# Reactive oxygen species promote endurance exercise-induced adaptations in skeletal muscles

**Article** · May 2024

DOI: 10.1016/j.jshs.2024.05.001



**4 authors**, including:

Scott Powers
University of Florida
**505** PUBLICATIONS **33,788** CITATIONS
SEE PROFILE

Zsolt Radak
Hungarian University of Sport Science
**313** PUBLICATIONS **14,926** CITATIONS
SEE PROFILE

Li Li Ji
University of Minnesota Twin Cities
**255** PUBLICATIONS **16,240** CITATIONS
SEE PROFILE

HOSTED BY

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Journal of Sport and Health Science xxx (2024) xxx-xxx

Review

# Reactive oxygen species promote endurance exercise-induced adaptations in skeletal muscles

Q1 Scott K. Powers [a,*], Zsolt Radak [b], Li Li Ji [c], Malcolm Jackson [d]

[a] *Department of Applied Physiology, University of Florida, Gainesville, FL 32608, USA*
[b] *Research Institute of Sport Science, Hungarian University of Sport Science, Budapest 1123, Hungary*
[c] *Department of Kinesiology, University of Minnesota, St. Paul, MN 55455, USA*
[d] *Department of Musculoskeletal and Ageing Science, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool L7 8TX, UK*

Received 14 June 2022; revised 26 October 2023; accepted 9 November 2023
Available online xxx



**Abstract**

The discovery that contracting skeletal muscle generates reactive oxygen species (ROS) was first reported over 40 years ago. The prevailing view in the 1980s was that exercise-induced ROS production promotes oxidation of proteins and lipids resulting in muscle damage. However, a paradigm shift occurred in the 1990s as growing research revealed that ROS are signaling molecules, capable of activating transcriptional activators/coactivators and promoting exercise-induced muscle adaptation. Growing evidence supports the notion that reduction-oxidation (redox) signaling pathways play an important role in the muscle remodeling that occurs in response to endurance exercise training. This review examines the specific role that redox signaling plays in this endurance exercise-induced skeletal muscle adaptation. We begin with a discussion of the primary sites of ROS production in contracting muscle fibers followed by a summary of the antioxidant enzymes involved in the regulation of ROS levels in the cell. We then discuss which redox-sensitive signaling pathways promote endurance exercise-induced muscle adaptation and debate the strength of the evidence supporting the notion that redox signaling plays an essential role in muscle adaptation to endurance exercise training. In hopes of stimulating future research, we highlight several important unanswered questions in this field.

**Keywords:** Antioxidants; Mitochondrial biogenesis; Radicals; Redox signaling

## 1. Introduction

The term oxidative stress was coined by Helmut Sies[1] in 1985 and was originally defined as "the imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to cellular damage." In this context, oxidant-mediated damage was commonly documented by the appearance of oxidized cellular components (e.g., oxidized proteins and/or biomarkers of lipid peroxidation). The first evidence that endurance exercise promoted oxidative stress in humans was reported in 1978.[2] This ground-breaking observation revealed that prolonged submaximal exercise is associated with increased lipid peroxidation; nonetheless, the cells responsible for this exercise-induced oxidant production were unclear.[2] Four years later, Davies et al.[3] discovered that contracting skeletal muscles produce free radicals, and this important finding was quickly confirmed by an independent study demonstrating that skeletal muscle contractions promote both radical production of and damage to rodent muscle fibers; these observations were later confirmed in both rodent and human skeletal muscles.[4–6] Collectively, these milestone studies launched the field of exercise and muscle reduction-oxidation (redox) biology.

Although Davies et al.[7] hypothesized that contraction-induced radical production provides a stimulus for exercise-induced muscle adaptation, the prevailing view in the 1980s was that exercise-induced radical production promotes muscle damage. However, a paradigm shift occurred in the 1990s as accumulating evidence revealed that reactive oxygen species (ROS) are signaling molecules, capable of activating transcriptional activators and promoting cellular adaptation.[7] This notion was highlighted in a seminal review detailing the Q2

Peer review under responsibility of Shanghai University of Sport.
*Corresponding author.
*E-mail address:* spowers@ufl.edu (S.K. Powers).

https://doi.org/10.1016/j.jshs.2024.05.001

evidence that ROS can stimulate transcriptional activators to promote protein synthesis.[8] Studies during the past decades have improved our understanding of the mechanisms behind exercise-induced redox signaling. More specifically, recent advances in methodology have significantly expanded our knowledge of the molecular interactions between ROS with redox-sensitive targets in cells. Therefore, this review provides a current synopsis of our present understanding of the sources of muscle contraction-induced ROS production and the roles that ROS play as signaling molecules to promote endurance exercise-induced adaptations in skeletal muscles. Because hydrogen peroxide ($H_2O_2$) is recognized as a major ROS in redox regulation of cell signaling activities,[9–11] this report will focus on the role that $H_2O_2$ plays in exercise-mediated redox signaling via post-translational modifications.

## 2. ROS are a family of biological signaling molecules

Reactive chemical species are often grouped into categories depending upon the reactive atom.[12] ROS is an umbrella term that includes several ROS formed by redox reactions or electronic excitation. Since ROS is a term referring to several chemical species, the name "ROS" does not denote a specific molecule. Nonetheless, because of the technical challenges in the detection of specific ROS in cells, it is common in redox biology to use the label "ROS" to refer to all ROS (both radical and non-radical).[10,13] Table 1 provides an overview of key ROS, including both radical and non-radical species.

The parent molecule of all ROS is the superoxide radical ($O_2^{\cdot-}$), and while numerous ROS exist, $H_2O_2$ is recognized as a key ROS player in redox control of biological signaling in mammals.[9,11,14-16] Indeed, $H_2O_2$ is a versatile and pleiotropic signaling molecule.[10] It has been over 50 years since the discovery that $H_2O_2$ levels are regulated in cells.[17] Similar to other key signaling molecules (e.g., calcium), $H_2O_2$ is typically controlled in resting skeletal muscle fibers at low levels (e.g., 0.01−0.1 μM); however, muscular contractions can increase the intracellular concentrations up to 0.2 μM.[18] Moreover, because ROS production occurs at specific locations within muscle fibers, the concentration of $H_2O_2$ differs across cellular compartments; the physiological significance of regional differences in ROS concentration will be addressed later.

Table 1
Overview of key reactive ROS including both radical and non-radical species.

Non-radical ROS
- $H_2O_2$: $H_2O_2$ is commonly produced by the dismutation of $O_2^{\cdot-}$ via superoxide dismutases. Although $H_2O_2$ is a relatively strong 2 electron oxidant, it's reactivity with biological targets is limited because of the high activation energy and the low cellular concentrations of $H_2O_2$.
- Organic hydroperoxides: This class of ROS includes hydroperoxides formed from lipid peroxidation of both polyunsaturated fatty acids and sterols (cholesterol).
- Singlet oxygen: Singlet oxygen is an electronically excited form of oxygen that can be formed by photoexcitation.

Free radical ROS
- Superoxide anion radical: The $O_2^{\cdot-}$ is formed when molecular oxygen accepts a single electron.
- HO: Most reactive ROS. The HO is formed from H2O2 by reduction via a metal-catalyzed reaction (e.g., Fe2+).
- Peroxyl radical: Peroxyl radicals are formed by reactions between molecular oxygen and carbon-centered radicals.

Abbreviations: HO = hydroxyl radical; $H_2O_2$ = hydrogen peroxide; $O_2^{\cdot-}$ = superoxide anion radical; ROS = reactive oxygen species

## 3. Sources of ROS production in contracting muscles

ROS are produced via numerous sources in resting and contracting skeletal muscle fibers. Indeed, in human cells, a total of 41 enzymes are capable of producing $O_2^{\cdot-}$ and/or $H_2O_2$.[19] Of these ROS producing enzymes, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) complexes are the only enzyme family known to produce ROS as their primary function.[20] Although the cellular sites of muscle contraction-induced ROS production have been investigated for decades, debate continues about which ROS generating locations are dominant during exercise. In this regard, several sources of ROS production exist in contracting muscle fibers, including mitochondria, xanthine oxidase, phospholipase A2 (PLA2), and NADPH oxidases (Fig. 1).

Mitochondria were first proposed to be a major source of ROS in contracting muscle fibers over 40 years ago.[3] Nonetheless, several lines of evidence indicate that mitochondria are not the dominant source of ROS during acute exercise. For example, assessment of ROS emission from both isolated skeletal muscle mitochondria and permeabilized muscle fibers reveals that mitochondria produce significantly more ROS in State 4 respiration (i.e., resting conditions) compared to State 3 respiration (ADP-driven, muscle contractions).[21,22] Furthermore, studies using a mitochondrial targeted fluorescent indicator (i.e., MitoSOX) to identify superoxide production during muscular contractions show that mitochondrial superoxide production does not increase during muscular contractions lasting up to 10 min.[23,24] Similarly, a study using a mitochondrial-targeted redox-sensitive green fluorescent protein to measure mitochondrial redox potential concluded that mature, single myocytes do not increase their mitochondrial ROS production during short periods of muscle contractions.[25] Laker et al.[26] developed a mitochondrial reporter gene (pMito-Timer) to measure skeletal muscle mitochondrial oxidation *in vivo*. This work revealed that 90 min of treadmill running does not increase mitochondrial ROS production in active skeletal muscles.[27] Together, these studies indicate that mitochondria are not a prominent source of ROS during a single acute bout of exercise. However, while mitochondrial ROS production is not elevated during a single bout of exercise, a subsequent bout of muscle contractions (20 min after first exercise session) does increase mitochondrial ROS production.[24] These intriguing results suggest that repeated bouts of exercise can modify mitochondrial ROS production during successive exercise. To further complicate this issue, while acute exercise does not increase mitochondrial ROS production, emerging evidence reveals that basal mitochondrial ROS production is elevated at 3−12 h post exercise.[27] While the mechanisms

Please cite this article as: Scott K. Powers et al., Reactive oxygen species promote endurance exercise-induced adaptations in skeletal muscles, Journal of Sport and Health Science (2024), https://doi.org/10.1016/j.jshs.2024.05.001

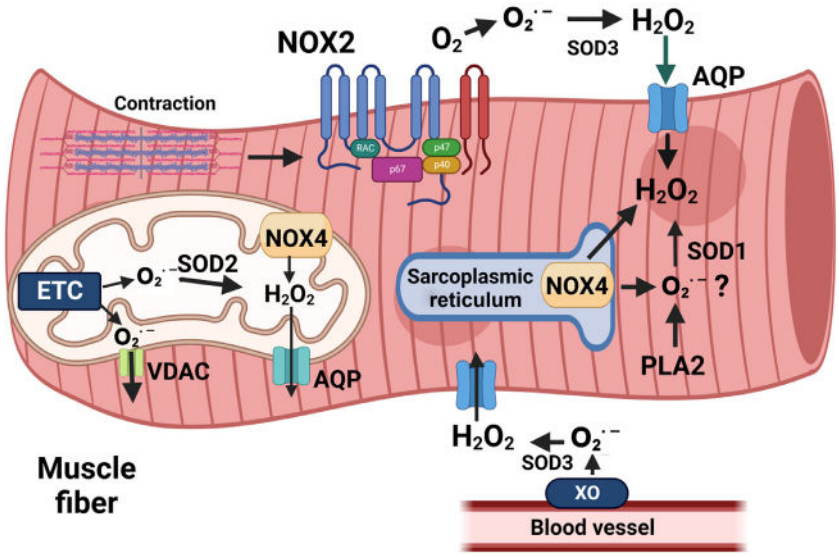


Fig. 1. Illustration of the sources of ROS in contracting skeletal muscles. See text for details. AQP = aquaporin; ETC = electron transport chain; $H_2O_2$ = hydrogen peroxide; NOX2 = nicotinamide adenine dinucleotide phosphate oxidase 2; NOX4 = nicotinamide adenine dinucleotide phosphate oxidase 4; $O_2$ = oxygen; $O_2^{\cdot-}$ = superoxide anion radical; PLA2 = phospholipase A2; ROS = reactive oxygen species; SOD1= superoxide dismutase 1; SOD2= superoxide dismutase 2; SOD3= superoxide dismutase 3; VDAC = voltage dependent anion channel; XO = xanthine oxidase.

responsible for this post-exercise increase in mitochondrial ROS production in contracting muscle remain unclear, recent data reveal that a single bout of exercise results in an increase in NOX4 expression in muscle fibers that could contribute to post-exercise ROS production and redox signaling.[28,29] More will be said about this important topic later.

Enzymes of the PLA2 super family catalyze the hydrolysis of ester bonds on phospholipids within the cell membrane, sarcoplasmic reticulum, and mitochondrial membranes to produce arachidonic acid and other fatty acids.[30,31] Notably, arachidonic acid is a substrate for lipoxygenases to produce ROS.[30,31] The PLA2 family consists of 16 members categorized into several groups that include both calcium and calcium-independent enzymes.[30,31] Skeletal muscles express both calcium-dependent and calcium independent PLA2 enzymes that modulate oxidant production in the cytosol and mitochondria during muscular contractions.[32–34] Although muscle contraction results in PLA2-mediated $O_2^{\bullet-}$ production, whether or not PLA2 production of ROS is a dominant source of cytosolic ROS in skeletal muscle during exercise remains an open question.

Xanthine oxidase has been proposed as another source of exercise-induced ROS production. Xanthine oxidase is an oxidoreductase that produces $O_2^{\bullet-}$ by oxidizing hypoxanthine to form xanthine; the oxidation of xanthine to uric acid follows with the resultant production of $O_2^{\bullet-}$.[35] Studies investigating the abundance of xanthine oxidase in skeletal muscle reveal that xanthine oxidase is either absent or expressed at low levels in human skeletal muscle.[36–38] However, xanthine oxidase is found within capillary endothelial cells surrounding muscle fibers.[36,37] Moreover, muscle contractions activate xanthine oxidase within capillary endothelial cells resulting in increased $O_2^{\bullet-}$ production.[39–42] Following the conversion of $O_2^{\bullet-}$ to $H_2O_2$ via extracellular superoxide dismutase, $H_2O_2$ can cross the sarcolemma contributing to an increase in ROS within the contracting muscle fibers.[10] Therefore, it is feasible that xanthine oxidase-induced ROS production outside the muscle fiber can impact the intracellular redox status of contracting muscle fibers.

Although 5 isoforms of NOX exist in skeletal muscle, NOX2 and NOX4 have received the most experimental attention, and both play a role in production of ROS.[20,43] The NOX2 isoform is located within the sarcolemma and the T-tubules.[20,43] In contrast, NOX4 is found in the mitochondrial inner membrane and colocalized with the ryanodine receptor in the sarcoplasmic reticulum.[20,43] Interestingly, both NOX2 and NOX4 exhibit a fiber type-dependent expression with mRNA levels of both NOX2/NOX4 being higher in slow, type I muscle fibers compared to fast, type II fibers.[44]

Active NOX2 is a multimeric enzyme composed of several subunits.[43] Activation of NOX2 in skeletal muscle involves contraction-induced phosphorylation of key subunits (p47phox or p67phox); these post-translational events lead to these subunits binding to the NOX complex located in the sarcolemma to form a functionally active complex.[43]

The NOX4 isoform is 39% homologous to NOX2 but notably, NOX4 can produce both $O_2^{\bullet-}$ and $H_2O_2$; nonetheless, which of these species is the dominant ROS remains a debate.[43] Historically, it has been believed that NOX4 is constitutively active and, therefore, the levels of ROS production from this isoform are transcriptionally regulated.[43] Nonetheless, select proteins (e.g., p22phox) have been reported to modulate NOX4 activity; therefore it appears feasible that NOX4 may be allosterically regulated.[45] Future studies are required to provide definitive evidence as to whether NOX4 is constitutively active or responds to activators.

While it is unclear whether NOX4 is a source of contraction-induced ROS production, a growing number of reports

Please cite this article as: Scott K. Powers et al., Reactive oxygen species promote endurance exercise-induced adaptations in skeletal muscles, Journal of Sport and Health Science (2024), https://doi.org/10.1016/j.jshs.2024.05.001

indicate that active NOX4 is required for muscle adaptation to endurance exercise.[28,46,47] In this regard, as discussed earlier, exercise training has been shown to increase NOX4 expression following acute exercise. Therefore, if NOX4 is constitutively active, an increased abundance of NOX4 would increase NOX4-mediated production of $H_2O_2$ following exercise. More will be said about NOX4 and exercise-induced muscle adaptations later.

It is now clear that contraction-induced activation of NOX2 is a key source of ROS production during exercise. For example, electrical stimulation-induced contraction of myotubes results in ROS production, which is blocked when NOX2 activity is pharmacologically inhibited.[48,49] Moreover, studies stimulating single muscle fibers have reported that NOX2 is a dominant source of ROS production during muscular contractions.[25,50] Notably, technological advances now permit the detection of NOX2 activation and ROS production within muscle fibers *in vivo*. Using recently developed techniques, *in vivo* studies reveal that both continuous exercise and moderate/high intensity interval exercise activate NOX2 and that active NOX2 plays a key role in muscle contraction-induced ROS.[51–53]

In summary, muscular contractions result in increased cytosolic ROS production in myotubes (*in vitro*), isolated mature muscle fibers (*in vitro*), and skeletal muscle fibers (*in vivo*). Although the primary sites of ROS production in contracting muscle remain a topic of debate, growing evidence suggests that contraction-induced activation of NOX2 plays an important role in ROS production in skeletal muscle during exercise. While evidence also implicates both PLA2 and xanthine oxidase in exercise-induced production of ROS, additional research is required to clarify the roles that PLA2 and xanthine oxidase play in muscle ROS production during exercise. For details about the sources of ROS production during exercise, the reader is referred to comprehensive reviews on this topic.[18,20,54–57]

## 4. Regulation of ROS in muscle fibers via redox sinks and relays

A detailed discussion of all enzymatic and non-enzymatic antioxidants in muscle fibers exceeds the scope of this review. Nonetheless, for readers unfamiliar with cellular antioxidants, we summarize key cellular enzymatic antioxidants and introduce the concept of redox relays. For more details about cellular antioxidants, readers are referred to the following reviews.[12,58–60]

Cellular levels of ROS are the sum of production and removal of each species. Superoxide radicals produced inside cells are dismutated into $H_2O_2$ via 2 isoforms of superoxide dismutase (SOD). SOD1 is found in both the cytosol and the mitochondrial intermembrane space whereas SOD2 is located within the mitochondrial matrix. In healthy cells, levels of $H_2O_2$ are maintained in the low nanomolar range; this control occurs because catalase, glutathione peroxidases (GPX), and peroxiredoxins (PRDX) catalyze the removal of $H_2O_2$.[12] Eight isoforms of GPX exist (GPX1−8) and 6 isoforms of PRDX (PRDX1−6) are found in humans; both enzymes are located in the cytosol and the mitochondrion to facilitate removal of $H_2O_2$ within different cellular compartments.[61,62]

While both GPX and PRDX remove $H_2O_2$, PRDX are expressed in higher levels than GPX, leading to the view that PRDX play the dominant role in elimination of $H_2O_2$ within cells.[62] Notably, PRDX are also hypothesized to play a key role in transmitting oxidizing equivalents to other target proteins.[10] This transfer of oxidizing equivalents from PRDX to molecular targets is an example of a relay that contributes to redox signaling in cells.[10] Indeed, the ability of PRDX to transfer oxidizing equivalents to specific target proteins is predicted to be important in cellular redox signaling because the reaction rate constants of $H_2O_2$ with many proteins is relatively low. Examples of PRDX acting as a redox relay can be demonstrated for PRDX2 and the transcription factor signal transducer and activator of transcription 3.[63] Similarly, PRDX2 can also serve as a redox relay for a key kinase in the ROS responsive p38 mitogen-activated kinase (p38) signaling pathway.[64]

## 5. Overview of redox signaling

Again, $H_2O_2$ is recognized as a key player in redox control of biological signaling.[9,11,14–16] In healthy cells, the steady-state physiological flux of $H_2O_2$ leads to reversible oxidation of target proteins; this process alters protein activity leading to a physiological level of redox signaling referred to as "oxidative eustress."[10,13,65] In contrast to the levels of $H_2O_2$ that support normal redox signaling during oxidative eustress, higher (i.e., supraphysiological) levels of ROS lead to widespread oxidation of both proteins and lipids, resulting in cellular damage; this condition is labeled "oxidative distress."[10,13,65]

As an oxidant, $H_2O_2$ is a versatile molecule that participates in numerous signaling events. As discussed earlier, cellular levels of $H_2O_2$ are regulated by a group of efficient antioxidant enzymes. During oxidative eustress, cellular $H_2O_2$ levels are predicted to be maintained within 0.01−0.1 μM.[10,18] During muscular contractions, intracellular $H_2O_2$ levels can double, reaching 0.1−0.2 μM (Fig. 2).[55] Note, however, these numbers serve only as an estimate because measurement of $H_2O_2$ levels in cells is technically difficult and the cellular levels of $H_2O_2$ likely differ between cell types and across varying subcellular locations.[10]

The primary mechanism by which $H_2O_2$ achieves specificity to promote biological signaling is via the oxidation of sulfur (thiolate groups) in target proteins; notably, thiolate groups in target proteins show rates of reactions with $H_2O_2$ that are significantly higher than those of protein thiols.[15] To promote biological signaling, $H_2O_2$-induced thiol oxidation must target select proteins by oxidation of specific cysteines.[66] Unfortunately, explaining how $H_2O_2$ achieves this signaling goal has been challenging.[66] For example, at the predicted cellular levels of $H_2O_2$ during oxidative eustress, $H_2O_2$ reacts with redox-related signaling proteins (e.g., kinases, phosphatases, and transcription factors).[67] Furthermore, because of the

Please cite this article as: Scott K. Powers et al., Reactive oxygen species promote endurance exercise-induced adaptations in skeletal muscles, Journal of Sport and Health Science (2024), https://doi.org/10.1016/j.jshs.2024.05.001

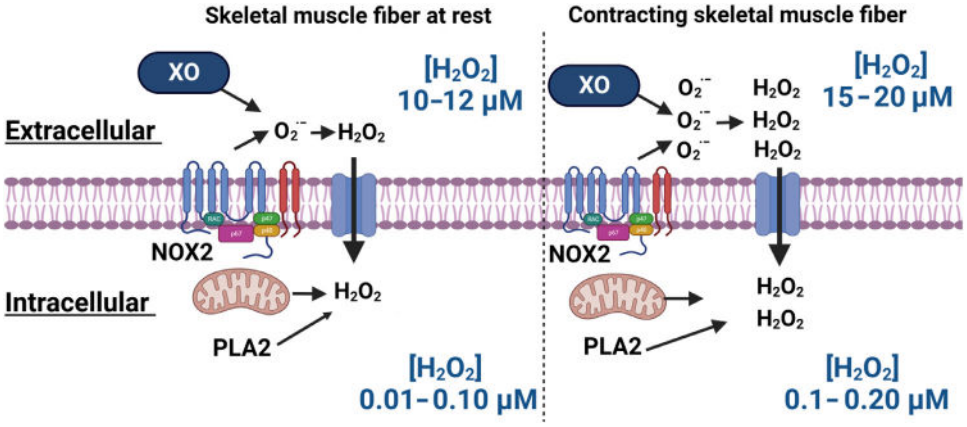


Fig. 2. Illustration of the predicted extracellular and intracellular concentrations of $H_2O_2$ in skeletal muscle fibers at rest and during contractions. $H_2O_2$ = hydrogen peroxide; NOX2 = nicotinamide adenine dinucleotide phosphate oxidase 2; $O_2^{\cdot-}$ = superoxide anion; PLA2 = phospholipase A2; XO = xanthine oxidase.

high abundance and reactivity of PRDX with $H_2O_2$, PRDX are projected to capture most of the $H_2O_2$ produced within cells.[66] Therefore, a conundrum arises: how are redox signaling proteins oxidized by $H_2O_2$? Although there is no consensus answer to this question, 2 schools of thought have emerged.

One school of thought is that $H_2O_2$ reacts directly with thiols on redox-regulated proteins. Specifically, this position hypothesizes that in cellular locations near the site of $H_2O_2$ production, the local concentrations of $H_2O_2$ are elevated, resulting in the direct interaction of $H_2O_2$ with the target protein. A potential contributor to local increases in the $H_2O_2$ concentration in cells is that the interaction between PRDX and $H_2O_2$ can oxidize PRDX, resulting in a reversible decrease in PRDX activity. It follows that inhibition of PRDX activity enables the accumulation of $H_2O_2$ in localized areas, leading to the direct oxidation of protein thiols to promote redox signaling in pathways involved in skeletal muscle adaptation to endurance exercise.[66,67] For example, mitogen-activated kinases, protein tyrosine phosphatases, peroxisome proliferator-activated receptor gamma, nuclear factor-kappa B (NF-κB), and nuclear factor erythroid-derived 2-like 2 (Nrf2) are all redox-sensitive signaling proteins that are activated in response to endurance exercise to promote functional adaptations in skeletal muscle fibers.[68–71] Fig. 3 provides a schematic representation of how local increases in $H_2O_2$ levels can result in direct oxidation of redox-regulated signaling proteins.

The second school of thought postulates that PRDX serve as redox relays to transfer oxidizing equivalents from $H_2O_2$

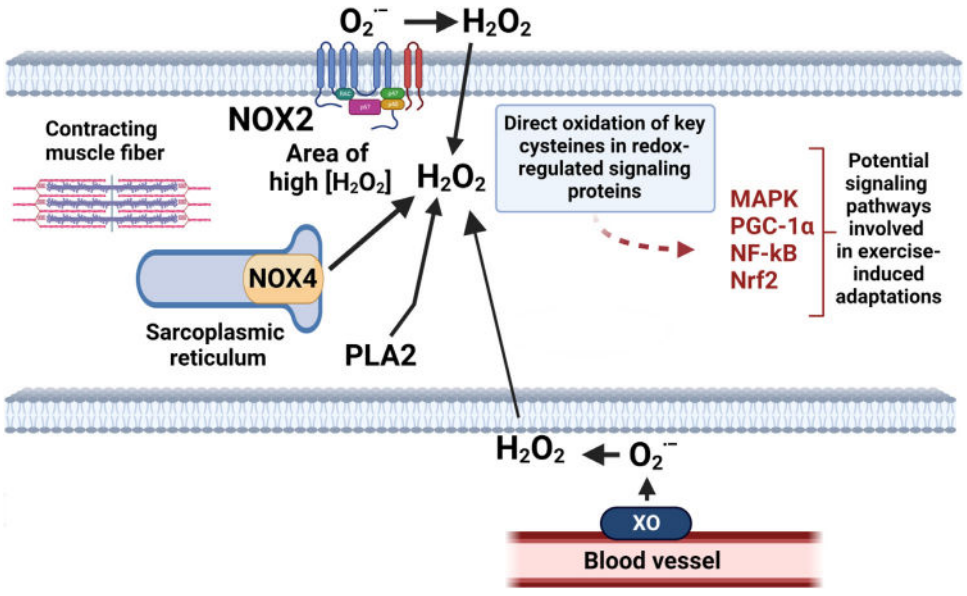


Fig. 3. Diagram illustrating a potential mechanism responsible for exercise-induced redox signing leading to adaptation in skeletal muscles. Specifically, repetitive muscular contractions result in $H_2O_2$ production from several sources; this local increase in $H_2O_2$ results in direct oxidation of redox-sensitive proteins linked to key signaling pathways. See text for more information. Illustration modified from Jackson et al.[18] $H_2O_2$ = hydrogen peroxide; MAPK = mitogen activated kinase; NF-κB = nuclear factor kappa beta; NOX2 = nicotinamide adenine dinucleotide phosphate oxidase 2; NOX4 = nicotinamide adenine dinucleotide phosphate oxidase 4; Nrf2 = nuclear factor erythroid-derived 2-like 2; $O_2^{\cdot-}$ = superoxide anion radical; PGC-1α = Peroxisome proliferator-activated receptor-gamma coactivator alpha; PLA2 = phospholipase A2; XO = xanthine oxidase.

Please cite this article as: Scott K. Powers et al., Reactive oxygen species promote endurance exercise-induced adaptations in skeletal muscles, Journal of Sport and Health Science (2024), https://doi.org/10.1016/j.jshs.2024.05.001

into a disulfide bond that can be transmitted to redox-sensitive signaling proteins via the formation of intermolecular disulfides.[18,55,63] Indeed, this line of reasoning speculates that PRDX are not competitors of protein thiol oxidation but, rather, promote protein oxidation by relaying oxidizing equivalents to redox-regulated target proteins (Fig. 4). In this school of thought, it is postulated that local concentrations of $H_2O_2$ are insufficient to directly oxidize signaling proteins. However, it is predicted that $H_2O_2$ reacts with highly sensitive PRDX that serve as redox relays to oxidize redox-signaling proteins via disulphide exchange, leading to activation of signaling pathways. For details about these 2 schools of thought on $H_2O_2$ signaling in cells, see Stocker et al.,[66] Sies and Jones,[10] and Jackson et al.[18]

Finally, it is noteworthy that these 2 schools of thought concerning how $H_2O_2$ signaling occurs in cells are not mutually exclusive. Indeed, it is feasible that $H_2O_2$ signaling can occur in dissimilar ways under differing cellular conditions. For example, whether $H_2O_2$ acts directly or indirectly via redox relays to oxidize target proteins will likely depend upon the cellular locations of $H_2O_2$ production and the duration of $H_2O_2$ production.[66] For instance, $H_2O_2$ signaling propagation could occur differently during oxidative eustress and oxidative distress conditions. Further, it is also possible that a plurality of $H_2O_2$ signaling exists during both eustress and oxidative distress conditions.[66] The next segment highlights several key cellular targets of redox signaling in skeletal muscles during endurance exercise training.

## 6. Cellular targets of redox signaling

Endurance exercise training results in numerous adaptations in muscle fibers including: increased abundance of heat shock protein 72 (HSP72); mitochondrial biogenesis; and increased expression of numerous antioxidant enzymes. These exercise-induced adaptations occur in skeletal muscles due to the activation of several signaling pathways, many of which are under redox control. The next sections highlight 4 redox-sensitive signaling pathways that promote endurance exercise-induced increases in gene expression of heat shock proteins, mitochondrial biogenesis, and the synthesis of antioxidant enzymes in skeletal muscles.

### 6.1. Redox control of exercise-induced expression of HSP72

It is well-known that endurance exercise training increases the expression of numerous stress proteins in both cardiac and skeletal muscle, including HSP72.[72–77] An increased abundance of HSP72 in heart and skeletal muscle fibers is protective against a variety of stressors. For example, increased expression of HSP72 in skeletal muscle can slow the progression of muscular dystrophy, increase insulin sensitivity, and protect muscle fibers against several different conditions that promote muscle wasting.[78–83] Further, elevated levels of HSP72 in cardiac myocytes protect against ischemia-reperfusion injury.[84]

The heat shock factor protein 1 (HSF1) acts as the primary transcription factor for the expression of HSP72 in humans and other mammals. In an unstressed skeletal muscle fiber, HSF1 is an inactive monomer in the cytoplasm and is complexed with regulatory proteins (e.g., HSP70 and HSP90).[85] Activation of HSF1 in skeletal muscle by endurance exercise, heat, or other stressors is a multistep process that includes the dissociation of the regulatory proteins, followed by trimerization of the HSF monomer, nuclear localization, DNA binding, and transcription of target genes (e.g.,

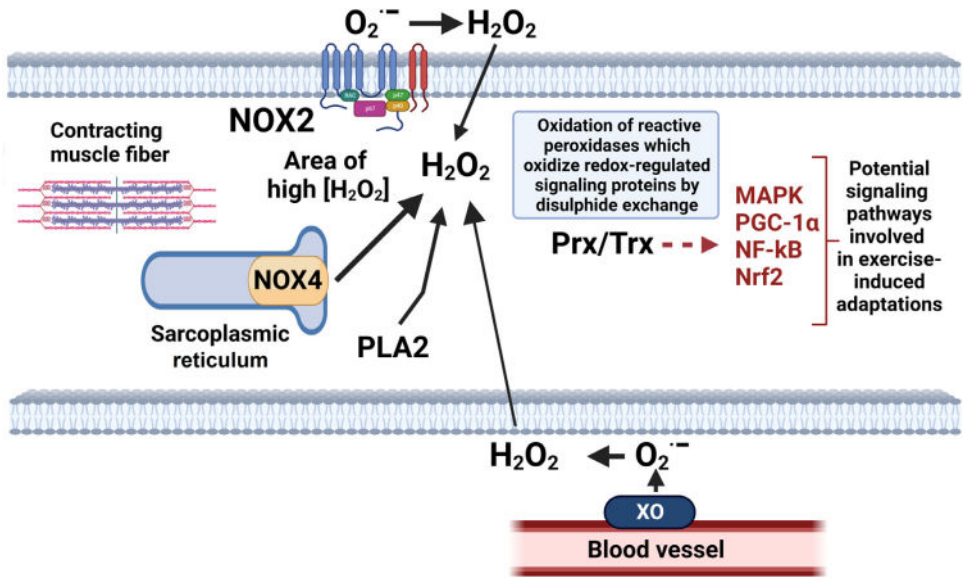


Fig. 4. Diagram illustrating a potential route by which exercise-induced production of $H_2O_2$ reacts with highly sensitive peroxidases that oxidize redox-sensitive signaling pathways via disulfide exchange. See text for more information. Figure modified from reference.[18] $H_2O_2$ = hydrogen peroxide; MAPK = mitogen activated kinase; NF-κB = nuclear factor kappa B; NOX2 = nicotinamide adenine dinucleotide phosphate oxidase 2; NOX4 = nicotinamide adenine dinucleotide phosphate oxidase 4; $O_2^{\cdot-}$ = superoxide anion radical; PGC-1α = peroxisome proliferator-activated receptor-gamma coactivator; PLA2 = phospholipase A2; Prx = peroxiredoxins; Trx = thioredoxins; XO = xanthine oxidase.

Please cite this article as: Scott K. Powers et al., Reactive oxygen species promote endurance exercise-induced adaptations in skeletal muscles, Journal of Sport and Health Science (2024), https://doi.org/10.1016/j.jshs.2024.05.001

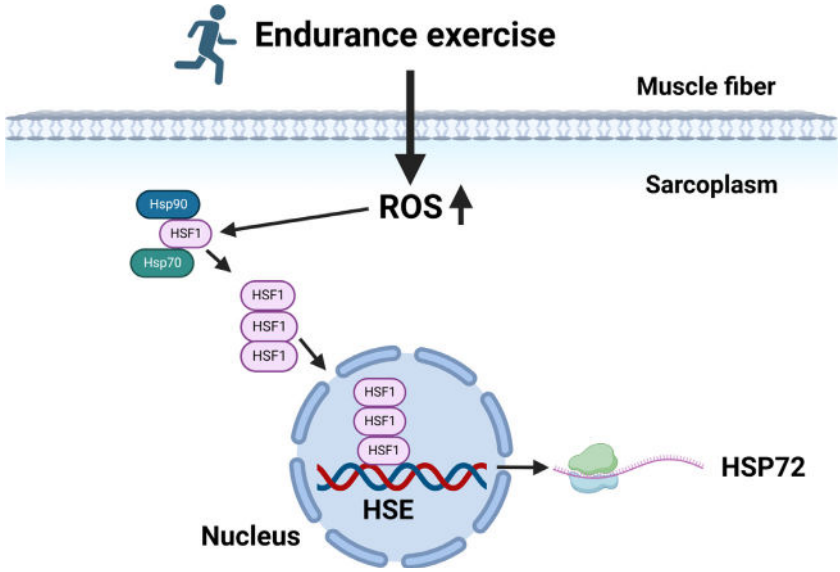


Fig. 5. Illustration of the impact of endurance exercise training on the production of ROS activation of HSF1 leading to the expression of HSP72 in skeletal muscle fibers. HSE = heat shock element; HSF1 = heat shock factor 1; HSP = heat shock protein; ROS = reactive oxygen species.

HSP72)[86] (Fig. 5). While the mechanisms that regulate HSP1 activation continue to be investigated, it is recognized that redox signaling plays a key role in exercise-induced activation of HSP72 gene expression by HSF1.[81,86–88] Indeed, supplementation with high levels of dietary antioxidants has been shown to blunt exercise-induced expression of HSP72 in both heart and skeletal muscle fibers.[89]

### 6.2. *Exercise and redox control of mitochondrial biogenesis*

Increased mitochondrial volume in skeletal muscle fibers is a hallmark of endurance exercise training. This exercise-induced increase in mitochondrial volume is mediated by transcriptional regulators that promote increased gene expression of both nuclear and mitochondrially encoded genes.[90] Mitochondrial biogenesis requires the expression of approximately 1200 gene products; notably, most of these genes are found within the myonucleus, with an additional 13 genes located in the mitochondria.[90] A short summary of the role that redox signaling plays in exercise-induced mitochondrial biogenesis follows.

The transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) is often labeled as the master regulator of mitochondrial biogenesis.[91] Indeed, PGC-1α drives the expression of respiratory complex subunits, mitochondrial import machinery, and several antioxidants via its interaction with select transcription factors, including nuclear respiratory factors 1 and 2.[90] Notably, both nuclear respiratory factors 1 and 2 regulate the expression of mitochondrial transcription factor A (TFAM), as well as nuclear-encoded mitochondrial proteins.[92,93] The control of TFAM by PGC-1α[92,93] provides a mechanism to coordinate mitochondrial gene expression with nuclear gene expression to complete mitochondrial biogenesis.

Exercise-induced activation of PGC-1α in skeletal muscle involves the coordination of several kinases, including calcium/calmodulin-dependent protein kinases (e.g., CaMKII and CaMKIV), adenosine monophosphate-activated protein kinase (AMPK), and p38.[90,94] In this regard, contraction-induced production of ROS has been implicated in the activation of CaMKII, AMPK, and p38.[95,96] For example, physiologically relevant concentrations of $H_2O_2$ can activate AMPK through oxidative modification of the AMPK subunit; hence, in addition to responding to changes in energy availability (i.e., AMP/ATP ratio), AMPK activity is also directly influenced by redox status.[97] Moreover, it is established that $H_2O_2$ is an activator of p38 signaling.[64] Therefore, based on the oxidant-mediated regulation of both AMPK and p38 activity, it follows that mitochondrial biogenesis is controlled, at least in part, by a redox-sensitive mechanism that stimulates both PGC-1α activation and TFAM signaling (Fig. 6).[98,99] Complete details of the redox regulation of mitochondrial biogenesis exceeds the scope of this review. For additional information, the reader is referred to recent reviews on the topic.[54,90]

### 6.3. *Endurance exercise and Nrf2 signaling*

An additional hallmark of endurance exercise training is an increased abundance of numerous antioxidant enzymes in the trained skeletal muscles.[60] In this regard, Nrf2 is a transcriptional activating factor responsible for the control of >250 genes providing cellular defense against oxidative stress and numerous other stressors.[100–102] Indeed, Nrf2 is considered the master regulator of antioxidant defenses in cells.[103] In response to increased cellular ROS production, activated Nrf2 interacts with the antioxidant response elements to promote the expression of numerous cellular antioxidant enzymes, including isoforms of both GPX and PRDX, along with thioredoxin and glutathione reductase.[103–105] Although the regulation of Nrf2 is complex, key elements involved in the regulation of Nrf2 activity are well-known.[104] During resting

Please cite this article as: Scott K. Powers et al., Reactive oxygen species promote endurance exercise-induced adaptations in skeletal muscles, Journal of Sport and Health Science (2024), https://doi.org/10.1016/j.jshs.2024.05.001

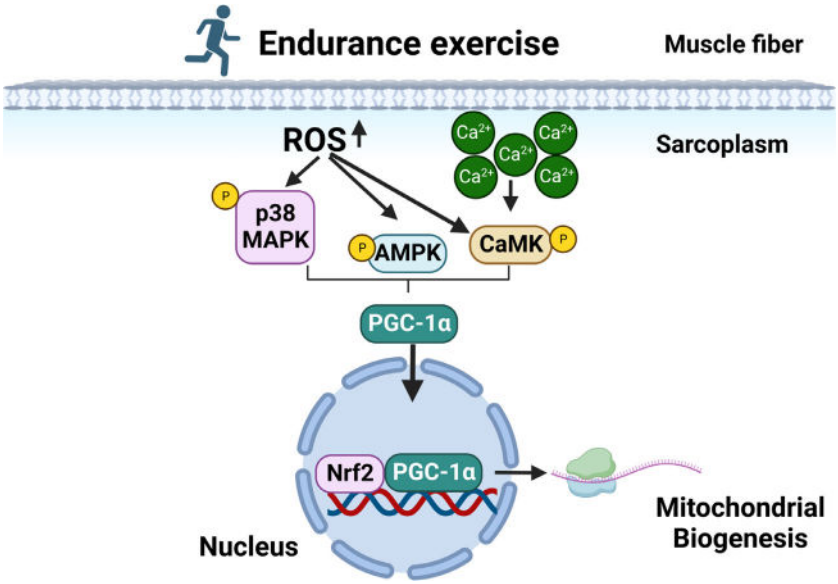


Fig. 6. Illustration of the impact of endurance exercise training on the production of ROS activation of the transcriptional coactivator PGC-1α, leading to mitochondrial biogenesis in skeletal muscle fibers. AMPK = adenosine monophosphate-activated protein kinase; CaMK = calcium/calmodulin kinases; Nrf2 = nuclear factor erythoid-derived 2-like 2; p38 MAPK = p38 mitogen activated kinase; PGC-1α = peroxisome proliferator-activated receptor-gamma coactivator alpha; ROS = reactive oxygen species.

conditions, Nrf2 in skeletal muscle fibers is sequestered in the cytoplasm by the regulatory protein Kelch-like ECH-associated protein 1 (KEAP1).[104,106] However, during exercise-induced oxidant production, KEAP1 and Nrf2 dissociate, allowing Nrf2 to translocate into the nucleus to bind with antioxidant response elements and promote the expression of antioxidant genes.[104] Specifically, KEAP1 can prevent Nrf2 from entering the nucleus in at least 2 ways: (1) KEAP1 binds to Nrf2 in the cytoplasm to prevent Nrf2 from moving into the nucleus[102]; and (2) the KEAP1/Nrf2 interaction in the cytoplasm targets Nrf2 for polyubiquitination and degradation via the ubiquitin-proteasome system.[102] Thus, during resting conditions, the relatively low level of Nrf2 in the nucleus maintains basal expression of antioxidant enzymes in skeletal muscle. However, during bouts of endurance exercise, the contraction-induced increase in ROS production results in both oxidant and electrophilic stress that modifies redox-sensitive cysteine residues on KEAP1, resulting in Nrf2 movement into the nucleus to promote the expression of antioxidant genes (Fig. 7).[71,104]

### 6.4. *Exercise and NF-κB signaling*

The transcriptional activating factor NF-κB comes from a family of 5 transcriptional factors, including p65, Rel B, c-Rel, p52, and p50.[107,108] To gain transcriptional capability, 2 of these family members must dimerize to achieve transcriptional competency.[108] Though all 5 NF-κB family members are expressed in skeletal muscle, it is predicted that the p50−p65 heterodimer accounts for most of the NF-κB activity in muscle.[109] Although NF-κB is regulated, in part, by redox influences, the control of NF-κB activity is subject to complex regulation. During unstressed conditions, NF-κB transcriptional factors remain in the cytoplasm bound to the inhibitory protein, IκB; this IκB binding prevents the dimerization of p50−p65 and therefore prevents NF-κB from moving into the nucleus.[110] However, an increase in cellular production of ROS can promote the dissociation of IκB, resulting in p50−p65 movement into the nucleus and the associated increase in gene expression[108] (Fig. 8). Depending on the specific NF-κB heterodimer formed, NF-κB has many gene targets, including the key antioxidant enzymes SOD1, SOD2, catalase, and GPX1.[111]

Note that although an increase in cellular ROS production can stimulate NF-κB−mediated gene expression, exceptionally high levels of ROS in cells can impair the capacity of NF-κB to bind to DNA.[108,112] Indeed, oxidation of NF-κB dimers can inhibit NF-κB binding with DNA and therefore, redox signaling can both promote and inhibit NF-κB−mediated gene expression.[112] However, whether contraction-induced levels of ROS can reach the levels required to depress NF-κB binding to DNA remains unknown. Nonetheless, recent evidence indicates that incremental exercise to exhaustion activates NF-κB signaling in human skeletal muscle and regulates the expression of several antioxidant enzymes.[113]

## 7. Contribution of redox signaling in endurance exercise-induced skeletal muscle adaptation

The preceding section highlights evidence that redox signaling contributes to mitochondrial biogenesis, expression of HSP72, and the increased synthesis of cellular antioxidant enzymes. The key question becomes: how robust is the evidence that exercise-induced production of ROS is essential to achieve the full benefits of endurance exercise-induced adaptations in skeletal muscle? The next paragraphs highlight 3 lines of evidence supporting the position that exercise-

Please cite this article as: Scott K. Powers et al., Reactive oxygen species promote endurance exercise-induced adaptations in skeletal muscles, Journal of Sport and Health Science (2024), https://doi.org/10.1016/j.jshs.2024.05.001

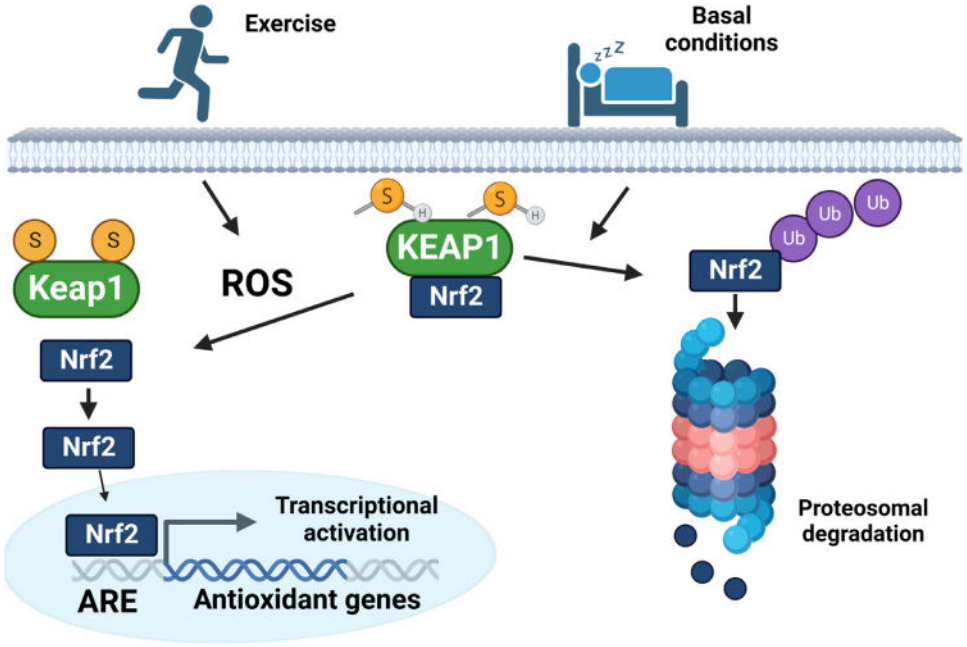


Fig. 7. Illustration of the impact of endurance exercise and resting conditions on the activation of the transcription factor, nuclear regulatory factor 2, leading to the expression of antioxidant enzymes in skeletal muscle fibers. ARE = antioxidant response element; KEAP1 = Kelch-like ECH-associated protein 1; Nrf2 = nuclear factor erythoid-derived 2-like 2; Ub = ubiquitin.

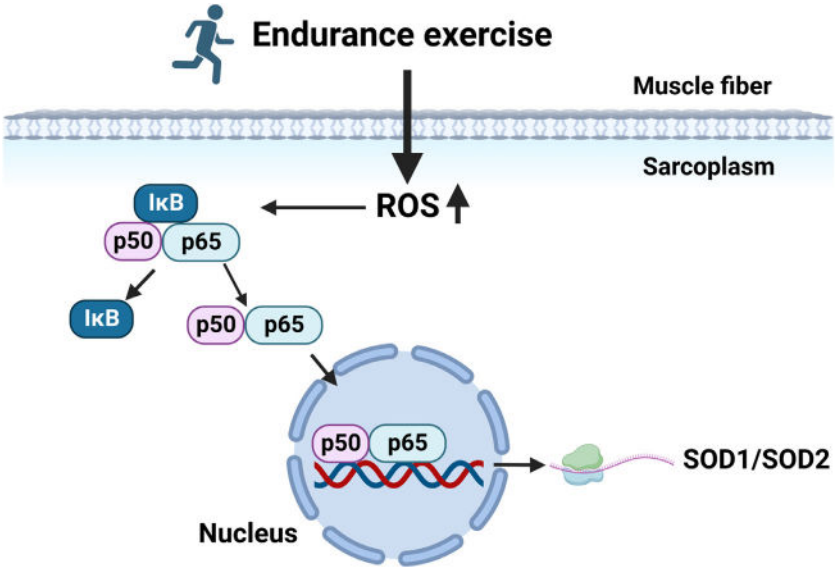


Fig. 8. Illustration of the impact of endurance exercise on the activation of the transcription factor NF-κB, leading to the expression of antioxidant enzymes (e.g., SOD1 and 2) in skeletal muscle fibers. IkB = inhibitory protein B; p50 = member of nuclear kappa beta family of transcriptional factors; p65 = member of nuclear kappa beta family of transcriptional factors; ROS = reactive oxygen species; SOD = superoxide dismutase;

induced ROS production plays a key role in skeletal muscle adaptations following endurance exercise.

The observation that supplementation with high doses of antioxidants (e.g., 400 i.u. vitamin E/1000 mg vitamin C) blunts some of the endurance exercise-induced adaptations in skeletal muscles supports the view that exercise-induced ROS production and redox signaling is essential for endurance training-induced adaptation to skeletal muscles. Specifically, numerous studies conclude that dietary supplementation with select antioxidants blunts the endurance training-induced adaptations in skeletal muscles of humans and other animals.[89,114–120] Nonetheless, not all studies concur with this conclusion.[121–124] The explanation for these divergent findings remains unclear but may be related to the dose and specific antioxidants used as well as the duration/intensity of exercise training.

In contrast to these antioxidant supplementation studies, uniform evidence indicates that NOX2- and/or NOX4-derived

ROS production is required for exercise-induced adaptations in skeletal muscles.[28,46,51,52,125] For example, pharmacological inhibition of NOX2 blunts exercise-induced gene expression in skeletal muscle following a bout of endurance exercise. Similarly, muscle specific knockout of NOX2 diminishes the training response to both endurance exercise and high intensity interval training.[52,53] For a detailed review of the evidence that NOX2 signaling plays a key role in exercise-induced adaptations in skeletal muscles see Henriquez-Olguin et al.[20] in the selected readings.

It is worth noting the evidence also indicates that exercise-induced activation of NOX4 in skeletal muscles is required for certain exercise-induced muscle adaptations.[28,125] In particular, knockout of muscle-specific NOX4 diminishes the exercise training-induced increase in insulin sensitivity.[28] These results provide cause and effect evidence to connect NOX4-derived ROS production in skeletal with the exercise-induced adaptations that promote increased insulin sensitivity.[28] As discussed earlier, the increase in NOX4-mediated ROS production in skeletal muscle may occur after the exercise bout due to increased expression of NOX4 in muscle fibers.

In addition to the NOX4 located in skeletal muscle, NOX4 is also expressed in the capillary endothelium and recent evidence suggests that several exercise responsive genes in skeletal muscle are dependent upon ROS production by endothelial NOX4.[125] Explicitly, deletion of endothelial NOX4 decreases the expression of several metabolic genes following exercise. In particular, although vascular NOX4 is not required for the exercise-induced increase in PGC-1$\alpha$, endothelial NOX4 is required for the exercise-induced expression of both hexokinase and pyruvate dehydrogenase; these results suggest that a ROS crosstalk exists between the endothelium and skeletal muscle in response to exercise.[125]

In summary, numerous studies using a variety of experimental approaches have addressed the question of whether exercise-induced production of ROS is required to attain the maximum benefits of endurance exercise-induced metabolic adaptations in skeletal muscle. Together, the available evidence supports the concept that exercise-induced ROS production is essential to achieve the full benefit of exercise-induced adaptation in skeletal muscles.

## 8. Summary and future directions

Muscular contractions result in an acute increase in ROS production from several cellular locations, including NOX2 and PLA2. Moreover, evidence indicates that a bout of endurance exercise results in increased mitochondrial ROS production within 3−6 h post-exercise. Collectively, this exercise-induced ROS production triggers signaling pathways regulating mitochondrial biogenesis and the expression of numerous genes (HSP72, mitochondrial oxidative enzymes, antioxidant enzymes, *etc*.) associated with muscle adaptation to endurance exercise. Growing evidence reveals that ROS production from NOX2 in skeletal muscle along with muscle and endothelial NOX4 contributes to these exercise-induced adaptations; collectively, these data support the concept that exercise-induced ROS production is essential to achieve the full benefit of exercise-induced adaptation in skeletal muscles.

Although progress has been made in our understanding of the role that ROS play in exercise-induced muscle adaptations, several questions remain unanswered. For example, although PLA2 can produce ROS during muscular contractions, the relative role that PLA2 ROS production plays in exercise-induced redox signaling is unknown.

Furthermore, although $H_2O_2$ is known to be an intracellular messenger in signal transduction, how muscle contraction-induced production of $H_2O_2$ leads to selective oxidation of specific thiols on signaling proteins remains unclear. In regard to $H_2O_2$ signaling, it is important to determine the relative contributions of PRDX-mediated oxidation or oxidation through other intermediary effectors *versus* direct thiol oxidation.

Another important area for future research is the investigation of the ROS signaling crosstalk that occurs between NOX4/vascular ROS production and NOX2/NOX4 ROS production within the contracting muscle fibers. Moreover, many unanswered questions remain regarding the role that NOX4 plays in promoting post-exercise ROS production and the potential role that this post-exercise ROS production plays in stimulating muscle adaptations to exercise training. Indeed, there is much more to be learned about this exciting topic.

## Uncited References Q3

[126]

## Declaration of competing interests

The authors declare that they have no competing interests.

## Authors' contributions

SKP assisted in conceptualization, writing original draft, review of literature, and editing; LLJ, ZR, and MJ assisted in conceptualization, review of literature and editing. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

## Acknowledgments

This work was supported by a grant from the National Institutes of Health (R21AR063956 to SKP). Figures were created with BioRender.com.

## References

1. Sies H. *Oxidative stress*. New York, NY: Academic Press; 1985.
2. Dillard CJ, Litov RE, Savin WM, Dumelin EE, Tappel AL. Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. *J Appl Physiol Respir Environ Exerc Physiol* 1978;**45**:927–32.
3. Davies KJ, Packer L, Brooks GA. Exercise bioenergetics following sprint training. *Arch Biochem Biophys* 1982;**215**:260–5.
4. Jackson MJ, Edwards RH, Symons MC. Electron spin resonance studies of intact mammalian skeletal muscle. *Biochim Biophys Acta* 1985; **847**:185–90.

5. Bailey DM, Lawrenson L, McEneny J, et al. Electron paramagnetic spectroscopic evidence of exercise-induced free radical accumulation in human skeletal muscle. *Free Radic Res* 2007;**41**:182–90.
6. Bailey DM, Davies B, Young IS, et al. Epr spectroscopic detection of free radical outflow from an isolated muscle bed in exercising humans. *J Appl Physiol (1985)* 2003;**94**:1714–8.
7. Powers SK, Radak Z, Ji LL. Exercise-induced oxidative stress: Past, present and future. *J Physiol* 2016;**594**:5081–92.
8. Sen CK, Packer L. Antioxidant and redox regulation of gene transcription. *FASEB J* 1996;**10**:709–20.
9. Rhee SG. Redox signaling: Hydrogen peroxide as intracellular messenger. *Exp Mol Med* 1999;**31**:53–9.
10. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat Rev Mol Cell Biol* 2020;**21**:363–83.
11. Winterbourn CC. Biological production, detection, and fate of hydrogen peroxide. *Antioxid Redox Signal* 2018;**29**:541–51.
12. BaJG Halliwell. *Free radicals in biology and medicine*. Oxford, UK: Oxford, Academic; 2015.
13. Sies H, Ursini F. Homeostatic control of redox status and health. *IUBMB Life* 2022;**74**:24–8.
14. Forman HJ, Maiorino M, Ursini F. Signaling functions of reactive oxygen species. *Biochemistry* 2010;**49**:835–42.
15. Sies H. Role of metabolic $H_2O_2$ generation: Redox signaling and oxidative stress. *J Biol Chem* 2014;**289**:8735–41.
16. Stone JR, Yang S. Hydrogen peroxide: A signaling messenger. *Antioxid Redox Signal* 2006;**8**:243–70.
17. Sies H, Chance B. The steady state level of catalase compound I in isolated hemoglobin-free perfused rat liver. *FEBS Lett* 1970;**11**:172–6.
18. Jackson MJ, Stretton C, McArdle A. Hydrogen peroxide as a signal for skeletal muscle adaptations to exercise: What do concentrations tell us about potential mechanisms? *Redox Biol* 2020;**35**: 101484. doi:10.1016/j.redox.2020.101484.
19. Go YM, Chandler JD, Jones DP. The cysteine proteome. *Free Radic Biol Med* 2015;**84**:227–45.
20. Henriquez-Olguin C, Boronat S, Cabello-Verrugio C, Jaimovich E, Hidalgo E, Jensen TE. The emerging roles of nicotinamide adenine dinucleotide phosphate oxidase 2 in skeletal muscle redox signaling and metabolism. *Antioxid Redox Signal* 2019;**31**:1371–410.
21. Kavazis AN, Talbert EE, Smuder AJ, Hudson MB, Nelson WB, Powers SK. Mechanical ventilation induces diaphragmatic mitochondrial dysfunction and increased oxidant production. *Free Radic Biol Med* 2009;**46**:842–50.
22. Powers SK, Hudson MB, Nelson WB, et al. Mitochondria-targeted antioxidants protect against mechanical ventilation-induced diaphragm weakness. *Crit Care Med* 2011;**39**:1749–59.
23. Aydin J, Andersson DC, Hanninen SL, et al. Increased mitochondrial $Ca^{2+}$ and decreased sarcoplasmic reticulum $Ca^{2+}$ in mitochondrial myopathy. *Hum Mol Genet* 2009;**18**:278–88.
24. Pearson T, Kabayo T, Ng R, Chamberlain J, McArdle A, Jackson MJ. Skeletal muscle contractions induce acute changes in cytosolic superoxide, but slower responses in mitochondrial superoxide and cellular hydrogen peroxide. *PLoS One* 2014;**9**:e96378. doi:10.1371/journal.pone.0096378.
25. Michaelson LP, Shi G, Ward CW, Rodney GG. Mitochondrial redox potential during contraction in single intact muscle fibers. *Muscle Nerve* 2010;**42**:522–9.
26. Laker RC, Xu P, Ryall KA, et al. A novel mitotimer reporter gene for mitochondrial content, structure, stress, and damage *in vivo*. *J Biol Chem* 2014;**289**:12005–15.
27. Laker RC, Drake JC, Wilson RJ, et al. Ampk phosphorylation of Ulk1 is required for targeting of mitochondria to lysosomes in exercise-induced mitophagy. *Nat Commun* 2017;**8**:548. doi:10.1038/s41467-017-00520-9.
28. Xirouchaki CE, Jia Y, McGrath MJ, et al. Skeletal muscle NOX4 is required for adaptive responses that prevent insulin resistance. *Sci Adv* 2021;**7**:eabl4988. doi:10.1126/sciadv.abl4988.
29. Osorio Alves J, Matta Pereira L, Cabral Coutinho do Rêgo Monteiro I, et al. Strenuous acute exercise induces slow and fast twitch-dependent NADPH oxidase expression in rat skeletal muscle. *Antioxidants (Basel)* 2020;**9**:57. doi:10.3390/antiox9010057.
30. Prunonosa Cervera I, Gabriel BM, Aldiss P, Morton NM. The phospholipase A2 family's role in metabolic diseases: Focus on skeletal muscle. *Physiol Rep* 2021;**9**:e14662. doi:10.14814/phy2.14662.
31. Six DA, Dennis EA. The expanding superfamily of phospholipase A(2) enzymes: Classification and characterization. *Biochim Biophys Acta* 2000;**1488**:1–19.
32. Gong MC, Arbogast S, Guo Z, Mathenia J, Su W, Reid MB. Calcium-independent phospholipase A2 modulates cytosolic oxidant activity and contractile function in murine skeletal muscle cells. *J Appl Physiol (1985)* 2006;**100**:399–405.
33. Nethery D, Callahan LA, Stofan D, Mattera R, DiMarco A, Supinski G. PLA(2) dependence of diaphragm mitochondrial formation of reactive oxygen species. *J Appl Physiol (1985)* 2000;**89**:72–80.
34. Nethery D, Stofan D, Callahan L, DiMarco A, Supinski G. Formation of reactive oxygen species by the contracting diaphragm is PLA(2) dependent. *J Appl Physiol (1985)* 1999;**87**:792–800.
35. Hille R, Nishino T. Flavoprotein structure and mechanism. 4. Xanthine oxidase and xanthine dehydrogenase. *FASEB J* 1995;**9**:995–1003.
36. Hellsten Y, Frandsen U, Orthenblad N, Sjodin B, Richter EA. Xanthine oxidase in human skeletal muscle following eccentric exercise: A role in inflammation. *J Physiol* 1997;**498**:239–48.
37. Hellsten-Westing Y. Immunohistochemical localization of xanthine oxidase in human cardiac and skeletal muscle. *Histochemistry* 1993;**100**:215–22.
38. Wajner M, Harkness RA. Distribution of xanthine dehydrogenase and oxidase activities in human and rabbit tissues. *Biochim Biophys Acta* 1989;**991**:79–84.
39. Ryan MJ, Jackson JR, Hao Y, Leonard SS, Alway SE. Inhibition of xanthine oxidase reduces oxidative stress and improves skeletal muscle function in response to electrically stimulated isometric contractions in aged mice. *Free Radic Biol Med* 2011;**51**:38–52.
40. Gomez-Cabrera MC, Borras C, Pallardo FV, Sastre J, Ji LL, Vina J. Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. *J Physiol* 2005;**567**:113–20.
41. Heunks LM, Vina J, van Herwaarden CL, Folgering HT, Gimeno A, Dekhuijzen PN. Xanthine oxidase is involved in exercise-induced oxidative stress in chronic obstructive pulmonary disease. *Am J Physiol* 1999;**277**:R1697–704.
42. Vina J, Gimeno A, Sastre J, et al. Mechanism of free radical production in exhaustive exercise in humans and rats; role of xanthine oxidase and protection by allopurinol. *IUBMB Life* 2000;**49**:539–44.
43. Ferreira LF, Laitano O. Regulation of NADPH oxidases in skeletal muscle. *Free Radic Biol Med* 2016;**98**:18–28.
44. Loureiro AC, do Rego-Monteiro IC, Louzada RA, et al. Differential expression of NADPH oxidases depends on skeletal muscle fiber type in rats. *Oxid Med Cell Longev* 2016;**2016**: 6738701. doi:10.1155/2016/6738701.
45. Chen F, Haigh S, Barman S, Fulton DJ. From form to function: The role of Nox4 in the cardiovascular system. *Front Physiol* 2012;**3**:412. doi:10.3389/fphys.2012.00412.
46. Vogel J, Figueiredo de Rezende F, Rohrbach S, Zhang M, Schroder K. Nox4 is dispensable for exercise induced muscle fibre switch. *PLoS One* 2015;**10**: e0130769.
47. Vogel J, Kruse C, Zhang M, Schroder K. Nox4 supports proper capillary growth in exercise and retina neo-vascularization. *J Physiol* 2015;**593**:2145–54.
48. Espinosa A, Leiva A, Pena M, et al. Myotube depolarization generates reactive oxygen species through NAD(P)H oxidase; ROS-elicited $Ca^{2+}$ stimulates ERK, CREB, early genes. *J Cell Physiol* 2006;**209**:379–88.
49. Pattwell DM, McArdle A, Morgan JE, Patridge TA, Jackson MJ. Release of reactive oxygen and nitrogen species from contracting skeletal muscle cells. *Free Radic Biol Med* 2004;**37**:1064–72.
50. Sakellariou GK, Vasilaki A, Palomero J, et al. Studies of mitochondrial and nonmitochondrial sources implicate nicotinamide adenine dinucleotide phosphate oxidase(s) in the increased skeletal muscle superoxide


Please cite this article as: Scott K. Powers et al., Reactive oxygen species promote endurance exercise-induced adaptations in skeletal muscles, Journal of Sport and Health Science (2024), https://doi.org/10.1016/j.jshs.2024.05.001

generation that occurs during contractile activity. *Antioxid Redox Signal* 2013;**18**:603–21.
51. Henriquez-Olguin C, Diaz-Vegas A, Utreras-Mendoza Y, et al. NOX2 inhibition impairs early muscle gene expression induced by a single exercise bout. *Front Physiol* 2016;**7**:282. doi:10.3389/fphys.2016.00282.
52. Henriquez-Olguin C, Knudsen JR, Raun SH, et al. Cytosolic ROS production by NADPH oxidase 2 regulates muscle glucose uptake during exercise. *Nat Commun* 2019;**10**:4623. doi:10.1038/s41467-019-12523-9.
53. Henriquez-Olguin C, Renani LB, Arab-Ceschia L, et al. Adaptations to high-intensity interval training in skeletal muscle require NADPH oxidase 2. *Redox Biol* 2019;**24**: 101188.
54. Bouviere J, Fortunato RS, Dupuy C, Werneck-de-Castro JP, Carvalho DP, Louzada RA. Exercise-stimulated ROS sensitive signaling pathways in skeletal muscle. *Antioxidants (Basel)* 2021;**10**:537. doi:10.3390/antiox10040537.
55. Jackson MJ, Pollock N, Staunton C, Jones S, McArdle A. Redox control of signalling responses to contractile activity and ageing in skeletal muscle. *Cells* 2022;**11**:1698. doi:10.3390/cells11101698.
56. Jackson MJ, Vasilaki A, McArdle A. Cellular mechanisms underlying oxidative stress in human exercise. *Free Radic Biol Med* 2016;**98**:13–7.
57. Sakellariou GK, Jackson MJ, Vasilaki A. Redefining the major contributors to superoxide production in contracting skeletal muscle. The role of NAD(P)H oxidases. *Free Radic Res* 2014;**48**:12–29.
58. Powers SK, Deminice R, Ozdemir M, Yoshihara T, Bomkamp MP, Hyatt H. Exercise-induced oxidative stress: Friend or foe? *J Sport Health Sci* 2020;**9**:415–25.
59. Powers SK, Jackson MJ. Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production. *Physiol Rev* 2008;**88**:1243–76.
60. Powers SK, Goldstein E, Schrager M, Ji LL. Exercise training and skeletal muscle antioxidant enzymes: An update. *Antioxidants (Basel)* 2022;**12**:39. doi:10.3390/antiox12010039.
61. Flohe L, Toppo S, Orian L. The glutathione peroxidase family: Discoveries and mechanism. *Free Radic Biol Med* 2022;**187**:113–22.
62. Rhee SG, Kil IS. Multiple functions and regulation of mammalian peroxiredoxins. *Annu Rev Biochem* 2017;**86**:749–75.
63. Sobotta MC, Liou W, Stocker S, et al. Peroxiredoxin-2 and STAT3 form a redox relay for $H_2O_2$ signaling. *Nat Chem Biol* 2015;**11**:64–70.
64. Barata AG, Dick TP. A role for peroxiredoxins in $H_2O_2$- and MEKK-dependent activation of the p38 signaling pathway. *Redox Biol* 2020;**28**: 101340. doi:10.1016/j.redox.2019.101340.
65. Sies H, Belousov VV, Chandel NS, et al. Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nat Rev Mol Cell Biol* 2022;**23**:499–515.
66. Stocker S, Van Laer K, Mijuskovic A, Dick TP. The conundrum of hydrogen peroxide signaling and the emerging role of peroxiredoxins as redox relay hubs. *Antioxid Redox Signal* 2018;**28**:558–73.
67. Marinho HS, Real C, Cyrne L, Soares H, Antunes F. Hydrogen peroxide sensing, signaling and regulation of transcription factors. *Redox Biol* 2014;**2**:535–62.
68. Yu M, Blomstrand E, Chibalin AV, Krook A, Zierath JR. Marathon running increases ERK1/2 and p38 MAP kinase signalling to downstream targets in human skeletal muscle. *J Physiol* 2001;**536**:273–82.
69. Pilegaard H, Saltin B, Neufer PD. Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle. *J Physiol* 2003;**546**:851–8.
70. Ji LL, Gomez-Cabrera MC, Steinhafel N, Vina J. Acute exercise activates nuclear factor (NF)-kappaB signaling pathway in rat skeletal muscle. *FASEB J* 2004;**18**:1499–506.
71. Gallego-Selles A, Martin-Rincon M, Martinez-Canton M, et al. Regulation of Nrf2/keap1 signalling in human skeletal muscle during exercise to exhaustion in normoxia, severe acute hypoxia and post-exercise ischaemia: Influence of metabolite accumulation and oxygenation. *Redox Biol* 2020;**36**: 101627. doi:10.1016/j.redox.2020.101627.72.
72. Demirel HA, Powers SK, Naito H, Tumer N. The effects of exercise duration on adrenal HSP72/73 induction in rats. *Acta Physiol Scand* 1999;**167**:227–31.
73. Hamilton KL, Powers SK, Sugiura T, et al. Short-term exercise training can improve myocardial tolerance to I/R without elevation in heat shock proteins. *Am J Physiol Heart Circ Physiol* 2001;**281**:H1346–52.
74. Powers SK, Locke, Demirel HA. Exercise, heat shock proteins, and myocardial protection from I-R injury. *Med Sci Sports Exerc* 2001;**33**:386–92.
75. Kavazis AN, Smuder AJ, Min K, Tumer N, Powers SK. Short-term exercise training protects against doxorubicin-induced cardiac mitochondrial damage independent of HSP72. *Am J Physiol Heart Circ Physiol* 2010;**299**:H1515–24.
76. Naito H, Powers SK, Demirel HA, Aoki J. Exercise training increases heat shock protein in skeletal muscles of old rats. *Med Sci Sports Exerc* 2001;**33**:729–34.
77. Samelman TR. Heat shock protein expression is increased in cardiac and skeletal muscles of Fischer 344 rats after endurance training. *Exp Physiol* 2000;**85**:92–102.
78. Geiger PC, Gupte AA. Heat shock proteins are important mediators of skeletal muscle insulin sensitivity. *Exerc Sport Sci Rev* 2011;**39**:34–42.
79. Marshall JPS, Estevez E, Kammoun HL, et al. Skeletal muscle-specific overexpression of heat shock protein 72 improves skeletal muscle insulin-stimulated glucose uptake but does not alter whole body metabolism. *Diabetes Obes Metab* 2018;**20**:1928–36.
80. Smuder AJ, Morton AB, Hall SE, et al. Effects of exercise preconditioning and HSP72 on diaphragm muscle function during mechanical ventilation. *J Cachexia Sarcopenia Muscle* 2019;**10**:767–81.
81. Ahn SG, Thiele DJ. Redox regulation of mammalian heat shock factor 1 is essential for Hsp gene activation and protection from stress. *Genes Dev* 2003;**17**:516–28.
82. Gehrig SM, van der Poel C, Sayer TA, et al. Hsp72 preserves muscle function and slows progression of severe muscular dystrophy. *Nature* 2012;**484**:394–8.
83. Kennedy TL, Swiderski K, Murphy KT, et al. BGP-15 improves aspects of the dystrophic pathology in mdx and dko mice with differing efficacies in heart and skeletal muscle. *Am J Pathol* 2016;**186**:3246–60.
84. Powers SK, Smuder AJ, Kavazis AN, Quindry JC. Mechanisms of exercise-induced cardioprotection. *Physiology (Bethesda)* 2014;**29**:27–38.
85. Gomez-Pastor R, Burchfiel ET, Thiele DJ. Regulation of heat shock transcription factors and their roles in physiology and disease. *Nat Rev Mol Cell Biol* 2018;**19**:4–19.
86. Dayalan Naidu S, Dinkova-Kostova AT. Regulation of the mammalian heat shock factor 1. *FEBS J* 2017;**284**:1606–27.
87. Nishizawa J, Nakai A, Matsuda K, Komeda M, Ban T, Nagata K. Reactive oxygen species play an important role in the activation of heat shock factor 1 in ischemic-reperfused heart. *Circulation* 1999;**99**:934–41.
88. Kukreja RC, Kontos MC, Loesser KE, et al. Oxidant stress increases heat shock protein 70 mRNA in isolated perfused rat heart. *Am J Physiol* 1994;**267**:H2213–9.
89. Hamilton KL, Staib JL, Phillips T, Hess A, Lennon SL, Powers SK. Exercise, antioxidants, and HSP72: Protection against myocardial ischemia/reperfusion. *Free Radic Biol Med* 2003;**34**:800–9.
90. Slavin MB, Memme JM, Oliveira AN, Moradi N, Hood DA. Regulatory networks coordinating mitochondrial quality control in skeletal muscle. *Am J Physiol Cell Physiol* 2022;**322**:C913–C26.
91. Scarpulla RC. Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. *Biochim Biophys Acta* 2011;**1813**: 1269–78.
92. Dominy JE, Puigserver P. Mitochondrial biogenesis through activation of nuclear signaling proteins. *Cold Spring Harb Perspect Biol* 2013;**5**: a015008. doi:10.1101/cshperspect.a015008.
93. Wu Z, Puigserver P, Andersson U, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 1999;**98**:115–24.
94. Barbieri E, Sestili P. Reactive oxygen species in skeletal muscle signaling. *J Signal Transduct* 2012;**2012**: 982794. doi:10.1155/2012/982794.
95. Dent J, Stocks B, Campelj D, Philip A. Transient changes to metabolic homeostasis initiate mitochondrial adaptation to endurance exercise. *Semin Cell Dev Biol* 2023;**143**:3–16.

Please cite this article as: Scott K. Powers et al., Reactive oxygen species promote endurance exercise-induced adaptations in skeletal muscles, Journal of Sport and Health Science (2024), https://doi.org/10.1016/j.jshs.2024.05.001

96. Akimoto T, Pohnert SC, Li P, et al. Exercise stimulates PGC-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway. *J Biol Chem* 2005;**280**:19587–93.
97. Zmijewski JW, Banerjee S, Bae H, Friggeri A, Lazarowski ER, Abraham E. Exposure to hydrogen peroxide induces oxidation and activation of AMP-activated protein kinase. *J Biol Chem* 2010;**285**:33154–64.
98. Irrcher I, Ljubicic V, Hood DA. Interactions between ROS and AMP kinase activity in the regulation of PGC-1alpha transcription in skeletal muscle cells. *Am J Physiol Cell Physiol* 2009;**296**:C116–23.
99. Kang C, O'Moore KM, Dickman JR, Ji LL. Exercise activation of muscle peroxisome proliferator-activated receptor-gamma coactivator-1alpha signaling is redox sensitive. *Free Radic Biol Med* 2009;**47**: 1394–400.
100. Torrente L, DeNicola GM. Targeting Nrf2 and its downstream processes: Opportunities and challenges. *Annu Rev Pharmacol Toxicol* 2022; **62**:279–300.
101. Dodson M, de la Vega MR, Cholanians AB, Schmidlin CJ, Chapman E, Zhang DD. Modulating Nrf2 in disease: Timing is everything. *Annu Rev Pharmacol Toxicol* 2019;**59**:555–75.
102. Lacher SE, Lee JS, Wang X, Campbell MR, Bell DA, Slattery M. Beyond antioxidant genes in the ancient Nrf2 regulatory network. *Free Radic Biol Med* 2015;**88**:452–65.
103. Done AJ, Traustadottir T. Nrf2 mediates redox adaptations to exercise. *Redox Biol* 2016;**10**:191–9.
104. Yamamoto M, Kensler TW, Motohashi H. The KEAP1-NRF2 system: A thiol-based sensor-effector apparatus for maintaining redox homeostasis. *Physiol Rev* 2018;**98**:1169–203.
105. Hayes JD, Dinkova-Kostova AT. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. *Trends Biochem Sci* 2014;**39**:199–218.
106. Cuadrado A, Rojo AI, Wells G, et al. Therapeutic targeting of the Nrf2 and KEAP1 partnership in chronic diseases. *Nat Rev Drug Discov* 2019;**18**:295–317.
107. Ji LL, Gomez-Cabrera MC, Vina J. Role of nuclear factor kappaB and mitogen-activated protein kinase signaling in exercise-induced antioxidant enzyme adaptation. *Appl Physiol Nutr Metab* 2007;**32**:930–5.
108. Ji LL. Antioxidant signaling in skeletal muscle: A brief review. *Exp Gerontol* 2007;**42**:582–93.
109. Jackman RW, Kandarian SC. The molecular basis of skeletal muscle atrophy. *Am J Physiol Cell Physiol* 2004;**287**:C834–43.
110. Jackman RW, Cornwell EW, Wu CL, Kandarian SC. Nuclear factor-κB signalling and transcriptional regulation in skeletal muscle atrophy. *Exp Physiol* 2013;**98**:19–24.
111. Morgan MJ, Liu ZG. Crosstalk of reactive oxygen species and NF-κB signaling. *Cell Res* 2011;**21**:103–15.
112. Lingappan K. NF-κB in oxidative stress. *Curr Opin Toxicol* 2018; **7**:81–6.
113. Gallego-Selles A, Galvan-Alvarez V, Martinez-Canton M, et al. Fast regulation of the NF-κB signalling pathway in human skeletal muscle revealed by high-intensity exercise and ischaemia at exhaustion: Role of oxygenation and metabolite accumulation. *Redox Biol* 2022;**55**: 102398. doi:10.1016/j.redox.2022.102398.
114. Gomez-Cabrera MC, Domenech E, Romagnoli M, et al. Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. *Am J Clin Nutr* 2008;**87**:142–9.
115. Ristow M, Zarse K, Oberbach A, et al. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci U S A* 2009;**106**:8665–70.
116. Morrison D, Hughes J, Della Gatta PA, et al. Vitamin C and E supplementation prevents some of the cellular adaptations to endurance-training in humans. *Free Radic Biol Med* 2015;**89**:852–62.
117. Paulsen G, Cumming KT, Holden G, et al. Vitamin C and E supplementation hampers cellular adaptation to endurance training in humans: A double-blind, randomised, controlled trial. *J Physiol* 2014;**592**:1887–901.
118. Venditti P, Napolitano G, Barone D, Di Meo S. Vitamin E supplementation modifies adaptive responses to training in rat skeletal muscle. *Free Radic Res* 2014;**48**:1179–89.
119. Meier P, Renga M, Hoppeler H, Baum O. The impact of antioxidant supplements and endurance exercise on genes of the carbohydrate and lipid metabolism in skeletal muscle of mice. *Cell Biochem Funct* 2013;**31**:51–9.
120. Strobel NA, Peake JM, Matsumoto A, Marsh SA, Coombes JS, Wadley GD. Antioxidant supplementation reduces skeletal muscle mitochondrial biogenesis. *Med Sci Sports Exerc* 2011;**43**:1017–24.
121. Yfanti C, Akerstrom T, Nielsen S, et al. Antioxidant supplementation does not alter endurance training adaptation. *Med Sci Sports Exerc* 2010;**42**:1388–95.
122. Yfanti C, Nielsen AR, Akerstrom T, et al. Effect of antioxidant supplementation on insulin sensitivity in response to endurance exercise training. *Am J Physiol Endocrinol Metab* 2011;**300**:E761–70.
123. Cumming KT, Raastad T, Holden G, et al. Effects of vitamin C and E supplementation on endogenous antioxidant systems and heat shock proteins in response to endurance training. *Physiol Rep* 2014;**2**:e12142. doi:10.14814/phy2.12142.
124. Higashida K, Kim SH, Higuchi M, Holloszy JO, Han DH. Normal adaptations to exercise despite protection against oxidative stress. *Am J Physiol Endocrinol Metab* 2011;**301**:E779–84.
125. Specht KS, Kant S, Addington AK, et al. Nox4 mediates skeletal muscle metabolic responses to exercise. *Mol Metab* 2021;**45**: 101160. doi:10.1016/j.molmet.2020.101160.
126. Jackson MJ. Control of reactive oxygen species production in contracting skeletal muscle. *Antioxid Redox Signal* 2011;**15**:2477–86.


Please cite this article as: Scott K. Powers et al., Reactive oxygen species promote endurance exercise-induced adaptations in skeletal muscles, Journal of Sport and Health Science (2024), https://doi.org/10.1016/j.jshs.2024.05.001