Oxidants and Skeletal Muscle Function: Physiologic and Pathophysiologic Implications (44450)

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Abstract. **Previous studies have demonstrated that skeletal muscles generate considerable reactive oxygen during intense muscle contraction. However, the significance of this phenomenon and whether it represents normal physiology or pathology are poorly understood. Treatment with exogenous antioxidants suggests that normal redox tone during contraction is influencing ongoing contractile function, both at rest and during intense exercise. This could represent the influence of redox-sensitive proteins responsible for excitation-contraction coupling or redox-sensitive metabolic enzymes. Some conditions associated with intense exercise, such as local tissue hypoxia or elevated tissue temperatures, could also contribute to reactive oxygen production. Evidence that muscle conditioning results in upregulation of antioxidant defenses also suggests a close relationship between reactive oxygen and contractile activity. Therefore, there appears to be a significant role for reactive oxygen in normal muscle physiology. However, a number of conditions may lead to an imbalance of oxidant production and antioxidant defense, and these, presumably, do create conditions of oxidant stress. Ischemia-reperfusion, severe hypoxia, severe heat stress, septic shock, and stretch-induced injury may all lead to oxidant-mediated injury to myocytes, resulting in mechanical dysfunction.** [P.S.E.B.M. 1999, Vol 222]

Skeletal muscles are rarely thought of as primary targets of oxidative stress. In fact, they appear to be uniquely designed to withstand stresses of many kinds. During intense exercise they are exposed to levels of mechanical and metabolic insult that would seriously injure or kill most other cells. For example, no other tissue of the body undergoes such drastic incremental changes in O_2 metabolism during what would be considered "normal activity." O_2 flux through the mitochondria can increase 100 times, when going from rest to maximum exercise in highly trained oxidative muscle fibers (1). If we assume a fixed percentage of this O_2 [i.e., 1%–2% (2)] is reduced to superoxide ($O_2^{\bullet-}$), then exercising muscles could be potent generators of reactive oxygen species (ROS). Many previous investigators have associated measurements of oxidative stress with exercise, but the biological implications of these measurements are unclear. Is this normal physiology or is it pathology?

Evidence for ROS Production in Skeletal Muscle

Some of the earliest work suggesting that free radicals are produced in exercising muscle was reported by Davies *et al.* (3), who showed an electron paramagnetic resonance (EPR) signal consistent with free radical formation and evidence of lipid peroxidation. Our laboratory has demonstrated similar results in diaphragm, quickly frozen from animals undergoing mechanical loading of their respiratory system (4). These results are often quoted as direct evidence of free radical formation in exercise. However, it is just as likely that they reflect paramagnetic species generated from normal electron flow of mitochondrial electron transport (5). Nevertheless, many other forms of evidence suggest that skeletal muscles produce considerable quantities of ROS, as discussed below.

Resting muscles *in vitro* (6), or *in situ* (7) produce

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extracellular superoxide (O_2•-), as measured by the reduction of cytochrome-*c*. The significance of *extracellular* ROS production in muscle is not known, but it may reflect the myocyte's attempts to regulate intracellular ROS or to remove excessive reducing equivalents. Alternatively, it could represent ROS produced by the abundant capillary endothelium, which *in vivo* may involve responses to shear stress (8, 9). Resting muscle also generates substantial *intracellular* ROS, as evidenced by the use of various redox-sensitive fluorescent probes such as dichlorofluorescin (10) and hydroethidine (11).

The production of intracellular and extracellular ROS is markedly increased with muscle contraction (10, 11). In addition, our laboratory (12) and others (13) have shown that muscle contraction induces hydroxylation of aromatic compounds such as salicylate, as shown in Figure 1. For many years, this assay was thought to be specific for hydroxyl radical activity. However, recently we and others have shown that at physiologic pH, peroxynitrite may be as responsible for hydroxylation of aromatic compounds as is hydroxyl radical (14, 15). Nitric oxide ($^{\cdot}$NO), which would be required for peroxynitrite formation, is also produced in significant quantities in contracting skeletal muscle (16). Substantial constitutive nitric oxide synthase (NOS) exists both in the myocytes and in the associated vascular endothelium (16). The roles of $^{\cdot}$NO production in muscle contractile function, metabolic regulation, and blood flow distribution are under intense investigation in many laboratories and though related to oxidant stress, for purposes of brevity, $^{\cdot}$NO is not discussed in detail in this review.

The molecular sources of ROS in skeletal muscle are poorly understood, and they probably differ depending on the physiologic state of the tissue and possibly the fiber type of the muscle. One likely candidate is mitochondrial electron transport. For example, ROS may be formed at several sites (including complex I, II, and III) that are capable of single reduction of O_2 to $O_2^{\cdot-}$, depending on a number of conditions (17, 18). In heart mitochondria, exogenous NADH oxidoreductase may be another source of $O_2^{\cdot-}$ production under conditions of high NADH (19). Whether this source of ROS is common to mammalian skeletal muscle is not yet known. Other possibilities include various poorly understood oxidoreductases on the membrane and cytosol. It has also been suggested that xanthine oxidase is involved in ROS formation in skeletal muscle (20), particularly in the vascular compartment. Interestingly, substrates for its activity (i.e., hypoxanthine and xanthine) can be delivered to a muscle capillary bed *via* the circulation from other tissues. However, its role as a source of ROS in skeletal muscle remains unclear. Even in conditions of ischemia reperfusion injury, there appears to be species-specific and condition-specific aspects to its involvement (21, 22).

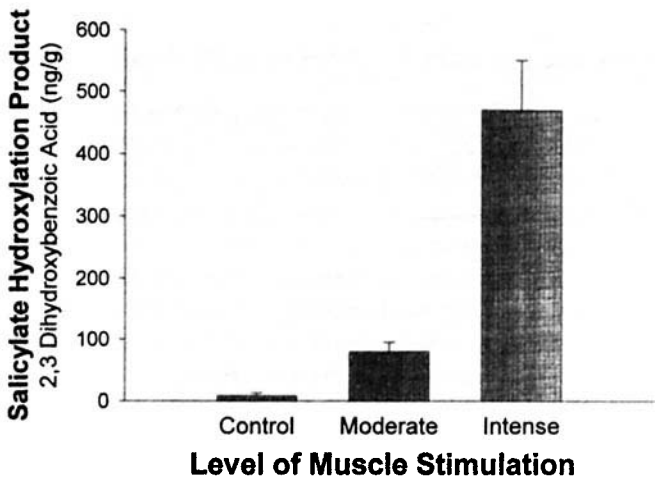

Figure 1. The salicylate hydroxylation product (2,3-dihydroxybenzoic acid) taken from muscle after various degrees of nerve-stimulated activation. (Derived from Ref. 12).

An interesting new hypothesis regarding the source of ROS in contracting skeletal muscle has originated from the laboratory of Supinski *et al.* (11) who has shown that ROS may be formed as a consequence of phospholipase A2 activation. Relatively specific phospholipase A2 inhibitors nearly eliminate the intracellular ROS produced during muscle contraction (11).

Despite the strong evidence that low levels of ROS are produced during various forms of contractile behavior in skeletal muscle, there is little particularly convincing data that this results in substantial oxidant "stress" under normal conditions of exercise. In exhaustive exercise, there are numerous reports of small elevations in oxidized glutathione, as well as increases in lipid and protein oxidation products, but these changes are, in general, small (3, 23, 24). However, oxidant stress has been demonstrated under numerous pathological conditions in skeletal muscle, some of which are described later in this review.

Effects of AOXs on Skeletal Muscle Function

A variety of exogenously applied intracellular and extracellular antioxidants (AOXs) cause alterations in the resting contractile characteristics of skeletal muscle. Maximum force development in response to high frequency tetanic stimulation is not particularly affected by AOXs, but responses to twitch stimulation and low frequencies of stimulation are changed appreciably. In general, a wide variety of exogenously applied AOXs, including *n*-acetylcysteine (25, 26), superoxide dismutase (SOD) (27), and catalase (27) cause faster twitch contractions, as illustrated in the top of Figure 2. As a consequence of these effects, AOX exposure also results in a net reduction in tetanic force development in response to low frequencies of stimulation (bottom of Fig. 2). On a theoretical basis, the reduction in twitch force and low frequency force probably reflects decreases in total Ca^{+2} available for cross-bridge activation; however, this has never been tested directly in intact muscle. The effect of mild oxidant treatment with H_2O_2 is opposite that of AOXs, suggesting again that resting redox tone is somehow modulating contractile function at rest (27, 28). The molecular mechanisms of how endogenous redox tone affects Ca^{+2}

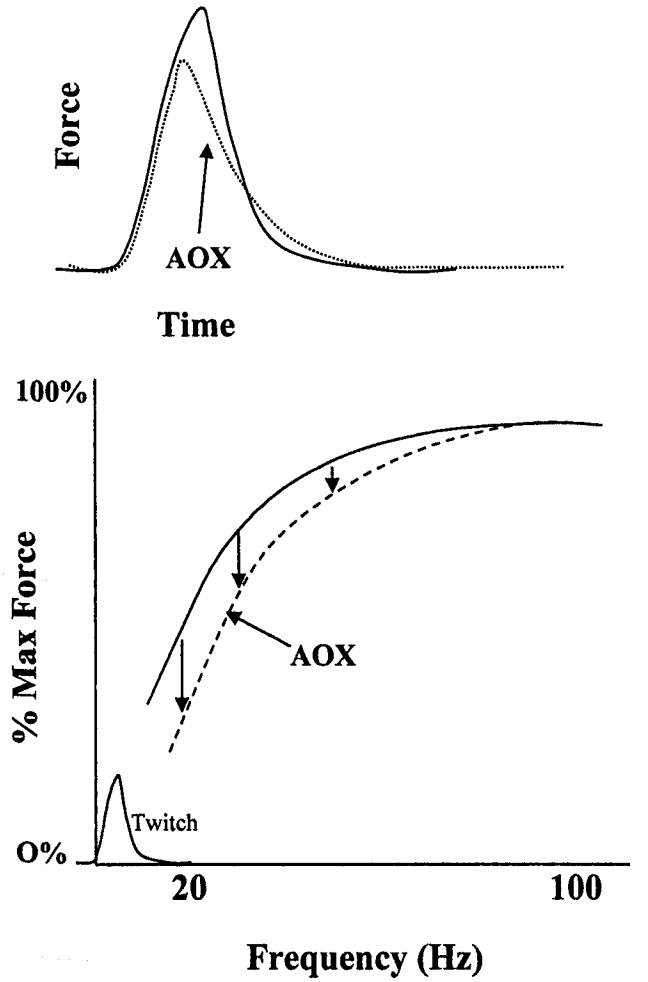

Figure 2. Schematic showing the influence of antioxidant (AOX) treatment on resting skeletal muscle force development. (Above) Most commonly seen effects of AOXs on single twitch contractions, causing lower twitch force. Note relative twitch force inset on the lower left of the bottom graph. (Below) The force response to increasing stimulation frequencies. AOX treatment lowers force development at low frequencies of stimulation, the range in which most contractions occur.

release and uptake are poorly understood, but represent an area under intense investigation in a number of laboratories. Interestingly, like AOXs, 'NO also decreases low frequency force production (16), and inhibition of NOS has the opposite effect (16). However, higher levels of H_2O_2 decrease Ca^{+2} release, presumably by direct oxidation of critical channel proteins (28, 29). Could 'NO be acting as an AOX in this environment by scavaging $O_2^{\bullet-}$? Probably not, since these effects appear to be related primarily to alterations in cyclic GMP activation (30).

AOXs and Skeletal Muscle Fatigue

One of the most seminal experiments that has driven much of the research related to oxidants, antioxidants, and muscle function came from the laboratory of Supinski *et al.* (31) nearly a decade ago. These investigators demonstrated that infusion of the antioxidant, N-acetylcysteine, in rabbits resulted in a remarkable preservation of force production, *in situ*, during fatiguing low-frequency stimulations of the diaphragm. This basic observation has been repeated with a variety of antioxidant treatments in limb muscle in animals (32), human limb muscle (33), human diaphragm (34), and rat diaphragm (25) with a variety of antioxidants (10, 35). Figure 3 illustrates typical responses to tiron (36), a superoxide scavenger, and this general augmentation of force during fatiguing stimulations is more-or-less typical of other kinds of AOX treatment in *in vitro* muscle. Interestingly, the effects are quite different from those seen during rest, where antioxidants lower the force response to low-frequency stimulation (Fig. 2). The underlying cause of this response is unknown, although one popular theory is that AOXs are preventing a low level of injury to the muscle. It has often been suggested that this modest injury represents the so-called low frequency fatigue, lasting 24 hr or more, but this has never been proven directly. Another hypothesis is that AOXs may change the redox tone of certain metabolic enzymes responsible for preservation of energy status within the cell, particularly during conditions of imbalance in energy supply and utilization, as might occur during fatigue. Candidates for redox-sensitive metabolic pathways include various components of electron transport within the mitochondria (37, 38), aconitase activity in the citric acid cycle (39, 40), mitochondrial and cytosolic creatine kinase (41, 42), and certain enzymes associated with glycolysis (43, 44).

Muscle Conditioning and Antioxidant Expression

An indication that ROS production is intimately tied to contractile or metabolic activity is suggested by the observations that AOXs are upregulated in response to prolonged muscle conditioning and downregulated with deconditioning. The molecular and genetic links between exercise, antioxidant mRNA, and antioxidant expression are still poorly understood, but much observational data regarding their as-

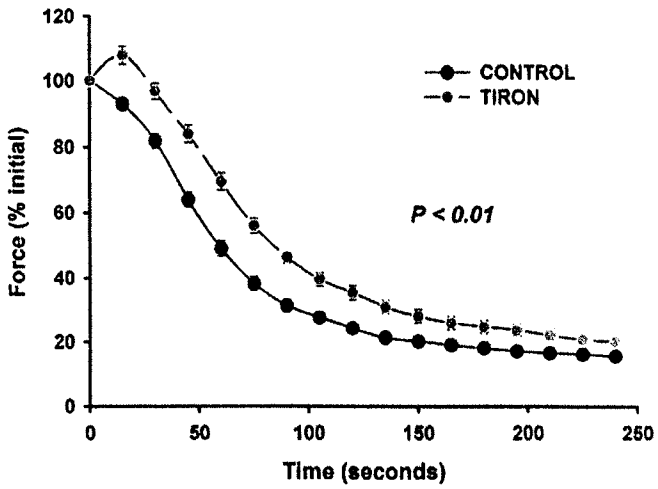

Figure 3. The influence of antioxidant treatment (tiron in this example) on the development of muscle fatigue in a 4-min *in vitro* fatigue protocol at 20 Hz stimulation; from Ref. 36). These results are typical of a wide range of different antioxidant treatments.

sociations have been presented in recent years, as discussed below.

A number of investigators have looked at changes in AOX activity secondary to exercise training and have reported varying results. It now appears that specific AOX responses depend on age (45, 46), muscle fiber type or muscle region (47, 48), exercise intensity or duration (47, 49), and probably species. Most investigators have observed increases in superoxide dismutase (SOD) activity in oxidative fibers in response to endurance training (47–51), and recent data suggest that both cytosolic (i.e., Cu/Zn SOD) and mitochondrial (MnSOD) isoforms are upregulated (51, 52). However, other investigators have shown little or no change in SOD activity (45, 53).

Hydrogen peroxide metabolism may have some unique features in skeletal muscle (e.g., in contrast to heart muscle) because catalase activity is not associated with isolated muscle mitochondria, and overall catalase activity is only about 1.4% of that in liver cells (54). Furthermore, in contrast to heart (55) or diaphragm muscle (51), catalase activity has not been shown to increase with exercise training in limb muscle (45, 47). In fact, several reports have demonstrated decreases in catalase activity in both oxidative and mixed fiber limb muscle (46, 53). Glutathione peroxidase enyzme is clearly upregulated in nearly every exercise paradigm (45–49, 51, 53) suggesting that the glutathione scavenging system may be primarily responsible for H_2O_2 metabolism in exercising muscle. Interestingly, Girten *et al.* (56) showed that with simulated weightlessness (limb suspension), AOX expression is decreased in rats. Furthermore, exercise conditioning prior to a period of simulated weightlessness increased levels of SOD and CAT activity in soleus muscle following weightlessness, and pharmacologic stimulation of metabolism with dobutamine during simulation also attenuated the subsequent decreases in AOX function (56).

ROS and Heat Stress

Muscles are tremendous heat generators during exercise, a fact that is largely overlooked in the free radical biology literature. Shortly after exhaustive exercise, rat limb muscles average temperatures in excess of 43°C, and core temperatures can exceed 42°C (57). Many of the results for the effects of exhaustive exercise (e.g., on antioxidant responses) may in fact relate to the influence of heat as a stimulus rather than increases in metabolic activity, but this has not been studied in detail.

Recent preliminary evidence from our laboratory has shown marked increases in intracellular ROS production in rodent diaphragm during brief exposure to 42°C, using the fluorescent probe, hydroethidine, as shown in Figure 4 (58, 59). Other researchers have also suggested that hyperthermia leads to oxidant production in skeletal muscle, resulting in increased heat shock protein (HSP) expression, mitochondrial production of $O_2^{\bullet-}$, progressive mitochondrial uncoupling, and increased mitchondrial ubisemiquinone concentration immediately following heat stress, induced by exercise (60). We have demonstrated that in response to a 15-min exposure to 42°C, heat shock proteins such as HSP_{70} are upregulated, and this can be blocked with superoxide or hydroxyl scavengers such as Tiron or DMSO (61). Thus, ROS may be partly responsible for the induction of HSP in heat stress (62), and these results illustrate that heat stress and ROS production are closely related. However,

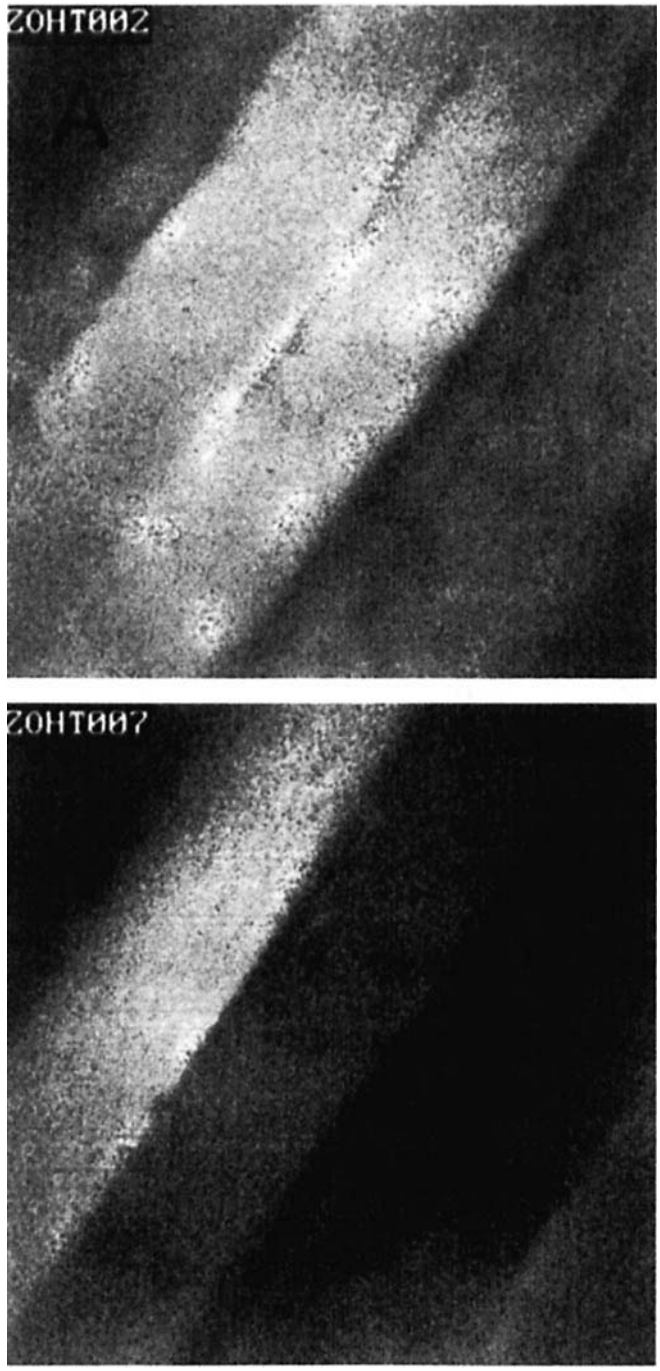

Figure 4. Confocal image of mouse diaphragm, before (A) and after (B) exposure to 42°C heat stress. Tissues preloaded with hydroethidine, a superoxide-sensitive fluorescent probe. The lighter areas are hydroethidine fluorescence which are diminished markedly after 25 min of heat stress. Ethidium formation, the product of hydroethidine-superoxide reactions was also increased in this experiment; however, it is not visible in this black and white image.

though we are reasonably certain ROS production is increased in heat, we are uncertain regarding the biological site of ROS production and by what specific mechanisms heat and or ROS can induce HSP expression and muscle contractile inhibition.

One important target for the influence of heat in skeletal muscle is the mitochondria. As shown in Figure 5 from work done in our laboratory, *in vitro* diaphragm muscle exposed to 42°C heat for 60 min results in marked swelling and disruption of subsacrolemmal and cytosolic mitochondria. For shorter periods of heat exposure, much more subtle damage is observed (not shown). Therefore, heat production during exercise, may have a tremendous impact on mitochondrial respiration, perhaps through oxidant mediated pathways. In support of this, in the 1970s, investigators found that O_2 consumption increased to high levels immediately after exercise, which may be related to the increased temperature of the muscle (57). At higher body temperature, skeletal muscles exhibited a lower level of phosphorylative efficiency (63), increased ATP utilization, and creatine phosphate depletion (64). In going from 25°C–45°C, resting mitochondrial oxygen consumption increased about 2.5-fold, the inhibition of respiratory rate by oligomycin increased about 2-fold, and the oligomycin-sensitive mitochondrial ATPase activity increased about 4-fold (63). Since the mitochondrion appears to be one of the major potential sources of ROS, and O_2 metabolism appears to be one of the major targets of the impact of heat stress, the specific effects of temperature on free radical production and mitochondrial function need to be further clarified.

Heat stress also influences the contractile machinery of skeletal muscles by several poorly understood mechanisms. Short heat exposure from 37°C to 42°C for 15 min reduces the force production at all stimulation frequencies, and this is inhibited by AOX treatment (61). In addition, heat increases passive stiffness of the muscle and alters the troponin-tropomyosin Ca^{+2} regulating system, causing Ca^{+2} -independent cross-bridge activation (65).

Hypoxia as a Stimulant for Oxidant Production

It is a paradox of free radical biology that excessive O_2 and decreased O_2 both result in increased ROS production in some tissues and under some conditions. The latter is often referred to as reductive stress and is believed to reflect the influence of an overabundance of reducing equivalents in the cell, driving one electron reduction of O_2 (66, 67). However, other mechanisms have been implicated in various tissues (66). In reductive stress we usually associate oxidant production with subsequent conditions of reoxygenation or reperfusion. However, elevated ROS formation can also occur in hypoxia alone. The most convincing evidence for this phenomenon has been demonstrated by Schumaker *et al.* (68), where in cardiac myocytes, *in vitro*, intracellular ROS production is markedly increased at PO_2 values of around 5–10 Torr. These investigators also have hypothesized that ROS produced during hypoxia play an important role as a molecular signaling agent in the cardiac myocyte. Whether similar mechanisms appear in skeletal muscle is not known at this time.

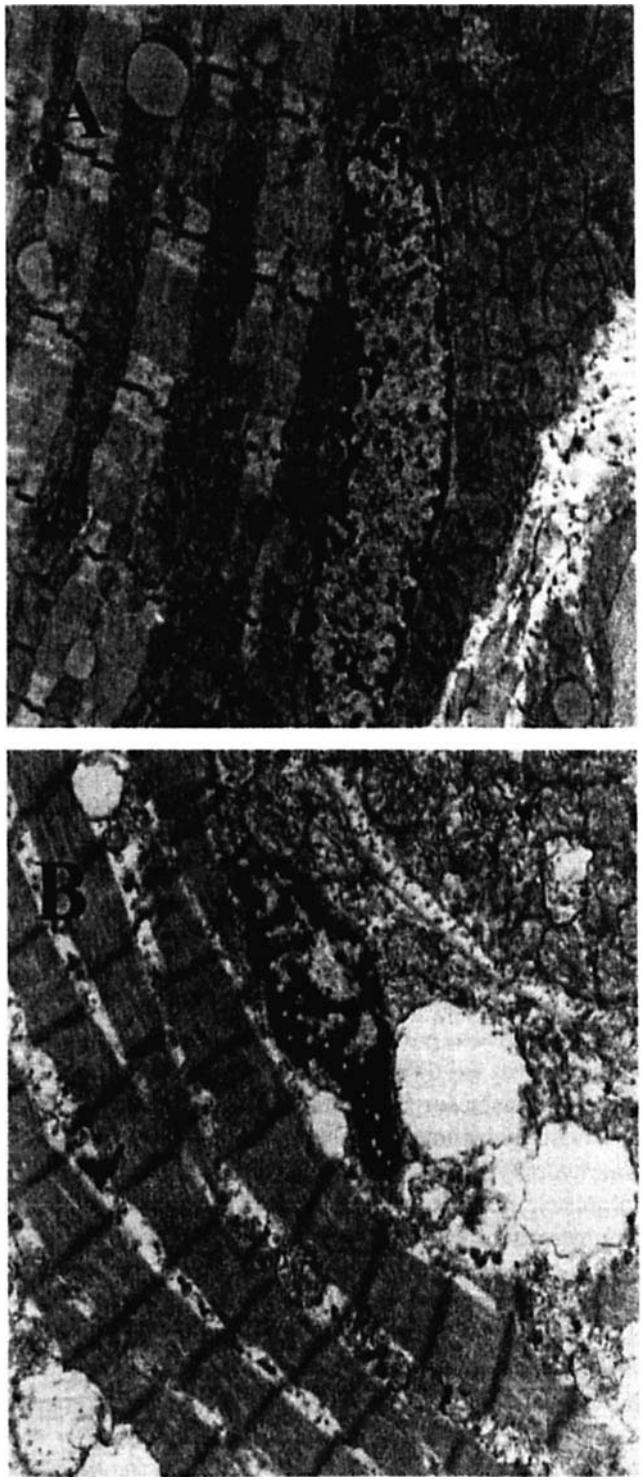

Figure 5. Electron microscopy of diaphragm muscle in: (A) matched control conditions at 37°C and (B) 60 min at 42°C. Note the mitochondrial swelling in both subsacrolemmal (around the nucleus) and cytosolic (between the myofibrils). Electron micrographs prior to 60 min showed more subtle evidence of mitochondrial injury.

Recently, our laboratory has demonstrated striking protective effects of a variety of AOXs on muscle during hypoxia, prior to reoxygenation (69). The effects on max force

of the superoxide scavenger, Tiron, are shown in Figure 6. These data suggest that low levels of oxidant production in conditions of hypoxia or reductive stress are contributing to the reduction of force in hypoxia. The extent to which individual skeletal muscle cells are exposed to regional hypoxia in any normal condition of exercise is poorly understood (70). However, it is likely that in extreme exercise, the delicate balance between oxygen supply and demand can be compromised, regionally, and that tissue oxygen can fall below a critical level in some portions of the cell, resulting in a buildup of reducing equivalents and enhanced ROS formation.

How AOXs affect muscle function in hypoxia is also not understood. However, recent data from our laboratory suggest that AOXs protect critical metabolic pathways during hypoxia, pathways that are responsible for preservation of creatine phosphate, the major energy shuttle system of contracting muscle (71).

The potential role of oxidant stress in ischemia/reperfusion injury is more established but still somewhat controversial. Ischemia is a considerably different stimulus from hypoxia because it is characterized by acidosis, accumulation of ·NO and NO-metabolites, accumulation of adenosine and other energy metabolites, and presumably accumulation of oxidants and oxidation products. In pure hypoxia, these conditions are less likely to occur. During reperfusion, not only is the tissue suddenly capable of generating large levels of reactive oxygen, but the metabolites are transiently changed, prior to the ability of the tissue to re-establish equilibrium. Muscle injury due to ischemia-reperfusion is of some practical importance in emergency medicine and surgery (e.g., revascularlization of severely injured tissues following trauma, following "encapsulation syndrome," or in surgical reconstruction). Most experimental models point to a definite role of hydroxyl radical or other hydroxylating species in ischemia/reperfusion of skeletal muscle because the injury can be attenuated by providing chelators of iron such as desferroxamine (22) or scavengers of hydroxyl radical (72, 73). Although early studies in rats (21, 73) suggested that a large part of the source of oxidative damage came from xanthine oxidase mechanisms, this has been difficult to show in humans and other species (22). A large portion of the xanthine oxidase activity in ischemia/reperfusion or in other forms of injury could come from the activity within inflammatory neutrophils or within endothelium during inflammation (74).

Reactive Oxygen and Muscle Injury

Muscle injury can occur in response to numerous stimuli (75), including exhaustive endurance exercise (76, 77), but it is most commonly seen in response to lengthening (i.e., eccentric contractions). Examples of lengthening contractions include the action of the quadriceps muscle while running downhill. Lengthening contractions lead to greater muscle injury than isometric or shortening contractions (78, 79), and this is probably related directly to strain absorbed by individual myofibrils (80). In the rodent model, there is a delayed onset of injury to the muscle, which peaks approximately 3 days after the initial insult (81). This coincides with the timing of secondary injury (82) and soreness (83) seen in humans. Faulkner *et al.* have shown that maximal muscle tension initially falls following eccentric contractions, then rises to 70% over the first day and falls again to 48% of initial force by Day 3.

What role do oxidants play in muscle injury? Recent evidence suggests that significant free radical activity and oxidative injury may occur as early as 24 hr after injury (84). Furthermore, AOXs may attenuate the injury in some conditions. For example, Zerba *et al.* (81) pretreated young, adult, and old mice with intraperitoneal injections of polyethylene glycol-superoxide dismutase (PEG-SOD) prior to a protocol of lengthening contractions. Three days postcontraction, untreated mice developed a maximum force of ≈ 60% in adult mice and ≈ 44% in aged mice, whereas PEG-SOD-treated mice demonstrated forces of 80%–90% in young and 70% in old mice. SOD-treated old mice also

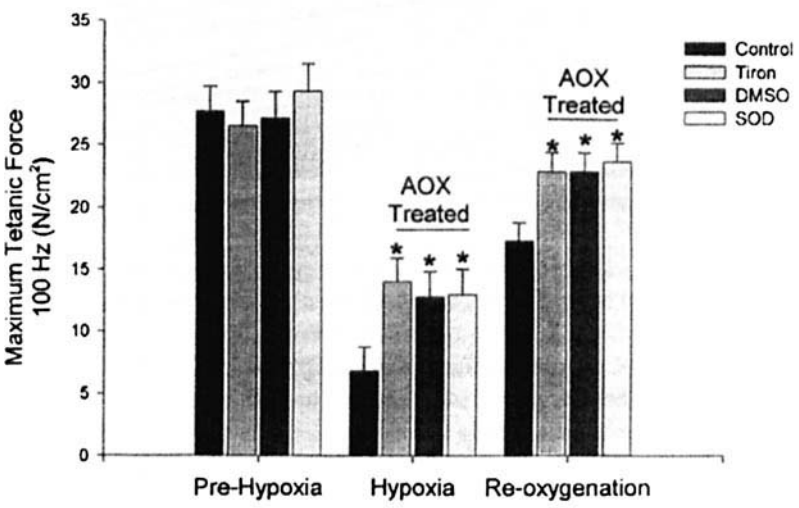

Figure 6. Effects of antioxidant (AOX) treatment in response to hypoxia and reoxygenation. All AOXs tested resulted in preservation of force production in hypoxia and following reoxygenation. (Redrawn from Ref. 69).

showed significant preservation of max force after 10 min of contraction, compared to control mice. Non-aged mice (i.e.,young and adult) showed no change. In the same study, histologic examination of the muscles was undertaken to determine the percentage of uninjured fibers in the young and adult mice. Uninjured fibers increased from 77%–96% with SOD treatment. These results were taken as evidence of a significant role for ROS in the delayed onset of muscle injury.

A similar study in rats was undertaken by the same group using vitamin E as the antioxidant instead of PEG-SOD (85). In contrast to PEG-SOD, vitamin E showed no protective effect in the muscles with regard to changes in maximum force or signs of histologic damage. However, increases in serum enzyme activities of creatine kinase (CK) and pyruvate kinase (PK), considered markers of cell damage, were decreased in treated muscle compared to control or vehicle-treated muscle. Similar failures by vitamin E to protect muscle from delayed onset injury were seen in studies by Jakeman *et al.* (86) and Warren *et al.* (87). Differences in effects of PEG-SOD and vitamin E are possibly due to differences in antioxidant solubility or cellular location (85). The most likely mechanism for AOX protection of contractile fibers and membranes is an inhibition of oxidation during inflammatory cell recruitment and activation associated with delayed onset of soreness (84).

ROS-Induced Respiratory Muscle Dysfunction and Respiratory Failure

Considerable interest has been shown in the potential role of oxidative stress in respiratory failure, particularly with respect to its influence on respiratory skeletal muscle. Respiratory arrest is a major cause of death and hospitalization. Conditions often associated with respiratory failure such as fever, hypoxia, respiratory acidosis, increased respiratory muscle mechanical loads, sepsis, inflammation, nutritional imbalance, and poor perfusion can all promote oxidative stress in some conditions. Anzuetto *et al.* (23, 88–90) have performed a series of studies in which anesthetized rats were brought to complete apnea by adding inspiratory resistive loads to the airway. Under these conditions increases in lipid peroxidation products and oxidized glutathione (GSSG) were seen in the diaphragm, particularly when the muscle's AOX defenses were compromised (89, 90). Our laboratory, using a similar model, but with supplemental O_2 and increased mechanical loads for more prolonged periods, has observed more modest evidence of oxidative stress, primarily a reduction in total GSH, with little or no increase in GSSG (91). We also saw no consistent change in lipid peroxidation. However, blood taken from animals undergoing prolonged resistive loading did show increases in carbon-based radical adducts from the circulation (92). Supinski *et al.* (93) have also done extensive work with these models, particularly using decerebrate, unanesthetized preparations. These investigators have shown that the amount of oxidative stress depends a great deal on supplemental O_2 and that treatment of the animals with antioxidants can, under some conditions, delay the onset of failure and protect the diaphragm from mechanical dysfunction.

One of the more common forms of respiratory failure is associated with septic shock, often leading to a condition referred to as acute respiratory distress syndrome (ARDS). Interestingly, septic shock results in diaphragm and other skeletal muscle dysfunction, with evidence of oxidative stress (94). The loss of diaphragm function in sepsis can be greatly attenuated by preadministration of AOXs (95, 96), suggesting a significant role of oxidant stress in this syndrome. Septic shock could influence respiratory muscle function through a number of pathways such as upregulation of inducible NOS activity (97), recruitment and activation of neutrophils into the capillary circulation, and direct or indirect effects of sepsis and various related cytokines on mitochondrial function.

Conclusions and Future Directions

Do normal skeletal muscles undergo oxidative stress or are the oxidants produced during exercise simply a pattern within the normal homeostatic environment of the cell? The answer is perhaps semantic and depends on one's definition of stress. We prefer to think of stress as a condition that seriously threatens cell survival. Furthermore, from this viewpoint, the minimal alterations of oxidized glutathione and lipid and protein oxidation products seen during exercise would be consequences of normal oxidant production and perhaps be important in cell signaling. Whatever the definition, skeletal muscles appear to function quite well in what appears to be relatively high oxidizing environments. There are conditions in which the delicate balance between oxidant production and antioxidant defense may be compromised, and these could include severe heat stress, infection or sepsis, severe ischemia, nutritional deficiencies, or wide variations in muscle activation for the given level of conditioning of the muscle, resulting in injury.

It seems to us that the more important questions are related to what reactive oxygen and products of oxidation chemistry are doing in *normal* cell function. We hypothesize, as others have, that oxidants are playing important roles in the normal contracting myocyte to regulate Ca^{+2} metabolism, contractile behavior, and perhaps utilization and control of energy substrates. Fatigue and hypoxia experiments suggest that they may be playing important roles as negative feedback signaling molecules, functioning to protect the muscle from overstimulation and subsequent injury. In many other systems growth factors induce reactive oxygen production (98, 99). Are ROS involved with muscle fiber adaptations and differentiation in response to exercise stimuli? How are antioxidant control enzymes orchestrated during conditioning to maximize cell function and minimize the potential damaging influences of ROS? These and many

other related questions lie in the frontiers of future free radical biology as it applies to skeletal muscle function in health and disease.

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