Abstract
The first report demonstrating that prolonged endurance exercise promotes oxidative stress in humans was published more than 4 decades ago. Since this discovery, many ensuing investigations have corroborated the fact that muscular exercise increases the production of reactive oxygen species (ROS) and results in oxidative stress in numerous tissues including blood and skeletal muscles. Although several tissues may contribute to exercise-induced ROS production, it is predicted that muscular contractions stimulate ROS production in active muscle fibers and that skeletal muscle is a primary source of ROS production during exercise. This contraction-induced ROS generation is associated with (1) oxidant damage in several tissues (e.g., increased protein oxidation and lipid peroxidation), (2) accelerated muscle fatigue, and (3) activation of biochemical signaling pathways that contribute to exercise-induced adaptation in the contracting muscle fibers. While our understanding of exercise and oxidative stress has advanced rapidly during the last decades, questions remain about whether exercise-induced increases in ROS production are beneficial or harmful to health. This review addresses this issue by discussing the site(s) of oxidant production during exercise and detailing the health consequences of exercise-induced ROS production.

Keywords: Hormesis; Oxidants; Radicals; Reactive oxygen species; Skeletal muscle

1. Introduction
The production of high levels of reactive oxygen species (ROS) in cells promotes redox disturbances leading to oxidative damage to cellular components. Indeed, it is clear that chronic oxidative damage is associated with the pathogenesis of cancer, cardiovascular disease, diabetes mellitus, hypertension, and several neurodegenerative diseases.1–4 Interestingly, while regular physical activity promotes health benefits, rigorous and/or prolonged exercise results in an acute increase in the production of ROS as evidenced by elevated biomarkers of oxidative damage in both blood and skeletal muscles.5 The fact that muscular exercise promotes ROS production appears enigmatic because regular exercise is the only health behavior associated with a decrease in all-cause mortality in humans.6 This review addresses this exercise/oxidative stress paradox by discussing the cellular consequences of exercise-induced ROS production. We begin with a review of the primary oxidants produced in cells followed by a summary of cellular antioxidant systems. We then discuss the sources of ROS production during exercise and debate the question of whether exercise-induced ROS production is beneficial or harmful to health.

2. Redox balance and oxidative stress
The term “oxidative stress” was first defined by Helmut Sies7 as “a disturbance in the pro-oxidant/antioxidant balance in favor of the former”. Although this simple definition has been widely applied in the literature for several decades, this account of oxidative stress has been criticized because of the lack of detail. In particular, given that cellular redox balance is complex, it has been argued that although the term “oxidative stress” does encompass a pro-oxidant vs. antioxidant condition in the cell, this definition does not provide sufficient details about the nature of the cellular redox imbalance. To address...
this concern, Helmut Sies collaborated with Dean Jones to generate a more detailed definition that defines oxidative stress as “an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control, and/or molecular damage.”

We have adopted this definition of oxidative stress for this review.

3. Biological radicals—an overview

The observation that living cells produce free radicals (radicals) was first reported in 1954. Since this early discovery, many studies have explored the sources and impact of radicals on cells. As a chemical species, radicals are defined as an atom/molecule that contains one or more unpaired electrons. The term ROS is a general label that refers to both oxygen-centered radicals and non-radicals that are reactive derivatives of oxygen (e.g., hydrogen peroxide (H2O2)).

A related term, reactive nitrogen species (RNS), refers to both radical (e.g., nitric oxide (NO)) and non-radical nitrogen species (e.g., peroxynitrite). A brief introduction to the major ROS and RNS follows. For more detailed information on ROS and RNS, the reader is directed to a comprehensive review on this topic.

Superoxide (O2−) is the parent molecule of all ROS and is formed by the one-electron reduction of molecular oxygen; this anion is negatively charged and relatively membrane impermeable, and compared to other radical species is relatively unreactive. The breakdown (dismutation) of O2− occurs spontaneously but can also be catalyzed by the enzyme superoxide dismutase (SOD). The dismutation of O2− provides a major source of the production of H2O2. H2O2 is a non-radical ROS that is permeable to membranes and has a relatively long half-life in the cell. Although H2O2 is considered a weak oxidizing agent, chronically high cellular levels of H2O2 are damaging to cell components and therefore cytotoxic. Importantly, because of the long half-life and high membrane permeability of H2O2, this molecule can diffuse considerable distances in/out of the cell. Together, these properties make H2O2 a primary candidate for ROS-mediated signaling in cells. More details about this topic will be provided elsewhere in this review.

The hydroxyl radical (·OH) has a strong oxidizing potential and is commonly formed by reactions involving both H2O2 and O2−. Because of the high reactivity, ·OH radicals typically oxidize molecules close to their site of production. By comparison to other reactive species, ·OH radicals are considered the most damaging ROS because of their high reactivity.

NO is the parent molecule of all RNS and is synthesized from the amino acid L-arginine through NO synthase (NOS). Four NOS isoforms exist, and 3 of these isoforms are found in skeletal muscle fibers. Specifically, both neuronal NOS (nNOS/NOS1) and endothelial NOS (eNOS/NOS3) exist in skeletal muscle fibers. Notably, 2 splice variants of NOS1 (i.e., nNOSβ and nNOSμ) exist in skeletal muscle fibers. Moreover, the inducible NOS (iNOS/NOS2) is also found in skeletal muscle during inflammatory conditions such as septic shock. These NOSs require several cofactors (e.g., 5,6,7,8-tetrahydrobiopterin and iron) and convert L-arginine into NO and L-citrulline utilizing nicotinamide adenine dinucleotide phosphate (NADPH). It is well-established that muscular contractions increase the production of NO within the contracting fibers.

Once produced, NO is a weak reducing agent that can bind with transition metals located within enzymes to serve as a positive allosteric activator. For example, NO binds to iron located in the enzyme guanyl cyclase; the result of NO binding to iron activates this enzyme, resulting in the formation of cyclic guanosine monophosphate (cGMP). Importantly, NO also reacts rapidly with O2− to form peroxynitrite (ONOO−). In fact, the reaction between NO and O2− occurs approximately 3 times faster than the dismutation of O2−; hence, the formation of ONOO− is the primary reaction of O2− when NO is present.

The formation of ONOO− has 2 important biological consequences. First, the formation of ONOO− results in the reduced bioavailability of NO. Second, the formation of ONOO− is also important because this RNS is a strong oxidizing agent that leads to the depletion of thiol groups and nitration of cellular proteins.

4. Cellular control of ROS

Since regulation of redox balance is critical for cellular health, all mammalian cells are equipped with control systems to regulate oxidation/reduction (redox) balance. A key component of redox control is the cellular antioxidant system. Antioxidants are commonly defined as any substance that significantly delays or prevents oxidation of a substrate. Because a detailed discussion of cellular antioxidants is outside the scope of this review, readers are directed to detailed reviews for more information about antioxidant systems. Nonetheless, to provide context for future discussions within this report, a brief overview of cellular antioxidant systems is provided here. Cells contain both enzymatic and non-enzymatic antioxidants that work as a complex regulatory network to control the levels of ROS. Indeed, throughout the cell, antioxidants are compartmentalized in both organelles and the cytoplasm to mitigate ROS and maintain redox balance. Further, antioxidants also exist in the interstitial fluid and blood, and these extracellular antioxidants play a key role in eliminating ROS that exist in extracellular fluids.

Three primary antioxidant strategies are used to protect cells against ROS-mediated damage. First, numerous low-molecular weight molecules capable of scavenging ROS exist in both the extracellular space and within cells. Second, some enzymatic antioxidants act by converting ROS into less reactive molecules; this limits oxidation and prevents the transformation of these ROS to more damaging species. A final antioxidant strategy involves the binding of pro-oxidant transition metals (e.g., iron and copper) via metal binding proteins; these chelating molecules prevent these transition metals from participating in ROS formation.

4.1. Antioxidant enzymes

SOD, glutathione reductase, and catalase (CAT) are the 3 primary antioxidant enzymes located in cells. Nonetheless, it is also clear that the thioredoxin (Trx) and peroxiredoxin (Prx) antioxidant systems also play a supportive role in maintaining redox balance in the cell. SOD is the first line of defense against O2− and dismutates O2− to form H2O2 and oxygen.
(O2). Three isofoms of SOD (SOD1, SOD2, and SOD3) exist in all mammals, and all require a redox active transition metal in the active site for the catalytic breakdown of the superoxide anion. Two of the SOD isoforms are located within cells, whereas the third SOD isofom is positioned within the extracellular space. SOD1 resides in both the cytosol and the mitochondrial intermembrane space whereas SOD2 is only situated in the mitochondrial matrix. In contrast, SOD3 is located outside the cell in the extracellular space.

Although superoxide radicals are not highly reactive, they remove electrons from cellular components (e.g., biological membranes), resulting in a series of radical-mediated reactions. As mentioned elsewhere in this article, superoxide radicals are also toxic because of their involvement in the generation of hydroxyl radicals. Further, recall that O2 can also react with NO to form ONOO−. It follows that elimination of superoxide radicals is critical to prevent cellular oxidative injury.

Five glutathione peroxidases (GPXs) exist in mammals (GPX1−GPX5). All of these GPX enzymes are responsible for the reduction of H2O2 or organic hydroperoxide to form water (H2O) and alcohol (ROH), respectively; this reaction requires an electron donor, and reduced glutathione (GSH) is the primary electron donor involved in GPX reactions.

Although all GPX isoforms reduce H2O2, the expression of multiple GPX isoforms is biologically logical because of the different cellular locations of the GPX isoforms. For example, GPX1 is located in both the cytosol and mitochondria. GPX2 is located exclusively in the cytosol, whereas GPX3 is found in both the cytosol and extracellular space. Hence, the family of GPX antioxidant enzymes play an important role in redox regulation due to both their substrate specificity and their dispersion across different cellular compartments; the varied cellular locations of GPX is advantageous because ROS are mitigated at their site of production.

The principal function of the antioxidant enzyme CAT is the breakdown of H2O2 into H2O and O2. Similar to GPX and SOD, CAT is also located within multiple compartments of the cell, including the cytosol and mitochondria. CAT differs from the GPXs in 2 major ways. First, CAT does not require an electron donor and, second, CAT only eliminates H2O2 and does not remove organic hydroperoxides.

The Trx antioxidant system is comprised of Trx and Trx reductase. Two isofoms of Trx exist: the cytosolic isofom (Trx1) and the mitochondrial form (Trx2). Functionally, Trx is the major ubiquitous disulfide reductase responsible for maintaining proteins in their reduced state. Trx maintains the reduced state of proteins via formation of a disulfide bond with the substrate protein and the transfer of 2 of its electrons to the target protein; this results in oxidation of the Trx protein and reduction of the target substrate. Oxidized Trx can then be reduced by electrons from NADPH via Trx reductase, allowing Trx to continue its role as a redox modulator. Trx serves several physiological roles: (1) protection against protein oxidation, (2) reduction of transcription factors, and (3) regulation of apoptosis. Moreover, Trx reductase also contributes as an antioxidant enzyme by reducing hydroperoxides and functioning as a NADPH-dependent dehydroascorbate reductase to recycle vitamin C.
exercise training increases several antioxidant enzymes, including SOD1, SOD2, and GPX1.\textsuperscript{38–46} In particular, these early studies show that exercise training increases both SOD1 and SOD2 in the trained skeletal muscles by 20%–110%.\textsuperscript{3} Similarly, studies also revealed that regular endurance exercise training increases skeletal muscle levels of GPX1 by 20%–180%.\textsuperscript{5} Although a few studies published in the 1980s and 1990s suggest that exercise training also increases muscle levels of CAT, other reports have failed to demonstrate an exercise-induced increase in CAT. Therefore, whether or not exercise increases CAT levels in cardiac and skeletal muscles remains controversial.\textsuperscript{5} Finally, to date, limited information exists about the impact of exercise training on the abundance of Prxs isoforms in skeletal muscle, and additional studies will be required to determine if exercise training impacts this antioxidant system in muscle fibers.

Another important discovery that occurred in the 1990s was the observation that skeletal muscle expresses 2 isoforms of NOS and that contracting skeletal muscles produce NO.\textsuperscript{12,47} This breakthrough paved the way for additional studies on the role that NO plays in skeletal muscle signaling.

In the late 1990s and early 2000s, a paradigm shift occurred in the thinking about the biological impact of exercise-induced ROS production. More specifically, during most of the 1980s, the ROS produced in skeletal muscles during exercise were considered damaging to muscle fibers without positive consequences. However, this view began to change after the recognition that NO was an important biological signaling molecule. Indeed, several important studies published in the early 2000s revealed that ROS are critical signaling molecules that increase gene expression in cultured myotubes, and many studies since have confirmed that ROS alter gene expression in skeletal muscle and other tissues.\textsuperscript{48–50} For a detailed overview of the history of research in exercise and oxidative stress.\textsuperscript{51}

6. Sources of oxidants in contracting skeletal muscles

Since the discovery that exercise-induced oxidative stress occurs, many investigators have probed the potential sources of ROS production in a number of tissues. Though oxidants could be produced in a variety of tissues during exercise, it has been established that skeletal muscle is the dominant source of ROS production during exercise.\textsuperscript{52} Possible sources of exercise-induced ROS production in muscle fibers has been widely investigated and include the following: (1) mitochondria, (2) phospholipase A\textsubscript{2} (PLA\textsubscript{2}), and (3) NADPH oxidases (NOX2 and NOX4) localized in 4 sites within fibers: mitochondria, sarclemma, sarcoplasmic reticulum, and T-tubules (Fig. 1).\textsuperscript{53–57} A brief discussion of each of these potential sites of oxidant production follows.

Although early studies proposed that mitochondria are the likely source of ROS production in muscle fibers during exercise, this prediction does not appear to be accurate given that ROS production in skeletal muscle mitochondria decreases during exercise. Specifically, based on studies performed in the 1970s, it was estimated that 2%–5% of molecular oxygen consumed in the mitochondria formed O$_2^•$. Based on this account, it was then hypothesized that increases in oxidative phosphorylation in the mitochondria within contracting skeletal muscles would result in a proportional increase in O$_2^•$ production. Nonetheless, contemporary studies reveal that mitochondria actually produce less O$_2^•$ during active State 3 respiration compared to basal State 4 respiration.\textsuperscript{59–61} Therefore, the available evidence indicates that mitochondria are not the major site of ROS production in skeletal muscle during exercise.

PLA\textsubscript{2} is an enzyme that cleaves membrane phospholipids to release arachidonic acid; free arachidonic acid is a substrate for several ROS-generating enzyme systems, including the lipoxygenases.\textsuperscript{62} Importantly, activation of PLA\textsubscript{2} can activate NADPH oxidases,\textsuperscript{63} and augmented PLA\textsubscript{2} activity in skeletal muscle can also promote ROS production in mitochondria\textsuperscript{64} and the cytosol.\textsuperscript{65} Note that both calcium-dependent and calcium-independent forms of PLA\textsubscript{2} exist in skeletal muscle and both isoforms are capable of stimulating ROS generation in muscle.\textsuperscript{64,65} It is hypothesized that the calcium-independent enzymes regulate cytosolic oxidant activity skeletal muscle cells under resting conditions,\textsuperscript{65} whereas the calcium-dependent isoform of PLA\textsubscript{2} stimulates mitochondrial

![Fig. 1. Potential sites of the production or reactive oxygen species in contracting skeletal muscles. CAT = catalase; GPX = glutathione peroxidase; H$_2$O$_2$ = hydrogen peroxide; NOX = NADPH oxidase; O$_2^•$ = superoxide; -OH = hydroxyl radical; PLA$_2$ = phospholipase A$_2$; SOD = superoxide dismutase. Modified from Powers and Jackson.\textsuperscript{5}](image)
ROS generation during contractile activity. Nonetheless, additional research is required to confirm or deny this postulate.

Skeletal muscle expresses 2 isofoms of NADPH oxidase (NOX2 and NOX4). NOX2 is located within the sarcolemma and T-tubule, whereas NOX4 is located in both the sarcoplasmic reticulum and the mitochondria. NOX4 is constitutively active and does not require association with regulatory subunits. In contrast, NOX2 is activated by specific agonists (e.g., angiotensin II, mechanical/contractile stress, and cytokines). Therefore, it appears likely that NOX4 contributes to the basal rate of ROS production in muscle fibers, whereas NOX2 is the primary source of NADPH oxidase-mediated ROS production in contracting muscle. Indeed, several recent studies point to NOX as a primary contributor to contraction-induced ROS production. For example, investigators using a variety of experimental techniques to examine the subcellular sites responsible for O$_2^-$ production in muscle fibers concluded that mitochondria are not responsible for contraction-induced production of O$_2^-$ in muscle fibers; instead, they concluded that NOX is a major source of O$_2^-$ production both at rest and during contractions. Similar conclusions have been reached by others. Nonetheless, at present, concluding that NOX is the dominant source for ROS in contracting skeletal muscle is complicated by the complexities associated with the study of NOX activity in cells. Clearly, improved methodologies and additional studies are required to clarify this issue. For more information on this topic, we refer the reader to the following references.

### 7. Cellular consequences of exercise-induced oxidant production in skeletal muscle fibers

It is established that ROS are continually produced in skeletal muscle at both rest and during exercise; importantly, ROS modulate a variety of physiological processes, including regulation of blood flow, muscle force production, and muscle adaptation to exercise training. Investigations into the consequences of ROS production in skeletal muscle fibers have now spanned 4 decades, resulting in a voluminous amount of research. Because of space limitations, we will limit our discussion to 3 issues related to the consequence of endurance exercise-induced ROS production in skeletal muscle: (1) exercise-induced oxidative stress, (2) ROS impact on muscle force production, and (3) ROS influence on muscle adaption to exercise training. We begin with a discussion of the impact of exercise-induced oxidative damage to macromolecules.

#### 7.1. Exercise-induced oxidative stress

Although short-duration (i.e., <1 min) and low-intensity (~30% maximal oxygen consumption (VO$_{2\text{max}}$) exercise does not appear to promote oxidative stress, it is well-established that acute bouts of prolonged and high-intensity endurance exercise in untrained humans and animals results in increases in biomarkers of oxidative stress (e.g., increased protein oxidation and lipid peroxidation) both in blood and in the active skeletal muscles. However, both short-term (5 consecutive days) and long-term (12 weeks) endurance exercise training increases antioxidant enzyme activities in the trained muscles and eliminates contraction-induced oxidative stress due to an acute bout of exercise. Further, a recent meta-analysis concludes that DNA damage occurs in white blood cells immediately following an acute bout of endurance exercise and the damage persists for up to 24 h. However, this exercise-induced DNA damage is not detectable several days post-exercise; this is likely attributable to the exercise-induced up-regulation of DNA repair mechanisms.

#### 7.2. ROS impact on muscle force production

The impact of ROS on muscle force production has been shown to be biphasic and dependent upon the level of ROS within the fiber. Again, the parent molecule in the ROS cascade is the superoxide radical that is dismutated to H$_2$O$_2$, and it appears likely that both O$_2^-$ and H$_2$O$_2$ influence muscle contractile function. At rest, superoxide radicals are produced at low rates in skeletal muscle fibers. During exercise, the rate of O$_2^-$ production in muscle is markedly increased; the amount of total O$_2^-$ production in the muscle fiber is dependent upon both the intensity and duration of exercise as well as the temperature of the contracting muscle. In general, relatively high-intensity, prolonged aerobic exercise (i.e., 65%–75% VO$_{2\text{max}}$) results in greater ROS production compared to low-intensity (i.e., <40% VO$_{2\text{max}}$), short duration exercise. Moreover, increased muscle temperature results in higher levels of ROS during contractions.

As discussed elsewhere in this review, ROS are eliminated by an array of enzymatic and non-enzymatic antioxidants in the muscle fiber. Thus, the impact of ROS production on skeletal muscle function is the balance between the rate of ROS production and the rate of ROS removal by antioxidants.

During the 1990s and early 2000s, Reid et al. published a landmark series of experiments demonstrating that ROS have a biphasic influence on skeletal muscle force production (Fig. 2). Their work reveals that, in unfatigued muscle,
an optimum level of ROS is required for muscle fibers to generate 100% of their maximal isometric force production. For example, their work reveals that selective removal of O$_2^-$ or H$_2$O$_2$ from the fiber using SOD or CAT, respectively, results in a decrease in muscle maximal force production. Conversely, increasing the ROS levels in the fiber above the optimal point results in a decrease in the muscles’ ability to generate force. The fact that administration of the antioxidant N-acetylcysteine delays the rate of muscle fatigue during prolonged exercise provides further support for the concept that high levels of ROS impairs maximal muscle force production. Note that, while these experiments measured maximal isometric force production in muscle, it is likely that an optimum level of ROS is also required for maximal force production during concentric contractions as well.

What are the mechanisms responsible for the observation that muscle force production can be increased or decreased in response to redox disturbances? This issue remains a debated topic, and a definitive answer to this question does not currently exist. Nonetheless, it is feasible that changes in both free calcium levels in the muscle and myofibrillar sensitivity to calcium contribute to the redox impact on muscle force production. Further, it is possible that a ROS-mediated decrease in Na$^+$/K$^+$ pump activity may also contribute to the decrease in muscle force production that occurs during prolonged endurance exercise. In reference to the role that calcium sensitivity plays in muscle force production, well-controlled studies performed on single skeletal muscle fibers confirm that high levels of oxidants (i.e., H$_2$O$_2$) decrease myofibrillar sensitivity to calcium resulting in decreased muscle force production at any given level of free calcium in the fiber. This consistent observation explains, at least in part, why high levels of oxidants depress muscle force production. In contrast, the impact of high oxidant levels on cytosolic levels of free calcium during muscle contraction is less clear. Specifically, while it is established that calcium release channels on the sarcoplasmic reticulum (i.e., ryanodine receptors) are redox sensitive, the precise impact of redox modulation on these channels remains unclear. For example, evidence exists both for and against the notion that high levels of oxidants disrupt calcium release from the sarcoplasmic reticulum. The explanation for this experimental discrepancy is unclear but may be due to differences in experimental conditions across numerous studies (e.g., muscle temperature, oxidant levels). Nonetheless, taken together, the experimental evidence indicates that the elevated levels of oxidants in skeletal muscle associated with prolonged exercise is capable of damaging one or more proteins involved in excitation-contraction coupling, resulting in a reduction in muscle force production (i.e., fatigue).

Finally, it is possible that a ROS-mediated decrease in Na$^+$/K$^+$ pump activity also contributes to the reduction in muscle force production that occurs during prolonged endurance exercise. Specifically, muscular exercise results in a loss of intracellular K$^+$ and an increase in intracellular Na$^+$ despite a decrease in Na$^+$/K$^+$ pump activity. This decrease in intracellular K$^+$ and the reduced trans-sarcolemmal Na$^+$ gradient impairs membrane excitability and, therefore, decreases muscle force production. Experimental evidence to support the concept that ROS-mediated depression of Na$^+$/K$^+$ pump activity contributes to muscle fatigue is derived from human experiments confirming that the antioxidant N-acetylcysteine attenuates exercise-induced muscle fatigue, in part by improved regulation of intracellular K$^+$ levels.

7.3. ROS influence on muscle adaption to exercise training

As discussed elsewhere in this review, it is clear that exercise-induced increases in oxidants contribute to muscle fatigue. However, the production of ROS in skeletal muscle during prolonged endurance exercise also plays an important role in cell signaling pathways involved in muscle adaptation to exercise. Indeed, both human and animal studies demonstrate that prevention of exercise-induced redox signaling blunts the training-induced changes in skeletal muscle fibers. A complete discussion of this topic exceeds the scope of this review, and the reader is referred to recent reviews for specific details about redox signaling and skeletal muscle adaptation. Nonetheless, a brief overview of the link between exercise-induced production of ROS and skeletal muscle adaptation to endurance exercise is appropriate.

Skeletal muscle is a highly plastic tissue that undergoes sizeable phenotypic changes in response to endurance exercise training. Remarkably, as few as 5–10 consecutive days of endurance exercise results in substantial increases in both the oxidative and antioxidant capacity of skeletal muscle fibers. During the past 20 years, our understanding of the signaling mechanisms responsible for these changes has increased markedly. Importantly, many of these cell-signaling pathways are initiated, or at least potentiated, by redox signals. Indeed, redox-sensitive pathways result in changes in transcription factor activity, either increasing or decreasing the transcription of target genes. In this regard, it is now clear that exercise-induced production of ROS plays an important role in exercise-induced signaling via nuclear factor-kappa B (NF-κB) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) in skeletal muscle fibers. This is important because both NF-κB and PGC-1α play a required role in exercise-mediated increases in skeletal muscle antioxidants and mitochondrial biogenesis. In addition to NF-κB and PGC-1α redox activation of nuclear factor erythroid 2-related factor 2 plays an important role in promoting exercise-induced expression of many key components involved in the endogenous antioxidant system. Indeed, nuclear factor erythroid 2-related factor (Nrf2) controls the expression of key components of the glutathione and Trx antioxidant systems as well as enzymes involved in NADPH generation. Together, the available evidence indicates that exercise-induced production of ROS is clearly a requirement for skeletal muscle adaptations induced by endurance exercise training.

Further, evidence exists that ROS signaling is also involved in resistance training-induced hypertrophy. For example, in a cell culture model, H$_2$O$_2$-induced oxidative stress can activate protein kinase B, which promotes protein synthesis in cells via...
down-stream activation of mammalian target of rapamycin (mTOR).\textsuperscript{101} mTOR activation stimulates protein synthesis via increased translation of contractile protein mRNA. Furthermore, experimental evidence in a plantaris muscle overload model reveals that production of ONOO\textsuperscript{−}, a reaction product of NO and O2\textsuperscript{−}, triggers a signaling cascade resulting in the direct activation of mTOR.\textsuperscript{102} Hence, it appears that contraction-induced ROS production is a key signaling molecule in resistance training-induced muscle hypertrophy.

8. Exercise and free radicals: friend or foe?

The question, “Is exercise-induced ROS production beneficial or a detriment to health?” has been debated for more than 3 decades without resolution. The following segment will discuss this perplexing question by examining the (1) concept of exercise-induced hormesis and the (2) association between regular exercise and the risk of chronic diseases.

8.1. Exercise and hormesis

Whether exercise-induced ROS production is damaging or beneficial to health likely depends on the balance between the levels of ROS production during exercise and the competency of the cellular antioxidant systems to protect cells against an oxidant challenge. In this regard, several recent reviews have concluded that regular exercise training does not result in chronic oxidative stress in the active muscles.\textsuperscript{17,103–106} Conceptually, this conclusion is supported by the notion of exercise-induced hormesis. The term hormesis is used in biology to describe a biphasic dose–response curve where a transient increase in low levels of a stressor (e.g., radiation, radicals) provides a beneficial adaptive effect on cells whereas a chronic and/or high dose of the stressor results in damage to the cells (Fig. 3A).\textsuperscript{107,108} Although hormesis research has a long history in biology, the first description of exercise-induced hormesis appeared in 2005.\textsuperscript{109} This report concluded that low to moderate levels of exercise-induced ROS production plays an essential role in exercise-induced adaptation of skeletal muscle. In contrast, high levels of ROS production results in damage to the muscle and a decline in the physiological benefits associated with low to moderate ROS production. This exercise-induced hormesis concept has received recent support from several investigators.\textsuperscript{17,106,109,110} As discussed elsewhere in this article, it has been established that exercise-induced increases in the production of ROS in skeletal muscle plays a required role in skeletal muscle adaptation to training. The bell-shaped hormesis curve predicts that increases in exercise-induced production of ROS promotes significant physiological benefits until an optimum level of ROS production is reached. However, if exercise results in a true hormetic effect on the body, after reaching this zenith of physiological benefit, any further increase in exercise-induced ROS production would result in tissue damage and a decline in the exercise-induced adaptations.

Recently, Ji et al.\textsuperscript{17} have questioned whether prolonged, high-intensity exercise reaches the level of ROS production required to result in the downward slope on the hormesis curve illustrated by the right-hand side of Fig. 3A. These researchers support this position by noting that the existing human and animal literature does not provide persuasive evidence that prolonged, high-intensity exercise results in extreme oxidant-mediated damage in cells and a decrease in the antioxidant capacity of the trained muscles.\textsuperscript{17} Ji et al.\textsuperscript{17} also support this position by arguing that high-intensity exercise is unlikely to generate extreme levels of oxidant damage in the working muscle fibers for several reasons. First, the exercise intensity that can be sustained for long durations is influenced by both the cardiovascular system’s ability to provide blood to the working muscles and the impact of ROS production on muscle fatigue. Thus, together, the cardiovascular limitation, along with exercise-induced muscle fatigue, would ultimately limit the intensity and duration of exercise that can be sustained. It follows that this limitation for exercise would also limit the total production of muscle ROS during an exercise bout. Second, although mitochondria are a known source of muscle ROS production, compared to State 4 (resting) respiration, mitochondrial coupling is higher during State 3 (exercise) respiration, reducing electron spill and ROS production by the mitochondria during exercise. Third, acute exercise has been reported to increase the expression of uncoupling proteins in skeletal muscle mitochondria; this increase in uncoupling proteins moves protons from the intermembrane space into the matrix, diminishing a high mitochondrial proton gradient that favors O2\textsuperscript{−} formation. Last, regular exercise training results in significant increases in the abundance of key antioxidant enzymes in skeletal muscles and therefore elevates the fibers’ ability to remove ROS during exercise. Together, these observations support the forecast that during exercise, skeletal muscles are not exposed to extreme levels of ROS-mediated damage, and therefore the exercise-induced ROS production hormesis curve is predicted to follow the pattern illustrated in Fig. 3B.\textsuperscript{17} Although this argument is logical, the thesis that exercise does not result in high

Fig. 3. (A) Relationship between cellular levels of ROS and physiological function. This biphasic bell-shaped curve represents the ROS hormesis curve. (B) Relationship between the exercise-induced muscle fiber levels of reactive oxygen species and physiological function. This figure predicts that training-induced increases in muscle fiber levels of ROS does not reach a detrimental level because of exercise-induced fatigue. ROS = reactive oxygen species.
levels of oxidative damage in muscles during exercise does not directly answer the question of whether exercise-induced ROS production in muscles increases the risk of chronic diseases. This issue is addressed in Section 8.2.

8.2. Exercise training, oxidative stress, and chronic disease

Scientific interest in the question of whether exercise-induced ROS production is friend or foe has been fueled by the knowledge that oxidative stress is associated with numerous chronic diseases including cancer, cardiovascular disease, hypertension, Alzheimer’s disease, and Parkinson’s disease.113,114,115,117 Moreover, the exercise-induced reduction in cardiovascular risk factors follows a clear dose–response relationship, with higher volumes and intensities of exercise providing greater health benefits.120 While it is possible that exercise-induced protection against hypertension and cardiovascular disease is directly linked to an exercise-induced reduction in oxidative stress, proving cause and effect is experimentally challenging. Therefore, it remains unclear as to whether exercise-mediated changes in cellular redox balance is the primary factor responsible for exercise-induced protection against heart disease and hypertension.111,119

Oxidative stress has been implicated in several neurodegenerative diseases, including both Alzheimer’s disease and Parkinson’s disease.112,121–123 Although ROS may not be the trigger for induction of these neurological disorders, oxidative stress is predicted to exacerbate both disorders.123 The influence of exercise training on the risk of these diseases is clear, since numerous epidemiological studies conclude that an inverse relationship exists between physical activity and the incidence of both Alzheimer’s disease and Parkinson’s disease.112 Furthermore, exercise training has been reported to slow the progression of both disorders.112 Several mechanisms have been proposed for this exercise-induced benefit, including increased cerebral blood flow and increased antioxidants within the brain.112,124

Given that regular exercise has been shown to reduce the risk of several types of cancers, cardiovascular disease, hypertension, Alzheimer’s disease, and Parkinson’s disease, it is not surprising that an inverse dose–response relationship exists between the volume of physical activity and all-cause mortality.125–127 Indeed, the evidence that regular physical activity reduces all-cause mortality has been widely accepted for more than 2 decades. Although the mechanism(s) responsible for exercise-induced protection against chronic disease remains a topic of debate, the undeniable evidence that regular exercise protects against all-cause mortality supports the conclusion that exercise-induced production of ROS is a “friend” and not a “foe” of good health.

9. Conclusions

The fact that high-intensity and/or prolonged exercise promotes oxidative stress in humans was discovered more than 4 decades ago. The tissues most responsible for ROS production during exercise remain debated, but it is clear that contracting skeletal muscles are an important source of ROS production during exercise. The intracellular sites of ROS production in contracting skeletal muscles continue to be an active area of research, but mounting evidence implicates NADPH as an important source of ROS production during exercise.

The consequences of exercise-induced oxidative stress continue to be a controversial topic. In theory, exercise-induced ROS production could be a double-edged sword, whereby a moderate level of ROS production during exercise promotes positive physiological adaptation in the active skeletal muscles (e.g., mitochondrial biogenesis, synthesis of antioxidant enzymes, and stress proteins), whereas high levels of ROS production result in damage to macromolecular structures (e.g., proteins, lipids, and DNA). Although the impact of exercise-induced ROS production in skeletal muscle has been postulated to be a bell-shaped hormesis curve, there is not convincing evidence that prolonged, high-intensity exercise results in tissue damage and impaired physiological function. Indeed, research consistently demonstrates that long duration and high-intensity exercise provides the greatest health benefits. Therefore, based on the available evidence, it appears unlikely that rigorous and prolonged exercise results in an oxidative stress level that is detrimental to human health.

Authors’ contributions

SKP developed the outline of the review, reviewed the literature, and wrote the manuscript; TY also reviewed the literature, contributed to figure preparation, and provided editorial suggestions during review; RD, MPB, and HH read the manuscript and provided editorial suggestions during revision, and contributed to figure preparation; MO read the manuscript and provided editorial suggestions during revision. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.
Competition interests

The authors declare that they have no competing interests.

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