Redox signaling in skeletal muscle: role of aging and exercise

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Ji LL. Redox signaling in skeletal muscle: role of aging and exercise. Adv Physiol Educ 39: 352–359, 2015; doi:10.1152/advan.00106.2014.—Skeletal muscle contraction is associated with the production of ROS due to altered O2 distribution and flux in the cell. Despite a highly efficient antioxidant defense, a small surplus of ROS, such as hydrogen peroxide and nitric oxide, may serve as signaling molecules to stimulate cellular adaptation to reach new homeostasis largely due to the activation of redox-sensitive signaling pathways. Recent research has highlighted the important role of NF-κB, MAPK, and peroxisome proliferator-activated receptor-γ coactivator-1α, along with other newly discovered signaling pathways, in some of the most vital biological functions, such as mitochondrial biogenesis, antioxidant defense, inflammation, protein turnover, apoptosis, and autophagy. There is evidence that the inability of the cell to maintain proper redox signaling underlies some basic mechanisms of biological aging, during which inflammatory and catabolic pathways eventually predominate. Physical exercise has been shown to activate various redox signaling pathways that control the adaptation and remodeling process. Although this stimulatory effect of exercise declines with aging, it is not completed abolished. Thus, aged people can still benefit from regular physical activity in the appropriate forms and at proper intensity to preserve muscle function.

aging; antioxidant; exercise; muscle; redox signaling; reactive oxygen species

EXERCISE PHYSIOLOGY is a discipline to study physiological response and adaptation to physical activity. The traditional approach has been primarily focused on systemic and organismic levels such as cardiovascular, neural, musculoskeletal, endocrinal, and pulmonary systems. During the past three decades, exercise physiology has undergone a revolution represented by two general trends: first, research has broken the disciplinary boundaries and become more integrated. Researchers often adopt a wide range of approaches such as biochemistry, molecular biology, immunology, and genetics, among others, to study physiological problems of interest. Second, investigation of cellular mechanisms has taken the central stage in the endeavor to understand and resolve main issues affecting people’s health, many of which have an etiological foundation related to the lack of sufficient exercise. In this context, signal transduction in the cell (often abbreviated as signaling) represents both of the aforementioned trends (12). While not being a discipline per se, signaling encompasses a body of knowledge pertaining to how extra- and intracellular signals are transmitted into the cell or within the cell to bring about either metabolic changes via enzyme activation/inhibition or changes of protein (mostly enzymes and transcription factor/cofactors) content via gene expression or both. While most of the signaling pathways results in anabolic processes such as growth, differentiation, and remodeling, controlled catabolic pathways such as apoptosis, proteolysis, and autophagy (including mitophagy) are also of high relevance.

As a subset of signaling pathways, redox signaling refers to a unique signal transduction pattern wherein some ROS, mostly H2O2 and nitric oxide (NO), serve as signaling molecules to modulate specific residues of the targets causing changes in enzyme activity, transcription factor/cofactor association/dissociation, DNA binding, and gene expression (3, 11, 12, 48). Although cysteine modification and the subsequent change of enzyme/protein activity constitute a fundamental mechanism of redox signaling, the concurrent definition of redox signaling is broader, including modifications of protein function due to the electron transfer process. Phosphorylation/dephosphorylation via kinases and phosphatases by tyrosine kinases and serine/threonine kinases are important consequences of redox signaling, but sulfhydryl/disulfide exchange, acetylation/deacetylation, methylation, and sulfoxidation are also potential covalent modulations in the process of redox signaling.

It is now well known that physical exercise increases ROS production in the body. However, exercise has a tremendous health implication and provides a host of benefits in preventing and reducing risks of some of the most prevailing diseases and disorders threatening human health. Thus, the American College of Sport Medicine has advocated the slogan “Exercise is Medicine.” A complete review of this paradigm is beyond the scope of this short review. Instead, the author will discuss several major exercise-induced redox signaling pathways including the key enzymes and transcription factors/cofactors, potential gene targets, major physiological consequences, and relevance to health issues. Pertaining to the focus of the American Physiological Society symposium on aging, the present review will focus on age-related changes in redox signaling capacity and its implications in metabolic and antioxidant functions in skeletal muscle.

Contraction-Mediated Activation of Redox Signaling

ROS generation during muscle contraction. During prolonged muscle contraction, the ATP demand for energy is increased. After the initial short-term supply by phosphocreatine and anaerobic glycolysis, the majority of ATP is generated by the mitochondrial electron transport chain (ETC) through oxidative phosphorylation. A small portion of the electrons will “leak” out of the ETC and be taken up by molecular oxygen, forming O2•−. With the enzyme SOD, O2•− is reduced to H2O2, which can be further reduced to water in the presence of catalase and/or glutathione peroxidase (GPX). However, not all O2•− and H2O2 are completely removed in the cell, thus raising the possibility of generating ‘OH due to the

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reaction between the two oxygen species (Haber-Weiss reaction) or between H$_2$O$_2$ and a metal ion that donates an electron (Fenton reaction). O$_2^-$, H$_2$O$_2$, and ‘OH are collectively called ROS, although sometimes NO is also included in this category (some researchers prefer to call ROS and NO reactive oxygen and nitrogen species or simply reactive species). The steady-state concentration of ROS is determined by their rate of generation and the activities of the antioxidant enzyme SOD, catalase, and GPX (47). It is estimated that 2% of the tissue’s total oxygen consumption can be converted to ROS (10), although recent estimates put this number at a much lower level (0.15%) (29). As the intensity of exercise increases, oxygen flux through the ETC also increases, but this does not always result in greater ROS generation due to a better coupling of oxidative phosphorylation and activation of uncoupling proteins (UCPs). Nevertheless, some other ROS-generating pathways may be activated during heavy exercise, such as NADPH oxidase (NOX), xanthine oxidase, and membrane-borne oxidases such as cyclooxygenase (COX)-2, lipooxygenase, and phospholipase A$_2$ (47). It is particularly noteworthy that cytosolic NOX appears to be a major source of ROS during muscle contraction (29). Furthermore, rigorous muscle contraction stimulates the body’s immune response and produces various cytokines (and chemokines), some of which also promote ROS generation (46).

**Exercise activation of redox signaling pathways.** Redox signaling by nature is a paracrine (also called autocrine) system wherein the signals produced in the cell activate a pathway or multiple pathways in adjacent (target) cells through a chain of local mediators. ROS produced in contracting muscle exactly serve that role. A large number of redox signaling pathways have been discovered over the decades, but the most relevant that have a significant impact on exercise physiology are NF-κB (44), MAPKs (3), and peroxisome proliferator-activated receptor (PPAR)-γ coactivator 1α (PGC-1α) (64). It is noteworthy that most signaling pathways do not operate independently but interact with each other to process and transfer signals, called “cross talk.” Target genes contain gene regulatory sequences (DNA-binding sites) in their promoter and/or intron regions that can interact with transcription factors to modulate the transcription rate and/or posttranscriptional processes of gene expression (3). Dimerization, covalent modification, and other conformational changes of transcriptional factors are often required before binding to DNA.

Muscle contraction poses a challenge to a wide range of intracellular homeostasis such as energy fuels, ATP, Ca$^{2+}$, and ROS as well as reducing powers such as NAD(P)H, GSH, and thioredoxin. These changes are caused by altered blood flow, oxygen flux, hormones, neural activity, and cytokine levels, all of which can have an impact on cell signaling. Not all signaling pathways are redox sensitive; NF-κB, MAPK, and PGC-1α are activated by exercise, and a major mechanism is due to increased H$_2$O$_2$ levels. Figure 1 shows the representative redox-sensitive signaling pathways and their roles in cellular adaptations in response to exercise and other physiological stimuli.

**NF-κB.** NF-κB is a dimeric transcription factors composed of members of the Rel family. In mammals, these proteins include p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), RelB, c-Rel, p105, and p100 (44). NF-κB is activated by a variety of stimulants, such as H$_2$O$_2$, proinflammatory cytokines (such as TNF-α and IL-1B), lipopolysaccharide, and phorboesters. These signals activate IκB kinase (IKK), which phosphorylates IκB and releases p50/p65 subunits from the NF-κB complex to enter the nucleus. Upstream activators of IKK include PKC and NF-κB-inducing kinase (NIK; a family of MAPKKKs), which are both redox-sensitive, i.e., their activities increase in response to increased intracellular oxidants (3). The best-known proteins and enzymes that require consensus binding of p65 in their promoter are mitochondrial SOD (SOD2), glutamylcysteine synthetase (GCS; the rate-limiting enzyme for glutathione synthesis), inducible NO synthase (iNOS), TNF-α, IL-6, COX-2, and VCAM-1. These genes are involved in a wide variety of biological functions, such as antioxidant defense, inflammation, immunity, and antiapoptosis (16).

An acute bout of exercise has been shown to activate the NF-κB pathway marked by phosphorylation of IKK, an increased phosphorylated IκB-to-IκB ratio, and nuclear p65 concentration (18, 26, 31, 37). Several characteristics of exercise-induced NF-κB activation are worth mentioning. First, the activation is muscle fiber specific, such that during prolonged exercise only oxidative fibers demonstrated increased NF-κB-DNA binding, suggesting that the ROS source was mitochondria (26). Second, time-course experiments revealed that IKK activation and IκB phosphorylation happen immediately after an acute bout of exercise, whereas peak NF-κB-DNA binding is at 2 h postexercise, as shown by a gel mobility shift assay and p65 nuclear accumulation (31). Finally, NF-κB activation is redox sensitive. The drug allopurinol, a xanthine oxidase inhibitor, was found to reduce NF-κB and abolish SOD2 and iNOS transactivation in response to sprinting exercise (18).

**MAPK.** MAPK pathways contain JNK, ERK1/2, and p38MAPK, each of which is controlled by upstream kinases called MAPKKKs (MEKs), which, in turn, are controlled by MAPKKKKs (MKK), forming a hierarchy (3). The primary function of the MAPK pathway is to modulate growth, metabolism, differentiation, transcription, translation, and remodeling. This broad MAPK function is outside the scope of this minireview, and the readers are referred to several excellent reviews (23, 56). The primary extracellular stimulators of the MAPK pathway are growth factors, inflammatory cytokines, and phorbol esters, whereas ROS are primary intracellular activators. There are considerable functional overlaps and cross talk between NF-κB and MAPK pathways (25). For example, NIK and IKK are members of the MKK/MEK family, and ERK and p38 have been shown to play an important role in the temporal regulation of NF-κB activation by IL-1β and H$_2$O$_2$ (7). It is noteworthy that several important exercise adaptations in skeletal muscle, such as mitochondrial biogenesis, hypertrophy, and fiber transformation, are regulated primarily by MAPK signaling, and MAPK is involved in exercise-induced activation of antioxidant enzymes such as MnSOD and iNOS (18, 30).

MAPK pathways have been reported to be activated during exercise, including ERK1/2 and p38 (23, 56). The signals triggering MAPK activation have been attributed to a variety of physiological stimuli associated with exercise, including growth factors, Ca$^{2+}$, neural activity, and certain cytokines. Growth factor (such as insulin) activation appears to rely on Ras protein, whereas PKC serves as the master controller for signals ranging from various extracellular sources (Fig. 1). H$_2$O$_2$ is a strong activator of PKC; thus, exercise-induced activation of MAPK has shown clear redox sensitivity. For example,
activation of p38 and ERK1/2 stimulated by an acute bout of sprinting exercise in rats was abolished or severely hampered by treatment with allopurinol, as were target genes for these signaling pathways, MnSOD and iNOS (18).

PGC-1α was first identified as a functional activator of PPAR-γ in brown adipose tissue (49). Subsequent research revealed that it is a master transcription cofactor participating in almost all aspects of mitochondrial functions ranging from energy fuel selection, muscle fiber differentiation and transformation, antioxidant gene expression, and mitochondrial fusion and fission dynamics (39, 64). PGC-1α has been identified in other mitochondria-rich tissues, including red skeletal muscle and the heart, as well as in the kidney, liver, and brain (15). PGC-1α is known to activate several nuclear transcription factors, such as PPAR, nuclear respiratory factor (NRF)-1 and NRF-2, and estrogen-related receptor-α, as well as mitochondrial transcription factor A (Tfam), which directly stimulates mitochondrial DNA replication and transcription (64). In addition, a PGC-1α isoform resulting from splicing of primary PGC-1α (PGC-1α4) has been found to stimulate muscle hypertrophy due to its ability to activate the IGF-1/ Akt/mechanistic target of rapamycin pathway and suppress myostatin (54).

In skeletal muscle, PGC-1α expression is activated during muscle contraction through several potential pathways. Increased Ca²⁺ signaling during muscle contraction can activate Ca²⁺/calmodulin-dependent protein kinase and calcineurin A, which, in turn, enhance expression of cAMP response element-binding protein (CREB) and myocyte enhancer factor-2, two required nuclear factor-binding proteins for PGC-1α transactivation (22, 61). Exercise-induced upregulation of PGC-1 also involves p38, which activates myocyte enhancer factor-2 and activating transcription factor 2 (ATF2). ATF2-CREB dimerization appears to be an early event in PGC-1α-mediated signaling processes (61). In addition to transcriptional activation, p38 also increases PGC-1α coactivating capacity by phosphorylation in response to cytokine stimulation in muscle cells (2). Furthermore, as a metabolic energy deprivation sensor, AMP-activated protein kinase (AMPK) is activated by exercise due to the increased AMP-to-ATP ratio and/or hypoxia and Ca²⁺ flux during muscle contraction, enhancing PGC-1α transcription as well as activity. It is important to note that PGC-1α is considered a redox signaling pathway because ROS produced during exercise provide a positive feedforward stimulus. In cultured myocytes, H₂O₂ has been shown to promote PGC-1α expression along with all gene products

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Fig. 1. Schematic illustration of the major redox signaling pathways in skeletal muscle, including major sources of ROS generation in the cell in response to physiological stimuli, and a schematic illustration of the affected nuclear protein-binding sites on DNA. ArA, arachidonic acid; ATF2, activating transcription factor-2; COX2, cyclooxygenase-2; CRE, cAMP-response element; DAG, diacylglycerol; Egr-1, early growth-responsive-1 protein; Gα, G protein α-subunit; IP₃, inositol triphosphate; LOX, lipooxygenase; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; PLC, phospholipase C; Trx, thioredoxin; XO, xanthine oxiase.
controlled by PGC-1α (59). Rats subjected to a repeated sprinting exercise were found to have six times higher PGC-1α content than rested control rats, but rats injected with allopurinol and subjected to the same exercise protocol showed only three times increase, indicating that ROS produced by xanthine oxidase were required cellular signals for the observed response (36). Furthermore, endurance training-induced upregulation of PGC-1α in rats was abolished by chronic administration of pyrrolidine dithiocarbamate, an antioxidant that inhibits NF-κB (14).

Chronic exercise training has been consistently shown to increase PGC-1α levels in skeletal muscle (2, 13, 14, 19, 38, 54). Along with PGC-1α upregulation are increased NRF-1 and Tfam levels, although some studies have shown no change in these PGC-1α-controlled transcription factors. Different exercise protocols with variable intensity and tissue sampling time may explain the discrepancies. However, endurance training increases mitochondrial oxidative capacity and ATP production, increasing expression of tricarboxylic acid cycle and ETC enzymes and enhancing fatty acid oxidation and mitochondrial morphological changes (64). A cross-sectional study (38) showed that endurance-trained human subjects had seven times higher PGC-1α, five times higher Tfam, and more than twofold higher NRF-1 protein contents in vastus lateralis muscle biopsies than their sedentary counterparts. There is little doubt that the training adaptations were dependent on intact PGC-1α signaling, as PGC-1α knockout (KO) mice that underwent endurance training showed virtually no change in mitochondrial markers (13).

**Antioxidant function of redox signaling.** Besides the clearly defined role of PGC-1α signaling, as mentioned above, one of the most important functions of redox signaling during exercise is to regulate cellular antioxidant defense, composed of antioxidant enzymes and low-molecular antioxidants such as GSH, thioredoxin, and UCPs. Adequate antioxidant capacity not only minimizes potential oxidative damage caused by exercise but also controls the formation and release of ROS so as to influence a wide range of physiological functions in response to exercise (32).

There is an abundance of literature reporting muscle antioxidant adaptations to chronic exercise training. SOD activity has consistently been shown to increase with exercise training in an intensity-dependent manner, and MnSOD (i.e., SOD2) is primarily responsible for the observed increase in SOD activity. GPX activity has also shown to increase after endurance training. The observed training adaptation is due to altered gene expression, with both mRNA and enzyme protein levels being upregulated (24, 26). The training adaptation of antioxidant enzymes is heavily influenced by a number of physiological and environmental factors, such as sex, age, diet, and medication state. For a thorough review on this topic, the readers are referred to several previous reviews (47, 52).

Besides SOD and GPX, the rate-limiting enzyme for GSH synthesis, GCS, can be induced by training in the muscle and liver (51). As a result, muscle and hepatic GSH contents, as well as GSH export, are increased. There is evidence that GCS upregulation is dependent on NF-κB activation at least in some, but not all, cell types (9).

NO production can have dual consequences. It can react with superoxide to form peroxynitrite, one of the most reactive ROS, and cause damage to macromolecules in myocytes and endothelial cells (11, 52). On the other hand, muscle contraction results in an increase in NO production via increased NO synthase expression, which enhances blood flow due to NO-induced vasodilation and indirectly enhances muscle antioxidant defense during exercise. iNOS expression has been shown to be enhanced in response to heavy exercise, and there is evidence that ROS and inflammatory cytokines play a role through activation of NF-κB and MAPK (1, 18). These data were consistent with the finding that the iNOS mRNA level was increased after an acute bout of exercise (5), whereas inhibition of ROS production by allopurinol treatment abolished this induction (18). It is noteworthy that although activation of NF-κB and MAPK is a necessary event in response to oxidative stress to induce antioxidant adaptation, prolonged hyper-activity of these signaling pathways also promotes the gene expression of proinflammatory cytokines and chemokines, leading to potential ROS generation, inhibition of PGC-1α function, and degradation processes such as proteolysis and apoptosis (53, 55). This could occur during rigorous muscle contraction, especially eccentric exercise, and subsequent muscle injury. Chronic NF-κB activation could lead to systemic inflammation, which is a major etiological mechanism for many chronic diseases such as rheumatoid, atherosclerosis, obesity, and certain types of cancer (12).

**Redox Signaling in Aging Skeletal Muscle.**

Because of the extensive role redox signaling plays in cell life, it is not surprising that skeletal muscle health and functionality are intrinsically dependent on the integrity of redox signaling during aging. In this section of the review, the author attempts to address two questions: 1) does aging attenuate signaling transduction in certain pathways in skeletal muscle and 2) how does exercise affect redox signaling in the muscle.

As skeletal muscle ages, there is a progressive loss of muscle mass, contractile force, and other functions, termed “sarcopenia.” Sarcopenia is not a disease but a syndrome associated with advanced age caused by both intrinsic and extrinsic factors (33). The most widespread report on senescent muscle is a decline of aerobic capacity caused by both loss of mitochondrial population density and oxidative phosphorylation, as reflected by a lower respiratory capacity and reduction of ATP production (8). These observations of mitochondrial enzyme profiles are related to decreased mitochondrial DNA and mitochondrial protein expression with aging. This “mitochondrial theory of sarcopenia” has been advocated by several research groups with an abundance of evidence (6). Limited by the scope of the article and personal experience, the following section will focus on PGC-1α, one of the most important signaling pathways, in the age-related disruption of mitochondrial homeostasis.

**Effect on PGC-1α and mitochondrial biogenesis.** Recent data suggest that the age-related downregulation of PGC-1α may play an important role in the decline of mitochondrial biogenesis and turnover and contribute to the etiology of sarcopenia (6, 60). Several recent studies have indicated that both PGC-1α mRNA and protein levels were significantly decreased in senescent muscle in rats and that the reduction was greater (up to >50%) in oxidative muscle fibers (8, 62). Decreased cytochrome c and a mitochondrial DNA-to-nuclear DNA ratio were also reported, but the effect of aging on Tfam,
the major controller of mitochondrial DNA replication and mitochondrial proliferation, has been inconsistent. In agreement with rodent studies, older human subjects (>65 yr) showed a lower PGC-1α mRNA level in leg muscles compared with young controls, along with decreased NFR-1 and Tfm protein content (17). Direct evidence supporting a crucial role of PGC-1α in aging muscle was provided in a transgenic mouse model study wherein muscle-specific overexpression of PGC-1α ameliorated a wide range of age-related physiological and cellular deteriorations, such as insulin resistance, body fat accumulation, neuromuscular junction integrity, and systemic inflammation at old age (62). Lean body mass was also increased in PGC-1α versus wild-type mice, indicating that PGC-1α has a direct role in preserving muscle mass. However, some studies showed no age difference in the protein levels of PGC-1α, Nrf-1, or Tfm in old compared with young human subjects, thus shedding some doubt as to the true function of PGC-1α signaling in age-associated muscle degradation (38). Potential explanations for the discrepancy could be the difference in the fiber composition of the muscle samples obtained and the methods with which the data were normalized.

The exact mechanism for the age-related decline of PGC-1α signaling is still unclear. However, aging is known to alter several important upstream enzymes and transcription factors that control PGC-1α expression and posttranslational modification. One such regulators is CREB, a mandatory step for PGC-1α transactivation that has been shown to decline in both content and DNA binding in aged muscle (34). Among the enzymes that control CREB expression and phosphorylation, the AMPK protein level and phosphorylation (activation) have been reported to decrease with aging (34, 63). An age effect on p38 has been controversial with both an increase and no change being reported (27, 50), whereas the effects of aging on Ca2+/calmodulin-dependent protein kinase and calcineurin A, the other potential activators of CREB, have not been reported.

Several redox-sensitive signaling pathways are known to interact with PGC-1α and demonstrate age-related alterations. A common signaling molecule that may impact on multiple signaling activities is H2O2, which is increased in aged skeletal muscle (42). Increased ROS are known to activate NF-κB and the forkhead family of transcription factors (FoxO), which are two redox-sensitive signaling pathways that can downregulate PGC-1α (12, 55). Inversely, PGC-1α has demonstrated inhibitory effects on FoxO3, which promotes inflammatory cytokine expression and downregulates antioxidant enzyme expression (22). Thus, PGC-1α KO mice have shown elevated TNF-α and IL-6 levels in skeletal muscle as well as higher serum IL-6 levels than wild-type mice. In contrast, PGC-1α overexpression suppressed the age-associated elevation of TNF-α and IL-6 in skeletal muscle and blood (62). Activated NF-κB and FoxO pathways along with upregulation of inflammatory cytokine expression have been shown to promote muscle protein degradation during disuse atrophy and sarcopenia, largely due to enhanced ubiquitin proteolysis (57). Taken together, PGC-1α may be regarded to have an anti-inflammatory function, which could significantly impact on aging muscle.

Finally, the NAD-dependent deacetylase sirtuin (SIRT)1 can be a potential regulator of PGC-1α transcriptional activity (21). SIRT1 has been reported to directly affect PGC-1α transcriptional activity by physical interactions, deacetylating and activating PGC-1α both in vitro and in vivo (45). Aging has been shown to decrease the expression of SIRT3, a mitochondrial analog of SIRT1, in human skeletal muscle, and this was interpreted as a potential mechanism for the age-related downregulation of mitochondrial biogenesis (38). It is noteworthy that as a master regulator of mitochondrial morphological and metabolic integrity, the role of PGC-1α is not limited to the above-mentioned paradigms but participates in other major signaling pathways such as apoptosis, autophagy (mitophagy), mitochondrial fusion/fission dynamics, and neuromuscular junction preservation, which could all undergo age-related deterioration and dysfunction. It is impossible to review all aspects as such in this article. Readers are referred to recently published reviews for details (6, 20, 22, 33, 40–43).

**Effect on NF-κB and MAPK and its implications.** Besides PGC-1α, aging-related alteration of several other redox signaling pathways may have a significant impact on enzymes controlling both muscle protein synthesis and degradation (32). For example, two muscle-specific ubiquitin ligases, atrogen-1/MAFbx and MuRF1, which control the final step of ubiquitination for muscle protein degradation, can be activated by NF-κB and FoxO3, thus exerting detrimental effects on muscle mass and structural integrity (35, 57). FoxO3 is known to be a strong activator of atrogen-1, leading to protein degradation, and it is also known to downregulate antioxidant enzymes and increase ROS generation, subsequently activating NF-κB (55). NF-κB induces pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6, whereas PGC-1α reduces the rate of protein breakdown in muscle wasting by inhibiting the transcriptional activity of Foxo3 and NF-κB (22, 55). Thus, preservation of a higher PGC-1α level has multiple advantages in promoting mitochondrial integrity, reducing ROS production, suppressing inflammation, and reducing protein loss in senescent muscle.

In skeletal muscle, antioxidant enzyme activities are increased with old age, whereas protein and mRNA levels of CuZnSOD, MnSOD, and GPX were found to be either decreased or unaltered in aged muscle (30). In addition, aged muscle exhibits a reduced antioxidant adaptation to training compared with young muscle. These findings have raised the possibility that redox signaling may be altered during aging, but data in this area have been far from consistent. Several authors have reported a decreased NF-κB-binding capacity and MAPK (ERK1/2 and p70S6K) activities in the plantaris and tibialis anterior muscles of aged rats at rest and in response to stimulated contraction (for a review, see Ref. 30). However, no difference in p38, p70S6K, and JNK activities was found in extensor digitorum longus muscles between young and old rats (27). Furthermore, Williamson et al. (63) reported higher resting activities of several MAPK enzymes in leg muscles of old men compared with young men, but the amount of protein in the MAPK pathway was unaltered with age. The above discrepancies derived from different muscle types and species are not surprising as muscle antioxidant singing is highly fiber specific due to differential intrinsic rates of ROS generation. The varied antioxidant defense capacity among different muscle types may also alter the sensitivity of cells to ROS.

Another potentially important confounding factor in studying antioxidant signaling, especially NF-κB signaling, is chronic inflammation due to minor injury and/or immobility, as often seen in senescent muscles (65). NF-κB is believed to be constitutively activated at old age, which leads to the higher
basal expression of proinflammatory cytokines, chemokines, and ROS-generating enzymes such as iNOS and COX-2. Chronic activation of NF-κB has been identified as a major reason for aged-related muscle wasting and sarcopenia (6). It has been reported that that 4-hydroxyphenxenal, a lipid peroxidation product often found in aged muscle, could activate NF-κB by activating the NIK/IKK signaling cascade due to ERK and p38 activation. Since NF-κB activation often leads to increased proinflammatory cytokine expression, this vicious cycle was hypothesized as the basis for the inflammation theory of aging (65).

**Antiiaging effect of exercise on muscle redox signaling.** It is widely perceived that aging can attenuate the magnitude of training adaptation seen at a younger age, but a clear explanation of this attenuation is still lacking. Since a broad range of training adaptation in muscle cells is mediated by redox signaling, as discussed above, one might speculate as to whether aging can reduce the ability of muscles to respond to redox signals. Derbre et al. (13) showed that muscle PGC-1α, NRF-1, and cytochrome c contents from aged rats did not respond to endurance training as those of young rats did and that the lack of the training response was notably identical to PGC-1α KO mice. Furthermore, mitochondrial biogenic markers in aged muscle did not respond to cold exposure or thyroid stimulation, the classic PGC-1α stimulators. These findings raised the possibility that loss of sensitivity to exercise-induced redox changes might underlie the mechanism for sarcopenia. However, the majority of the studies to date have not support such a view and have pointed out that aged muscle still maintains the ability of exercise-induced redox signaling seen in younger muscles (Refs. 17, 34, and 38; for a thorough review, see Ref. 40). For example, Kang et al. (34) showed that whereas PGC-1α mRNA and protein levels were 35% and 80% lower in soleus muscles of 24- versus 3-mo-old rats, 12 wk of endurance training resulted in a 2.7-fold higher PGC-1α content along with increased Tfam, cytochrome c, and mitochondrial DNA contents in old rats. CREB phosphorylation and its DNA-binding capacity in old rats were also increased. These studies support a view that the critical regulators of mitochondrial biogenesis were functional despite old age, which explains the metabolic adaptations observed in trained old individuals.

Because the magnitude of the training adaptation in older skeletal muscle reported in the literature displays a wide range of difference, one might speculate that aged muscle is less responsive to the exercise stimulus compared with young muscle, i.e., training sensitivity may be reduced with advanced age. However, a recent study directly challenged this perception. Iversen et al. (28) compared endurance-trained or untrained elderly subjects (71 yr old) in response to an acute bout of bicycle exercise at 75% of their matched maximal O2 consumption in muscle biopsies obtained from the vastus lateralis muscle before, immediately after, and 2 h after exercise. Although both trained and untrained subjects increased PGC-1α mRNA expression at 2 h, a surprise finding was that untrained subjects displayed twice as high PGC-1α responses (~12- vs. 6-fold increase) as trained subjects. Both groups showed remarkable increases in the phosphorylation level of AMPK and p38, the two major upstream enzymes that activate PGC-1α expression. It was also shown that whereas older subjects had lower basal muscle PGC-1α mRNA and NRF-1 and Tfam protein contents than young subjects, training increased PGC-1α mRNA by 2-fold and NRF-1 content by 1.5-fold (17). Furthermore, in a cross-sectional study, Lanza et al. (38) showed that mitochondrial protein levels, mitochondrial DNA, and PGC-1α signaling markers (NRF-1 and Tfam) were significantly higher in trained old subjects than their sedentary counterparts, although the magnitude of improvement was lower than in young subjects after training. These findings clearly indicate that the skeletal muscle of elderly subjects maintains the ability of responding to acute exercise and that aging does not abolish muscle plasticity at least as far as mitochondrial function is concerned. However, despite decades of investigation, controversy still exists in the literature as to whether or not training adaptation is attenuated with age and what role redox signaling plays in contributing to this controversy. A clear consensus on these issues is yet to be obtained.

**Conclusions**

Despite a highly efficient antioxidant defense system in the cell, a small surplus of stable ROS (H2O2 and NO) produced during muscle contraction may serve as signaling molecules to stimulate cellular adaptations to reach new homeostasis due to the activation of redox-sensitive signaling pathways. NF-κB, MAPK, and PGC-1α have been identified as some of the most important signaling pathways and, through their cross talk, participate in several critical cellular functions such as mitochondrial biogenesis, antioxidant defense, inflammation, protein turnover, apoptosis, and autophagy. Despite the nature of aging as a degenerative process, aged muscle is capable of activating redox signaling pathways and upregulating many metabolic functions in response to acute and chronic exercise. Although this stimulatory function of exercise declines with aging, it is not completed abolished. Thus, aged people can still benefit from regular exercise in appropriate forms and at proper intensity to preserve muscle function.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: L.L.J. conception and design of research; L.L.J. performed experiments; L.L.J. analyzed data; L.L.J. interpreted results of experiments; L.L.J. prepared figures; L.L.J. drafted manuscript; L.L.J. edited and revised manuscript; L.L.J. approved final version of manuscript.

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