Chapter 7
Physical Activity: A Strong Stimulant for Hormesis During Aging

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Introduction: Physical Activity and Successful Aging

In the animal world physical exercise is an intimate part of life cycle to pursue food, escape predators, and ensure reproduction. Aged individuals with reduced fitness and mobility are subjected to natural selection. In rodents, volunteer wheel runners show an increase in both maximal life span and 50% survival rate compared to their sedentary counterparts, indicating physical activity can change aging process (Holloszy 1993). In human population, morbidity is concentrated in the last 2 decades of life, beginning on the average at age 55 and increasing in frequency until the average age of death at 75. The benefit of exercise is highlighted by the increase of approximately 2 years in longevity in physically active people as compared to less active people (Paffenbarger et al. 1993). Disability levels in a vigorously exercising population are below that of non-exercisers and age-related increases in disability are delayed by approximately 15 years (Fries 1996). These data indicate that engaging in regular physical activity would increase the age of onset of chronic illness and shorten the time between the onset of morbidity and death. Furthermore, this compression of the period of morbidity as a result of physical exercise would represent a significant improvement in the quality of life and result in major reductions in the health care for the elderly.

Despite these clear benefits of participating in physical exercise, there is a concern that aged individuals are more susceptible to some of the harmful effects of rigorous exercise as a result of increased exposure to reactive oxygen species (ROS) (Davies et al. 1982). The free radical theory of aging (Harman 1956) has allowed for the establishment of a powerful link between exercise and aging research. A fundamental premise for this theory is that ROS generated in normal metabolic processes are the underlying reason for cell and tissue oxidative damage seen throughout the aging process. Since exercise increases metabolic rate reflected by

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a greater amount of oxygen uptake, ROS production is increased during physical exertion. One may naturally ask this question: is physical exercise more beneficial or harmful to the elderly population? A complete and unequivocal conclusion is still forthcoming; however, there is enough evidence to demonstrate that a mild oxidative stress associate with exercise may not be a bad thing during aging.

Although aging may cause increased ROS generation and oxidative stress probably in all cell types, which could be influenced by physical activity overall, in this chapter the author chose to focus on skeletal muscle for the obvious reasons that (a) skeletal muscle health is vital for mobility and normal life; (b) deterioration of skeletal muscle mass and functionality (sarcopenia) is an important issue in medical gerontology (Thomas 2007); and (c) skeletal muscle has displayed some unique characteristics during aging.

Exercise and Oxidative Stress

The most prominent biological changes occurring during exercise is the increased metabolic rate, matched by an enhanced rate of mitochondrial respiration and oxidative phosphorylation to provide ATP for contracting muscles. It is estimated that during maximal workload in men oxygen consumption at the local muscle fibers can reach as high as 100-fold of the resting levels, while the whole body oxygen consumption (VO_2) increases by ~20-fold. The electron transport chain (ETC) consumes >85% of all the O_2 utilized in the cell whereas ~1–5% of oxygen can form $O_2^{\bullet-}$ and eventually other ROS as byproducts (Meydani and Evans 1993). A large number of studies reported elevated $O_2^{\bullet-}$ level in skeletal muscle following contractile activity (see Ji 1999). For example, using dichlorofluorescin (DCFH), a non-specific intracellular ROS probe Bejma and Ji (1999) showed that an acute bout of treadmill running at 75% VO_2 max for 1 h can increase ROS production by 30–40%, corresponding to 20–30 pmol/min/mg protein in rat quadriceps muscles. Using a microdialysis technique, McArdle et al. (2001) reported a mean amount of 1.2 nmol $O_2^{\bullet-}$ in contracting mouse gastrocnemius muscle during the 15 min period of electrically stimulated contraction. It is important to point out that the observed elevation of ROS was in spite of the continuous removal by endogenous antioxidant defense systems, composed of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), as well as ROS scavengers such as vitamin E, C and GSH.

Mitochondria are the main, but not the only source of ROS during muscle contraction. Depending on the intensity of exercise and the physiological conditions, several other cellular components and pathways can contribute to ROS production. Xanthine oxidase (XO) plays a major role in $O_2^{\bullet-}$ production in skeletal muscle during intermittent hypoxia and reoxygenation, which occurs during ischemic contraction, heavy weight lifting and sprinting exercise (Gomez-Cabrera et al. 2005). Member-borne NADPH oxidase can be activated to generate $O_2^{\bullet-}$ during muscle contraction (Bejma and Ji 1999; Pattwell et al. 2004) and injury-associated inflammation (see below).

Another important source of ROS during muscle contraction is nitric oxide (NO) (Reid 2001). Several authors demonstrated increased NO production in cultured myotubes in vitro (Pattwell et al. 2004), in isolated contracting muscle (Balon 1999), and in muscle subjected to passive stretching (Tidball et al. 1998). The major enzyme producing NO in muscle cells are endothelial NO synthase (eNOS); however, inducible NOS (iNOS) may increase its importance when muscle undergoes lengthening contraction (LC), inflammation and healing (Sakurai et al. 2005). While the increased NO can scavenge $O_2^{\bullet-}$ to reduce $^{\bullet}OH$ formation via Haber-Weise reaction, it risks the generation of peroxynitrite, another highly reactive species that targets selective amino acids such as phenoalanine and tyrosine (Leeuwenburgh et al. 1999).

Physical activity at high intensity sometimes leads to mechanical injury such as stretching or muscle-soft tissue injury followed by inflammation. Inflammatory cells in the injured tissues can generate ROS (Cannon and Blumberg 1994). Blood-borne polymorphoneutrophils (PMN) play a critical role in defending tissues from viral and bacterial infection by producing $O_2^{\bullet-}$ via activation of NADPH oxidase during a respiratory burst (Pyne 1994; Aoi et al. 2004). Cytoplasmic (CuZn) SOD converts $O_2^{\bullet-}$ to H_2O_2, which is further converted to $^{\bullet}OH$ in the presence of ferrous ions, or to hydrochloric acid (HOCl) catalyzed by myleoperoxidase. While these ROS are considered critical in the healing process, they can also cause secondary damage to healthy muscle cells. Figure 1 illustrates major cellular sources of ROS during exercise or inflammation.

Aging Increases Exercise-Induced Oxidative Stress

Aging increases ROS production in the cell especially in the mitochondria (Sohal and Sohal 1991). In skeletal muscle and heart ROS production is also increased with age along with increased oxidative damage markers. ROS production was shown to be 77% higher in the muscle of 25 month-old rats than 8 month-old rats, and increased 50% and 38%, respectively after 1 h treadmill running (Bejma and Ji 1999). In the above study running speed and grade were adjusted to impose a similar workload (~75% VO_2max) to the young and old rats. In the heart, ROS production was also increased with age, but the acute exercise bout increased ROS generation only in the old rat (Bejma et al. 2000). These data clearly reveal that as animals grow older, a smaller work task can provoke a greater ROS-generating effect in the heart and skeletal muscle.

The reason for the aged animals to increase ROS production during exercise is not entirely clear. Age-related defects in mitochondrial ETC are considered a major mechanism (Nolh et al. 1978; McArdle and Jackson 2000). Lowered cytochrome c oxidase (complex IV) with age favors a greater electron “leakage” and formation of $O_2^{\bullet-}$ in the senescent organism. Peroxidative modification of mitochondrial membrane lipids may be another major change at old age, such as elevated malondialdehyde and 4-hydroxynonenol levels, decreased membrane fluidity and enhanced fatty acid unsaturation (Kim et al. 1996; Yu and Chung 2006). These changes may

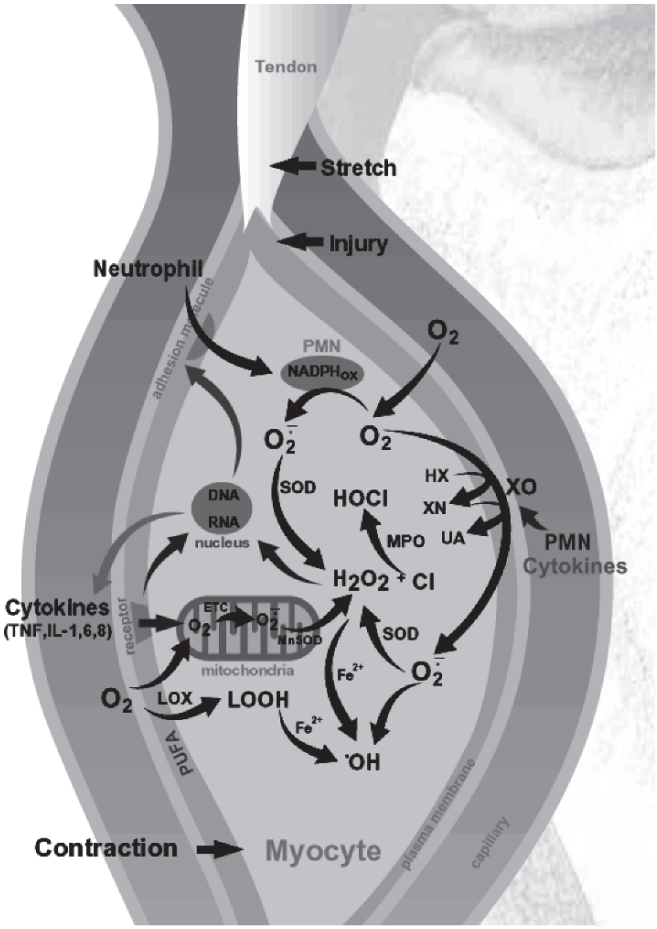

Fig. 1 Sources of reactive oxygen species (ROS) in muscle cells during exercise and contraction-mediated injury. Abbreviations: ETC, election transport chain; HOCl, hypochorous acid; HX, hypoxanthine; IL, interleukine; LOX, lipooxygenase; MPO, melyeoperoxidase; PMN, polymorphoneutrophil; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; UA, uric acid; XN, xanthine; XO, xanthine oxidase

cause further ROS generation via ETC and enzymatic pathways involving cyclooxygenase (COX), NADPH oxidase and XO (Sawada et al. 1992).

Chronic muscle inflammation is a common problem associated with old age. Endothelial cells from injured muscle are known to release cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1 and 6 with distinct individual time courses (Pedersen et al. 1998). These pro-inflammatory molecules can stimulate signal transduction pathways (see below) to promote de novo expression of the above cytokines and vascular cellular adhesion molecules (VCAM). Increased iNOS expression and subsequent NO production may cause vasodilation that

further facilitates PMN and cytokine infiltration, forming a vicious cycle (Gath et al. 1996). All these events may occur several hours to a few days after an acute bout of strenuous exercise, especially when involving LC (Best et al. 1999). Aged individuals are more susceptible to muscle injury as a much smaller workload can produce mechanical injury. In humans, stretch injury and fall constitute a majority of incidence for subsequent muscle inflammation.

Antioxidant Defense in Aging Muscle: A Hormetic Response?

Aging is associated with a deterioration of protein synthesis and cell differentiation capacity in most tissues, particularly in the postmitotic tissues. Therefore, antioxidant utilization and degradation probably are not adequately replenished at old age. However, antioxidants have demonstrated considerable plasticity in response to oxidant exposure. Localized oxidative stress in specific organelles and cell compartments may stimulate cellular uptake and synthesis of certain antioxidants under highly regulated signaling process (Ji 2007). According to the free radical theory of aging one might expect to see a general decline of cellular antioxidant defense capacity at old age. Available data suggest that these changes are not uniform across all species and all cell types, and some even show an increased antioxidant defense with age (Matsuo 1993). Skeletal muscle is a unique organ that exhibits marked increases in antioxidant enzyme activity with aging (Ji et al. 1990; Lawler et al. 1993; Luhtala et al. 1994). Activities of all major antioxidant enzymes, such as SOD, CAT and GPX, as well as GSH sulfur-transferase and glutathione reductase, were significantly higher in the hindlimb muscles of old vs. young rats. In order to know whether gene expression of antioxidant enzymes are more active in skeletal muscle of age animals, mRNA levels and enzyme protein contents were measured for the various enzymes. All studies did not find significant changes of the relative abundance of mRNA for either SOD isozyme or GPX, except for CAT mRNA which showed an increase with age in soleus muscle (see Ji 2001). Age-adaptation of antioxidant enzymes appears to be muscle fiber specific, with the most prominent increases found in type 1 (slow-twitch oxidative) muscles, followed by type 2a (fast-twitch oxidative), whereas type 2b muscles showed little effect. Luhtala et al. (1994) reported that elevation of muscle antioxidant enzymes during aging was markedly affected by dietary restriction in Fischer 344 rats. The progressive increases in CAT and GPX activities from 11 to 34 month of age were prevented by a 30% reduction of food intake, while an age-related increase in MnSOD was also attenuated.

Aging is associated with a decline of cellular thiol reserve in most tissues (Matsuo 1993). However, skeletal muscle and heart may be spared of this effect. Leeuwenburgh et al. (1994) showed that aging caused no significant alteration of GSH content or GSH/GSSG ratio in rat deep vastus lateralis (DVL) muscle, whereas in soleus there was a 37% increase in GSH content in old rats along with a higher GSH/GSSG ratio. Activity of γ-glutamyl transpeptidase (GGT), the first

enzyme in the γ-glutamyl cycle, was elevated in the aged muscle indicative of a greater potential for muscle GSH transport. Fiebig et al. (1996) showed a significant increase in total glutathione content (GSH + GSSG) in the heart of 27- vs. 5-month-old rats. The elevated myocardial GSH content was associated with a twofold increase in GGT activity.

The mechanism responsible for the increased antioxidant enzyme activities in aging skeletal muscle is still elusive. Mitochondria from aged muscles produce more ROS that may stimulate antioxidant enzyme gene expression. This scenario is consistent with the finding that mitochondrial fractions of antioxidant enzyme activity showed a greater increase in the senescent skeletal muscle (Ji et al. 1990; Luhtala et al. 1994). Age-related muscle inflammation and ROS production through NADPH oxidase and NOS may also play a role (Yu and Chung 2006). The lack of uniformity in mRNA elevation of antioxidant enzymes suggests that age-related adaptation is complicated and subject to both transcriptional and post-transcriptional mechanisms yet to be understood. Recent findings of age-related alteration in cell signaling may shed some light on this issue (see below).

Muscle Contraction: A Powerful Stimulant for Antioxidant Adaptation

Although aged muscles demonstrated higher levels of ROS generation when they were subjected to an acute bout of exercise at a given workload, animals or humans involved in chronic exercise training demonstrate lower levels of oxidative stress and damage at the organ, tissue and cell levels as documented by numerous research (Sen 1995; Meydani and Evans 1993; Powers et al. 1999). For example, "trained" muscles have shown lower levels of lipid peroxidation, protein oxidation, DNA damage and disturbance of redox status both at rest and in response to an acute bout of exercise. Muscle mitochondria isolated from trained rats showed greater resistance to imposed oxidants and improved respiratory function (Chandwaney et al. 1998; Tonkonogi and Sahlin 2002). Hearts of animals involved in endurance training are less susceptible to ischemia-reperfusion insult either in vitro (i.e., isolated perfused model), in situ (e.g., open heart surgery model) or in vivo (Bowles et al. 1992; Ramires and Ji 2001). The benefits of chronic exercise are not limited to skeletal muscle and heart. Liver, brain, erythrocytes and other tissues from animals or humans engaged in routine exercise demonstrate a lesser extent of oxidative damage as compared to their sedentary counterparts (Goto et al. 2007). Interestingly, these changes are associated with increased muscle and whole body oxygen consumption, increased utilization of fat as energy fuel, and increase muscle mitochondrial population, which all seem to favor a higher level of ROS generation.

Numerous studies have shown that antioxidant enzyme activities are elevated in skeletal muscle after endurance training involving repeated bouts of prolonged exercise, and this is probably the most important reason as to why training reduces oxidative stress in young as well as old individuals. Due to the abundance of

reviews on this subject (see Power et al. 1999; Ji 1999, 2007; Reid 2001; Jackson 2005), only a brief summary of the findings is provided below. (a) Among antioxidant enzymes in skeletal muscle, SOD activity has consistently been shown to increase with exercise training in an intensity-dependent manner. MnSOD is primarily responsible for the observed increase in SOD activity, whereas CuZn SOD activity is little affected. GPX activity has also shown an increase after endurance training by most authors. Training effect on CAT activity is inconsistent and controversial. Muscle fiber type is an important factor in determining whether and how much training can influence antioxidant enzyme activity, reflecting both fiber recruitment patterns during exercise and intrinsic antioxidant capacity within a given fiber. Myocardial and diaphragm SOD and GPX have also been shown to increase with treadmill running and swim training in rats. (b) There is considerable evidence that the observed training adaptation of antioxidant activity is due to altered gene expression, with both mRNA and enzyme protein levels being upregulated. For example, resting MnSOD activity and protein content were increased with endurance training in several rat muscles. Even though resting mRNA level for MnSOD was not affected by training status, it was elevated immediately following an acute bout of exercise. Since mRNA generally has a short half-life and muscle tissues in the above studies were harvested 24–48 h post-exercise, it can be concluded that mRNA may increase only transiently following acute exercise bouts. In contrast to SOD, available data indicate that steady state GPX and CAT mRNA levels in trained rat muscles are not different from the sedentary controls, whereas their acute response to exercise is little known.

Training can elicit whole-body adaptations that benefit individual organs and tissues to battle against deleterious ROS. This notion could be supported by two important systems, i.e., GSH and cytokines modulation. GSH is a required antioxidant for all cells, whereas de novo synthesis of GSH occurs only in the liver. Thus, most organs including skeletal muscle and heart import GSH from the circulation via the γ-glutamyl cycle (Meister and Anderson 1983). Exercise training has been shown to induce hepatic γ-glutamylcysteinyl synthetase (GCS), the rate-limiting enzyme for GSH synthesis (Ramires and Ji 2001). GGT activity has also been shown to increase with training in rat heart and himdlimb muscles (Sen et al. 1992). An enhanced hepatic synthesis and output, coupled with a more vigorous transport, result in higher levels of GSH in the organs. Recent research suggests that GSH: GSSG homeostasis is a key factor in muscle inflammation and other pathogenic conditions associated with aging. For example, high levels of GSH prevent inflammatory process partially by inhibiting VCAM-1 expression (Kevil et al. 2004). Interestingly, oxidants (such as menadione) could only transiently decrease GSH levels in the endothelium, as GSH content measured 6–12 h after withdrawal of the oxidant stress increased by twofold accompanied by a twofold increase in GCS activity and 1.3–1.6-fold increase in GCS mRNA expression (Ray et al. 2002). GSH content was also reported to be higher in rabbit tibialis muscle 24 h after an isokinetic stretch injury, accompanied with elevated GPX and GR activities (Best et al. 1999).

Adaptation of body immune system to exercise may have a mixed impact on the oxidative stress level at senescence (Gleeson et al. 2006; Petersen and Pedersen

2006). Training has been shown to attenuate circulatory levels of pro-inflammatory cytokines such as TNFα, IL-1 and 6. Higher levels of habitual physical activity are associated with lower mitogen-stimulated inflammatory cytokine production and lower levels of skeletal muscle inflammatory protein. This suppression is a huge benefit for reducing oxidative stress and cell damage, since aging is often associated with a chronic low level inflammation especially in aged skeletal muscle due to minor injury and/or immobility (Bar-Shai et al. 2005). NFκB is believed to be constitutively activated at old age, which leads to the higher basal expression of pro-inflammatory cytokines, chemokines, adhesion molecules (ICAM-1, VCAM) and ROS-generating enzymes (iNOS and COX-2). In fact, chronic activation of NFκB has been identified as a main etiological reason for aged-related muscle wasting and sarcopenia (Cai et al. 2004). Yu and Chung (2006) demonstrated that 4-hydoxyhenxenal, a lipid peroxidation product often found in aged muscle, could activate NFκB by activating NIK/IKK signaling cascade due to ERK and p38 activation. Since NFκB activation often leads to increased pro-inflammatory cytokine expression, this vicious cycle was hypothesized as the basis for the inflammation theory of aging.

It is noteworthy that the hormetic effect of exercise on inducing antioxidant defense and reducing oxidative damage can be accomplished only at moderate, but not damaging intensity. "Non-damaging" is important but difficult to define in absolute terms because it depends on many factors such as prior training level, gender, age and nutritional status. Generally, it means the oxidative-antioxidant homeostasis is not overwhelmed by ROS resulting in irreversible changes. Past research has well established that strenuous exercise could cause high levels of ROS generation associated with cell damage in the skeletal muscle, heart and liver. Many of the benefits mentioned in previous sections can be achieved by participating in mild to moderate intensity of exercise. Several recent studies in rodents showed that voluntary wheel running could elicit protective effects on mitochondrial biogenesis (Akimoto et al. 2005) and prevent apoptosis (Phillips and Leeuwenburgh 2005). In humans, few studies could document a significant training effect on antioxidant enzymes (Tonkonogi and Sahlin 2002). The variability of prior physical activity levels and the difficulty to obtain homogenous muscle biopsy samples are potential confounding factors.

Molecular Mechanism of Exercise-Induced Hormesis

Hormesis is a pharmacological term meaning low dosage of toxins may increase body's tolerance for greater toxicity (Finkel and Holbrook 2000). Exercise-induced hormetic effects are conferred primarily by an upregulation of antioxidant enzymes through redox signaling (Ji 2007). The key to understanding exercise-induced hormetic response lies on the fact that mammalian cells are endowed with signaling pathways that are sensitive to intracellular redox environment and can be activated by oxidative stress. Those include NFκB, heat-shock transcriptional factor 1 (HSF-1), and P53 pathways, as well as mitogen-activated protein kinase (MAPK) and PI(3)K/

Akt that regulate the first three pathways through phosphorylation. Although all of these pathways are important in regulating normal growth and metabolism, NFκB and MAPK are considered the most critical for the cells to cope with oxidative stress (Allen and Tresini 2000). An acute bout of exercise has been shown to activate MAPK and NFκB signal transduction pathways in both animal and human studies (Ji 2007). The three main MAPK pathways, JNK, ERK1/2 and $p38^{MAPK}$, were activated in rat skeletal muscle after an acute bout of treadmill running (Goodyear 1996). ERK1/2 was reportedly activated after bicycle exercise in human muscle, along with activation of MEK1 and Raf-1 (MEKK), two upstream enzymes controlling ERK, as well as downstream enzyme p90 ribosomal S6 kinase (RSK). The signals triggering the MAPK activation have been attributed to a variety of physiological stimuli associated with exercise including hormones, calcium ion, neural activity, and mechanical force. Biological implications of MAPK activation are widespread including such important functions as glucose transport, muscle and heart hypertrophy, angiogenesis, and vascular adaptation (Hawley and Zierath 2004; Sakamoto and Goodyear 2002).

NFκB is activated by a variety of external stimulants, such as H_2O_2, pro-inflammatory cytokines (TNF-α, IL-1, IL-6), lipopolysaccharide (LPS), phorboester PMA, ionizing irradiation, and viral infection (Baeuerle and Baltimore 1988; Li and Karin 1999). These signals result in the phosphorylation and activation of IκB kinase (IKK) which phosphorylates two critical serine residues in IκB and primes IκB for ubiquitination and proteolytic degradation by the 26S proteasome. IκB dissociation unleashes P50/P65 to dimerize and translocate into the nucleus and bind the κB consensus sequence of the target genes. During and shortly after an acute bout of prolonged exercise, NFκB and AP-1 binding was significantly elevated in rat skeletal muscle in a fiber-specific manner (Hollander et al. 2001; Ji et al. 2004; Ho et al. 2005). NFκB binding was accompanied with increased IKK activity, IκB phosphorylation and degradation, and P50 nuclear translocation. These findings were confirmed in several different muscle fibers such as DVL, soleus and red gastrocnemius. Time course studies reveal that IKK activation and IkB phosphorylation could occur as early as 15–60 min, whereas P50/65 nuclear translocation and maximal NFκB binding was at 2–3 h. Noticeably, application of p38 and ERK inhibitors reduced IKK activation, suggesting MAPK and NFκB might work synergistically during exercise (Ho et al. 2005). The reliance of NFκB activation on MAPK is probably explained by the fact that NFκB activating kinase (NIK) is a member of MEKK family. Li and Engelhardt (2006) reported that IL-1β stimulation of NFκB is partially regulated by H_2O_2 -induced activation of NIK due to the inhibition of NIK phosphatase with the oxidation of a critical cysteine residue. Interestingly, activation of NIK can only be conferred within a narrow range of H_2O_2 concentration of 1–10 μM, close to physiological range within the cell.

Activation of redox-sensitive signaling pathways are overtures to upregulation of gene expression of antioxidant enzymes and other important proteins for the maintenance of oxidant-antioxidant homeostasis, which is deemed essential during aging. Some of these proteins may be double-edged sword, i.e., whereas adequate levels of expression offer extra protections, excessive expression may exacerbate oxidative stress. Several major gene targets of antioxidant signaling are introduced below.

MnSOD MnSOD promoter contains NFκB and AP-1 binding sites, which are sensitive to ROS, TNFα and IL-1 stimulation (Das et al. 1995). During heavy exercise, mitochondrial production of H_2O_2 is increased due to elevated ETC respiration. TNFα and IL-1 levels can also increase especially during strenuous muscle contraction and LC. These external stimuli may activate protein kinase C (PKC) and NIK as the distal key enzymes leading to the activation of NFκB cascades. However, even though NFκB binding at the promoter region constitutes a required condition, it does not guarantee a MnSOD upregulation. Jones et al. (1997) identified a 238-bp region of intron 2 that was responsive to TNFα and IL-1. This TNFα response element (TNFRE) contained both NFκB and 5′-CCAAT enhancer binding protein (C/EBP) motifs, shown to be both necessary and sufficient for TNF responsiveness. Guo et al. (2003) further explored the mechanism of MnSOD gene expression and elucidated two regulatory regions on MnSOD DNA, the proximal promoter region (PPR) and the above-mentioned TNFRE. Furthermore, MnSOD expression was shown to be activated by platelet-derived growth factor (PDGF) due to early growth-responsive-1 (egr-1) protein binding to a putative GC-rich region within the second intron of MnSOD gene, which may be controlled by MEK1 and ERK1/2 signaling (Maehara et al. 2001). Since MEK1 and ERK1/2 are activated in response to exercise, these regulatory sites may provide additional mechanisms for MnSOD transactivation. Indeed, ERK1/ERK2 and p38 ^{MAPK} activation was shown to accompany NFκB activation and a twofold increase in MnSOD mRNA level in rats subjected to an acute bout of progressive treadmill running (Gomez-Cabrera 2005). Figure 2 depicts the intracellular redox signaling pathways that potentially can induce MnSOD (For details see Ji 2007).

GPX GPX is a homotetramer with each 22-kDa subunit bound to a selenium atom existing as a selenocysteine (Holliwell and Gutteridge 1989). Two oxygen response elements (ORE) located at −1232 to −1213 and −282 to −275 in the 5′-flanking region of human GPX gene have been identified (Cowan et al. 1992). The expression of the GPX gene, *hgpx1*, occurs in a wide range of tissues controlled by development, hormones, and oxygen tension. In the myocardium, GPX activity induced by oxygen tension was found to be proportional to the mRNA levels, suggesting a transcriptional mechanism (Cowan et al. 1993). GPX promoter contains both NFκB and AP-1 binding sites. Zhou et al. (2001) showed that H_2O_2 and paraquat-induced GPX mRNA expression in C2C12 cell culture was dependent on functional NFκB signaling. Introduction of IκB mutant abolished GPX mRNA expression. Both cytosolic and mitochondrial fraction of GPX activity has also shown to increase after endurance training in skeletal muscle (Ji 1995). However, steady state GPX mRNA levels in trained rat muscles were found to be similar to those from the sedentary animals (Gore et al. 1998). Given the importance of GPX in muscle cells to remove H_2O_2 and lipid peroxide, more research is needed in elucidating GPX gene regulation in response to exercise.

GCS GSH plays a critical role in muscle antioxidant defense during exercise by providing substrate for GPX, maintaining proper redox status and scavenging $^{\bullet}OH$ and $O_2^{\bullet -}$. Surprisingly, little is known about the gene regulation of GCS in skeletal muscle. In mammalian cells, GCS is a heterodimer consisting of the catalytic

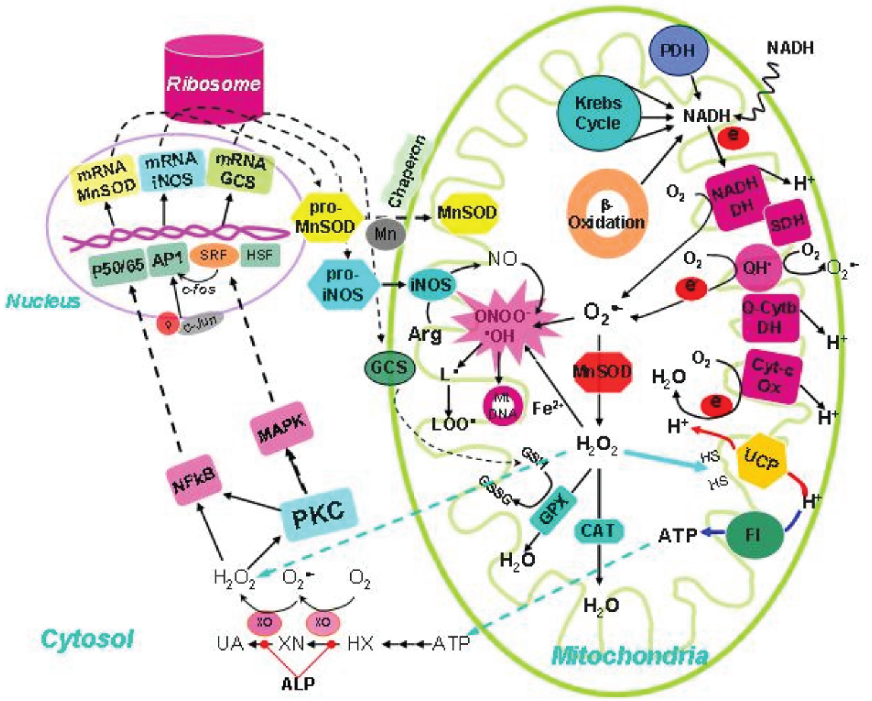

Fig. 2 The role of MnSOD and iNOS in mitochondrial antioxidant defense against oxidative damage and hypothetical signal transduction pathways of MnSOD and iNOS gene expression in the muscle cell. Abbreviations: 26S, 26S proteasome; CAT, catalase; GCS, γ-glumatylcysteine synthetase; GPX, glutathione peroxidase; HX, hypoxanthine; LOO•, lipid peroxyl radical; MEK, MAP/ERK kinase; MKK, MAP kinase kinase; Nox, NAPDH oxidase; PKC, protein kinase C; SDH, succinate dehydrogenase; QH•, ubisemiquinone; SRF, serum response factor; UA, uric acid; UCP, uncoupling protein. Other abbreviations: see text

heavy-chair subunit (GCS-HS) and regulatory light-chair subunit (GCS-LC) (Rahman and McNee 2000). GCS-HS expression is known to be regulated by redox-sensitive mechanism via a variety of oxidants, phenolic antioxidants and pro-inflammatory cytokines (TNFα and IL-1β). Both GCS-HC and GCS-LC promoters contain antioxidant response element (ARE) and NRF-2 binding seems to play a critical role in oxidative stress-induced GCS upregulation. GCS-HC also has NFκB binding sites that are essential for GCS expression in some, but not all cell types (Chan and Kwong 2000; Haddad 2002). If these signaling pathways are also operational in muscle cells, they could be potential mechanism for training-induced upregulation of GCS and GSH biosynthesis.

iNOS NO at low concentration exerts an antioxidant function by removing $O_2^{-\bullet}$ (Reid 2001). Its vasodilative effect increases blood flow to the working muscle thereby improving the availability of blood-borne energy substrates and antioxidants. Thus, an increase in NO production via the regulation of NOS may be

viewed as indirectly enhancing muscle antioxidant defense during exercise. Unlike the other forms of NOS, iNOS is not regulated by calcium ion and instead responsive primarily to ROS and inflammatory cytokines through activation of NFκB and MAPK (Adams et al. 2002). In rat skeletal muscle myoblasts, the IL-1β-mediated iNOS induction was reduced by blocking ERK1/2 activation and completely abolished by the inhibition of NFκB. Moreover, a linear correlation was observed between NFκB activation and iNOS expression in human skeletal muscle (Adam et al. 2003). iNOS mRNA level has been reported to elevate after an acute bout of exercise in rat skeletal muscle (Balon 1999; Gomez-Cabrera et al. 2005). However, while chronic exercise training successfully increased nNOS and eNOS activity and protein expression, it failed to induce iNOS in rat gastrocnemius and diaphragm muscles (Vassilakopoulos et al. 2003). The role of iNOS is largely viewed as being catabolic and it is often co-expressed with pro-inflammatory cytokines and adhesion molecules during muscle injury and wasting (Schulze et al. 2002). High levels of NO production also lead to the formation of peroxynitrite, a highly reactive species contributing to muscle oxidative damage. Therefore, regulation of iNOS expression is a delicate process and requires further investigation.

Increased muscle mitochondrial number and protein may be the ultimate adaptation in reducing muscle oxidative stress, as postulated by Davies et al. (1982). Almost 3 decades ago, Chance et al. (1979) pointed out that cytochrome c oxidase might be considered the most important antioxidant enzyme as it secures electron flow to oxygen in ETC thereby decreasing potential formation of $O_2^{\bullet -}$. These foresights have been highlighted by the recent advances in studying exercise-induced mitochondrial biogenesis. The interactions of ROS, MAPK signaling and peroxisome proliferator-activated receptor-γ coactivator 1 (PGC-1), the master transcription factor of mitogenesis, have recently been shown to play a vital role in mitochondrial biogenesis of rodent muscle (Akimoto et al. 2005). Excitingly, PGC-1 expression has recently been shown to be associated with enhanced antioxidant gene expression and reduced oxidative stress (St-Pierre et al. 2006). It is not unthinkable that increased PGC-1 signaling may be instrumental in protecting skeletal muscle from sarcopenia.

Aging and Hormesis: A LifeTime Race

It has long been suspected that senescent skeletal muscle may compromise its ability to adapt to oxidative stress due to structural and functional impairment (Ji 2001; McArdle et al. 2002). Several relevant questions may be asked. (1) Does aged muscle have decreased level of protein components in the various redox signaling pathways? (2) Does aged muscle exhibit reduced sensitivity to oxidative challenge and diminished redox signaling potential? (3) What is the functional implication of this impairment and the potential strategy to reverse it? (4) Can exercise-induced hormetic effects be substituted by antioxidant supplementation? Due to the limited data available on this subject, only a few highlights will be

mentioned. Readers are referred to several expert reviews for more insights (de Magalhaes and Church 2006; Yu and Chung 2006; Jackson 2005),

Hollander et al. (2000) reported NFκB and activating protein (AP)-1 binding at rest was attenuated in several types of skeletal muscle fibers of senescent rats. This led the authors to speculate that aging may attenuate cell's signal transduction capacity for antioxidant enzymes. This hypothesis was supported by other studies regarding NFκB activity in aged muscles (Radak et al. 2004). Broome et al. (2006) showed that NFκB binding was lower in senescent mouse muscle in response to stimulated contraction. Parkington et al. (2004) measured ERK1/2 and $p70^{S6K}$ activities in the plantaris and tibialis anterior muscles of young and old rats in response to electric stimulation and concluded that signaling of anabolic response to contractile stimulus is attenuated with aging, which may contribute to reduced exercise-induced muscle hypertrophy. In contrast, Hornberger et al. (2005) found no difference in p38, $p70^{S6K}$ and JNK2 activities in EDL muscle between young and old rats. Williamson et al. (2003) even reported higher resting activities of ERK ½, $p90^{RSK}$, $p38^{MAPK}$ and JNK/SAPK in the leg muscle of old compared to young men. However, aged muscles had decreased MAPK enzyme activities after an acute bout of resistance contraction, whereas young ones increased these enzyme activities. Total amount of protein expression in the MAPK pathway was found unaltered with age. The above discrepancies derived from different species and muscle types are not surprising as muscle antioxidant signaling is highly fiber specific due to differential intrinsic rate of ROS generation. Varied antioxidant defense capacity among different muscle types may also alter the sensitivity of cells to ROS. It is also possible that senescent muscles are often subjected to chronic inflammation and higher levels of pro-inflammatory cytokines may be a confounding factor. Thus, it is still premature to draw a conclusion as to how aging affects signaling and hormetic response to oxidative stress in skeletal muscle.

In conclusion, it can be said that ROS generated during muscle contraction either from mitochondrial respiratory chain or other oxidases play a critical role in muscle adaptation to exercise-induced oxidative stress by activating redox-sensitive signal transduction of antioxidant enzymes and other proteins vital to cell survival and functionality. NFκB and MAPK are two major signaling pathways that can be activated in response to ROS stimulation. These hormetic effects could be an important mechanism to protect senescent skeletal muscle which is subjected to increased intrinsic ROS generation and oxidative stress. However, there is a delicate balance between oxidative stress and muscle adaptability hinged on redox signaling in senescence. The word "mild" oxidative stress may be the key to achieving this balance.

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