[Frontiers in Bioscience 12, 4826-4838, September 1, 2007]

Response and adaptation of skeletal muscle to exercise – the role of reactive oxygen species

Andreas Michael Niess, Perikles Simon

Medical Clinic, Department of Sports Medicine, University of Tuebingen, Germany

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1. ABSTRACT

In the last 30 years, the role of reactive oxygen species (ROS) in exercise physiology has received considerable attention. Acute physical exertion has been shown to induce an augmented generation of ROS in skeletal muscle via different mechanisms. There is evidence that ROS formation in response to vigorous physical exertion can result in oxidative stress. More recent research has revealed the important role of ROS as signaling molecules. ROS modulate contractile function in unfatigued and fatigued skeletal muscle. Furthermore, involvement of ROS in the modulation of gene expression via redox-sensitive transcription pathways represents an important regulatory mechanism, which has been suggested to be involved in the process of training adaptation. In this context, the adaptation of endogenous antioxidant systems in response to regular training reflects a potential mechanism responsible for augmented tolerance of skeletal muscle to exercise-induced stress. The present review outlines current knowledge and more recent findings in this area by focussing on major sources of ROS production, oxidative stress, tissue damage, contractile force, and redox-regulated gene expression in exercising skeletal muscle.

2. INTRODUCTION

Research over the last 30 years has revealed a complex link between exercise and reactive oxygen species (ROS). Initial research in this area has focussed on the potential of exercise-related ROS formation to induce oxidative stress. However, a number of more recent findings have emerged, which show that ROS also participate in the modulation of a variety of cellular functions including gene expression via redox-sensitive transcription pathways. In this regard, skeletal muscle is of special interest, as it is simultaneously a generation site and a target of ROS in the context of exercise. Function and adaptation of skeletal muscle seems to be closely affected and controlled by stress induced b by exercise. Adaptation of skeletal muscle to repeated exercise not only augments tolerance to exercise, but may also limit exercise-induced oxidative stress by enhancing protective systems. The present review will outline current knowledgement and more recent findings in this area by focussing on major sites of exercise-induced ROS production, oxidative stress, tissue damage, contractile force and redox-regulated gene expression in skeletal muscle.

3. EVIDENCE AND SITES OF ROS GENERATION IN EXERCISING SKELETAL MUSCLE

By definition, free radicals are atoms or molecules that have one or more unpaired electrons in their orbitals and present very pronounced chemical reactivity as a result. Further oxidating derivates without unpaired electrons are classified as so-called non-radicals (1, 2). Chemical reactivity and resulting toxicity to cellular targets vary between the different types of ROS. Superoxide is the most thoroughly investigated among the biologically relevant oxygen-centered reactive species. It can be converted to hydrogen peroxide by a reaction catalyzed by superoxide dismutase (SOD). In turn, further conversion of hydrogen peroxide to the by far most reactive ROS, the hydroxyl radical, can occur via an iron-dependent reaction. A non-oxygen centered free radical is nitric oxide. Nitric oxide can oppose effects of ROS at low levels. However, interaction of nitric oxide with superoxide generates peroxynitrite, a highly toxic radical that promotes nitrosative injury.

Acute exercise has been shown to induce a complex stress response, which involves reactions on the cardiocirculatory, metabolic, hormonal, and immunological levels. There is further evidence that physical exercise leads to the enhanced formation of ROS. While indirect methods such as detection of lipid peroxidation products initially suggested an exercise-induced formation of free radicals (3-6), direct evidence of increased ROS formation using electron paramagnetic resonance spectroscopy was found in rat and cat muscle (7-9) and by Ashton *et al.* (10) in human serum after exhausting aerobic exercise. More recently, Bailey *et al.* (11) documented direct analytic evidence for ROS outflow across the skeletal muscle bed during isolated quadriceps muscle exercise at different intensities by assessing electron paramagnetic resonance spectra in arterial and venous femoral blood. The augmenting effect of exercise on ROS production is further supported by changes in the thiol/disulfide redox state, as shown by decreases in skeletal muscle as well as serum reduced/oxidized glutathione (GSH/GSSG) ratios in response to strenuous exercise (12). Skeletal muscle contains different mechanisms potentially responsible for an augmented formation of ROS in response to exercise. These include mitochondrial respiration, plasma membrane nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidase, and iron-related reactions as described in the following.

Many publications in the area of oxidative stress have traditionally considered mitochondrial respiration as the most important source of ROS formation during exercise (1, 2, 13). Inadequate coupling of electron transfer, in particular at complexes I, II, and III, has been supposed to cause leakage of electrons to oxygen resulting in the formation of superoxide radicals. Since exercise increases the muscle metabolic rate up to 100 times higher than during resting conditions, a marked increase of oxygen flux through mitochondria has been considered as the major mechanism of exercise-induced formation of superoxide (13). Earlier estimations concerning the extent to which total electron flux in the mitochondria shows this leakage and leads to the formation of superoxide ranged between 1 and 5% (2). However, more recently, it has been suggested that a drop in mitochondrial oxygen partial pressure rather than an augmented oxygen flux, causes enhanced formation of superoxide in muscle during exercise. Furthermore, a more recent study gives rising evidence that mitochondrial respiration has been overestimated as a major site of ROS formation during exercise (14), and the rate of ROS formation had to be corrected to an upper limit of only 0.15% of the electron flux (15). Moreover, Kozlov *et al.* (16) report that the generation of ROS by isolated skeletal muscle mitochondria from rats is restricted to old animals. An intrinsic control mechanism that gets impaired by age seems to be responsible for this limited ROS formation in mitochondria. There is strong evidence from animal studies that uncoupling proteins (UCPs), such as UCP3, attenuate and regulate mitochondrial ROS production via a control of uncoupling in mitochodria (17). Inhibition of UCPs by guanosine diphosphate (GDP) augments mitochondrial ROS formation (18), and UCP3 knock out mice show enhanced ROS formation (19). In turn, sources other than mitochondria gain attention as important sites for augmented ROS formation in exercising skeletal muscle.

Superoxide formation via NADPH oxidase isoforms is most prominent in phagocytotic cells but has been implicated to play a role in a variety of other cell types, including cardiac and skeletal myocytes (20-22). In these cell species the extent of ROS formation by NADPH oxidase plays an important role in the modulation of redox-sensitive signaling pathways. Experiments on rats have shown that a non-mitochondrial NADPH oxidase enzyme complex is present in skeletal muscle fibers and is localized to the region of the plasma membrane (22). The relevance of plasma membrane NADPH oxidases for contraction-induced ROS generation is not completely confirmed.But there is initial evidence that this mechanism contribute to superoxide generation in working muscle as inhibition of NADPH oxidase leads to a suppressed formation of superoxide in stimulated myotubes (23).

Xanthine oxidase is not present in myocytes, but is predominantly localized in the associated vascular endo-thelium. It catalyzes the conversion of hypoxanthine to xanthine, and from xanthine to uric acid. Xanthine oxidase uses molecular oxygen and forms superoxide radicals during catalytic action (24). This ROS-generating process has been examined during metabolic stress, and occurs during cellular hypoxia–reoxygenation injury (25). Xanthine oxidase does not seem to play a role in superoxide formation at rest. However, the so-called adenosine triphosphate (ATP) breakdown occurring during high-intensity exercise leads to an augmented formation of adenosine monophosphate (AMP) via adenylate kinase, which is further degraded to inosine monophosphate (IMP), inosine, and hypoxanthine. Increased levels of hypoxanthine and uric acid (26) indirectly indicate that xanthine oxidase is active in response to exercise, which has been confirmed recently by direct measurements of xanthine oxidase activity in the plasma of rats (27). In addition, treatment with allopurinol, an xanthine inhibitor,

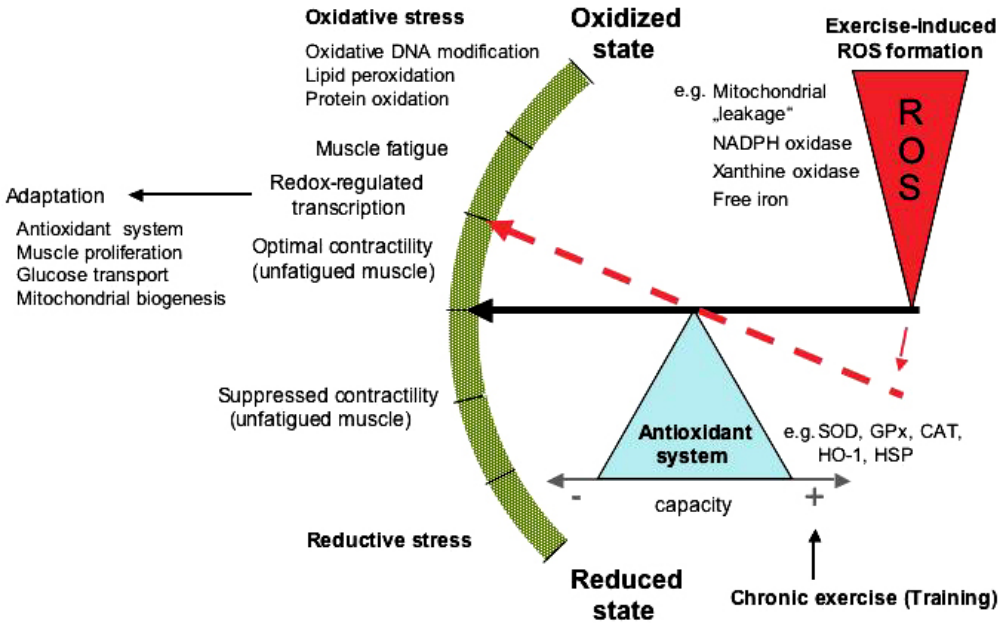

Figure 1. Schematic model depicting the potential determinants and the consequences of exercise-related changes in the cellular redox balance in skeletal muscle. Increasing levels of ROS, generated in working muscle, lead to a change in the redox-balance towards a more pro-oxidant state. The extent of redox changes resulting from a given amount of generated ROS is mainly determined by the capacity of the antioxidant system. While moderate changes in the cellular redox state as reflected by the red arrow exert primarily more regulating properties, excessive ROS generation without adequate compensation result in damaging oxidative stress (For details see text). ROS: reactive oxygen species; CAT: Catalase; GPx: glutathione peroxidase; NADPH: nicotinamide adenine dinucleotide phosphate (reduced form); HO-1: heme oxygenase-1; HSP: heat shock proteins; SOD: superoxide dismutase.

prevents oxidation of GSH in rats running to exhaustion in a progressive exercise test. This provides evidence that xanthine contributes to ROS formation in response to exercise (28).

Free iron has been shown to catalyze the formation of ROS. A well-known mechanism of iron-catalyzed ROS generation is the Fenton reaction, which converts hydrogen peroxide to the much more reactive hydroxyl radical (29). An augmented release of iron-containing heme proteins, such as hemoglobin or myoglobin, as a result of exercise-induced damage to muscle tissue has been proposed as one mechanism by which exercise enhances ROS via free iron (2). Jenkins *et al.* (30) found an increased concentration of loosely bound iron in the muscle tissue of rats exercised to exhaustion, and simultaneously, evidence of increased lipid peroxidation. However, only little data is available at present, which confirm a relevant contribution of hem-related mechanisms to ROS formation in context with exercise (31).

4. EXERCISE-INDUCED OXIDATIVE STRESS

Maintenance of cellular redox homeostasis requires a balance between the generation rate of ROS and the capacity of the antioxidant system. The current paradigm proposes that cellular redox homeostasis is mainly regulated by redox-sensitive signaling mechanisms, which respond to an augmented formation of ROS by inducing scavenger systems. However, if the generation of ROS is exaggerated or rapid, the system may not react sufficiently. Indeed, several observations confirm that enhanced formation of ROS in response to exercise can lead to oxidative modifications of lipids, proteins, nucleic acid, and other cellular compounds (13, 32-34). The functional consequences of exercise-induced oxidative stress are only partly understood. Importantly, features of oxidative stress in response to exercise are usually transient. Furthermore, exercise-induced oxidative damage does not exhibit a magnitude comparable to that observed for several pathophysiological conditions. On the other hand, alterations in redox homeostasis in response to exercise may have functional consequences beyond oxidative stress as described more detailed in the latter sections (Figure 1).

4.1. Lipid peroxidation

The initial step of non-enzymatic lipid peroxidation is reflected by ROS abstracting hydrogen from a polyunsaturated fatty acid side chain. In turn, resulting lipid radicals and oxygen are necessary for the propagation steps in this chain reaction. Additional availability of metal ions decomposes lipid peroxides to peroxyl and alkoxyl radicals, which in turn abstract hydrogen and start new peroxidation cycles (2). Accumulating lipid peroxides exert destabilizing effects on cell membranes. This scenario gives rise to disturbances in cell integrity and results in further ROS-mediated reactions. Peroxyl radicals

are capable of removing hydrogen not only from polyunsaturated fatty acids, but also from nucleic acids and amino acids, explaining the occurrence of oxidative modifications of DNA and membrane proteins during the process of lipid peroxidation.

Dillard *et al.* (4) were the first to detect an increase in expired pentane after 20-min cycling ergometry at moderate intensity. In numerous further studies, exercise-induced lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances, malondi-aldehyde, lipid hydroperoxides, and F2-isoprostane in blood, urine, expired air and various tissues, including skeletal muscle (3, 7, 35-37). Although the majority of the studies could show that exercise exerts an inducing effect on lipid peroxidation in animal models, as well as in humans, the results vary considerably (31). This is likely to be due to the variability of exercise protocols und methods used for assessing oxidative stress in the different studies.

4.2. Protein oxidation

A major mechanism of oxidative protein modification is represented by initial $\cdot OH^-$-induced abstraction of hydrogen from amino acid residues forming carbon-centered free radicals. Further reactions lead to the formation of alkyl, peroxyl, and alkoxyl radicals, which in turn may also abstract hydrogen from amino acid residues (38). The most frequently studied markers of oxidative protein damage are reactive carbonyl derivates. The modification of proteins can impair their physiological function and accelerate proteolytic degradation, which can be demonstrated by using glutamine synthetase as an example (39). The inhibition of enzymes like mitochondrial ATP synthetase is elicited via the oxidation of sulfhydryl groups by ROS (40). Accumulation of oxidized proteins occurs progressively during the aging process and correlates with the severity of a number of diseases (38).

Initial results from Reznik *et al.* (41) demonstrated that rats subjected to exhaustive exercise accumulated reactive carbonyl derivates in skeletal muscle, indicating an increased rate of oxidative damage to proteins. Similar results were observed in the hind legs of rats following a three-month period of endurance exercise, which consisted of treadmill running lasting two hours, 3 days a week (42). Interestingly, immobilization similarly results in elevated protein oxidation (43). At the moment, the picture of the functional significance of exercise-induced protein oxidation in skeletal muscle is still incomplete. Nevertheless, there is initial evidence that protein oxidation may have the potential to exert suppressive effects on exercise performance by affecting contractile elements (see below) or by inhibiting enzymes, such as glycerinaldehyde phosphate-dehydrogenase or mitochondrial ATP synthetase (40).

4.3. Oxidative DNA modifications

DNA has been assumed to be the most biologically significant target of oxidative damage. Oxidative damage to nuclear DNA is considered a potential pathophysiological factor in the development of cancer. Oxidative modifications of mitochondrial DNA lead to an accumulating rate of mutations, which in turn result in deficient mitochondrial respiratory function and disturbances in cellular energy supply (44). For rapid removal of DNA lesions, cells are employed by a complex system of DNA repair enzymes (45). There is growing evidence that exercise is also capable of inducing oxidative modifications of DNA. Most indices of exercise-induced oxidative DNA damage were revealed by studies in peripheral leukocytes (33, 46). In addition, detection of elevated excretion rates of 8-hydroxy-deoxy-guanosine (8-OhdG) in urine after vigorous or cumulative endurance exercise confirm the occurrence of oxidative DNA damage due to physical exertion (47, 48). However, analyzing urinary excretion of 8-OHdG does not allow any conclusions regarding the cells, where DNA damage occurs. Nevertheless and as reflected by augmented levels of 8-OHdG in muscle 24 h after eccentric muscle exercise (49), exercise-related DNA damage does not seem to be restricted to immunocompetent cells. The functional significance of exercise-induced DNA damage is not completely understood and needs to be further clarified. From a more hypothetical point of view, it can be speculated that accumulating damaging effects on mitochondrial DNA in muscle cells due to repeated unaccustomed exercise may affect mitochondrial function and therefore result in disturbances in cellular energy supply.

5. EXERCISE-INDUCED MUSCLE DAMAGE AND ROS: THE UNRESOLVED LINK

Damage to skeletal muscles occurs in response to unaccustomed intense, prolonged, and in particular eccentric exercise (50). The precise mechanisms leading to contraction-induced muscle injury are not completely understood (51). Current knowledge proposes that the process of muscle damage is initiated when myofibrils are stretched while contracting. During the process of repeated contractions, a rising number of sarcomeres are over-stretched and become disrupted. This kind of structural distortion goes along with enhanced damage to membrane structures of the myocyte and includes damage to membranes of the sarcoplasmic reticulum (SR) and transverse tubules (51). Damage to membrane structures in this area is the basis for an uncontrolled influx of calcium into the sacroplasm. In turn, enhanced resting intracellular calcium affects functional aspects, such as passive and active muscle tension, but also triggers further damaging mechanisms, such as proteolysis and the breakdown of muscle fibers. During this process, acute inflammation occurs and damaged areas are invaded by neutrophils and macrophages (52). In addition, this process includes the release of inflammatory mediators and markers, such as histamine, serotonin, prostaglandin, substance P, and pro-inflammatoy cytokines (51, 53). The sensitization of noci-receptors by some of these substances and an occurring edema are the basis for localized sensations of pain and discomfort, a symptomatology which is termed delayed onset of muscle soreness.

There is indirect evidence that ROS are involved in the process of exercise-induced muscle damage (54-57).

In the animal model and more recently in humans, eccentric exercise has been shown to be followed by an increased free radical signal in muscle measured using electron paramagnetic resonance spectroscopy (8, 57). Secondary indices of increased oxidative stress, such as elevated parameters of lipid peroxidation and protein oxidation, could also be detected in muscle tissue after heavy exercise. In humans, a delayed increase of lipid peroxidation products in plasma after eccentric running exercise (58) was postulated as an argument for the involvement of ROS in the process of muscle damage. In addition, a relationship was seen between muscle damage and the extent of immigrating neutrophils, which can release ROS in excess during the process of inflammation (59). Enhanced expression of inducible nitric oxide synthase as shown after stretch injury in rabbit muscle may contribute to the additional formation of nitric oxide (60). However, a causal linkage between ROS and the onset of contraction-induced muscle injury can only be drawn through the inhibition of ROS during the process of muscle damage. Using muscle glutathione status as a marker of oxidative stress in a mouse model, Duarte *et al.* (61) showed that by using colchicine to inhibit the the function of neutrophils invading the muscle, the extent of oxidative stress and damage to muscle can be reduced. While some, but not all, studies show a lower extent of myocellular enzyme outflow or a slightly attenuated degree of delayed onset of muscle soreness through antioxidant supplementation (56, 62), no treatment effect was observed with respect to ultrastructural damage after eccentric damage (63, 64). Similarly, neither torque deficit, Z-band disruption, nor macrophage cell invasion into muscle after an ultramarathon run (65) or eccentric exercise protocol (66) were affected by alpha-tocopherol supplementation. It was recently shown that ROS formation due to eccentric exercise occurs after the maximal peak of muscle soreness, when muscle function is already returning to normal (57). Thus, it is not unequivocally established whether exercise-induced oxidative stress plays a significant causal role or is merely a by-product of contraction-induced muscle injury.

6. CELLULAR REDOX STATE AND CONTRACTILE FORCE OF SKELETAL MUSCLE

Continuously produced ROS and nitric oxide play a critical role in the modulation of contractile force in skeletal muscle (67). Under unfatigued or basal conditions, ROS and nitric oxide exhibit different effects on contractile force. Contractility is enhanced by a moderate shift of the redox homeostasis toward a more pronounced pro-oxidant state. In this situation, the presence of muscle-derived ROS is necessary for optimal contractility. Conversly, treatment of an unfatigued muscle with antioxidants can lead to a suppression of force production (68). However, if the presence of ROS is more extensively and exceeds a threshold range, ROS have the opposite effect. Several studies demonstrate a decrease in maximal force production and an increase in fatigue in skeletal muscle exposed to higher amounts of oxidating molecules (69), e.g. during chronic inflammation or inactivity. Similarly, enhanced formation of ROS during strenuous exercise can negatively affect contractility and contribute to the development of fatigue (70). Under such conditions, antioxidants have been shown to exert a preserving effect on force production during repeated muscle contractions in several experimental settings (69-72). In endurance-trained cyclists, high dosaged N-acetylcysteine (NAC) administered intravenously via an initial loading dose followed by constant infusion rate during exercise was sufficient to delay time to fatigue during submaximal cycling ergometry (73). In contrast, near-maximal exercise does not seem to be affected by NAC (74). Taking these dose- and situation-related effects into account, Reid and co-workers proposed a model, which depicts a biphasic relation between the cellular redox state and isometric force (67).

The mechanisms by which ROS affect contractile force in skeletal muscle are still only partly understood. Suppressive effects of ROS result, in part, from modifications of redox-sensitive proteins, particularly myofilaments and components of the SR. Administering oxidants have been shown to negatively affect regulatory proteins of the SR, enhance the probability of calcium channel opening, and inhibit the activity of the SR calcium-dependent ATPase (67, 69). These effects of ROS on SR seem to suppress contractile force via a loss of calcium homeostasis. Furthermore, direct oxidative modification of myofilaments (75) can contribute to a loss in function as shown in isolated skeletal muscle fibers of mice (76). More recent research yields evidence that ROS additionally accelerate fatigue by exerting a suppressive effect on myofilament sensitivity to calcium (77, 78).

In contrast to oxygen centered free radicals, nitric oxide has been shown to diminish force production in both fatigued and unfatigued muscle (67, 69, 79). This effect is quite similar to the influence of nitric oxide on vascular smooth muscle. Inversely, treatment with nitric oxide synthase inhibitors, such L-arginine methyl ester (L-NAME), leads to an increase of force production in muscle during exercise, while the administration of nitric oxide donors has the opposite effect (80). Although great progress has been made in this area, further and more detailed understanding of the mechanisms by which ROS modulate contractile forces of skeletal muscle is necessary.

7. REDOX-SENSITIVE TARGETS IN SIGNALING CASCADES

By activating redox-sensitive transcription factors and cellular signaling cascades, ROS can take on the role of important intracellular messengers. Typical cellular components sensitive to redox changes are nuclear factor-kappaB (NF-kappaB), activator protein-1 (AP-1), mitogene activated proteine kinases (MAPKs), heat shock transcriptional factor-1 (HSF-1), and insulin receptor kinase by inhibiting protein tyrosine phosphatases (81-84). Examples of genes in which NF-kappaB acts as an inducible transcriptional activator are cell adhesion molecules, inducible nitric oxide synthase, acute phase proteins, cytokines, and hematopoietic growth

Table 1. Effects of antioxidant treatment on exercise-induced changes in MAPK phosphorylation, NF-kappaB binding, expression of HSP60 and HSP70 protein, and glucose uptake in skeletal muscle

	Exercise protocol	Antioxidant treatment	Effects	Reference
M. gastrognemius (rat)	Exhaustive incremental exercise (55 min)	Allopurinol 32 mg/kg i.p.	Exercise-induced increase of NFκB binding ↓ and MAPK phosphorylation ↓	28
M. extensor digitorum longus (rat)	Concentric exercise	Dithiotreitol or N-Acetylcysteine (NAC)	Exercise-induced increase of MAPK hosphorylation ↓	97
M. vastus lateralis (rat)	Exhaustve treadmill exercise (60 min)	Pyrrolidine dithiocaramate (PDTC) 100 mg/kg i.p.	Exercise-induced increase of NFκB binding ↓	89
M. vastus lateralis (humans)	Constant cycling ergometry (45 min) at 80% VO_{2max}	Alpha-tocopherol 400 mg/d or Beta-Carotine 15 mg/d or Ascorbic acid 500 mg/d	Exercise-induced increase of HSP60 and HSP70 protein ↓	104, 138
M. vastus lateralis (humans)	Knee extensor exercise (3 h)	Alpha-tocopherol 290 IU/d + Gamma-tocopherol 130 IU/d + Ascorbic acid 500 mg/d	Exercise-induced increase of HSP72 mRNA and protein ↓	139
Isolated M. extensor digitorum longus (mouse)	Tetanic contractions (10 min) induced by in-vitro stimulation (50 Hz)	N-Acetylcysteine (NAC)	Contraction-induced increase of glucose uptake ↓	102

factors (81). In this context, sustained activation of NF-kappaB by oxidative stress has been suggested to play a central role in inflammatory processes and sarcopenia (79). On the other hand, changes in gene expression through regulatory transcription factors are crucial components of the machinery that determines cellular protective responses to oxidative perturbations. Several proteins of the antioxidant network contain NF-kappaB and AP-1 binding sites in their gene promoter (85). Superoxide dismutase (SOD) and glumatylcysteine synthetase (GCS) are potential targets for NF-kappaB-signaled gene expression. The gene of the antioxidant stress protein heme oxygenase-1 (HO-1) contains binding sites for several redox-sensitive transcription factors, and responds to the activation of NF-kappaB, AP-1, MAPKs, and hypoxia-inducible factor-1 (86).

Growing evidence exists that acute exercise can activate redox-sensitive intracellular signaling cascades in skeletal muscle. Hollander *et al.* (87) investigated the effect of exhaustive treadmill running lasting 1 h on NF-kappaB and AP-1 binding activity in rat vastus lateralis muscle. NF-kappaB binding activity increased 2 h post exercise and remained elevated up to 48 h after exercise. AP-1 binding was also increased, but reached baseline levels within a few hours. MnSOD mRNA increased in parallel, which may point to an involvement of NF-kappaB and AP-1 in the modulation of the antioxidant defense in response to exercise. In another study, NF-kappaB binding activity increased by 50% in rat muscle up to 3 h after 1 h of treadmill exercise, returning to baseline by 5 h (88). Ji *et al.* (89) confirmed these findings by detecting contraction-induced activation of the NF-kappaB signaling cascade in muscle nuclear extracts of rats. Furthermore, treatment with pyrrolidine dithiocarbamate, an antioxidant inhibitor of NF-kappaB, partially suppresses activation of this signaling pathway (Table 1). In a similar manner, suppression of exercise-induced ROS generation via xanthine oxidase by allopurinol was sufficient to attenuate NF-kappaB binding in rat gastrognemius muscle. This effect was paralleled by a complete block of exercise-induced up-regulation of Mn-SOD mRNA expression (28). Thus, it appears that a useful and important up-regulation of antioxidant mechanisms in response to exercise requires a certain degree of a pro-oxidant milieu.

Based on structural differences, the MAPK family of proteins is represented by four parallel cascades: stress-activated protein kinase p38 ($MAPK^{p38}$), c-jun N-terminal kinases ($MAPK^{jnk}$), extra-cellular signal-regulated kinase 1 and 2 ($MAPK^{erk1/2}$), and the extra-cellular signal-regulated kinase 5 ($MAPK^{erk5}$) (83). MAPK activation can be triggered by hormonal changes, lowered pH, or mechanical and oxidative stress (90). Hydrogen peroxide has been shown to be a potent activator of MAPKs in myoblasts (91) and augments glucose transport in isolated skeletal muscle (92). Studies on human trained and untrained skeletal muscle revealed that acute exercise can activate MAPKs, such as $MAPK^{erk1/2}$ (93-96). As found in isolated rat skeletal muscle, concentric exercise induces phosphorylation of $MAPK^{erk1/2}$ but not $MAPK^{p38}$, whereas eccentric contractions or highly intensive exercise activate both kinases (97). Growing evidence exist that activation of MAPKs play a putative role in the metabolic and adaptive response to exercise (90). With respect to training adaptation, it is important to note that MAPKs are involved in the mitogenic and metabolic plasticity of skeletal muscle. Moreover, increased uptake and oxidation of fatty acids in rodent skeletal muscle during low-to-moderate exercise seem to be mediated in parts by $MAPK^{erk1/2}$ (98). Recent research implicate $MAPK^{erk1/2}$ phosphorylation in regulation of important key components of lipid metabolism, including hormone sensitive lipase and acetyl-CoA carboxylase (99, 100). In contrast, insulin-independent glucose-uptake during exercise does not seem to be mediated in large parts by MAPKs (90).

Exercise has been shown to stimulate expression of peroxisome proliferators-activated receptor γ co-activator 1alpha (PGC 1alpha) concomitant with phosphorylation of $MAPK^{p38}$ (101). PGC 1alpha is known to have potential functional roles in exercise-induced fiber-type transformation and mitochondrial biogenesis. There is some evidence that MAPKs are involved in the process of muscle adaptation as inhibition of $MAPK^{p38}$ exerts a blocking effect on the promoter activity of PGC 1alpha. Antioxidant treatment reduced contraction-induced phosphorylation of $MAPK^{erk1/2}$ from a fivefold to only a 1.5-fold increase in rat muscle (97). The potent antioxidant NAC has been shown to attenuate contraction-induced glucose transport in isolated mouse muscle (102). It is discussed that NAC suppress glucose uptake via a partial

inhibition of the contraction-induced activation of AMP-activated protein kinase, which has been recently shown to be sensitive to ROS (103).

Similarly to NF-kappaB, MAPKs represent a potential redox-sensitive pathway involved in the process of training adaptation (90). Clearly, future research is necessary to deliniate the specific role of redox-regulated pathways in exercise-induced adaptations. From a more hypothetical point of view, it cannot be excluded that inhibition of redox-regulated pathways by large doses of antioxidants is at risk to prevent adaptational effects of regular training (28, 89, 104-106).

8. THE ANTIOXIDANT SYSTEM

The effects of ROS are counteracted y a complex network of antioxidant systems and endogenous, as well as alimentary, antioxidants (107). Beyond their considerable role in ameliorating the harmful effects of ROS, antioxidant defense systems also modulate redox-sensitive signaling processes. Enzymatic antioxidant systems include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and thioredoxin. Additionally, heat shock proteins (HSP) and heme oxygenase-1 (HO-1) yield cellular protection against oxidative damage (2, 108).

Superoxide dismutase (SOD) is a metalloprotein that catalyzes the reaction of superoxide to hydrogen peroxide. The three existing isoenzymes of mammalian SOD can be characterized by their metal ions and cellular locations. Cu- and Zn-SOD are cytosolic enzymes, whereas Mn-SOD is found in mitochondria (109). The third isoenzyme EC-SOD is represented by a Zn- and Cu-containing form and predominatly localized in the extracellular space. It has been estimated that up to 80% of the superoxide formed in the mitochondria are reduced by SOD. Catalase (CAT) is mainly present in the peroxisomes of most mammalian cells, but mitochondria and other intracellular organelles, such as endoplasmic reticulum, also contain this enzyme. CAT catalyzes the decomposition of hydrogen peroxide to water and oxygen. Similar to the situation with SOD, the activity of CAT is highest in the liver and rather low in skeletal muscle (110). The selenoprotein GPx is located in both the cytosol and mitochondrial matrix in close vicinity to cellular sources of hydrogen peroxide formation. GPx catalyzes the reduction of hydrogen peroxide and a wide range of complex organic hydroperoxides to water and alcohol, respectively, using reduced glutathione (GSH) as the electron donor. In turn, oxidized glutathione (GSSG) can be quickly recycled to GSH by GSSG reductase in the presence of NADPH. As a result, in most tissues, levels of GSSG are very low and the ratio of GSH to GSSG is normally kept much higher (107, 111). GSH fulfills various biological functions, which include detoxifying electrophilic xenobiotics, storing and transporting cysteine, modulating redox-sensitive signaling processes, reducing ascorbate or tocopheroxyl radicals, and other antioxidant functions (112).

Representatives of heat shock proteins (HSP) are HSP27, HSP60 and the family of HSP70 , whose expression is induced by heat shock and several other stressors, including oxidative stress (113, 114). The family of HSP70 is represented by four members, the constitively expressed isoform heat shock cognate (HSC70), the inducible isoform HSP70 (HSP72) and two glucose-regulated isoforms GRP75 and GRP78. An augmented pro-oxidant state results in HSF-1 activation, HSP gene expression, and HSP synthesis (115). Upregulation of HSP72has been shown to provide protection from ischemia–reperfusion-induced lipid peroxidation in rat myocardium (116). Furthermore, over expression of mouse HSP25 and human HSP27 exerts augmenting effects on cellular levels of GSH by increasing the activity of glutathione reductase and glucose-6-phosphatase dehydrogenase (117). One specific antioxidant stress protein is heme oxygenase, which exists in two isoforms: the inducible HO-1 (32 kDa) and the constitutive HO-2 (36 kDa) (86). HO-1 protects the cell against oxidative stress by reducing the intracellular pool of free iron via induction of ferritin synthesis. In addition, HO-1 catalyzes the initial step in the degradation of heme to bilirubin, which is known to be a potent water-soluble antioxidant. HO-1 is strongly induced by ROS, hypoxia, ultra violet irradiation, and various other inducers of oxidative stress. Regulation of HO-1 expression at the RNA level involves transcription factors such as NF-kappaB, AP-1, MAPKs, and HIF-1 (86).

9. EXERCISE AND THE ANTIOXIDANT SYSTEM

Acute strenuous exercise has been shown to increase SOD activity in various rodent tissues including skeletal and heart muscle (85, 110). The little available data from studies in humans show variable results, with no acute effects in runners' muscles after a marathon (118) but an elevated SOD protein content in M. vastus lateralis of untrained subjects after 45 min of cycling ergometry (119). It appears that substantial upregulation of the SOD system in trained subjects requires cumulative stimulation, as it typically occurs during chronic exercise training. Indeed, regular endurance training has been shown to induce activity and increase protein levels of SOD in rodent skeletal muscle (107, 109). This training effect seems to be restricted to oxidative muscle fibers and is not associated with increased steady state mRNA levels (120). Thus, exercise-induced activation of SOD gene expression may be a transient process limited to the post-exercise period (87). In humans, SOD activity has been shown to be enhanced in skeletal muscle and red blood cells in response to a sufficient training stimulus (109, 118, 119, 121).

While acute exercise temporarily decreases the GSH/GSSG ratio in terms of a shift toward an augmented pro-oxidant state, high-intensity and long-duration training seems to be capable of increasing muscle GSH content in the animal model (107). In contrast, eight weeks of moderate endurance training consisting of 35-min cycling, three times a week, failed to affect the GSH/GSSG ratio in human vastus lateralis muscle (122). Data regarding the stimulating impact of regular training on GPx activity in skeletal muscle are reasonably consistent (110).

Furthermore, it was shown that upregulation of GPx activity is related to training volume. However, it appears that increased GPx activity due to regular exercise is limited to oxidative skeletal muscles and that the response of mitochondrial GPx is more pronounced than that of its cytosolic fraction (107, 111). More recent research shows that exercise training may be sufficient to double the expression of SOD and GPx genes in skeletal muscle of patients with chronic heart failure (123). Compared to SOD and GPx, activity of CAT does not seem to respond to regular training and even exhibited a down-regulation in some studies (111).

With respect to stress proteins with antioxidant properties, Essig *et al.* (124) demonstrated an increased expression of HO-1 at the RNA level in muscles of mice stressed by acute exercise. Instant activation of HO-1 expression in working muscle has also been shown in men subjected to 60 to 90 min one-legged knee extensor exercise or 4 h cycling ergometry performed at 50% to 60% of maximal oxygen consumption (125). Further, the stimulation of muscle HO-1 by acute exercise has been confirmed in a rat model, in which the animals completed low-intensity exercise for 180 min and high-intensity exercise for 45 min (126). Thus, HO-1 appears to be highly inducible by exercise and represents a major factor acting against exercise-induced oxidative stress. Data regarding training effects on HO-1 expression in skeletal muscle are not available as yet. Current data, however, show that HO-1 mRNA and protein content was increased in the rat myocardium after a 14-week training program, and upregulation of HO-1 was paralleled by myocardial protection against ischemia/reperfusion injury (127).

Compared to data on HO-1, there is considerably more knowledge regarding the effects of exercise on inducing expression of proteins of the HSP70 family in skeletal muscle (for more extensive information see (114, 128)). Acute exercise has been shown to upregulate expression of HSP70 on mRNA and at the protein level. The response of HSP was shown after different types of exercise, such as cycling, rowing, and leg extensions. Initial data from humans show that in untrained subjects, 30 min of treadmill running augments HSP70 mRNA in muscle, while corresponding protein content did not change within 3 hours post-exercise (129). However, subsequent research further revealed that maximal stimulation of HSP60, HSP70 and HSC70 on protein level after a single bout of exercise such as treadmill running or cycling ergometry for 45 min occurs at a later time point, peaking 2 – 4 days post-exercise (119, 130). Up-regulation of HSP70, as well as of the GSH-increasing HSP27, has been shown on RNA and protein levels 48 hours after high-force eccentric exercise in biceps brachii muscle of untrained human subjects (131). In the same investigation, a mixed contraction exercise protocol, such as downhill running, did not induce increased HSP protein content.

In well-trained rowers, a progressive increase of training load and intensity has been shown to augment HSP70 protein in the M vastus lateralis (132). Also in rowers, HSP70 protein content only increased after a 3 week period of high-intensity training, while low-intensity endurance training was not sufficient to yield a similar effect (133). Interestingly, HSP72 content in the M. biceps brachii of well trained males decreased by 46% after 12 weeks of high force concentric weight training (134). This decrease did not occur if the training was performed using an eccentric protocol. Thus, for trained subjects, it appears that upregulation or stabilization of HSP protein content depends more on intensity than the duration of the training program. Furthermore, stimulation predominantly through eccentric exercise suggests that mechanical stress is more involved in the process of the training-induced HSP response than metabolic factors.

In this context, the contribution of exercise-induced oxidative stress on the upregulation of HSP awaits further clarification (135-137). In addition, it is still controversial as to how exercise-generated ROS induce upregulation of HSP. It is most likely that ROS exert stimulating effects via cellular protein damage, which in turn provides signals for HSP expression. On the other hand, alpha-tocopherol, beta-carotene as well as ascorbic acid supplementation has recently been shown to abolish the increase of HSP70 protein in human skeletal muscle after exercise (56, 138, 139) (Table 1), although these antioxidants do not reduce the severity of contraction-induced muscle damage.

Similar to the effects of antioxidants on signaling pathways, such as MAPKs or NF-kappaB, attenuation of ROS formation by antioxidants may also have the potential to blunt the exercise-induced adaptation of stress proteins useful for antioxidant defense. Whether this lower expression of protective systems due to antioxidant supplementation mirrors only a lower stress situation in muscle or whether it reflects a relevant suppression of useful adaptational mechanisms warrants further research.

10. CONCLUSIONS

Skeletal muscle produces ROS during contractile activity and there is evidence that exercise-induced changes in the cellular redox state clearly have functional significance. Nevertheless there are several unresolved issues, but existing knowledge provides a basis for future research to gain additional and more detailed information about the role of ROS in the response and adaptation of skeletal muscle to exercise. More insight into redox mechanisms and oxidative stress in skeletal muscle will yield a clearer picture of contractile regulation and will broaden our understanding of the process of muscle fatigue and muscle damage. Similarly, the growing link between redox-regulated gene expression and adaptation to regular exercise training is at an exciting stage and merits further clarification. In this context, further knowledgement regarding the functional significance of an exercise-induced adaptation of the antioxidant system under physiological and pathophysiological is required.

11. ACKNOWLEDGEMENTS

We are grateful to Mrs. Lisa Neumann for her assistance in the preparation of the manuscript.

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Abbrevations: AMP: adenosine monophosphate; AP-1: activator protein-1; ATP: adenosine triphosphate (ATP); CAT: catalase; GDP: guanosine diphosphate; GPx: glutathione peroxidase; IMP: inosine monophosphate; MAPK: mitogene activated proteine kinase; NAC: N-acetylcysteine; NADPH: nicotinamide adenine dinucleotide phoasphate (reduced form); NF-kappaB: nuclear factor-kappaB; 8-OHdG: 8-hydroxy-deoxy-guanosine; PGC 1alpha: peroxisome proliferator-activated receptor g co-activator 1alpha; HO-1: heme oxygenase-1; HSF-1: heat shock transcriptional factor-1; HSP: heat shock protein; GSH: reduced glutathione; GSSG: oxidized glutathione; SOD, superoxide dismutase; SR: sarcoplasmic reticulum

Key Words: Skeletal Muscle, Exercise, Training, Reactive Oxygen Species, Ros, Oxidative Stress, Redox-Regulation, Adaptation, Review

Send correspondence to: Professor Dr. Andreas M. Niess, Medical Clinic, Department of Sports Medicine, University of Tuebingen, Silcher Str. 5, D-72076 Tubingen, Germany, Tel: 49-7071-2986493, Fax: 49-7071-29-5162, E-mail: andreas.niess@med.uni-tuebingen.de

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