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Effects of Exercise and Training on Oxidative Stress and Antioxidants

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Abstract

The first report showing that long-term endurance exercise increases oxidative stress in humans was published more than 4 decades ago. Since this discovery, many subsequent studies have confirmed the fact that muscle activity increases the production of reactive oxygen species (ROS) and leads to oxidative stress in multiple tissues, including blood and skeletal muscle. Although several tissues may contribute to exercise-induced ROS production, muscle contractions are predicted to stimulate ROS production in active muscle fibers, and skeletal muscle is the major source of ROS production during exercise. This contraction-induced ROS production is associated with 1.oxidant damage in several tissues (eg, increased protein oxidation and lipid peroxidation), 2.accelerated muscle fatigue, and 3.activation of biochemical signaling pathways leading to training adaptation. While our understanding of exercise and oxidative stress has advanced rapidly over the past decades, questions remain as to whether exercise-induced increased ROS production is beneficial or detrimental 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, antioxidants, oxidative stress, reactive oxygen species.

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INTRODUCTION

Regular exercise has many health benefits, including reducing the risk of death from all causes and reducing the risk of cardiovascular diseases, cancer and diabetes (S. K. Powers et al., 2020). Paradoxically, it is also clear that skeletal muscle contraction produces free radicals, and prolonged and intense exercise can lead to oxidative damage to cellular components (Scott K Powers & Jackson, 2008). Over the past three decades, our knowledge of the biological consequences of exercise-induced oxidative stress has expanded rapidly. Indeed, it is now recognized that while high levels of free radicals can damage cellular components, low to moderate levels of oxidants play multiple regulatory roles in cells, such as controlling gene expression, regulating cell signaling pathways, and modulating skeletal muscle force production (Kaur, Allahbadia, & Singh, 2022). Due to the recent advances in the field of exercise and oxidative stress, now is the right time to summarize some of the main principles of exercise-induced oxidative stress and its effect on skeletal muscle function. In the present study, we will start with an overview of oxidant species, antioxidant systems and the concept of oxidative stress, then a summary of the history of researches in the field of oxidative stress caused by exercise, cellular sources of oxidants during exercise and also in Specific aspects of exercise and oxidative stress will be discussed. We will also discuss redox modulation of muscle force production/fatigue and redox sensitive targets in skeletal muscle.

The searches for literature review was done based on scientific databases like Scopus ،Web of Science ،PubMed and Google Scholar. The search terms "oxidative stress", "antioxidants", "reactive oxygen species", "sports performance" and their variants were applied and the identified articles were used. To ensure that all key empirical studies were included, comprehensive review articles were subsequently identified and their references checked with the primary articles. Finally, the most recent articles in quality peer-reviewed journals were identified by citing these review articles. Although this review will focus on a wide range of issues related to exercise-induced oxidative stress, it is impossible for a single report to address all aspects of this vast field of study.

2. Redox balance and oxidative stress

The term oxidative stress was first defined in 1985 as "a disturbance in the pro-oxidant balance in favor of the former" (Sies, 2018). Because of the complexity associated with assessing cellular redox balance, it has been argued that the term oxidative stress defies a simple definition of pro-oxidant versus antioxidant, and that the description of an "oxidative stress" is only useful when the molecular details of the imbalance are known. In an attempt to refine the meaning of oxidative stress, Dean Jones has suggested that the term should be redefined as "disorders in redox signaling and control" (Jones, 2006). Regardless of how oxidative stress is defined, a stable pro-oxidant environment in cells can change molecules sensitive to oxidation/reduction. A common approach to assess oxidative stress in biological systems involves measuring the increase or decrease of an oxidative-sensitive molecule that responds to oxidative stress. In general, reliable markers of oxidative stress have the following characteristics: 1) chemically unique and detectable, 2) increase or decrease during periods of oxidative stress, 3) relatively long half-lives, and 4)) are not affected by other cellular processes (for example, cell cycle, energy metabolism, etc.) (Halliwell & Gutteridge, 2015).

During periods of oxidative stress, pro-oxidants overcome the antioxidant defense in cells and damage cellular components. Therefore, oxidative stress in biological systems is often associated with parameters including: 1) increased formation of radicals and other oxidants, 2) reduction of small molecular weight and/or lipid-soluble antioxidants, 3) disruption of cellular redox balance, and 4) oxidative damage to cellular components (i.e., lipids, proteins or DNA), are determined. Therefore, oxidative stress biomarkers are usually placed in one of these four categories (Fig 1).

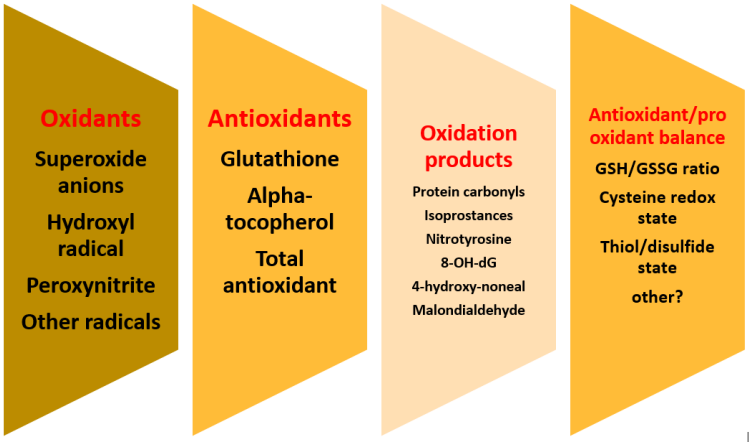

Figure 1: The four broad classes of biomarkers used to assess cellular oxidative stress in tissues. These categories include the measurement of oxidant production, cellular levels of antioxidants, oxidation products, and the antioxidant/pro-oxidant balance. 8-OH-dG, 8-hydroxydeoxyguanosine; GSH/GSSG, ratio of reduced glutathione to oxidized glutathione (Scott K Powers & Jackson, 2008).

In summary, several approaches to assess oxidative stress in biological systems have been reported in studies. Unfortunately, each category of oxidative stress biomarkers has limitations. Therefore, although there are many parameters to quantify oxidative stress, it is difficult to establish a single and ideal biomarker. Therefore, it seems that no single biomarker evaluates oxidative stress in the best way and in most cases, the measurement of multiple biomarkers is required to confirm the presence of oxidative stress in tissues (Marrocco, Altieri, & Peluso, 2017).

3. Biological radicals

The observation that living cells produce free radicals (radicals) was first reported in 1954 (Commoner, Townsend, & Pake, 1954). Since this initial discovery, many studies have investigated the sources and effects 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 generic term that refers to both oxygen-based radicals and non-radicals that are reactive derivatives of oxygen (such as hydrogen peroxide (H_2O_2). A related term, reactive nitrogen species (RNS), refers

to both radical species (eg, nitric oxide (NO)) and non-radical nitrogen species (eg, peroxynitrite) (S. K. Powers et al., 2020).

Superoxide ($O_2^{\cdot-}$) is the main molecule of all ROS and is formed by the reduction of one electron of molecular oxygen. This anion has a negative charge and is relatively impermeable to the membrane and is relatively inactive compared to other radical species (S. K. Powers, Ji, Kavazis, & Jackson, 2011). Decomposition (parallel change) of superoxide occurs spontaneously, but it can also be catalyzed by the enzyme superoxide dismutase (SOD). Dismutation of superoxide provides the main source of H_2O_2 production (Wang, Branicky, Noë, & Hekimi, 2018). H_2O_2 is a non-radical ROS that penetrates membranes and has a relatively long half-life in the cell. Although H_2O_2 is considered a weak oxidizing agent, chronic high cellular levels of H_2O_2 damage cellular components and thus lead to cytotoxicity. The important point is that due to the long half-life and high permeability of the H_2O_2 membrane, this molecule can diffuse significant distances inside or outside the cell. Together, these properties make H_2O_2 a prime candidate for ROS-mediated signaling in cells.

Hydroxyl radical ($\cdot OH$) has a strong oxidizing potential and is usually formed by reactions involving both H_2O_2 and O_2. Due to their high reactivity, hydroxyl radicals usually oxidize molecules close to their production site. Compared to other active species, hydroxyl radicals are considered the most harmful ROS due to their high reactivity (Hensley & Floyd, 2002).

NO is the main molecule of all RNS and it is synthesized from amino acid L-arginine through NO synthase (NOS). There are four isoforms of NOS and 3 of these isoforms are found in skeletal muscle fibers. Specifically, both neuronal NOS (nNOS/NOS_1) and endothelial NOS (eNOS/NOS_3) have been identified in skeletal muscle fibers. Notably, there are 2 types of NOS1 binding (i.e., nNOSb and nNOSm) in skeletal muscle fibers (Balke, Zhang, & Percival, 2019). In addition, inducible NOS (iNOS/NOS_2) is also found in skeletal muscle under inflammatory conditions such as septic shock (Adams et al., 2002). These NOSs require several cofactors (eg, 5, 6, 7, 8-tetrahydrobiopterin and iron) and use nicotinamide adenine dinucleotide phosphate (NADPH) to convert L-arginine to NO and L-citrulline (Förstermann & Sessa, 2012). It has been

well proven that muscle contractions increase the production of NO in the contractile fiber (Reid, 2016).

After production, NO is a weak reducing agent that can act as a positive allosteric activator with intermediate metals located in enzymes. For example, NO binds to iron located in the enzyme guanylyl cyclase. The result of binding NO to iron activates this enzyme and as a result cyclic guanosine monophosphate (cGMP) is formed. Importantly, NO also rapidly reacts with superoxide and forms peroxynitrite (ONOO). In fact, the reaction between NO and $O_2^{\cdot -}$ occurs approximately 3 times faster than the dismutation of $O_2^{\cdot -}$. Hence, the formation of $ONOO^-$ is the primary reaction of $O_2^{\cdot -}$ in the presence of NO (Fukai & Ushio-Fukai, 2011). The formation of $ONOO^-$ has two important biological consequences. First, the formation of ONOO- leads to a decrease in the 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

Because the regulation of redox balance is critical to cellular health, all mammalian cells are equipped with control systems to regulate oxidation/reduction (oxidation) balance. One of the main components of redox control is the cellular antioxidant system. Antioxidants are usually defined as any substance that significantly delays or prevents the oxidation of a substrate. Cells contain enzymatic and non-enzymatic antioxidants that act as a complex unit to regulate ROS. Inside the cell, these antioxidants are strategically distributed throughout the cytoplasm and in different organelles (eg, mitochondria). In addition, enzymatic and non-enzymatic antioxidants exist both in the extracellular space and in the vascular space. Collectively, these antioxidants protect muscle fibers from oxidative damage during periods of increased oxidant production (eg, intense or prolonged exercise).

There are many antioxidant strategies and they can be used to protect cells from damage caused by ROS. For example, some agents (eg, catalase) convert ROS into less active molecules and prevent the conversion of these less active species into more harmful forms. Another antioxidant strategy is to minimize the availability of pro-oxidants such as iron and copper ions through metal binding proteins. In addition,

several low molecular weight agents are able to scavenge ROS species. Examples of this antioxidant strategy include endogenous synthesis molecules such as glutathione, uric acid, and bilirubin along with dietary factors such as ascorbic acid and vitamin E.

4-1. Enzymatic antioxidants

SOD, glutathione reductase and catalase (CAT) are 3 basic antioxidant enzymes that are present in cells (Weydert & Cullen, 2010). Nevertheless, it is also clear that the antioxidant systems thioredoxin (Trx) and peroxiredoxin (Prx) also have a supporting role in maintaining the redox balance in the cell. SOD is the first line of defense against $O_2^{\cdot -}$ is and $O_2^{\cdot -}$ changes and forms H_2O_2 and oxygen (O_2) (Younus, 2018). Three isoforms of SOD (SOD_1, SOD_2, and SOD_3) exist in all mammals, and all require a redox-active intermediate metal at the active site for the catalytic breakdown of superoxide anion. Two isoforms of SOD are located inside the cells, while the third isoform of SOD is located in the extracellular space. SOD1 is located both in the cytosol and in the mitochondrial intermembrane space, while SOD_2 is located only in the mitochondrial matrix. In contrast, SOD_3 is located outside the cell in the extracellular space (Wang et al., 2018). Although superoxide radicals are not highly reactive, they remove electrons from cellular components (e.g., biological membranes), leading to a series of radical-mediated reactions. Superoxide radicals are also toxic due to their involvement in the production of hydroxyl radicals. In addition, recall that $O_2^{\cdot -}$ can also react with NO to form $ONOO^-$. The result is that the removal of superoxide radicals is vital to prevent cellular oxidative damage.

There are five glutathione peroxidases (GPXs) in mammals (GPX_1 GPX_5). All these GPX enzymes are responsible for the reduction of H_2O_2 or organic hydroperoxide to form water (H_2O) and alcohol (ROH), respectively (Brigelius-Flohé, 2006). This reaction requires an electron donor, and reduced glutathione (GSH) is the main electron donor involved in GPX reactions (Björnstedt, Kumar, Björkhem, Spyrou, & Holmgren, 1997). Although all GPX isoforms reduce H_2O_2, expression of multiple GPX isoforms is biologically reasonable due to the different cellular locations of GPX isoforms. For example, GPX1 is located in both the cytosol and mitochondria. GPX_2 is located exclusively in the cytosol, while GPX_3 is found in both the cytosol and the extracellular space (Buday & Conrad, 2021). Therefore, the GPX family of

antioxidant enzymes play an important role in redox regulation due to their substrate specificity and distribution in different cell parts. The diverse cellular locations of GPX are beneficial because ROS are reduced at the site of their production.

The main function of the antioxidant enzyme CAT is to decompose H_2O_2 into H_2O and O_2. Similar to GPX and SOD, CAT is located in several compartments of the cell, including the cytosol and mitochondria. CAT differs from GPX in two ways. First, CAT does not need an electron donor, and secondly, CAT only removes H_2O_2 and does not remove organic hydroperoxides (Radovanovic, Banjac, Obradovic, & Isenovic, 2021).

The Trx antioxidant system consists of Trx and Trx reductase. There are two isoforms of Trx, which include: the cytosolic isoform (Trx_1) and the mitochondrial form (Trx_2) (Anzueto et al., 1992). Functionally, Trx is the main ubiquitous disulfide reductase responsible for maintaining proteins in their reduced state (Appell, Duarte, & Soares, 1997). Trx maintains the reduced state of proteins by forming a disulfide bond with the substrate protein and transferring its 2 electrons to the target protein. This leads to the oxidation of the Trx protein and the reduction of the target substrate. Oxidized Trx can then be reduced by NADPH electrons via Trx reductase, allowing Trx to continue its role as a redox modulator. Trx has several physiological roles, including (1) protecting against protein oxidation, (2) reducing transcription factors, and (3) regulating apoptosis. In addition, Trx reductase also serves as an antioxidant enzyme by reducing hydroperoxides and acting as an NADPH-dependent dehydroascorbate reductase to recover vitamin C (Arend, Zilmer, Vihalemm, Selstam, & Sepp, 2000).

Finally, Prxs are a family of peroxidases of mammalian cells that express 6 different isoforms. Using the electrons provided by Trx, Prxs catalyzes the reduction of H_2O_2, alkyl hydroperoxides and peroxynitrite. Recent investigations into the function of Prxs in cells have concluded that Prxs appear to be more than simple peroxide-scavenging enzymes and may play an important role in cell signaling in various cell types, including skeletal muscle (Arnér & Holmgren, 2000). However, the full details of how Prxs interact with cellular proteins to regulate redox signaling remain largely unclear.

4-2. Non-enzymatic antioxidants

There are many non-enzymatic antioxidants in cells (such as GSH, uric acid, bilirubin, vitamin E, and vitamin C), and a detailed discussion on this issue is beyond the scope of this review. Nevertheless, due to the importance of GSH in the control of redox balance, we provide a brief overview of the critical role of GSH in the prevention of oxidative stress. In fact, as a cellular antioxidant, GSH plays several important roles. For example, GSH directly reacts with a variety of radicals by donating a hydrogen atom. This results in a less reactive species and less damage. In addition, an important antioxidant action of GSH is electron donation for GPX to destroy H_2O_2 and organic hydroperoxides. In addition, GSH is also important because it reduces the antioxidant vitamins E and C. This action of GSH is central because it helps maintain limited cellular sources of vitamin E and C in a reduced state. This reduction of vitamins E and C allows these molecules to act as cellular antioxidants (Kurutas, 2016).

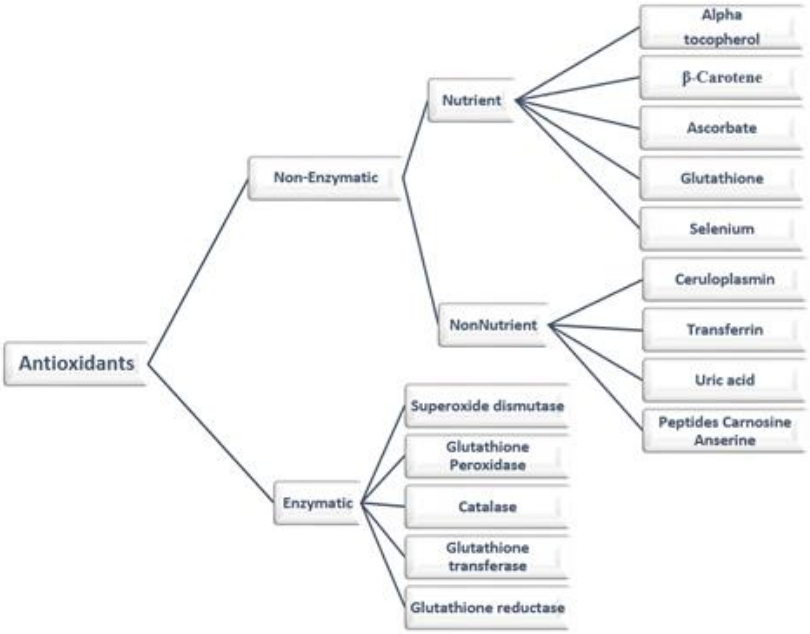

Figure 2: Antioxidants classified as enzymatic and non-enzymatic (Shastri, Srivastava, Jyoti, & Gupta, 2016).

5. An overview of the history of studies conducted in relation to oxidative stress caused by exercise

The first report that exercise was associated with increased biomarkers of oxidative damage appeared in 1978 studies. This preliminary report showed that long-term endurance exercise in humans leads to an increase in oxidative stress biomarkers. Four years later, this work was followed by the discovery that skeletal muscle contraction produces ROS (A. Thirupathi et al., 2021). Using similar techniques (eg, electron spin resonance), this finding was confirmed by Jackson et al., and numerous studies in the last 4 decades have confirmed that intense exercise is associated with oxidative stress in both humans and animals (Lu, Wiltshire, Baker, & Wang, 2021). In particular, many types of exercise (long-duration endurance exercise, resistance exercise, high-intensity anaerobic exercise, and eccentric exercise) lead to oxidative stress, as evidenced by increased oxidative biomarkers in skeletal muscle and blood (Anand Thirupathi et al., 2021).

Several important studies conducted in the 1990s showed that endurance exercise or high-intensity interval training increases the antioxidant capacity of cardiac and skeletal muscle myocytes. Specifically, these studies showed that exercise increases several antioxidant enzymes, including SOD_1, SOD_2, and GPX_1 (S. K. Powers et al., 2020).

Specifically, these preliminary studies show that exercise increases SOD_1 and SOD_2 in trained skeletal muscles by 20% to 110%. Similarly, research has also shown that regular endurance exercise increases GPX1 levels in skeletal muscle by 20% to 180%.Although a few studies published in the 1980s and 1990s suggested that exercise training also increased muscle CAT levels, other reports have failed to demonstrate an exercise-induced increase in CAT. Therefore, whether exercise increases CAT levels in cardiac and skeletal muscles is controversial (S. K. Powers, Radak, & Ji, 2016). Finally, to date, there is limited information on the effect of exercise training on the abundance of Prxs isoforms in skeletal muscle, and further studies are needed to determine whether exercise training affects 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 (Kobzik, Stringer, Balligand, Reid, &

Stamler, 1995). This development paved the way for further studies on the role of NO in skeletal muscle signaling.

6. Sources of oxidants in skeletal muscle contraction

Since the discovery of oxidative stress caused by exercise, many researchers have investigated the potential sources of ROS production in a number of tissues. Although oxidants can be produced in various tissues during exercise, skeletal muscle has been found to be the main source of ROS production during exercise. Possible sources of exercise-induced ROS production in muscle fibers have been extensively investigated and include: (1) mitochondria, (2) phospholipase A2 (PLA_2), and (3) NADPH oxidases (NOX_2 and NOX_4), which there are 4 tissue areas: mitochondria, sarcolemma, sarcoplasmic reticulum and T-tubes (Figure 3) (Espinosa et al., 2006).

Although initial studies suggested that mitochondria are a possible source of ROS production in muscle fibers during exercise, but considering that ROS production in skeletal muscle mitochondria decreases during exercise, this prediction does not seem accurate. Specifically, based on studies conducted in the 1970s, it was estimated that 2% to 5% of the molecular oxygen consumed in mitochondria is O_2 (Boveris & Chance, 1973). According to these results, it was then hypothesized that the increase in oxidative phosphorylation in the mitochondria in skeletal muscles would lead to a proportional increase in the production of O_2. However, contemporary studies show that mitochondria actually produce less $O_2^{\cdot-}$ during active mode 3 respiration compared to basal mode 4 respiration (S. K. Powers, Hudson, et al., 2011). Therefore, the available evidence shows that mitochondria are not the main site of ROS production in skeletal muscle during exercise.

PLA_2 is an enzyme that cleaves membrane phospholipids to release arachidonic acid. Free arachidonic acid is a substrate for several enzyme systems that generate ROS, including lipoxygenases (Adibhatla & Hatcher, 2008). Most importantly, the activation of PLA_2 can activate NADPH oxidases and the increased activity of PLA_2 in skeletal muscles can also increase the production of ROS in mitochondria and cytosol (Gong et al., 2006). Note that both calcium-dependent and calcium-independent forms of PLA_2 exist in skeletal muscle, and both isoforms are capable of stimulating ROS production in muscle. It is assumed that calcium-independent enzymes regulate cytosolic oxidant activity of

skeletal muscle cells in resting conditions, while calcium-dependent isoform PLA_2 stimulates mitochondrial ROS production during contractile activity (Nethery, Stofan, Callahan, DiMarco, & Supinski, 1999). However, more research is needed to confirm or reject this assumption.

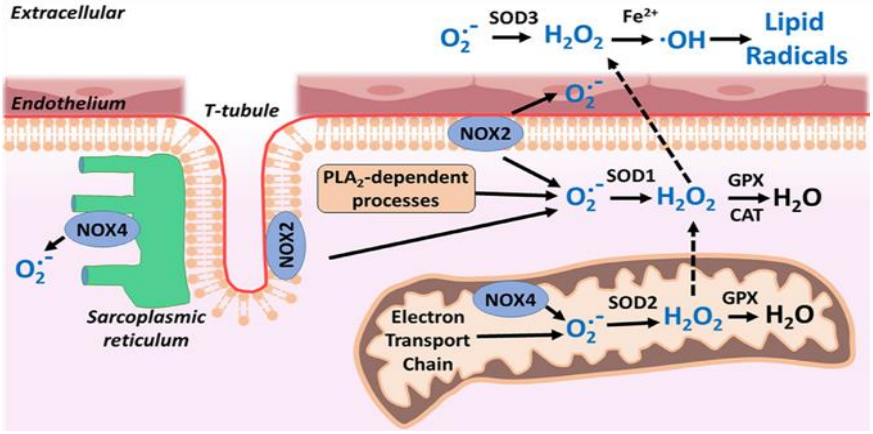

Figure 3: Potential sites of the production or reactive oxygen species in contracting skeletal muscles. CAT = catalase; GPX = glutathione peroxidase; H_2O_2 = hydrogen peroxide; NOX = NADPH oxidase; $O_2^{\cdot-}$ = superoxide; ·OH = hydroxyl radical; PLA_2 = phospholipase A_2; SOD = superoxide dismutase (S. K. Powers et al., 2020).

Skeletal muscle expresses 2 NADPH oxidase isoforms (NOX_2 and NOX_4). NOX_2 is located within the sarcolemma and the T channel, while NOX_4 is located in both the sarcoplasmic reticulum and mitochondria (Ferreira & Laitano, 2016). NOX_4 is constitutively active and does not require association with regulatory subunits. In contrast, NOX_2 is activated by specific agonists (such as angiotensin II, mechanical/contractile stress, and cytokines). Therefore, it seems that NOX_4 contributes to the basal amount of ROS production in muscle fibers, while NOX_2 is the main source of NADPH oxidase-mediated ROS production in muscle contraction. Indeed, several recent studies point to NOX as a major factor in contraction-induced ROS production (Ward, Prosser, & Lederer, 2014). For example, researchers using

various experimental techniques to investigate the intracellular locations responsible for the production of O2.- in muscle fibers concluded that mitochondria are not responsible for the production of O2.- due to contraction in muscle fibers. Instead, they concluded that NOX is the main source of O2- production at rest and during contraction (Sakellariou et al., 2013). However, at present, the conclusion that NOX is the dominant source of ROS in skeletal muscle contraction is complicated due to the complications associated with the study of NOX activity in cells. Obviously, improved methods and more studies are needed to clarify this issue.

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

It has been shown that ROS are continuously produced in skeletal muscle both at rest and during exercise. Most importantly, ROS modulates a variety of physiological processes, including regulation of blood flow, muscle force production, and muscle adaptation to exercise. Studies on the consequences of ROS production in skeletal muscle fibers have now lasted for 4 decades, which has led to extensive research.

Due to space limitations, We limit our discussion to the issue related to the consequences of ROS production due to exercise in skeletal muscles, oxidative stress caused by exercise, the effect of ROS on muscle force production, and the effect of ROS on muscle adaptation to exercise.

7-1. Oxidative stress caused by exercise

Although short duration (i.e. less than 1 minute) and low intensity exercise (31% VO2max) does not seem to increase oxidative stress, however, it is well established that an acute bout of long-term, high-intensity endurance exercise in untrained humans and animals leads to increased biomarkers of oxidative stress (e.g. increased protein oxidation and lipid peroxidation) in both blood and active skeletal muscle (Quindry, Stone, King, & Broeder, 2003). However, both short-term (1 consecutive day) and long-term (12 weeks) endurance training increases the activity of antioxidant enzymes in trained muscles and eliminates the oxidative stress caused by contraction due to an acute period of exercise (Jîtcă et al., 2022). In addition, a recent meta-analysis concluded that DNA damage in white blood cells occurs immediately after an acute endurance exercise and the damage persists for up to 24 hours. However, this exercise-induced DNA damage is not detectable for several days

after exercise. This is most likely attributed to the up-regulation and down-regulation of DNA repair mechanisms induced by exercise (Moreno-Villanueva et al., 2019).

7-2. The effect of ROS on muscle force production

The effect of ROS on muscle force production has been shown to be biphasic and depends on the level of ROS in the fiber. Again, the parent molecule in the cascade is the superoxide radical ROS, which converts to H_2O_2, and both $O_2^{-\cdot}$ and H_2O_2 appear to affect muscle contractile function. At rest, superoxide radicals are produced at a low rate in skeletal muscle fibers. During exercise, significantly the production rate of $O_2^{-\cdot}$ in the muscle increases. The total amount of O_2 production in the muscle fiber depends on the intensity and duration of the exercise as well as the temperature of the contracting muscle.

In general, long-term aerobic exercise with relatively high intensity (i.e. 61-71% VO2max) causes more ROS production compared to low-intensity exercise (i.e. less than 41% VO2max) with short duration. In addition, increased muscle temperature leads to higher levels of ROS during contractions (Clanton, Zuo, & Klawitter, 1999). As stated, ROS are removed by a set of enzymatic and non-enzymatic antioxidants in muscle fiber. Therefore, the effect of ROS production on skeletal muscle function is the balance between the rate of ROS production and the rate of ROS removal by antioxidants (S. K. Powers, Ji, et al., 2011). During the 1881s and early 1980s, Reid et al. published a series of experiments showing that ROS has a biphasic effect on skeletal muscle force production (Figure 4). Their work shows that in unfatigued muscles, optimal levels of ROS are required for muscle fibers to produce 100% of maximal isometric force. For example, their work shows that selective deletion $O_2^{-\cdot}$ or H_2O_2 from fiber using SOD or CAT, respectively, leads to a decrease in maximum muscle force production. Conversely, increasing the level of ROS in the fiber above the optimal point leads to a decrease in the muscle's ability to produce force.

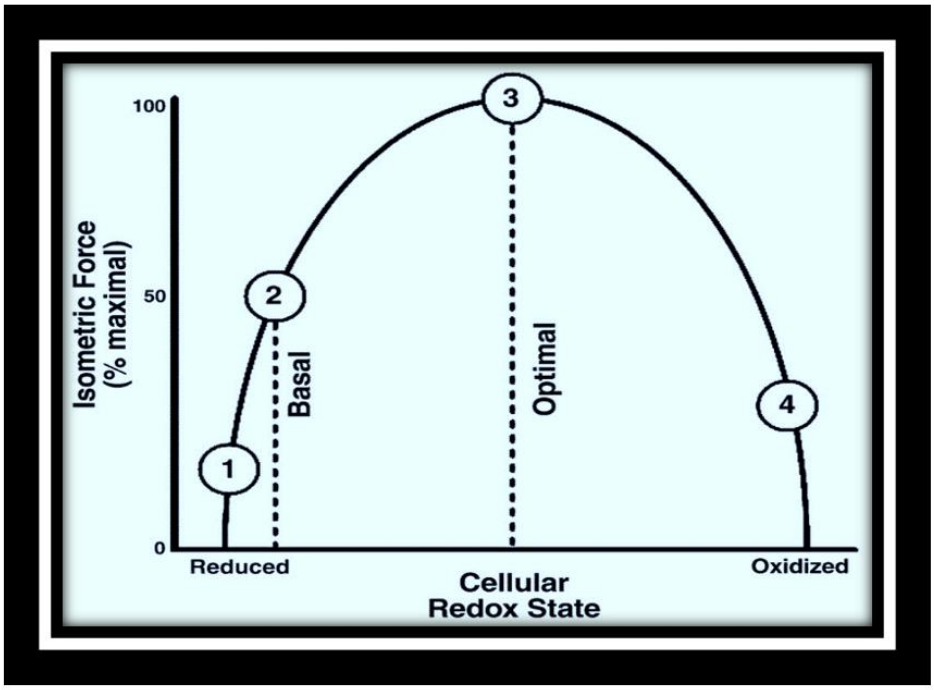

Figure 4: Relationship between cellular redox state and skeletal muscle force production. Note that maximal force production in skeletal muscle requires an optimal redox state. Movement away from the optimal redox state (i.e., an increase in reduction or oxidation) results in a decrease in maximal isometric force production. ROS = reactive oxygen species (Scott K Powers & Jackson, 2008).

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 impair maximal muscle force production (Radak et al., 2017). Note that, while these experiments measured maximal isometric force production in the muscle, it is likely that optimal levels of ROS are also required for maximal force production during concentric contractions. However, it is possible that changes in free calcium levels in muscle and myofibrillar sensitivity to calcium contribute to the redox effect on muscle force production. In addition, ROS-mediated reduction in Na^+/K^+ pump activity may also contribute to the reduction in muscle force production that occurs during prolonged endurance exercise. Regarding the role that calcium

sensitivity plays in muscle force production, well-controlled studies on monoskeletal muscle fibers confirm that high levels of oxidants (e. g, H_2O_2) decrease myofibrillar sensitivity to calcium and thus decrease muscle force production in any level of free calcium is determined in the fiber (Moopanar & Allen, 2005).

This observation at least partially explains why high levels of oxidants reduce muscle force production. In contrast, the effect of high oxidant levels on cytosolic free calcium levels during muscle contraction is less clear. In particular, while calcium release channels in the sarcoplasmic reticulum (i.e., ryanodine receptors) are known to be redox sensitive, the precise effect of redox modulation on these channels is unclear (Xia, Stangler, & Abramson, 2000). For example, there is evidence that high levels of oxidants disrupt the release of calcium from the sarcoplasmic reticulum. The explanation for this experimental discrepancy is unclear, but may be due to differences in experimental conditions in multiple studies (e. g, muscle temperature, oxidant levels). However, taken together, experimental evidence suggests that high levels of oxidants in skeletal muscle associated with prolonged exercise can damage one or more proteins involved in excitation-contraction coupling, thereby reducing muscle force production (e. g, fatigue). Finally, ROS-mediated reduction in Na^+/K^+ pump activity may also contribute to the reduction in muscle force production that occurs during prolonged endurance exercise (Adrian, 1956). In particular, muscle exercise leads to a loss of intracellular K^+ and an increase of intracellular Na^+ despite the decrease in the activity of the Na^+/K^+ pump (Clausen & Nielsen, 2007). This decrease in intracellular K^+ and decrease in the transsarcolemma Na^+ gradient impairs membrane excitability and thus, decreases muscle force production. Experimental evidence to support the concept that ROS-mediated reduction in Na^+/K^+ pump activity contributes to muscle fatigue is derived from human experiments, which confirms that the antioxidant N-acetylcysteine reduces exercise-induced muscle fatigue, partially by improving the regulation of intracellular K^+ levels (McKenna et al., 2006).

7.3. The effect of ROS on muscle adaptation to sports training

It is clear that the increase in oxidants caused by exercise contributes to muscle fatigue. However, ROS production in skeletal muscle during

prolonged endurance exercise also plays an important role in cellular signaling pathways involved in muscle adaptation to exercise. Indeed, both human and animal studies show that prevention of exercise-induced redox signaling reduces exercise-induced changes in skeletal muscle fibers (Ristow et al., 2009). Skeletal muscle is a very flexible tissue that undergoes significant phenotypic changes in response to exercise. It is noteworthy that only 1 to 11 consecutive days of endurance training leads to a significant increase in the oxidative and antioxidant capacity of skeletal muscle fibers (Steinbacher & Eckl, 2015). Over the past 21 years, our understanding significantly of the signaling mechanisms responsible for these changes has increased. Importantly, many of these cell signaling pathways are initiated or at least amplified by redox signals. In fact, pathways sensitive to oxidation and reduction lead to changes in transcription factor activity, increasing or decreasing the transcription of target genes. In this regard, it is now clear that exercise-induced ROS production plays an important role in exercise-induced signaling through nuclear factor-kappa B (NF-kB) and peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1a) in skeletal muscle fibers (Di Meo, Napolitano, & Venditti, 2019). This is important because both NF-kB and PGC-1a play essential roles in exercise-mediated increases in skeletal muscle antioxidants and mitochondrial biogenesis. In addition to NF-κB and PGC-1a, nuclear factor erythroid-related redox activation 2 plays an important role in promoting exercise-induced expression of many key components involved in the endogenous antioxidant system (He, Ru, & Wen, 2020). In fact, nuclear factor erythroid 2 (Nrf2) controls the expression of key components of glutathione and Trx antioxidant systems, as well as enzymes involved in NADPH production (Jaganjac, Milkovic, Sunjic, & Zarkovic, 2020). Again, the available evidence shows that exercise-induced ROS production is clearly a requirement for skeletal muscle adaptations resulting from endurance exercise. In addition, there is evidence that ROS signaling is also involved in resistance training-induced hypertrophy (Mesquita et al., 2021), For example, in a cell culture model, H_2O_2-induced oxidative stress can activate protein kinase B, which increases protein synthesis in cells through downstream activation of the mammalian target of rapamycin (mTOR) (Oka et al., 2017). mTOR activation stimulates protein synthesis through increased

translation of contractile protein mRNA. In addition, experimental evidence in a plantaris muscle overload model shows that the production of ONOO, the reaction product of NO and -O2, creates a signaling cascade that leads to the direct activation of mTOR (Blaauw, Schiaffino, & Reggiani, 2013). Hence, it appears that ROS production results from the contraction of a key signaling molecule in resistance exercise-induced muscle hypertrophy (Mason & Wadley, 2014).

8. The effect of exercise training on antioxidant defense

Under normal physiological conditions, 1-5% of oxygen is converted into active oxygen species, which the body's antioxidant enzyme system can convert into harmless compounds. If the formation of free radicals exceeds the capacity of the antioxidant system and accumulates over time; they can cause oxidative damage, strand breaks, base changes and mitochondrial DNA mutations. This not only causes a disturbance in the production of ATP in this path; Rather, it produces more reactive oxygen species through electron leakage, which may cause more oxidative damage in mitochondrial biomolecules and lead to the creation of a vicious cycle that increases with age and ultimately decreases the bioenergetic function of cells of different tissues in It leads to the aging process (Fujii, Homma, & Osaki, 2022).

During intense sports activities, oxygen consumption increases 10 to 20 times compare to resting state. In addition, during intense sports activities, following anemia caused by physical activity, tissues such as the liver, kidney, and intestine experience a hypoxic environment due to more blood distribution to active muscles for more muscle work, which ultimately leads to an increase in free radicals. Some studies have shown that endurance training with moderate volume prevents the appearance of some signs of free radical production and against the damage caused by free radicals, it improves the tissue's antioxidant defense by increasing the activity of antioxidant substances (Lobo, Patil, Phatak, & Chandra, 2010).

However, some studies show that the high volume of endurance training that is normally used by elite athletes reduces the efficiency of the antioxidant system, increases the production of free radicals in skeletal muscle and other active tissues, and ultimately increases oxidative stress (Khan, Khan, & Sahreen, 2010). Body cells and tissues show oxidative

and antioxidant responses and adaptations to exercise, but not necessarily all tissues respond to the same level of exercise. Interestingly, the production levels of reactive oxygen species vary across tissues or cells, even at rest (Cantú-Medellín, Byrd, Hohn, Vázquez-Medina, & Zenteno-Savín, 2011). In sports training, the need for energy causes an increase in oxygen consumption in active tissues, so that oxygen consumption in general increases 10 to 20 times and in muscles 100 to 200 times. The increase in oxygen consumption leads to a substantial increase in the flow of electrons to the mitochondria, and as a result, the leakage of more reactive oxygen species from the mitochondria, and ultimately increases the production of reactive oxygen species, which plays a major role in the initiation and progression of muscle damage. Muscle injuries cause a decrease in sports performance and a person's strength. There is also a relationship between muscle damage indicators and lipid peroxidation (Gorzi, Ekradi, & Rahmani, 2018). One of the tissues prone to oxidative damage caused by reactive oxygen species such as superoxide, peroxide and hydroxyl is the heart muscle, which plays a role as an oxidative tissue with continuous activity during exercise. The increase in the metabolic activity of the heart during exercise provides the conditions for increasing the production of reactive oxygen species in the mitochondria and can lead to the loss of balance between the production of free radicals and their detoxification by antioxidant agents and the occurrence of oxidative stress (Siu, Bryner, Martyn, & Alway, 2004). On the other hand, the liver is the main metabolic organ in the body; for this reason, it is indirectly affected by the harmful metabolic products formed by other tissues such as the heart, skeletal muscle, and kidney through blood circulation (Finkler, Lichtenberg, & Pinchuk, 2014).

Heart and liver experience a hypoxic environment due to more blood distribution to active muscles during sports activities for more muscle work. Following anemia caused by exercise, the production of free radicals increases in the tissues (Afzalpour, Gharakhanlou, Gaeini, MOHEBI, & Hedayati, 2006). Intense aerobic physical activity affects the processes of oxidative stress by increasing the secretion of hormones such as epinephrine or other catecholamines, the metabolism of prostanoids, xanthine oxidase, NADH oxidase, and the activity of macrophages, and ultimately causes an increase in oxidative stress and lipid peroxidation (Cunningham, Geary, Harper, Pendleton, & Stover,

2005). In addition, many researches have shown that both types of aerobic and non-aerobic exercise with sufficient intensity and duration increase the activity of antioxidant enzymes and reduce the basic state of oxidative stress and strengthen the antioxidant capacity of organisms to continue physical activity. On the other hand, there is a belief that sports activities can act like a double-edged knife! Exercise and sports activity, as they are able to improve the antioxidant defense system, can also increase the production of free radicals that damage body molecules from different sources (Delavar, Mogharnasi, & Khoobkhahi, 2017).

In the study conducted on aged rats, induction of SOD protein was observed with exercise, while Laher showed in 2013 that short-term exercise increases oxidative stress in eight-month-old mice by removing glutathione (Laher et al., 2013). Regular long-term sports exercises it seems that with the feature of inducing anemia-reperfusion like intense exercises can lead to improvement of heart protection by improving antioxidant defense. The results of another study showed that the amount of superoxide desmutase and glutathione peroxidase enzymes increased after performing both types of intense intermittent exercise protocol and continuous exercise by rats compared to the control group, and this increase was greater in the intense intermittent exercise group. This led to the conclusion that doing intense interval training with less time and inducing more metabolic and free radicals and also oxygen consumption during the recovery period after intense training leads to the strengthening of the antioxidant system (Soori, Gerami, Pornemati, & Eskandari, 2019).

9. Effects of exercise on oxidative stress

9.1. Aerobic Exercise

9.1.1. Effect on oxidative stress and production of free radicals (FR)

Most studies show that endurance training reduces oxidative stress and muscle damage after exercise. These findings agree with the general idea that regular aerobic exercise leads to the fight against cellular aging and prevents the emergence of some cancers. This reduction can be so significant that oxidative stress is not increased in highly trained triathletes despite the significant inflammation induced by triathlon (Finaud, Lac, & Filaire, 2006). However, from these results, it is not yet clear whether this reduction in oxidative stress is due to a reduction in

FR production during exercise or due to an increase in the efficiency of the antioxidant system.

9.1.2. Effects on antioxidants

The effects of aerobic exercise on antioxidant enzymes are found in muscle, plasma, liver and heart levels. In muscle, some studies show that there is a specific antioxidant enzyme adaptation in muscle with a high percentage of type 1 fibers with strong oxidative power (Leeuwenburgh, Hansen, Holloszy, & Heinecke, 1999). In plasma and other tissues, increased antioxidant enzyme activity has been observed following a controlled endurance exercise protocol. However, this adaptation appears to be unrelated to the increase in VO_2max observed during these studies, and SOD and GPX increase more than CAT (Miyazaki et al., 2001). The results regarding the effects of endurance training on non-enzymatic antioxidants are more controversial with studies showing improvement or reduction of total antioxidant capacity or an isolated antioxidant in trained individuals compared to sedentary individuals. Some studies have also shown that antioxidant adaptation can be related to training volume or VO_2max (Askari, Ghiasvand, Feizi, Ghanadian, & Karimian, 2012). However, the training protocol it appears that must be sufficiently long and intense to produce an adaptive response. For example, a 8 week protocol increases VO_2max without increasing antioxidant potential, while an 10 week (longer and more intensive) protocol increases VO_2max and the activity of some antioxidants (Schneider & Tiidus, 2007). During such studies, it is essential to measure the nutritional antioxidant contribution because the efficiency of the antioxidant system is largely dependent on it.

9.2. Anaerobic exercise

9.2.1. Effect on oxidative stress and FR production

Little information is available on the effect of anaerobic exercise on oxidative stress. However, anaerobically trained individuals it has been shown that have less oxidative stress and muscle damage at rest or after exercise than non-trained individuals. Furthermore, these improvements are comparable to those observed in endurance-trained athletes (Teixeira, 2008). These results are controversial because other studies did not show a reduction in oxidative stress following an anaerobic exercise protocol (Vincent, Vincent, Braith, Lennon, & Lowenthal, 2002). Methodological

differences (population characteristics, exercise protocols, biological measurements) can explain some of these differences.

9.2.2. Effects on antioxidants

According to some studies, anaerobically trained people have better antioxidant enzyme activity in blood, tissues and especially in muscles during exercise (Ortenblad, Madsen, & Djurhuus, 1997). However, this improvement has not been observed in all studies. The difference between the results can be explained by the location of the doses and the training protocol. In fact, in the case of aerobic training, it seems that the duration of the protocol is important, because the phenomenon of adaptation appears only after several weeks of intense training (Hellsten, Apple, & Sjödin, 1996). For non-enzymatic antioxidants, it seems that anaerobic exercise increases their concentration. According to Cazzola and colleagues, this adaptation is the result of frequent production of FR during ischemia-reperfusion and the inflammation caused by this type of exercise at the muscle level (Cazzola, Russo-Volpe, Cervato, & Cestaro, 2003).

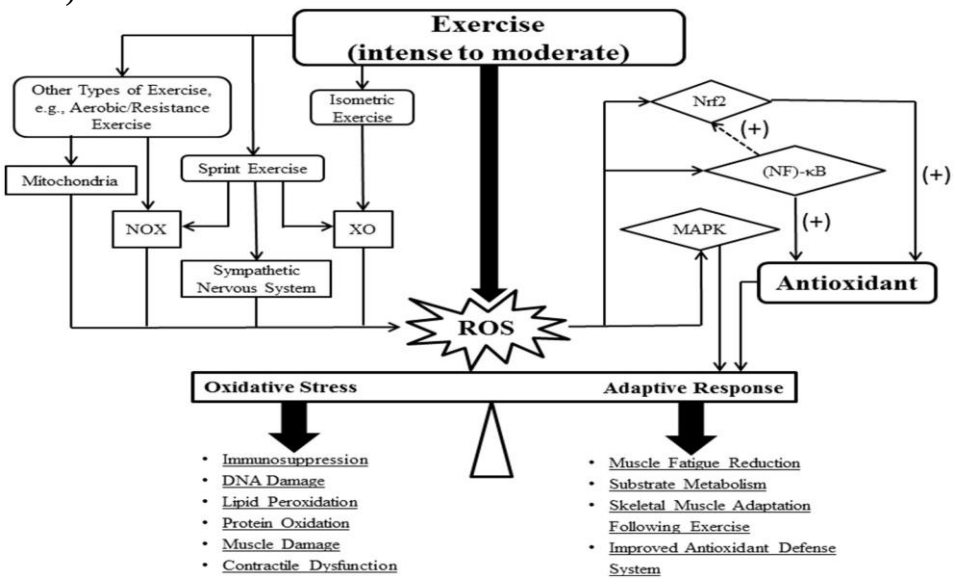

Figure 5: | Schematic illustrating ROS generation during different types of exercise and their associated roles in adaptive response. The dash arrow represents an indirect effect. Abbreviations: reactive oxygen species (ROS);

NADPH oxidase (NOX); xanthine oxidase (XO); mitogen-activated protein kinase (MAPK); nuclear erythroid 2 p45-related factor 2 (Nrf2); nuclear factor κB (NF-κB) (He et al., 2016).

9.3. Combined exercise

9.3.1. Effect on oxidative stress and FR production

Little research has been done on the effect of compound exercise on oxidative stress. However, recent studies show that trained soccer or rugby players have lower oxidative stress at rest than sedentary individuals (Metin, Gumustas, Uslu, Belce, & Kayserilioglu, 2003). Furthermore, after a rugby match, players with a high level of physical fitness have less oxidative stress compared to players with a lower level of physical fitness (Chang, Tseng, Hsuuw, Chan, & Shieh, 2002). Therefore, it seems that the level of exercise has an important effect on oxidative stress in this type of activity.

9.3.2. Effects on antioxidants

Studies have shown that football or rugby players have an increased enzymatic antioxidant system (Evelson et al., 2002). These results are confirmed in top athletes as well as in people with a lower level (BRITES et al., 1999). Combined exercise also increases the total antioxidant capacity and some non-enzymatic antioxidants such as vitamin C and vitamin E (Evelson et al., 2002). Therefore, improving the antioxidant system protects players from the harmful effects of oxidative stress. However, increasing the training and competition load can have the opposite effect, as shown in basketball players (Schröder, Navarro, Tramullas, Mora, & Galiano, 2000). The opposite results have been obtained in high-level soccer players (Cazzola et al., 2003). The differences can be explained by nutritional status; football players had sufficient nutritional antioxidants, while the diet of basketball players was not controlled.

CONCLUSIONS

The fact that intense and/or prolonged exercise increases oxidative stress in humans was discovered more than 4 decades ago. The tissues most responsible for ROS production during exercise are still debated, but it is clear that skeletal muscle contraction is an important source of ROS production during exercise. The intracellular sites of ROS production in

contracting skeletal muscle are still an active area of research, but increasing evidence points to NADPH as an important source of ROS production during exercise. The consequences of exercise-induced oxidative stress remain a controversial issue. In theory, exercise-induced ROS production can be a double-edged sword, Thereby, a moderate level of ROS production during exercise promotes positive physiological adaptations in active skeletal muscles (e. g, mitochondrial biogenesis, synthesis of antioxidant enzymes and stress proteins). While high levels of ROS production lead to damage to macromolecular structures (for example, proteins, lipids, and DNA). Although the effect of exercise-induced ROS production in skeletal muscle has been hypothesized as a bell-shaped hormesis curve, there is no convincing evidence that long-term, high-intensity exercise leads to tissue damage and physiological dysfunction. In fact, studies consistently show that long-term, high-intensity exercise has the greatest health benefits. Therefore, based on the available evidence, it is unlikely that intense and prolonged exercise leads to levels of oxidative stress that are harmful to human health.

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