is also activated in contractile skeletal muscle.[191,192] It is recognized that AMPK is sensitive to AMP/ADP levels, which are the primary messengers for activating AMPK.[193,194] Liver kinase B1 (LKB1),[195] calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ),[196] and calcium/calmodulin-dependent protein kinase II (CaMKII)[197] are potential upstream regulators of the AMPK activator, capable of inducing phosphorylation of the $AMPK\alpha_{1/2}$ phenotype during exercise (Figure 8). However, it is still unclear which factor has dominant control over the activation of the AMPK complex, leading to the activation of the PGC1α pathway. Furthermore, previous studies have demonstrated that increased intracellular ROS levels can stimulate AMPK activity without a decrease in cellular ATP. This work has proved that the oxidation of cysteine 299 and 304 sites on AMPKα by

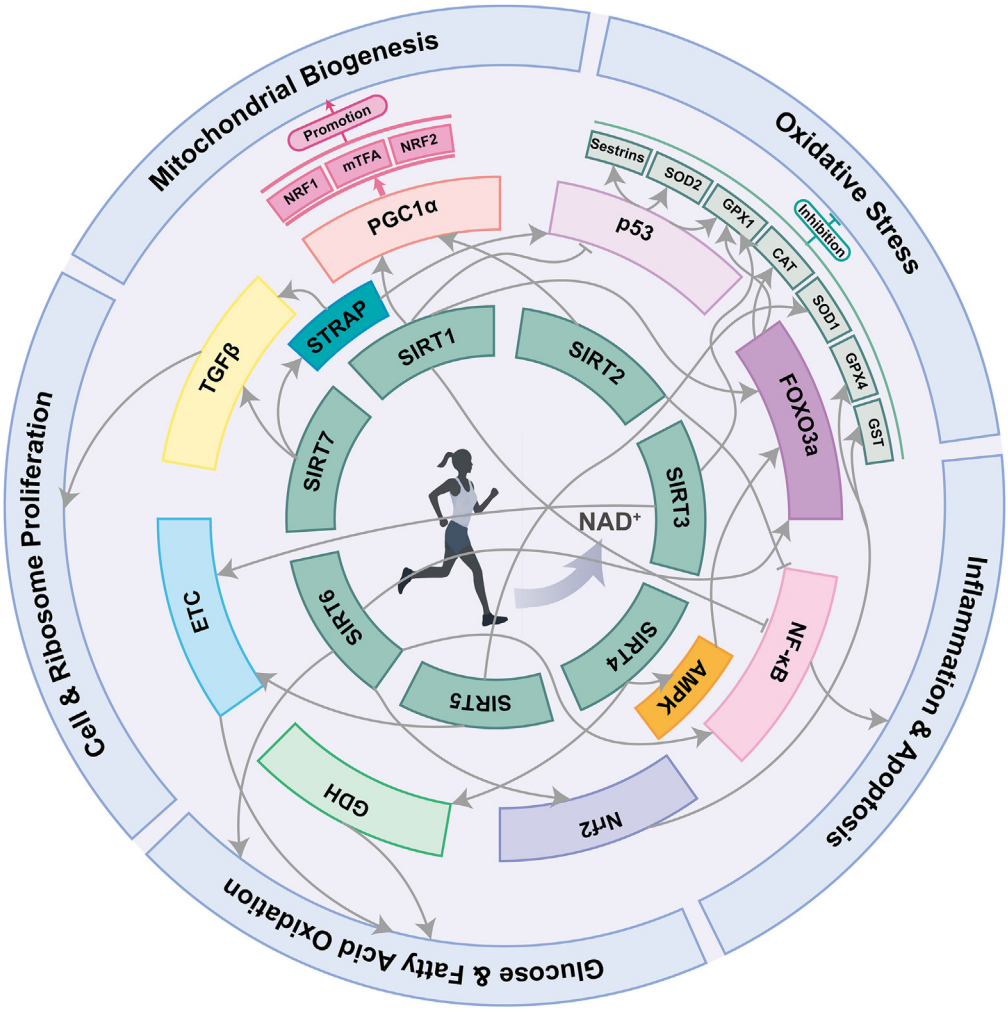


Figure 7. The modulatory functions of SIRT (1–7) involved in exercise-induced responses

SIRT (1–7) are involved in modulating mitochondrial biogenesis, oxidative stress, inflammation and apoptosis, glucose and fatty acid oxidation, and cell and ribosome proliferation through distinct pathways.

H_2O_2 can directly activate AMPK; alternative mutants in cysteine 299 or 304 resulted in diminished or abrogated AMPK activity.[72] However, excessive ROS results in the oxidation of Cys130/Cys174 in the AMPKα subunit, causing aggregation of AMPK through intermolecular disulfide bonds formation, which prevents Thr172-AMPKα phosphorylation by upstream kinases in cardiomyocytes.[198] Besides AMPK bridging between ROS and PGC1α, there is P38γ mitogen-activated protein kinase (P38γ MAPK), one of three isoforms of P38 involved in upregulating PGC1α transcription in skeletal muscle following endurance exercise.[199] Thus, the P38γ MAPK-PGC1α axis is one of the transduction pathways in starting mitochondrial biogenesis. Recently, Cho et al. found that PGC1α and ERR-induced muscle 1 regulator (Perm1) can promote CaMKII activation.[200] A knockdown of Perm1 showed defects in the activation of CaMKII and p38γ MAPK, and Perm1 knockdown muscles attenuated mitochondrial biogenesis following four weeks of voluntary exercise training.[200] Interestingly, one study showed that inhibition of cytosolic XO using allopurinol attenuated PGC1α expression and decreased PGC1α downstream factors, such as NRF-1 and mTFA.[201] Therefore, it is suggested that ROS are a critical chemical entity triggering mitochondrial biogenesis. However, what sources of ROS generated during exercise primarily affect mitochondrial biogenesis still needs further clarification.

As previously stated, the activation of PGC1α is mediated by SIRT1 through deacetylation,[202] which is dependent on the presence of NAD^+.[203,204] Besides, SIRT1 can be activated via phosphorylation in the catalytic domain by several kinases, such as cAMP-dependent PKA,[205,206] AMPK,[207] LKB1,[208] CaMMKβ,[209] protein kinase CK2,[210] and JNK1.[211] However, which kinase dominates SIRT1 phosphorylation in the context of aerobic exercise is required for further exploration. Simultaneously, under hypoxic conditions, the hypoxia factors 1α and 2α (HIF-1α and HIF-2α) can bind HIF-responsive elements located on the promoter of SIRT1 and trigger the transcription of SIRT1.[212] Furthermore, mice with muscle-specific knockout of SIRT1 did not increase PGC1α acetylation in the volunteer wheel-trained group compared with the control group,[213] suggesting that SIRT1 is not the only modulator for the PGC1α in exercise adaption. Krishnan et al. reported that SIRT2

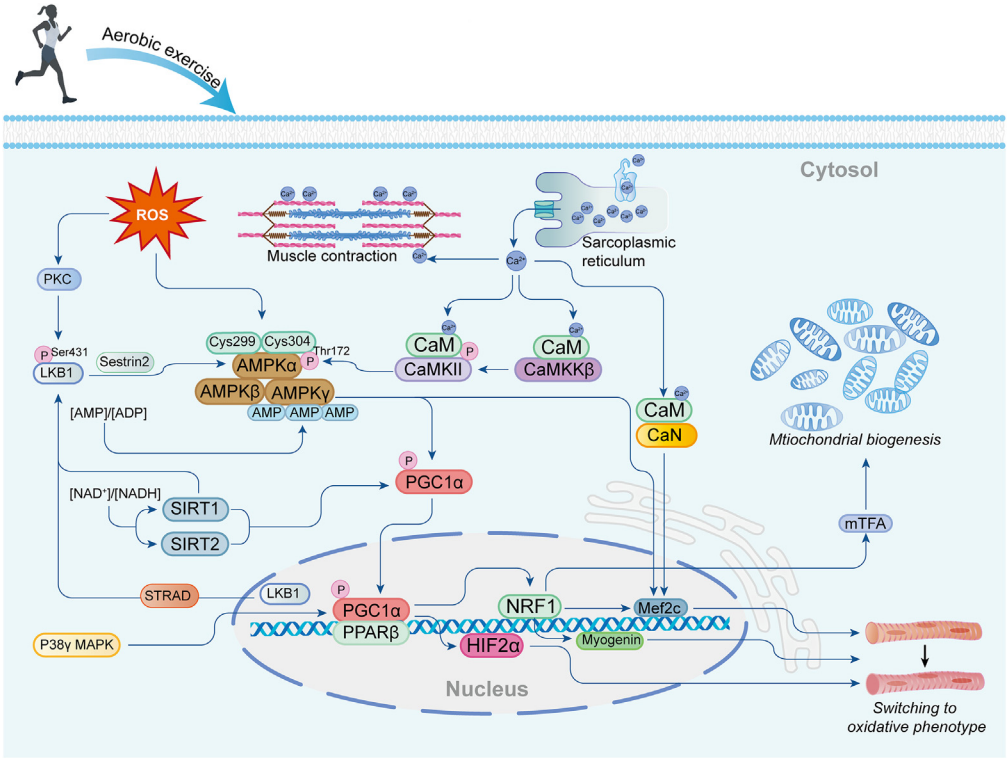


Figure 8. Pathways of exercise-induced adaptation in mitochondrial biogenesis and fibril switching

Physical activity can cause an increase in ROS, the release of calcium anions, and the elevation of the AMP/ATP ratio. The ROS (generally H_2O_2) and AMP can directly activate the AMP-activated protein kinase (AMPK) complex by oxidizing thiol groups of Cys 299 and 304 on AMPKα and binding to AMPKγ by AMP. STe20 Related Adaptor (STRAD) interacts with and phosphorylates LKB1, facilitating its translocation from the nucleus to the cytoplasm. Optimal levels of ROS induce the activation of protein kinase C (PKC), which in turn phosphorylates LKB1 at Ser431, leading to its activation. Furthermore, SIRT1 facilitates the deacetylation and subsequent activation of LKB1, while Sestrin2 plays a crucial role in enhancing the stability of the LKB1/AMPK complex. Subsequently, activated LKB1 phosphorylates AMPKα at Thr172, resulting in AMPK activation. The release of calcium ions from the sarcoplasmic reticulum during exercise, which binds to calmodulin (CaM), can induce the activation of CaMKKβ and CaMKII, subsequently leading to the activation of AMPKα by phosphorylation. The activation of the AMPK complex followingly stimulates PGC1α, which later combines with PPARβ to stimulate the transcription of NRF1 and HIF2α. The NRF1 starts the transcription of mTFA, Myogenin, and Mef2c. The mTFA is an essential transcription factor for mitochondrial biogenesis, and the Myogenin, HIF2α, and Mef2c stimulate to switch to slow muscle fibril.

could deacetylate PGC1α and activate PGC1α-mediated adipocyte fatty acid oxidation.[214] Therefore, SIRT1 and SIRT2 may synergistically regulate the PGC1α, subsequently activating mitochondrial biogenesis during exercise (Figure 8). Conversely, acetylation of PGC1α on the lysine site by general control of amino acid synthesis 5 (GCN5) inhibits its transcriptional activity.[215] It was recently suggested that peroxisome proliferator-activated receptor β (PPARβ) increases PGC1α by protecting it from degradation by binding to PGC-1α and limiting ubiquitination.[216] Additionally, knockout of PPAPβ leads to a decrease in PGC1α activity and attenuated mitochondrial biogenesis,[216] suggesting that PPAPβ should be essential for PGC1α in activating NRF1, leading to the transcript of the mTFA gene.

Mitochondrial fusion occurs in muscle cells to generate efficient energy. Aerobic exercise stimulation results in mitochondrial fusion, thereby enhancing mitochondrial efficiency. Mitofusin 1 and 2 (Mfn1 and 2) and optical atrophy 1 and 2 (Opa1 and 2) are key regulators of this process, with Mfn1 and 2 anchoring the outer mitochondrial membrane while Opa1 and 2 assist in inner membrane fusion.[217] Bell and his colleagues discovered that the deletion of Mfn1 and 2 in skeletal muscle impedes exercise performance.[218] In contrast, the association of dynamin with mitochondrial fission factor results in the induction of mitochondrial fission.[219] However, the mitochondrial fusion or fission mechanism during aerobic exercise still needs further elucidation.

Along with mitochondrial biogenesis in skeletal muscle, PGC1α serves as a key regulator in orchestrating the transition of muscle fibers toward a slow oxidative myogenic phenotype, characterized by the upregulation of myosin heavy chain (MyHC) 1 (encoded by *MYH7*), MyHC2a (encoded by *MYH2*), while concurrently suppressing the fast glycolytic isoforms of MyHC, namely MyHC2b and MyHC2x (encoded by *MYH4* and *MYH1*, respectively).[220] Transgenic overexpression of PGC1α results in the conversion to slow-twitch type I fibers.[178] Conversely, skeletal muscle-specific PGC1α knockout mice express more fast-twitch glycolytic type fibers (MyHC2b and MyHC2x).[221] Furthermore, SIRT1 participates in these processes, and the deacetylation of PGC1α by SIRT1 facilitates muscle remodeling toward slow and oxidative phenotypes.[222] Activation of PGC1α induces upregulation of NRF1, myocyte enhancer factor 2c (Mef2c), myogenin, troponin I (Tn I slow),

MyHC1, and myoglobin while concurrently downregulating the expression of Tn I fast, MyHC2b, FOXO1, and myostatin.[178,223] It is worth noting that NRF1 promotes the expression of CaMKKβ, which leads to the phosphorylation of AMPK and subsequent activation of Mef2c in skeletal muscle.[216]

Further research has demonstrated the crucial role of the PGC1α/HIF2α axis in regulating the transition of muscular fibers to slow-twitched fibers.[224] Hence, there exist multiple pathways that regulate the switching of muscular fibers during aerobic exercise (Figure 8). It can be inferred that PGC1α plays a pivotal role in regulating the transition of muscle fibers toward an oxidative phenotype. Meanwhile, calcineurin (CaN) synergistically activates Mef2c, causing the conversion of type II to type I fibers.[178] At the same time, CaN is stimulated to enhance calcium concentration in skeletal muscle.[225] In the presence of calcium anions, the calcium-sensing protein calmodulin (CaM) binding to CaN forms a complex of the active phosphatase, which triggers downstream signaling.[225] Alongside Mef2c, a range of mediators, including NFAT1c, MyoD, myogenin, and GATA transcription factors, are activated by CaN to facilitate the hypertrophy of slow-type muscle.[226–228] According to a report by Delling et al., CaN works with MyoD and NFAT1c to activate the differentiation of slow-oxidative MyHC.[229] According to the aforementioned evidence, it is suggested that a potential collaboration between PGC1α and CaN is implicated in the regulation of slow-oxidative MyHC transition during aerobic exercise.

Human skeletal muscle expresses three types of MyHC (I, IIa, and IIx).[230] Among the athletic population, individuals engaged in long and middle-distance running exhibited a fiber composition of 60–70% slow twitch fibers, whereas sprinters displayed an 80% predominance of fast twitch fibers.[231] Early findings indicate that 6-week aerobic training elicits a 12% increase in type I fibers, while concurrently inducing a 24% reduction in type IIx (previously classified as type IIb) fibers within the human skeletal muscle.[232] Moreover, the density of mitochondria increased by 35% in type I fibers, 55% in type IIa fibers, and 35% in type IIx fibers.[232] According to the latest study, novice runners who underwent 13 weeks of marathon training, followed by a 3-week taper, exhibited alterations in their fiber types. The proportion of type I fibers in the vastus lateralis increased from 42.6% to 48.6%, while I/IIa fibers increased from 5.1% to 8.2%. However, IIa fibers decreased from 40.1% to 35.8%, IIa/IIx fibers decreased from 11.9% to 6.4%, and IIx fibers increased from 0% to 1%.[233] In addition, previous reports have demonstrated that endurance exercise induces a transition from IIx to IIa fibers.[234,235] However, another early study demonstrated that six untrained participants who exercised 1 h d^{-1}, 4 days wk^{-1}, for five months, at 85–90% of VO_{2max} did not change the percentage of muscle in whole, however, two participants increased their percentage of slow twitch fibers by 9% during the study.[236] Furthermore, Gehlert et al. found that the transitions of muscle fibers induced by exercise are contingent upon the distribution of basal fiber types.[237] Therefore, the adaptational shift in muscle fiber types varies depending on the individual or training conditions. Moreover, the proliferation of mitochondria in the various muscle fibers may play a pivotal role in the adaptive response to aerobic exercise. To date, there is limited evidence supporting PGC1α involvement in muscle fiber transition during aerobic exercise in humans. However, a recent study has demonstrated that acute endurance training induces the upregulation of PGC1α expression in the human vastus lateralis muscle, with higher levels observed in type IIa fibers compared to type I fibers.[181]

Post-translational modifications of histone proteins play a crucial role in shaping chromatin structure and regulating gene expression.[238,239] Figure 9 outlines the potential transcriptional processes involving Mef2 within the nucleus. Histone acetylation is a crucial modification that occurs in the nucleus of muscle cells. Engaging in physical activity causes the acetylation of various lysine residues in the histone protein within human skeletal muscles. This results in the opening of chromatin, allowing for transcriptional activation.[240] In the nucleus, the class IIa histone deacetylases perform the task of repressing Mef2-dependent gene expression by recruiting a complex that consists of NCorI, SMRT, and HDAC3/4/5.[241] Moreover, exercise-induced AMPK activation leads to the phosphorylation of HDAC4 and HDAC5, resulting in their nuclear export, histone acetylation, and activation of Mef2-dependent transcription.[242] On the other hand, CaMKII is also activated during exercise and selectively phosphorylates HDAC4, resulting in nuclear export of both HDAC4 and HDAC5 because of the hetero-dimerization of these two class IIa HDAC isoforms.[243] This process prevents the formation of Mef2-HDACs complexes, promoting myogenesis.[244] Previous studies show that, in human skeletal muscle, the class IIa HDACs are phosphorylated and exported from the nucleus, which is associated with histone acetylation and the expression of exercise-responsive genes.[245] Moreover, transient expression of HDAC5 mutant in mice skeletal muscle hampers its phosphorylation and exportation from the nucleus, leading to suppressed expression of the exercise-responsive PGC1α gene.[246] On the other hand, the acetylation of histones is tuned by the reciprocal actions of histone acetyltransferases (HATs) and HDACs.[247] Three primary HAT families, including the GNTA, MYST, and P300/CBP, are involved in the acetylation of histones and non-histone proteins.[248] However, the activities of the HAT enzymes responsible for histone acetylation in response to exercise need further elucidation.

Alongside the acetylation/deacetylation process facilitated by HAT and HDACs, which are involved in the post-transcriptional modification of histone proteins within nucleosomes, histone methylation also plays a pivotal role in the regulation of gene expression.[249] Moreover, DNA methylation/demethylation on the gene promoter is a significant factor in regulating muscle growth, differentiation, and metabolism.[250–252] However, more research is necessary to understand the intricate relationship between DNA methylation and its function in transcriptional activation during exercise.

RONS and myogenesis

ROS play a crucial role in myogenesis during exercise, and the optimal level of ROS can stimulate muscular regeneration and differentiation. The generation of $O_2^{\cdot-}$/H_2O_2 by NOX2 and NOX4 is strongly linked to the proliferation of muscle stem cells (MuSCs) and myogenic differentiation *in vitro*; however, this process can be inhibited by certain antioxidants (N-acetylcysteine and apocynin).[253] Another report shows that mitochondrial H_2O_2 synergistically formed by ETC and SOD2 plays a pivotal role in muscular differentiation.[254] Additionally, NOX4-dependent ROS play a vital role in promoting muscle regeneration in mice that undergo exercise training. However, this positive effect

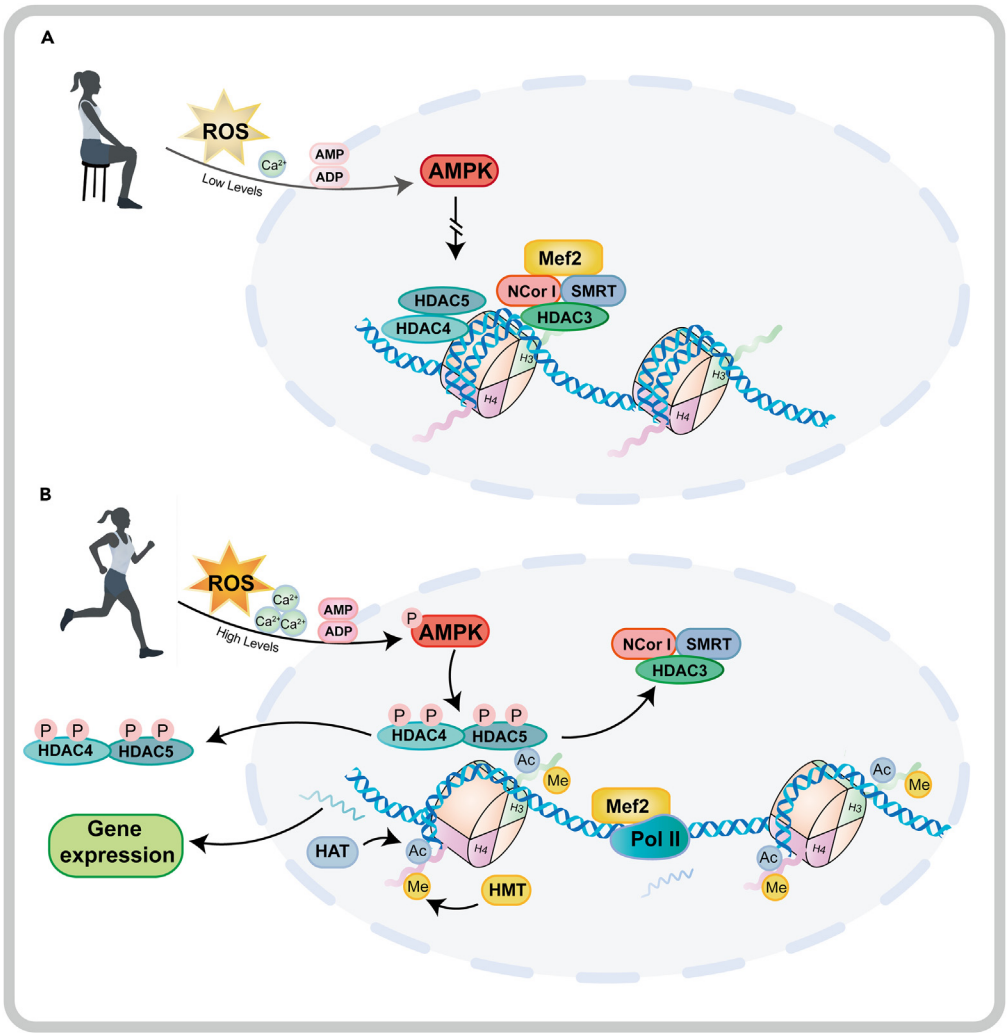


Figure 9. Class IIa HDAC in the regulation of MEF2-intermediated gene expression in skeletal muscle

(A) Myocyte enhancer factor 2 (Mef2) activity is repressed by the class IIa histone deacetylases (HDACs) at the quiescent stage by forming a co-repressor complex through recruiting SMRT, NCor I, and HDAC3, which keeps the periphery chromatin in a condense state.

(B) During exercise, AMP-activated protein kinase is activated and translocates to the nucleus, phosphorylating the HDACs, leading to their nuclear export, and releasing Mef2 to the nucleosome. Furthermore, histone acetylation and methylation by HAT and HMT, respectively, facilitate transcriptional accessibility of DNA.

was not observed in NOX4-knockout mice.[255,256] The increase in muscular fiber associated with PGC1α is understood to be facilitated by NOX4.[41,190] These pieces of evidence indicate that H_2O_2 produced during exercise is essential for the proliferation or differentiation of MuSCs.

Moreover, it has been demonstrated that NO exerts both stimulatory and inhibitory effects on myoblast proliferation in a dose-dependent manner in the *in vitro* setting.[257] Previous reports indicate that the increased NO promotes myoblast fusion, whereas the inhibited NO delays fusion.[258,259] Furthermore, it has been demonstrated that NO inhibiting a GTPase dynamin-related protein-1 mediated mitochondrial fission is critical for myogenic differentiation.[260] In other words, mitochondrial fusion is one of the essential processes for muscle differentiation. Additionally, the NOS activity experiences a transient increase during the differentiation process, which is dependent on the presence of Ca^{2+}, CaM, and NADPH. These processes are associated with NF-κB signaling since the promotor of the i/nNOS gene encodes NF-κB binding sites.[261] Meanwhile, the fine-tuned antioxidant regulators, Pitx2 and Pitx3, are involved in modulating myogenesis.[262] Deletion of Pitx2/3 genes in rat embryos exhibits impaired mitochondrial function and overproduction of ROS during differentiation, leading to defective skeletal muscle development and the apoptotic differentiating myoblast.[262]

However, some studies present different views on the effects of ROS on muscle differentiation. One study shows that dual oxidase maturation factor 1 (DUOXA1) overexpression in MuSCs declines myogenic differentiation. Conversely, suppression of DUOXA1 expression

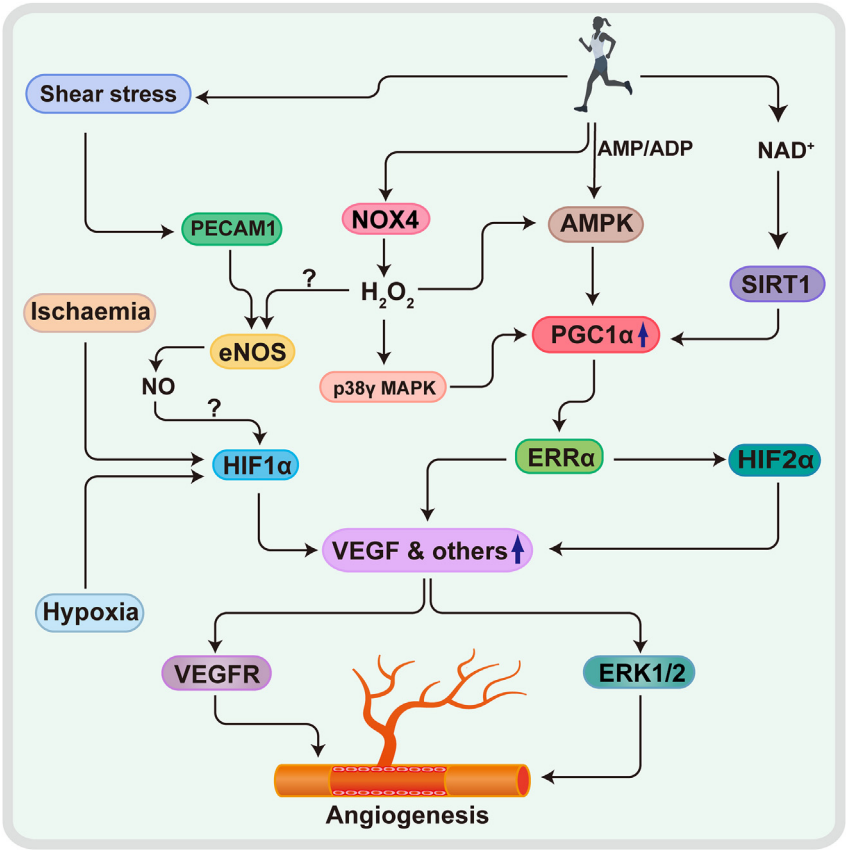


Figure 10. Angiogenesis in aerobic exercise-induced transduction pathway

Exercise can cause increases in AMP and H_2O_2, which subsequently activate AMPK. The activated AMPK followingly activates PGC1α, which is reinforced by the SIRT1 pathway. The activated PGC1α triggers the VEGF pathway and finally causes angiogenesis.

enhances *in vitro* the transcription of myogenic differentiating factors (i.e., MyoD, myogenin).[263] In addition, $p66^{shc}$, a redox protein in mitochondria, generates mtROS as signaling molecules for apoptosis.[264] *In vivo*, knockout of the $P66^{shc}$ gene results in lower mtROS production and better muscle regeneration.[265] Meanwhile, deleting an antioxidant protein, selenoprotein N, leads to differentiation defects in the MuSCs.[266] The carbonyl reductase (CBR1), regulated by Nrf2, operates in the same way to inhibit protein carbonylation and DNA damage while promoting MuSCs differentiation.[267]

In summary, the role of ROS in muscular differentiation is versatile, such as oxidative activation, MSCs proliferation, and differentiation. The roles of ROS in muscular adaptation during aerobic exercise are intricate, and several underlying mechanisms remain to be elucidated. We suggest that six family members of NADPH oxidase may play different roles in signaling muscular differentiation and coordinate with periphery factors under specific conditions, and the different NOXs may present various activities in distinct differentiation stages. The antioxidant proteins play a critical role in maintaining redox balance during muscular proliferation or differentiation processes, and distinct types of antioxidant enzymes are present in specific cellular compartments to deal with ROS under varying conditions.

Vascular angiogenesis

Oxygen consumption must be matched to the intensity of aerobic exercise. However, insufficient oxygen availability leads to an adaptation toward increased oxygen utilization in skeletal muscles. MuSCs and endothelial cells (ECs) collaborate to facilitate muscle regeneration.[268,269] The MSCs exhibit angiogenic properties by expressing various angiogenic factors, such as vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1α/2α (HIF1α/2α).[270] Figure 10 briefly outlines the pathways of vascular angiogenesis. Angiogenesis in response to hypoxia is a complex process requiring the coordination of multiple signals.[271] In this process, HIF1α/2α plays a critical role in stimulating the transcript of VEGF, leading to capillary generation.[272] Moreover, it seems HIF1α is more sensitive than HIF2α to hypoxia, subsequently enabling VEGF transcription.[272] One study has demonstrated that the amount of HIF1α transcripts in the body rises during a single session of exercise but decreases with extended endurance training.[273] Additionally, the activation of the PGC1α/ERRα pathway leads to exercise-induced angiogenesis, as evidenced by increased VEGF expression. Notably, this process occurs independently of HIF1α.[273] The concomitant factors concerning angiogenesis, such as platelet-derived growth factor (PDGF) and angiopoietin 2, are also upregulated via the

PGC1α/ERRα pathway. VEGF plays a dominant role in recruiting ECs, while PDGF-B recruits mural cells to provide support and encapsulation for the endothelium. VEGF and angiopoietin 2 facilitate the sprouting of new vessels from existing vessels.[274] At the same time, HIF2α can be transcriptionally stimulated by the PGC1α/ERRα pathway, increasing the expression of VEGF.[224] Meanwhile, p38γ-MAPK activated by ROS is also involved in the transcript of PGC1α, subsequently leading to increases in PGC1α.[199] Thus, the exercise-induced PGC1α/ERRα axis seems to be the leading pathway in neovascular regeneration. Further, exercise can stimulate the expression of NOX4, resulting in increased H_2O_2, which subsequently enables the increases in VEGF expression, leading to capillarization.[255] However, overexpression of CAT in transgenic mice suppressed angiogenesis and exhibited the impairment of neovascularization and reduced vessel sprouting.[275,276] Meanwhile, a finding shows that H_2O_2 generated by NOX4 triggers vascular angiogenesis by activating VEGF and transforming growth factor β1 (TGFβ1), stimulating their downstream factors expression, including VEGFR2 and eNOS.[277] As mentioned previously, an exercise-induced increase in $O_2^{\cdot-}$/H_2O_2 by NOX2 or NOX4 stimulates the AMPK/PGC1α/ERRα pathway and, subsequently, upregulates VEGF and PDGF, which enable the recruit of ECs leading to angiogenesis.

Furthermore, VEGF can further activate VEGFR2 and extracellular signal-regulated kinase 1/2 (ERK1/2) via activating NOX4, NOX2, and p66Shc, subsequently starting EC proliferation, migration, and angiogenesis.[278,279] In addition, angiopoientin1 transiently stimulates endothelial NOX4-induced H_2O_2 increase, leading to angiogenesis.[275] Hypoxia-induced NOX4 expression promotes endothelial proliferation, migration, and tube formation by activating eNOS.[280] Previous work suggests that NO can stabilize HIF1α activity and stimulate ECs to secrete VEGF and basic fibroblast growth factor.[281] However, the precise mechanisms by which NO influences HIF1α or VEGF secretion to modulate angiogenesis remain unclear. Meanwhile, exercise results in an increased cyclic circumferential strain, enhancing the stimulation of ECs.[282] Shear stress can also stimulate ECs and subsequently activate eNOS, releasing more NO to cause vascular adaption to exercise.[283] Furthermore, platelet endothelial cell adhesion molecule 1 (PECAM1) is involved in the expression of eNOS expression in response to shear stress.[284,285] However, further research is required to determine if PECAM1 is a contributing factor in the adaptation of muscular cells during exercise.

Neuronal regeneration

Besides vascular angiogenesis, neuronal growth is also essential in muscular remodeling. In previous findings, ROS were observed to be involved in neuronal polarity, regulation of connectivity, synaptic transmission, and the tuning of neuronal networks.[286,287] There is abundant evidence of H_2O_2 produced by NOXs contributing to axonal growth cone pathfinding, whereby neuronal growth depends on the physiological amount of H_2O_2.[288,289] Neurons gained from NOX2 knockout mice exhibit reduced neurite length compared to control cells.[288] Similarly, a genetic reduction in NADPH oxidase activity in embryonic hippocampal neurons through expressing the dominant-negative $P22^{phox}$ regulatory subunit can lead to delayed and reduced axon outgrowth.[289] On the contrary, overexpression of NADPH oxidase subunit $P47^{phox}$ promoted axonal growth.[290] The mechanism of ROS-induced neuronal outgrowth is associated with calcium release from intracellular stores.[291] Exercise-induced respiratory burst of NADPH oxidase-generated ROS stimulates calcium release by redox modification of ryanodine and inositol-3-phosphate receptor, which, in turn, results in increased expression of Rac1, a stimulator of the NOX2 complex, thus, causing sustained ROS generation.[290] This suggests that ROS might have a modulatory role required to establish neuronal polarity. In addition, insufficient H_2O_2 generation will cause spatial memory deficits in mice, whereas a pathological increase of H_2O_2 results in growth cone collapse and axonal degeneration.[287] Thus, a well-balanced amount of ROS, especially H_2O_2, within the physiological range is required for the development and performance of the nervous system. The optimum amount of H_2O_2 ranging from 1 nM to 10 nM was estimated to promote physiological promotion in neuronal growth; however, H_2O_2 exceeding 10nM causes oxidative damage in neurons, with any further increase in H_2O_2 (>100 nM) resulting in neuronal degradation.[5,287] So far, few studies have examined ROS contribution to muscular neuronal growth, but the mechanism may be similar as observed in other tissues. The amount of ROS determines neuromuscular junction development (most possibly: H_2O_2). The concentration of H_2O_2 ranging from 1 to 10 nM stimulates neuromuscular junction growth. However, an amount less than 1 nM may inhibit the development of the neuromuscular junction, and a number of more than 100 nM results in degeneration of the neuromuscular junction. Figure 11 shows the nerve growth or degradation models under different concentrations of cellular H_2O_2.

ROS AND GLUCOSE METABOLISM

Exercise intensity and duration are the primary determinants of muscle glucose uptake during exercise.[292] Glucose uptake by skeletal muscle occurs through diffusion, dependent upon the presence of glucose transporter 4 (GLUT4) on the cell surface membrane and an inward glucose diffusion gradient.[292] Increased AMPK activity can further enhance GLUT4 vesicle translocation to the muscle membrane's surface through phosphorylation in the site Ser237 of TBC1D1, a Rab GTPase-activating protein.[293–297] Thus, an exercise-induced increase of TBC1D1 phosphorylation in skeletal muscle has been correlated with AMPK activity. The phosphorylated TBC1D1 released GLUT4 vesicles to the muscle cell membrane, enhancing the transport of glucose.[296] Meanwhile, GLUT4 release can be activated by the insulin-Akt-AS160 axis, in which AS160 (known as TBC1D4) has a conserved RabGAP domain as TBC1D1.[298] Recently, Hatakeyama et al.[299] disclosed that TBC1D1 plays a dominant role in the liberation of static GLUT4 when both AS160 and TBC1D1 are present. They postulated that two RabGAP proteins cooperatively regulate GLUT4 release in response to exercise.[299] Furthermore, the exercise-induced AMPK-TBC1D1 pathway affects glucose uptake mainly following exercise and muscle contraction.[300] However, how these two RabGAPs cooperatively regulate GLUT4 transport requires further investigation.

In addition, recent findings showed that cytosolic ROS production by NOX2 was critical for GLUT4 translocation and glucose uptake during moderate-intensity exercise.[15] The authors found that mutants in $p47^{phox}$ or Rac1 of NOX2 subunits significantly decreased ROS levels in

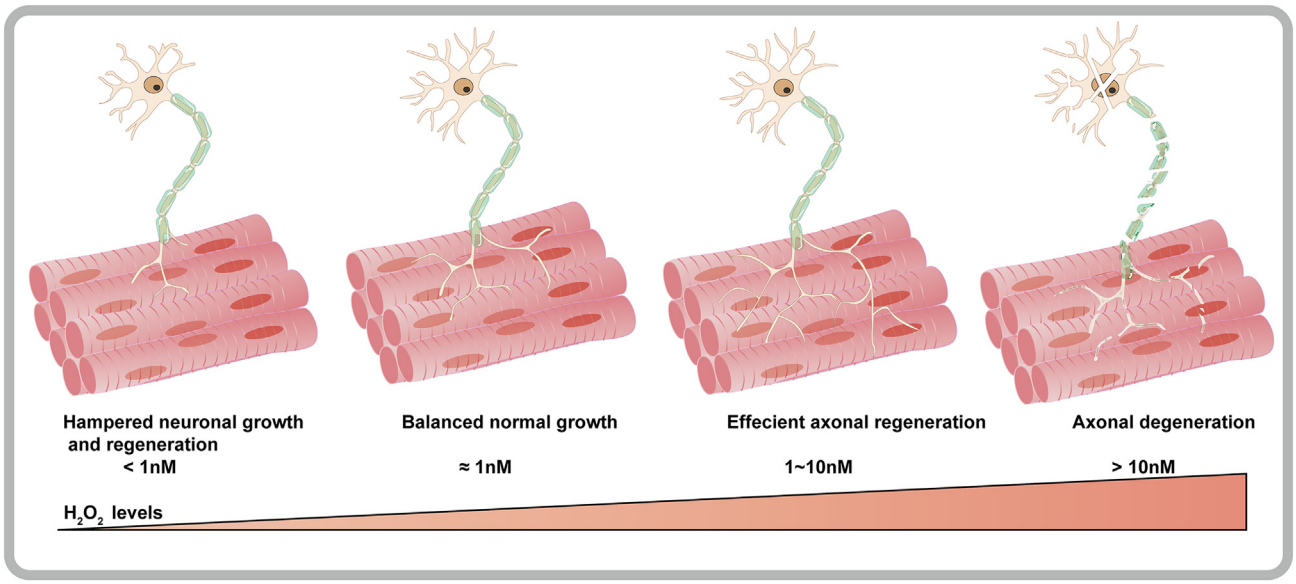


Figure 11. The relationship between exercise-induced H_2O_2 concentration and neuronal regeneration

Neuronal growth or regeneration is inhibited by low concentrations of H_2O_2 (≈0.1∼1 nM). Balanced concentrations of H_2O_2 (≈1 nM) are essential for maintaining normal neuronal development. The aerobic exercise-stimulated elevation of H_2O_2 (≈1∼10 nM) improves neuronal regeneration. However, an excessive amount of H_2O_2 leads to axonal degradation.

skeletal muscle and lowered GLUT4 transport to the cell membrane.[15] More recently, a study by Specht et al. shows that NOX4 is critical in muscle metabolic responses to exercise.[301] Their findings indicate that H_2O_2 generated by NOX4 is required to express mitochondrial proteins, such as uncoupling protein 3, hexokinase 2, and pyruvate dehydrogenase kinase 4. NOX4-deficient mice demonstrated impaired activity of skeletal muscle citrate synthase and beta-hydroxyalkyl-CoA-dehydrogenase.[301]

PHYSICAL ADAPTATION AND EXOGENOUS ANTIOXIDANTS IN EXERCISE TRAINING

Exogenous antioxidants are beneficial for health improvement during training. However, any supplementation with antioxidants needs to consider individual redox conditions. Excessive elimination of ROS by exogenous antioxidants may inhibit the beneficial effects of exercise training. Gomez-Cabrera et al. found that supplementation of a high dose of vitamin C inhibits the expression of PGC1α and blunts mitochondrial biogenesis, and hampers training-induced physical adaption to endurance performance.[302] Their study found that superoxide suppression using oxypurinol inhibits XO and diminishes muscle force generation.[10] In addition, Morrison et al. demonstrated that vitamin C and E supplements inhibited some cellular adaptions to human endurance training.[303] Our previous study showed that a high-dose astaxanthin supplement hampered the Nrf2-regulated pathway.[304] However, exogenous antioxidants are beneficial to exercise training when the endogenous antioxidant system is incapable of neutralizing the excessive amount of ROS. Our recent findings showed that astaxanthin supplementation improved mitochondrial biogenesis and antioxidant capacity during chronic HIIT in mice, but the beneficial effects were severely impaired in the training group without astaxanthin supplementation.[305] Figure 12 shows the relationship between exercise stimulation and antioxidants in physiological adaptation during aerobic exercise.

During long-term training, gradually increased exercise intensities synthesize more antioxidants and proliferate more mitochondria to reach a new level of homeostasis. In exercise training, it is crucial that endogenous antioxidants precisely protect critical enzymes or proteins from damage. Generic antioxidants may not benefit from exercise adaptation because the stimulating effect on redox signaling is diminished. Many authors have reviewed antioxidants and exercise adaptions and discussed the interactive mechanisms between antioxidants and exercise adaption,[306,307] and there are several different conclusions on the application of antioxidants during exercise. A systematic review by Ranchordas et al. showed that antioxidants could prevent or reduce muscle soreness after exercise.[308] Thus, optimum ROS can cause positive effects on physical adaption during aerobic exercise. We believe generic exogenous antioxidants may hamper exercise adaptation and may only be appropriate for overtrained athletes with a pathological phenotype or individuals who are deficient in a particular antioxidant.

FUTURE PERSPECTIVES

The roles of RONS in physiological function have been extensively investigated, particularly in the context of cellular adaptation to exercise training. However, there remain several lines of controversy relating to RONS-induced adaptation during exercise. NOXs and XO are the primary $O_2^{\cdot-}$/H_2O_2 generation sources with subsequent involvement in signaling transduction processes. Thus, they may play a critical role in

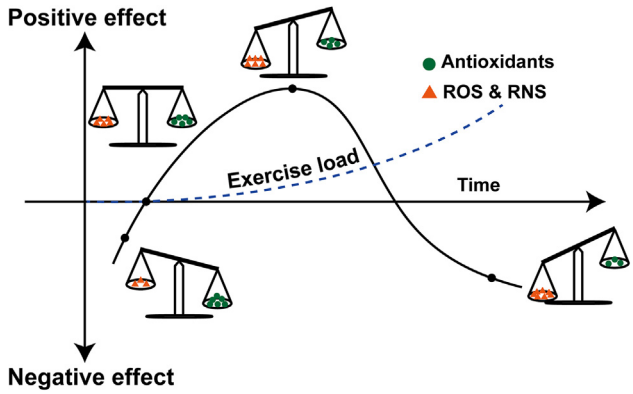


Figure 12. The balance between antioxidants and RONS in physiological adaptation during exercise

The beneficial effects of cellular adaptation during exercise occur only at an optimal level of reactive oxygen and nitrogen species (ROS & RNS).

muscle adaptation during exercise. Therefore, the upstream signaling of NOXs and XO activation for exercise training adaptation should be a future line of comprehensive enquiry. Also, the primary function of NOXs is worth exploring further. In addition, the mechanism of precise modulation of antioxidant genes to maintain redox balance in distinct exercise conditions still awaits disclosure, such as the pathways of Nrf2-Keap1 and NF-κB and their interactions. Finally, skeletal muscle biochemical and physiological adaptations during and following aerobic exercise are complicated. These adaptations, including the role of oxidative (eu)stress and antioxidants, require further detailed research.

AUTHOR CONTRIBUTIONS

All authors participated in the initial discussions and collaborated on formulating the manuscript. Y.Z. was responsible for the initial drafting of the article, which was reviewed and edited by all authors. Y.Z., J.S.B., and G.W.D. contributed to the conception, design, and composition of the manuscript. All authors reviewed the intellectual content and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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