Published in final edited form as:

Annu Rev Nurs Res. 2013 ; 31: 367–387. doi:10.1891/0739-6686.31.367.

ROS and RNS signaling in skeletal muscle: critical signals and therapeutic targets

Luke P Michaelson¹, Colleen Iler², and Christopher W Ward²

¹Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814

²University of Maryland School of Nursing, 655 W Lombard St, Baltimore, MD 21201

Abstract

The health of skeletal muscle is promoted by optimal nutrition and activity/exercise through the activation of molecular signaling pathways. Reactive oxygen species (ROS) or reactive nitrogen species (RNS) have been shown to modulate numerous biochemical processes including glucose uptake, gene expression, calcium signaling and contractility. In pathological conditions, ROS/RNS signaling excess or dysfunction contributes to contractile dysfunction and myopathy in skeletal muscle. Here we provide a brief review of ROS/RNS chemistry and discuss concepts of ROS/RNS signaling and its role in physiological and pathophysiological processes within striated muscle.

Introduction

Skeletal muscle is a complex organ system within the body. While we often consider skeletal muscle contraction in support of posture, locomotion or respiration as its main function, skeletal muscle contraction is also important for guarding body cavities (i.e., muscle sphincters), for maintaining body temperature (i.e., shivering thermogenesis), for maintaining glucose control and as a protein reserve. The health of muscle is promoted by optimal nutrition and activity/exercise that activate molecular signaling pathways to promote an increase in the functional and metabolic capacity of the muscle. Conversely, muscle health is negatively impacted by inactivity as well as disease (i.e., diabetes, muscular dystrophies, amyotrophic lateral sclerosis, sarcopenia, critical illness myopathy) often through a dysregulation through similar molecular pathways.

Reactive oxygen species (ROS) or reactive nitrogen species (RNS) have been shown to modulate numerous biochemical processes through the targeted modification of specific protein residues. In striated muscle, contractile activity and/or stretch increases ROS/RNS signaling to modulate a host of biochemical processes including glucose uptake (Chambers, Moylan, Smith, Goodyear, & Reid, 2009), gene expression, calcium signaling and contractility (Chambers, Moylan, & Reid, 2009). In pathological conditions, ROS/RNS signaling excess or dysfunction contributes to contractile dysfunction and myopathy in skeletal muscle.

It is clear that ROS and RNS are generated from numerous tissues and paracrine signaling to skeletal muscle is important in health and disease. In fact, several excellent reviews have been published that consider these pathways (Donoso, Sanchez, Bull, & Hidalgo, 2011; Ferreira & Reid, 2008; Kuster, Hauselmann, Rosc-Schluter, Lorenz, & Pfister, 2010; Lamb & Westerblad, 2011; Powers, Talbert, & Adhietty, 2011). Emerging research is now revealing pathways by which ROS/RNS are generated within the myocyte at rest and during contraction (i.e., exercise) (Jackson & McArdle, 2011; Powers, Smuder, & Judge, 2012) and novel mechanistic insights into mechanoactivation of ROS signaling have recently been reported (Khairallah et al., 2012; Prosser, Khairallah, Ziman, Ward, & Lederer, 2012). These pathways are being revealed with both *in vivo* and single cell studies enabled by new methods and reagents. These findings are providing unique insights into both the positive adaptations seen with exercise and the dysfunction seen with inactivity and disease. Here we provide a brief review of ROS/RNS chemistry and discuss concepts of ROS/RNS signaling and its role in physiological and pathophysiological processes within striated muscle.

Molecular Signaling in the context of classical nursing theory

The nurse theorist Mary Levine developed a Conservation Model emphasizing the practical understanding of physiological mechanisms identified in four key principles; Conservation of Energy, Conservation of Structural Integrity, Conservation of Personal Integrity, and Conservation of Social Integrity (Meleis, 2012). In general, these concepts identify attributes that require the nurse to help the client return to a homeostatic balance with respect to each concept. The Conservation Model indicates the nurse acts as an intermediary, helping the client to “adapt” to the life experiences involved with pathophysiological events (Levine, 1996). One of Levine’s assessments to evaluate the biological and physical systems responsible for disease processes was to evaluate the “body movement and positioning” of the client. Understanding the client’s activity with respect to their operational environment (Meleis, 2012) allows nurses to integrate a treatment plan to therapeutically “adapt” an ill client to his/her current condition. Levine’s Model forms a guide for the nurse to help the “ill” client reacquire a new homeostatic balance within their social, personal, and biological milieu. Then as a corollary, one may suggest this model substantiates the need for the modern nurse to consider molecular pathways, such as ROS and RNS and their homeostatic role in physiological signaling and their contribution to disease when the signaling is out of balance.

Reactive Oxygen Signaling and the ROS Paradox

Reactive oxygen species (ROS) are reactive molecules that contain oxygen. ROS are formed as natural byproducts of oxygen metabolism and by enzymatic activity. In both cases, ROS production is highly localized and spatially restricted which makes it a efficient system for cell signaling processes such as glucose transport (Chambers, Moylan, Smith, et al., 2009), eicosanoid (Spiteller, 2010) and cytokine production (Sigala et al., 2011), and mitochondrial biogenesis (Austin, Klimcakova, & St-Pierre, 2011; Kang & Li Ji, 2012). However, during times of environmental stress (e.g., UV or hyperthermia), metabolic stress (e.g., hyperglycemia), or in disease states (e.g., muscular dystrophy, unloaded diaphragm during mechanical ventilation) ROS levels can increase dramatically resulting in aberrant

signaling (D. G. Allen, Gervasio, Yeung, & Whitehead, 2010; Davidovich et al., 2013; Faist, König, Hoger, & Elmadfa, 2001; Kavazis et al., 2009).

In skeletal muscle, the functional consequences of this biphasic response are well described in experiments where small increases in ROS, as seen with mild exercise, increase muscle force generation. Yet, with excessive fatiguing exercise or with an experimental increase in exogenous ROS, muscle function decreases (Andrade, Reid, & Westerblad, 2001; Ferreira et al., 2012). This biphasic effect of ROS on cell function (Andrade, et al., 2001; Reid, 2001) forms the basis for the ROS “paradox” which acknowledges ROS as necessary for proper signaling pathways yet detrimental to function when in excess (Figure 1).

Reactive Oxygen Species

Reactive Oxygen Species (ROS) is a general term encompassing oxygen free radicals; oxygen containing molecules that contain one or more unpaired electrons. (Halliwell & Gutteridge, 2007). It is important to note that not all ROS need to be a radical species. As indicated in Formulas 1 and 2 below, hydrogen peroxide (H_2O_2) is a type of ROS but it is not a “free radical” because it does not contain unpaired electrons.

Superoxide ($\text{O}_2^{\cdot-}$) is a common ROS molecule within skeletal muscle (Reid, 2001). However, has limited intrinsic reactivity and its negative charge limits its membrane permeability, however $\text{O}_2^{\cdot-}$ has been shown to move through anion channels or aquaporin (Fisher, 2009; Ren, Raucci, Browe, & Baumgarten, 2008). Superoxide’s spontaneous dismutation or enzyme catalyzed dismutation (Formula 1) into the membrane permeable H_2O_2 (Cadenas & Packer, 2002) yields a potent and reactive signaling molecule.

Reactive Oxygen Species Reactions

The formation of H_2O_2 can initiate key redox reactions. H_2O_2 forms the substrate for the production of the extremely reactive hydroxyl radical ($\text{OH}^{\cdot}$) via a Fenton Reaction with divalent metal cations, but mainly with ferrous iron, Fe^{2+} (Formula 2). The sulfhydryl ($-\text{SH}$) group associated with a protein may initially be oxidized to a sulfenic acid (protein $-\text{SOH}$). The $\text{OH}^{\cdot}$ can then form irreversibly oxidized protein sulfhydryl groups, indicative of protein “damage” (Cadenas & Packer, 2002; Thomas & Mallis, 2001) (Formulas 3 and 4).

Irreversible Protein Oxidation

Reactive Nitrogen Species (RNS)

It is important to note other radical and non-radical species have been detected in skeletal muscle, which influences the overall reduction/oxidation (redox) homeostasis within the skeletal myocyte. The nitric oxide (NO[•]) molecule forms the parent molecule for reactive nitrogen species. In the presence of O₂^{•-}, NO[•] forms the highly reactive nitrogen intermediate, peroxynitrite, by the reaction in Formula 5.

Peroxynitrite Formation

ROS and RNS can refer to multiple radical and non-radical types of each species. This review primarily focuses on those species indicated below.

ROS/RNS Formation

As outlined above, skeletal muscle generates superoxide and nitric oxide (NO) as the primary ROS/RNS species. Once generated, each lead to the formation of secondary ROS or RNS species with specific signaling roles (Figure 2). They derive from several sources and the amount of each type is increased with contractile activity.

Nitric oxide synthase

In skeletal muscle cells, nitric oxide (NO) is generated by neuronal nitric oxide synthase 1 (nNOS; (Ferreira & Reid, 2008)) from the precursor L-arginine. nNOS contains a calmodulin (CaM) binding domain making it Ca²⁺ sensitive and therefore responsive to contractile activity (Supinski & Callahan, 2007). While NO is a potent signaling molecule, it readily reacts with superoxide to form peroxynitrite, which in-turn reacts potently with thiol groups on protein targets. The formation of peroxynitrite also decreases the bioavailability of NO and superoxide, thus modifying the redox balance in the myocytes (Judge, Kass, Thompson, & Wagner, 2011; Kass, Takimoto, Nagayama, & Champion, 2007).

Xanthine Oxidase

In skeletal muscle, xanthine oxidase (XO) exists in two forms whose primary purpose is to catabolize purines into uric acid (Corte & Stirpe, 1972; Harrison, 2002). Under basal activity conditions, XO primarily exists as the enzyme xanthine dehydrogenase (XDH). Increased oxidative stress can oxidize key cysteine amino acids inducing reversible changes of the XDH to XO. Proteolytic enzymes may also cleave the XDH irreversibly to form XO (McNally, Saxena, Cai, Dikalov, & Harrison, 2005). Both enzymes catabolize hypoxanthine to xanthine and then from xanthine to uric acid (Halliwell & Gutteridge, 2007; Harrison,

2002). The primary difference between the two enzymes is that the XDH uses NAD⁺ as the electron acceptor as opposed to XO that uses O₂. Therefore, generally XO forms the free radical O₂^{•-} while XDH does not. XO primarily resides on the surface of the endothelial cells lining the skeletal muscle. However, intracellular expression of this enzyme have also been detected (M.-C. Gomez-Cabrera, Domenech, & Viña, 2008; M. C. Gomez-Cabrera et al., 2005; M. C. Gomez-Cabrera, Pallardo, Sastre, Vina, & Garcia-del-Moral, 2003; Harrison, 2002; Houston et al., 1999). It is important to note XO can also form the free radical NO[•] under specific hypoxic conditions (Halliwell & Gutteridge, 2007; Harrison, 2002).

Phospholipase A2

Another source for ROS generation in skeletal muscle is from the phospholipase A₂ (PLA₂) enzyme family. These enzymes catalyze the hydrolysis reactions of membrane glycerophospholipids to form arachidonic acid (AA) (Murakami & Kudo, 2002). The enzymatic pathways for cyclooxygenase (COX1, COX2) and the lipoxygenase family then catalyze AA to form eicosanoid inflammatory mediator products such as prostaglandins, leukotrienes, and thromboxanes (Harris, 2007). In skeletal muscle the Ca²⁺ dependent cPLA₂ was detected along the sarcolemma and within the mitochondria while the Ca²⁺ independent constitutively active iPLA₂ isoform was detected within the cytosol.

Nethery and colleagues (1999) used excised rat hemidiaphragm preparations to evaluate cPLA₂ dependent ROS generation in contracting and non-contracting muscle. The cPLA₂ specific inhibitor manolide significantly decreased the ROS generation during contractile conditions when compared to the untreated control samples (Nethery, Stofan, Callahan, DiMarco, & Supinski, 1999). Furthermore, isolated mitochondrial preparations from the diaphragm muscle also suggested AA interacted with the electron transport chain most likely at Complex I to form ROS, as measured by a H₂O₂ assay (Nethery et al., 2000).

Mitochondria

The mitochondria are a source of free radical generation in muscle. In the process of oxidative phosphorylation, Complexes I–IV and the F₁F₀ ATP synthase protein form the primary quaternary structures to oxidize reducing equivalents. The stepwise reduction transfers electrons to O₂ forming water (H₂O) while establishing a H⁺ ion gradient between the inner and outer mitochondrial membranes. RS are generated along the electron transport chain (ETC), particularly O₂^{•-} which spontaneously dismutates to H₂O₂ or the enzyme manganese superoxide dismutase (Mn²⁺SOD) catalyzes the reduction of O₂^{•-} to H₂O₂ and O₂, Formula 6. The antioxidant Mn²⁺SOD is localized to the mitochondria, while CuZnSOD is located within the cytosol.

O₂^{•-} Dismutation Reaction

This reaction is important because the SOD enzymes remove the $O_2^{\cdot-}$ from the overall oxidant pool. Initial research estimated approximately 2% of the total oxygen within mitochondrial samples of rat liver, rat heart and pigeon heart generated H_2O_2 (Chance, Sies, & Boveris, 1979). Further insight into mitochondrial oxygen metabolism suggested less RS are generated from the electron transport during oxidative phosphorylation. Using specific Complex inhibitors, St- Pierre and colleagues estimated only 0.1% – 0.2% of the oxygen is consumed for RS formation. The amount and rate of RS generation are important. The $O_2^{\cdot-}$ is membrane impermeable, but the dismutation product H_2O_2 can form $OH^{\cdot}$ which acts as a significant oxidant.

NADPH Oxidase

Nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase or Nox) is a family of multi-protein enzymes first described in phagocytic cells (Hohn & Lehrer, 1975). In phagocytic cells, the Nox enzymes are responsible for generating a large amount of the free radical $O_2^{\cdot-}$ as a microbicidal mechanism. The active oxidase structure localized to the membrane includes the two membrane proteins, gp91 $phox$ and p22 $phox$ forming the b₅₅₈ reductase complex (Chowdhury et al., 2005). Three cytosolic proteins, p40 $phox$, p47 $phox$, p67 $phox$, and the small GTP-binding protein Rac form the remaining structure of the Nox enzyme. Upon stimulation, the cytosolic proteins translocate to the membrane to form a complex with the two membrane proteins which results in enzymatic activation and production of $O_2^{\cdot-}$. The variants of the catalytic subunit, gp91 $phox$, form the different isoforms: Nox 1–5, and the dual oxidase (Duox) Nox2 homologues, Duox 1 and 2 (Bedard & Krause, 2007; Brown & Griendling, 2009). Continued research of this enzyme family revealed these multiple isoforms are differentially expressed in tissues and do not only perform microbicidal functions. The ROS generated from Nox are key signaling intermediates within inflammatory, angiogenic (Vokurkova, Xu, & Touyz, 2007; Xu & Touyz, 2006), apoptotic, cell proliferation (Sauer, Klimm, Hescheler, & Wartenberg, 2001), and tumorigenic pathways (Arbiser et al.).

The Nox2 and Nox4 isoforms are the predominant Nox enzymes expressed in skeletal muscle. Both have been detected within the transverse tubule system and at the plasma membrane (Hidalgo, Sanchez, Barrientos, & Aracena-Parks, 2006; Javesghani, Magder, Barreiro, Quinn, & Hussain, 2002). It is believed that Nox4 is active at rest while Nox2 may be activated through several mechanisms. For example, Nox2 enzymes have been implicated in ROS formation with depolarization (Espinosa et al., 2006), osmotic stress (Martins, Shkryl, Nowycky, & Shirokova, 2008), as well as the exercise pressor reflex (H. J. Wang, Pan, Wang, Zucker, & Wang, 2009). Additionally, H_2O_2 has been shown to activate the tyrosine kinase Src, in mouse skeletal muscle (Gervasio, Whitehead, Yeung, Phillips, & Allen, 2008). Activation of Src is important because this kinase phosphorylates the cytosolic subunit p47 $phox$. Phosphorylated p47 $phox$ translocates to the plasma membrane and complexes with the membrane bound b₅₅₈ subunits. The subunit p67 $phox$ binds to the phosphorylated p47 $phox$ and is transported concurrently to the plasma membrane. The p40 $phox$ and Rac1 subunits then also bind to form a complete Nox2 enzyme. Osmotic stress has been shown to generate ROS, but the physiological importance of this type of mechanical stretch is debatable.

Recent work by our group and others has implicated the Nox pathway as the major source of superoxide ROS in the skeletal muscle cell during repetitive contractions (Michaelson, Shi, Ward, & Rodney, 2010; Sakellariou et al., 2012). ROS production with contraction has been shown to arise from the sarcolemma and t-tubules by activation of transmembrane Nox2 (Espinosa, et al., 2006; Khairallah, et al., 2012; Prosser, Ward, & Lederer, 2011; Sakellariou, et al., 2012). It was further suggested that Nox2 generated superoxide then promoted Nox4 activation within the mitochondria to generate ROS that was released into the cytosol (Whitehead, Streamer, Lusambili, Sachs, & Allen, 2006; Whitehead, Yeung, & Allen, 2006). However, experiments to directly address this hypothesis have not supported that idea (Sakellariou, et al., 2012).

Mechano-transduction activated NOX2 dependent ROS production

Recent investigation into the mechanisms by which exercise or activity regulates ROS/RNS production in the muscle has focused on how the mechanical stress of contraction is converted to a signal that activates ROS (i.e., the mechano-transduction dependent activation of ROS). Experiments and methods used to mechanically manipulate single enzymatically isolated muscle cells were pioneered by several groups (Calaghan, Belus, & White, 2003; Gannier, White, Lacampagne, Garnier, & Le Guennec, 1994; Le Guennec, White, Gannier, Argibay, & Garnier, 1991). These approaches have recently been coupled with high-speed fluorescence microscopy and have begun to reveal new mechanistic insight into the mechano-activation of ROS signaling in skeletal muscle.

The concept of cellular "tensegrity," proposes that the microtubule (MT) filament network resists mechanical perturbations in cells and in doing so acts as a mechano-transducer (Ingber, 2008; Stamenovic, Mijailovich, Tolic-Norrelykke, Chen, & Wang, 2002; N. Wang, Butler, & Ingber, 1993; N. Wang et al., 2001). The initial hypothesis that the MT network was a mechano-signaling element in heart arose from elegant work in the literature (Calaghan, et al., 2003; Calaghan, Le Guennec, & White, 2004; Iribe et al., 2009). Working with the knowledge that Nox2 is activated by either contraction (i.e., contraction/compression followed by relaxation/stretch), osmotic stress (stretch or compression) (Isaeva, Shkryl, & Shirokova, 2005; Martins, et al., 2008; Shkryl et al., 2009; Ullrich, Fanchaouy, Gusev, Shirokova, & Niggli, 2009), or stretch (Murdoch, Zhang, Cave, & Shah, 2006), our group focused attention on a hypothesis whereby a MT dependent mechano-transduction pathway could translate a mechanical signal to Nox2 to activate ROS. To address this question, we developed new tools and methods (Khairallah, et al., 2012; Prosser, et al., 2011) enabling us to establish a model of stretch activated mechano-transduction in striated muscle. This novel individual muscle fiber device removed the potential confounders of contraction, membrane damaging force (eccentric injury), or non-physiologic experimental stress such as osmotic shock.

Using these new methods coupled with high-speed confocal fluorescence imaging approaches, we revealed that in muscle cells loaded with the ROS indicator dichlorofluorescein (DCF), a brief, acute physiologic stretch elicits a burst of ROS production. As DCF is a non-specific ROS indicator, we used selective inhibitors and genetic approaches to reveal that the microtubule network acted as a mechano-transducer to

activate Nox2 dependent **ROS** generation, a pathway termed **X-ROS** signaling (Khairallah, et al., 2012; Prosser, et al., 2011). In heart cells, others reported that X-ROS directly or indirectly lead to post-translational modification of the RyR2s, increasing the sensitivity of RyR2s to $[Ca^{2+}]_i$ and thus promoting the fidelity of excitation-contraction (EC) coupling. We confirmed many of the features of X-ROS signaling in heart also in skeletal muscle (Khairallah, et al., 2012; Palomero, Pye, Kabayo, & Jackson, 2012), however we demonstrated that the signaling involves a stretch-activated sarcolemmal channel whose opening is enhanced by Nox2-derived ROS. Our ongoing experiments are defining the role of X-ROS in modulating signaling during the contraction cycle. However, we have firmly established that X-ROS is an important pathological component in Duchenne muscular dystrophy, where an increase in microtubule network density and Nox2 expression leads to a detrimental enhancement of X-ROS signaling (Khairallah, et al., 2012).

Targets of ROS/RNS in skeletal muscle

ROS/RNS have been shown to modulate multiple processes in skeletal muscle such as, transcription factor activity, ion transport, apoptosis, metabolism including numerous proteins critical for muscle cell function (R. G. Allen & Tresini, 2000; Barbieri & Sestili, 2012). Within proteins, sulfhydryl groups (–SH) are susceptible to either oxidation or the formation of disulfide bonds. Physiological reduction and oxidation of sulfhydryl moieties take part in the modulation of protein function (Thomas & Mallis, 2001). For example, oxidation, S-nitrosylation (Foster, McMahon, & Stamler, 2003; Sun, Xin, Eu, Stamler, & Meissner, 2001), or S-glutathionylation of key cysteine in the RyR1 calcium release channel of skeletal muscle can significantly alter the mean open time and the permeability of the channel (Aracena-Parks et al., 2006; Hamilton & Reid, 2000; Hidalgo, et al., 2006). In addition to the RyR1, the sarcoendoplasmic reticulum calcium ATPase pump (i.e., SERCA pump) (Lehotsky, Kaplan, Murin, & Raeymaekers, 2002; Squier, 2001; Trebak, Ginnan, Singer, & Jourdain, 2010) membrane lipids (Spiteller, 2010), or DNA (Ragu et al., 2007), CaMKinase (Franklin, Rodriguez-Mora, LaHair, & McCubrey, 2006; Pinto, de Sousa, & Sorenson, 2011) and contractile proteins (Ferreira, et al., 2012; Stasko, Hardin, Smith, Moylan, & Reid, 2013) have all been shown to be modified by ROS/RNS.

ROS has also been shown to act as a second messenger in the stretch dependent activation of the nf-KB pathway (Kumar & Boriek, 2003). This pathway, when in excess, promotes degeneration and atrophy of skeletal muscle (Reid & Li, 2001) across multiple disease states (Ferreira, et al., 2012). In addition, several inflammatory disorders/conditions (i.e., rheumatoid arthritis, heart failure, systemic lupus erythematosus) affect skeletal muscle function and new evidence supports cytokine receptor dependent activation and subsequent intracellular second messenger ROS signaling as a contributor in the functional deficits (See Figure 3).

Finally, ROS has been shown to be a critical modulator in several transcriptional pathways that respond to activity/exercise in muscle. Peroxisome Proliferator-activated Receptor- γ Coactivator-1 α (PGC1- α) is a transcriptional coactivator shown to activate mitochondria biogenesis (Spiegelman, 2007). As mitochondrial deficits contribute to numerous skeletal muscle diseases (diabetes, amyotrophic lateral sclerosis, Duchenne muscular dystrophy) this

pathway is thought to represent a novel therapeutic target (Guevel et al., 2011; Hsieh et al., 2011; Santos, Tewari, Goldberg, & Kowluru, 2011). The regulation of the PGC1- α pathway is in fact redox sensitive, which suggests that ROS plays a role in its regulation (Chen et al., 2011; Spiegelman, 2007). The transcriptional regulators histone deacetylase 4 and 5 (HDAC4, 5) are important regulating muscle plasticity and metabolism with activity. Recently, Nox2 dependent ROS has been shown to regulate the nuclear effluxes of the HDAC4, 5 after intense muscle activity (Liu, Hernandez-Ochoa, Randall, & Schneider, 2012). Finally, nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a transcription factor that regulates the transcription of over 200 genes responsible for antioxidant defenses in cell and antioxidant enzymes. Nrf2 is kept in the cytoplasm by a cluster of proteins that degrade it quickly. Under oxidative stress, Nrf2 is released from the protein cluster and it travels to the nucleus where it initiates transcription of the antioxidative genes and their proteins (Guerrero-Beltran, Calderon-Oliver, Pedraza-Chaverri, & Chirino, 2012; Mann et al., 2007).

Conclusion

Until recently, ROS/RNS have been considered toxic species resulting in cellular oxidative stress and leading to pathogenesis and premature aging (Amicarelli et al., 1999; Bejma & Ji, 1999; Fielding & Meydani, 1997; Lawler, Cline, Hu, & Coast, 1997; Pansarasa, Bertorelli, Vecchiet, Felzani, & Marzatico, 1999; Reid & Durham, 2002; Vasilaki et al., 2006). We now however understand that ROS/RNS are also important signaling molecules, which regulate physiological processes (Barbieri & Sestili, 2012). This apparent dichotomy in the role of ROS/RNS in skeletal muscle is a focus of much investigation.

It is reasonable to assume that the concentration of ROS/RNS distinguishes between physiological and pathological signaling (Barbieri & Sestili, 2012). In support, generalized ROS scavenging has been shown to be deleterious to acute exercise adaptation in healthy muscle (Petersen et al., 2012; Strobel et al., 2011) showing that a basal level of ROS signaling is important for positive adaptation. This same general ROS reduction strategies are also ineffective as therapeutic strategies in neuromuscular diseases (i.e., idebenone (Buyse et al., 2011), Coenzyme Q₁₀ (Spurney et al., 2011), tocopherol (Arthur, Austin, & Roberts, 1988)) where excess ROS is known to be a disease contributor. In these cases, the source and the species of ROS are likely disease specific and in this case, a targeted approach for therapy is likely needed.

Our understanding of ROS/RNS signaling is moving rapidly. This brief review of ROS/RNS chemistry and concepts of ROS/RNS signaling will hopefully spur the reader to explore this topic further in the reviews and primary literature cited here. We expect that this knowledge will rapidly make its way into clinical practice as novel treatments are developed to target diseases and disorders by ROS/RNS dependent mechanisms.

References

- Allen DG, Gervasio OL, Yeung EW, Whitehead NP. Calcium and the damage pathways in muscular dystrophy. *Can J Physiol Pharmacol*. 2010; 88(2):83–91. doi: y09-058 [pii] 10.1139/Y09-058. [PubMed: 20237582]

- Allen RG, Tresini M. Oxidative stress and gene regulation. *Free Radic Biol Med*. 2000; 28(3):463–499. doi: S0891-5849(99)00242-7 [pii]. [PubMed: 10699758]
- Amicarelli F, Ragnelli AM, Aimola P, Bonfigli A, Colafarina S, Di Ilio C. Age-dependent ultrastructural alterations and biochemical response of rat skeletal muscle after hypoxic or hyperoxic treatments. [Comparative Study Research Support, Non-U.S. Gov't]. *Biochim Biophys Acta*. 1999; 1453(1):105–114. [PubMed: 9989250]
- Andrade FH, Reid MB, Westerblad H. Contractile response of skeletal muscle to low peroxide concentrations: myofibrillar calcium sensitivity as a likely target for redox-modulation. *FASEB J*. 2001; 15(2):309–311. [pii]. [PubMed: 11156946]
- Aracena-Parks P, Goonasekera SA, Gilman CP, Dirksen RT, Hidalgo C, Hamilton SL. Identification of cysteines involved in S-nitrosylation, S-glutathionylation, and oxidation to disulfides in ryanodine receptor type 1. *J Biol Chem*. 2006; 281(52):40354–40368. doi: M600876200 [pii] 10.1074/jbc.M600876200. [PubMed: 17071618]
- Arbiser J, Petros J, Klafter R, Govindajaran B, McLaughlin E, Brown L. Reactive oxygen generated by Nox1 triggers the angiogenic switch. (0027-8424 (Print)).
- Arthur H, Austin L, Roberts LJ. A pilot trial of plasma infusions in Duchenne muscular dystrophy. *Aust Paediatr J*. 1988; 24(Suppl 1):24–30. [PubMed: 3202736]
- Austin S, Klimcakova E, St-Pierre J. Impact of PGC-1 α on the topology and rate of superoxide production by the mitochondrial electron transport chain. *Free Radical Biology and Medicine*. 2011; 51(12):2243–2248. doi: <http://dx.doi.org/10.1016/j.freeradbiomed.2011.08.036>. [PubMed: 21964033]
- Barbieri E, Sestili P. Reactive oxygen species in skeletal muscle signaling. *J Signal Transduct*. 2012; 2012:982794. [PubMed: 22175016]
- Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev*. 2007; 87(1):245–313. doi: 87/1/245 [pii] 10.1152/physrev.00044.2005. [PubMed: 17237347]
- Bejma J, Ji LL. Aging and acute exercise enhance free radical generation in rat skeletal muscle. *J Appl Physiol*. 1999; 87(1):465–470. [PubMed: 10409609]
- Brown DI, Griendling KK. Nox proteins in signal transduction. *Free Radic Biol Med*. 2009; 47(9):1239–1253. doi: 10.1016/j.freeradbiomed.2009.07.023 S0891-5849(09)00442-0 [pii]. [PubMed: 19628035]
- Buyse GM, Goemans N, van den Hauwe M, Thijs D, de Groot IJ, Schara U. Idebeneone as a novel, therapeutic approach for Duchenne muscular dystrophy: results from a 12 month, double-blind, randomized placebo-controlled trial. *Neuromuscul Disord*. 2011; 21(6):396–405. doi: S0960-8966(11)00061-7 [pii] 10.1016/j.nmd.2011.02.016. [PubMed: 21435876]
- Cadenas, E.; Packer, L. *Handbook of antioxidants*. 2nd. New York: Marcel Dekker; 2002.
- Calaghan SC, Belus A, White E. Do stretch-induced changes in intracellular calcium modify the electrical activity of cardiac muscle? *Prog Biophys Mol Biol*. 2003; 82(1–3):81–95. doi: S0079610703000075 [pii]. [PubMed: 12732270]
- Calaghan SC, Le Guennec JY, White E. Cytoskeletal modulation of electrical and mechanical activity in cardiac myocytes. *Prog Biophys Mol Biol*. 2004; 84(1):29–59. doi: S0079610703000579 [pii]. [PubMed: 14642867]
- Chambers MA, Moylan JS, Reid MB. Physical inactivity and muscle weakness in the critically ill. *Crit Care Med*. 2009; 37(10 Suppl):S337–S346. doi: 10.1097/CCM.0b013e3181b6e974 00003246-200910001-00008 [pii]. [PubMed: 20046119]
- Chambers MA, Moylan JS, Smith JD, Goodyear LJ, Reid MB. Stretch-stimulated glucose uptake in skeletal muscle is mediated by reactive oxygen species and p38 MAP-kinase. [In Vitro Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *J Physiol*. 2009; 587(Pt 13):3363–3373. [PubMed: 19403598]
- Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. *Physiol Rev*. 1979; 59(3):527–605. [PubMed: 37532]
- Chen SD, Yang DI, Lin TK, Shaw FZ, Liou CW, Chuang YC. Roles of Oxidative Stress, Apoptosis, PGC-1 α and Mitochondrial Biogenesis in Cerebral Ischemia. *Int J Mol Sci*. 2011; 12(10):7199–7215. [PubMed: 22072942]

- Chowdhury AK, Watkins T, Parinandi NL, Saatian B, Kleinberg ME, Usatyuk PV. Src-mediated tyrosine phosphorylation of p47phox in hyperoxia-induced activation of NADPH oxidase and generation of reactive oxygen species in lung endothelial cells. *J Biol Chem*. 2005; 280(21): 20700–20711. doi: M411722200 [pii] 10.1074/jbc.M411722200. [PubMed: 15774483]
- Corte ED, Stirpe F. The regulation of rat liver xanthine oxidase. Involvement of thiol groups in the conversion of the enzyme activity from dehydrogenase (type D) into oxidase (type O) and purification of the enzyme. *Biochem J*. 1972; 126(3):739–745. [PubMed: 4342395]
- Davidovich N, Dipaolo BC, Lawrence GG, Chhour P, Yehya N, Margulies SS. Cyclic Stretch-Induced Oxidative Stress Increases Pulmonary Alveolar Epithelial Permeability. *Am J Respir Cell Mol Biol*. 2013
- Donoso P, Sanchez G, Bull R, Hidalgo C. Modulation of cardiac ryanodine receptor activity by ROS and RNS. *Front Biosci*. 2011; 16:553–567. doi: 3705 [pii].
- Espinosa A, Leiva A, Pena M, Muller M, Debandi A, Hidalgo C. Myotube depolarization generates reactive oxygen species through NAD(P)H oxidase; ROSelicited Ca²⁺ stimulates ERK, CREB, early genes. *J Cell Physiol*. 2006; 209(2):379–388. [PubMed: 16897752]
- Faist V, Konig J, Hoger H, Elmadfa I. Decreased mitochondrial oxygen consumption and antioxidant enzyme activities in skeletal muscle of dystrophic mice after low-intensity exercise. *Ann Nutr Metab*. 2001; 45(2):58–66. [PubMed: 11359030]
- Ferreira LF, Moylan JS, Stasko S, Smith JD, Campbell KS, Reid MB. Sphingomyelinase depresses force and calcium sensitivity of the contractile apparatus in mouse diaphragm muscle fibers. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *J Appl Physiol*. 2012; 112(9):1538–1545. [PubMed: 22362402]
- Ferreira LF, Reid MB. Muscle-derived ROS and thiol regulation in muscle fatigue. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Review]. *J Appl Physiol*. 2008; 104(3): 853–860. [PubMed: 18006866]
- Fielding RA, Meydani M. Exercise, free radical generation, and aging. [Research Support, U.S. Gov't, Non-P.H.S. Review]. *Aging (Milano)*. 1997; 9(1–2):12–18. [PubMed: 9177582]
- Fisher AB. Redox signaling across cell membranes. *Antioxid Redox Signal*. 2009; 11(6):1349–1356. [PubMed: 19061438]
- Foster MW, McMahon TJ, Stamler JS. S-nitrosylation in health and disease. *Trends in Molecular Medicine*. 2003; 9(4):160–168. doi: [http://dx.doi.org/10.1016/S1471-4914\(03\)00028-5](http://dx.doi.org/10.1016/S1471-4914(03)00028-5). [PubMed: 12727142]
- Franklin RA, Rodriguez-Mora OG, LaHair MM, McCubrey JA. Activation of the Calcium/Calmodulin-Dependent Protein Kinases as a Consequence of Oxidative Stress. *Antioxidants & Redox Signaling*. 2006; 8(9–10):1807–1817. [PubMed: 16987033]
- Gannier F, White E, Lacampagne A, Garnier D, Le Guennec JY. Streptomycin reverses a large stretch induced increases in [Ca²⁺]_i in isolated guinea pig ventricular myocytes. *Cardiovasc Res*. 1994; 28(8):1193–1198. [PubMed: 7954622]
- Gervasio OL, Whitehead NP, Yeung EW, Phillips WD, Allen DG. TRPC1 binds to caveolin-3 and is regulated by Src kinase - role in Duchenne muscular dystrophy. *J Cell Sci*. 2008; 121(Pt 13):2246–2255. doi: jcs.032003 [pii] 10.1242/jcs.032003. [PubMed: 18544631]
- Gomez-Cabrera M-C, Domenech E, Viña J. Moderate exercise is an antioxidant: Upregulation of antioxidant genes by training. *Free Radical Biology and Medicine*. 2008; 44(2):126–131. doi: <http://dx.doi.org/10.1016/j.freeradbiomed.2007.02.001>. [PubMed: 18191748]
- Gomez-Cabrera MC, Borrás C, Pallardo FV, Sastre J, Ji LL, Vina J. Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. *J Physiol*. 2005; 567(Pt 1):113–120. doi: jphysiol.2004.080564 [pii] 10.1113/jphysiol.2004.080564. [PubMed: 15932896]
- Gomez-Cabrera MC, Pallardo FV, Sastre J, Vina J, Garcia-del-Moral L. Allopurinol and markers of muscle damage among participants in the Tour de France. *JAMA*. 2003; 289(19):2503–2504. 289/19/2503 [pii]. [PubMed: 12759321]
- Guerrero-Beltran CE, Calderon-Oliver M, Pedraza-Chaverri J, Chirino YI. Protective effect of sulforaphane against oxidative stress: recent advances. *Exp Toxicol Pathol*. 2012; 64(5):503–508. doi: S0940-2993(10)00183-1 [pii] 10.1016/j.etp.2010.11.005. [PubMed: 21129940]

- Guevel L, Lavoie JR, Perez-Iratxeta C, Rouger K, Dubreil L, Feron M. Quantitative proteomic analysis of dystrophic dog muscle. [Research Support, Non-U.S. Gov't]. *J Proteome Res.* 2011; 10(5): 2465–2478. [PubMed: 21410286]
- Halliwell, B.; Gutteridge, JMC. *Free Radicals in Biology and Medicine*. 4th. New York: Oxford University Press; 2007.
- Hamilton SL, Reid MB. RyR1 modulation by oxidation and calmodulin. *Antioxid Redox Signal.* 2000; 2(1):41–45. [PubMed: 11232598]
- Harris, RE., editor. *Inflammation in the Pathogenesis of Chronic Diseases*. Vol. 42. New York: Springer; 2007.
- Harrison R. Structure and function of xanthine oxidoreductase: where are we now? *Free Radical Biology and Medicine.* 2002; 33(6):774–797. doi: [http://dx.doi.org/10.1016/S0891-5849\(02\)00956-5](http://dx.doi.org/10.1016/S0891-5849(02)00956-5). [PubMed: 12208366]
- Hidalgo C, Sanchez G, Barrientos G, Aracena-Parks P. A transverse tubule NADPH oxidase activity stimulates calcium release from isolated triads via ryanodine receptor type 1 S-glutathionylation. *J Biol Chem.* 2006; 281(36):26473–26482. [PubMed: 16762927]
- Hohn DC, Lehrer RI. NADPH oxidase deficiency in X-linked chronic granulomatous disease. *J Clin Invest.* 1975; 55(4):707–713. [PubMed: 235560]
- Houston M, Estevez A, Chumley P, Aslan M, Marklund S, Parks DA. Binding of xanthine oxidase to vascular endothelium. Kinetic characterization and oxidative impairment of nitric oxide-dependent signaling. *J Biol Chem.* 1999; 274(8):4985–4994. [PubMed: 9988743]
- Hsieh CJ, Weng SW, Liou CW, Lin TK, Chen JB, Tiao MM. Tissue-specific differences in mitochondrial DNA content in type 2 diabetes. [Research Support, Non-U.S. Gov't]. *Diabetes Res Clin Pract.* 2011; 92(1):106–110. [PubMed: 21281975]
- Inger DE. Tensegrity and mechanotransduction. *J Bodyw Mov Ther.* 2008; 12(3):198–200. doi: S1360-8592(08)00078-8 [pii] 10.1016/j.jbmt.2008.04.038. [PubMed: 19083675]
- Iribe G, Ward CW, Camelliti P, Bollensdorff C, Mason F, Burton RA. Axial stretch of rat single ventricular cardiomyocytes causes an acute and transient increase in Ca²⁺ spark rate. *Circ Res.* 2009; 104(6):787–795. doi: CIRCRESAHA.108.193334 [pii] 10.1161/CIRCRESAHA.108.193334. [PubMed: 19197074]
- Isaeva EV, Shkryl VM, Shirokova N. Mitochondrial redox state and Ca²⁺ sparks in permeabilized mammalian skeletal muscle. *J Physiol.* 2005; 565(Pt 3):855–872. [PubMed: 15845582]
- Jackson MJ, McArdle A. Age-related changes in skeletal muscle reactive oxygen species generation and adaptive responses to reactive oxygen species. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. *J Physiol.* 2011; 589(Pt 9):2139–2145. [PubMed: 21320885]
- Javesghani D, Magder SA, Barreiro E, Quinn MT, Hussain SN. Molecular characterization of a superoxide-generating NAD(P)H oxidase in the ventilatory muscles. *Am J Respir Crit Care Med.* 2002; 165(3):412–418. [PubMed: 11818330]
- Judge DP, Kass DA, Thompson WR, Wagner KR. Pathophysiology and therapy of cardiac dysfunction in duchenne muscular dystrophy. *Am J Cardiovasc Drugs.* 2011; 11(5):287–294. [PubMed: 21812510]
- Kang C, Li Ji L. Role of PGC-1 α signaling in skeletal muscle health and disease. *Annals of the New York Academy of Sciences.* 2012; 1271(1):110–117. [PubMed: 23050972]
- Kass DA, Takimoto E, Nagayama T, Champion HC. Phosphodiesterase regulation of nitric oxide signaling. *Cardiovasc Res.* 2007; 75(2):303–314. doi: S0008-6363(07)00099-5 [pii] 10.1016/j.cardiores.2007.02.031. [PubMed: 17467673]
- Kavazis AN, Talbert EE, Smuder AJ, Hudson MB, Nelson WB, Powers SK. Mechanical ventilation induces diaphragmatic mitochondrial dysfunction and increased oxidant production. [Research Support, N.I.H., Extramural]. *Free Radic Biol Med.* 2009; 46(6):842–850. [PubMed: 19185055]
- Khairallah RJ, Shi G, Sbrana F, Prosser BL, Borroto C, Mazaitis MJ. Microtubules underlie dysfunction in duchenne muscular dystrophy. *Sci Signal.* 2012; 5(236):ra56. doi: 5/236/ra56 [pii] 10.1126/scisignal.2002829. [PubMed: 22871609]

- Kumar A, Boriak AM. Mechanical stress activates the nuclear factor-kappaB pathway in skeletal muscle fibers: a possible role in Duchenne muscular dystrophy. *FASEB J*. 2003; 17(3):386–396. 17/3/386 [pii]. [PubMed: 12631578]
- Kuster GM, Hauselmann SP, Rosc-Schluter BI, Lorenz V, Pfister O. Reactive oxygen/nitrogen species and the myocardial cell homeostasis: an ambiguous relationship. *Antioxid Redox Signal*. 2010; 13(12):1899–1910. [PubMed: 20698753]
- Lamb GD, Westerblad H. Acute effects of reactive oxygen and nitrogen species on the contractile function of skeletal muscle. *J Physiol*. 2011; 589(Pt 9):2119–2127. doi: jphysiol.2010.199059 [pii] 10.1113/jphysiol.2010.199059. [PubMed: 21041533]
- Lawler JM, Cline CC, Hu Z, Coast JR. Effect of oxidant challenge on contractile function of the aging rat diaphragm. [Research Support, Non-U.S. Gov't]. *Am J Physiol*. 1997; 272(2 Pt 1):E201–E207. [PubMed: 9124323]
- Le Guennec JY, White E, Gannier F, Argibay JA, Garnier D. Stretch-induced increase of resting intracellular calcium concentration in single guinea-pig ventricular myocytes. *Exp Physiol*. 1991; 76(6):975–978. [PubMed: 1768419]
- Lehotsky J, Kaplan P, Murin R, Raeymaekers L. The role of plasma membrane Ca²⁺ pumps (PMCA) in pathologies of mammalian cells. *Front Biosci*. 2002; 7:d53–d84. [PubMed: 11779702]
- Levine ME. The Conservation Principles: A Retrospective. *Nursing Science Quarterly*. 1996; 9(1):38–41. [PubMed: 8710302]
- Liu Y, Hernandez-Ochoa EO, Randall WR, Schneider MF. NOX2-dependent ROS is required for HDAC5 nuclear efflux and contributes to HDAC4 nuclear efflux during intense repetitive activity of fast skeletal muscle fibers. *Am J Physiol Cell Physiol*. 2012; 303(3):C334–C347. doi: ajpcell.00152.2012 [pii] 10.1152/ajpcell.00152.2012. [PubMed: 22648949]
- Mann GE, Niehueser-Saran J, Watson A, Gao L, Ishii T, de Winter P. Nrf2/ARE regulated antioxidant gene expression in endothelial and smooth muscle cells in oxidative stress: implications for atherosclerosis and preeclampsia. *Sheng Li Xue Bao*. 2007; 59(2):117–127. [PubMed: 17437032]
- Martins AS, Shkryl VM, Nowycky MC, Shirokova N. Reactive oxygen species contribute to Ca²⁺ signals produced by osmotic stress in mouse skeletal muscle fibres. *J Physiol*. 2008; 586(1):197–210. [PubMed: 17974587]
- McNally JS, Saxena A, Cai H, Dikalov S, Harrison DG. Regulation of Xanthine Oxidoreductase Protein Expression by Hydrogen Peroxide and Calcium. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2005; 25(8):1623–1628.
- Meleis, AI. *Theoretical Nursing: Development & Progress*. 5th. Philadelphia: Wolters Kluwer, Lippincott Williams & Wilkins; 2012.
- Michaelson LP, Shi G, Ward CW, Rodney GG. Mitochondrial redox potential during contraction in single intact muscle fibers. *Muscle Nerve*. 2010; 42(4):522–529. [PubMed: 20730875]
- Murakami M, Kudo I. Phospholipase A2. *J Biochem*. 2002; 131(3):285–292. [PubMed: 11872155]
- Murdoch CE, Zhang M, Cave AC, Shah AM. NADPH oxidase-dependent redox signalling in cardiac hypertrophy, remodelling and failure. [Research Support, Non-U.S. Gov't Review]. *Cardiovasc Res*. 2006; 71(2):208–215. [PubMed: 16631149]
- Nethery D, Callahan LA, Stofan D, Mattera R, DiMarco A, Supinski G. PLA2 dependence of diaphragm mitochondrial formation of reactive oxygen species. *Journal of Applied Physiology*. 2000; 89(1):72–80. [PubMed: 10904037]
- Nethery D, Stofan D, Callahan L, DiMarco A, Supinski G. Formation of reactive oxygen species by the contracting diaphragm is PLA(2) dependent. *J Appl Physiol*. 1999; 87(2):792–800. [PubMed: 10444641]
- Palomero J, Pye D, Kabayo T, Jackson MJ. Effect of passive stretch on intracellular nitric oxide and superoxide activities in single skeletal muscle fibres: influence of ageing. *Free Radic Res*. 2012; 46(1):30–40. [PubMed: 22103935]
- Pansarasa O, Bertorelli L, Vecchiet J, Felzani G, Marzatico F. Age-dependent changes of antioxidant activities and markers of free radical damage in human skeletal muscle. *Free Radic Biol Med*. 1999; 27(5–6):617–622. [PubMed: 10490283]
- Petersen AC, McKenna MJ, Medved I, Murphy KT, Brown MJ, Della Gatta P. Infusion with the antioxidant N-acetylcysteine attenuates early adaptive responses to exercise in human skeletal

- muscle. [Randomized Controlled Trial]. *Acta Physiol (Oxf)*. 2012; 204(3):382–392. [PubMed: 21827635]
- Pinto JR, de Sousa VP, Sorenson MM. Redox state of Troponin C Cysteine in the D/E helix alters the C-domain affinity for the thin filament of vertebrate striated muscle. *Biochimica et Biophysica Acta (BBA) - General Subjects*. 2011; 1810(4):391–397. [PubMed: 21145939]
- Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev*. 2008; 88(4):1243–1276. doi: 88/4/1243 [pii]10.1152/physrev.00031.2007. [PubMed: 18923182]
- Powers SK, Smuder AJ, Judge AR. Oxidative stress and disuse muscle atrophy: cause or consequence? *Curr Opin Clin Nutr Metab Care*. 2012; 15(3):240–245. doi: 10.1097/MCO.0b013e328352b4c2. [PubMed: 22466926]
- Powers SK, Talbert EE, Adhietty PJ. Reactive oxygen and nitrogen species as intracellular signals in skeletal muscle. *J Physiol*. 2011; 589(Pt 9):2129–2138. doi: jphysiol.2010.201327 [pii]10.1113/jphysiol.2010.201327. [PubMed: 21224240]
- Prosser BL, Khairallah RJ, Ziman AP, Ward CW, Lederer WJ. X-ROS signaling in the heart and skeletal muscle: Stretch-dependent local ROS regulates $[Ca^{2+}]_i$. *J Mol Cell Cardiol*. 2012
- Prosser BL, Ward CW, Lederer WJ. X-ROS signaling: rapid mechano-chemo transduction in heart. *Science*. 2011; 333(6048):1440–1445. doi: 333/6048/1440 [pii] 10.1126/science.1202768. [PubMed: 21903813]
- Ragu S, Faye G, Iraqui I, Masurel-Heneman A, Kolodner RD, Huang ME. Oxygen metabolism and reactive oxygen species cause chromosomal rearrangements and cell death. *Proc Natl Acad Sci U S A*. 2007; 104(23):9747–9752. doi: 0703192104 [pii]10.1073/pnas.0703192104. [PubMed: 17535927]
- Reid MB. Invited Review: redox modulation of skeletal muscle contraction: what we know and what we don't. [Research Support, U.S. Gov't, P.H.S. Review]. *J Appl Physiol*. 2001; 90(2):724–731. [PubMed: 11160074]
- Reid MB, Durham WJ. Generation of reactive oxygen and nitrogen species in contracting skeletal muscle: potential impact on aging. [Research Support, U.S. Gov't, P.H.S. Review]. *Ann N Y Acad Sci*. 2002; 959:108–116. [PubMed: 11976190]
- Reid MB, Li YP. Tumor necrosis factor- α and muscle wasting: a cellular perspective. *Respir Res*. 2001; 2(5):269–272. [PubMed: 11686894]
- Reid MB, Moylan JS. Beyond atrophy: redox mechanisms of muscle dysfunction in chronic inflammatory disease. *J Physiol*. 2011; 589(Pt 9):2171–2179. [PubMed: 21320886]
- Ren Z, Raucci FJ Jr, Browe DM, Baumgarten CM. Regulation of swelling-activated Cl^- current by angiotensin II signalling and NADPH oxidase in rabbit ventricle. *Cardiovasc Res*. 2008; 77(1):73–80. doi: cvm031 [pii] 10.1093/cvr/cvm031. [PubMed: 18006461]
- Sakellariou G, Vasilaki A, Palomero J, Kayani A, Zibrik L, McArdle A. Studies of mitochondrial and non-mitochondrial sources implicate NADPH oxidase(s) in the increased skeletal muscle superoxide generation that occurs during contractile activity. *Antioxid Redox Signal*. 2012
- Santos JM, Tewari S, Goldberg AF, Kowluru RA. Mitochondrial biogenesis and the development of diabetic retinopathy. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Free Radic Biol Med*. 2011; 51(10):1849–1860. [PubMed: 21911054]
- Sauer H, Klimm B, Hescheler J, Wartenberg M. Activation of p90RSK and growth stimulation of multicellular tumor spheroids are dependent on reactive oxygen species generated after purinergic receptor stimulation by ATP. *The FASEB Journal*. 2001
- Shkryl VM, Martins AS, Ullrich ND, Nowycky MC, Niggli E, Shirokova N. Reciprocal amplification of ROS and Ca^{2+} signals in stressed mdx dystrophic skeletal muscle fibers. *Pflugers Arch*. 2009; 458(5):915–928. [PubMed: 19387681]
- Sigala I, Zacharatos P, Toumpanakis D, Michailidou T, Noussia O, Theocharis S. MAPKs and NF- κ B differentially regulate cytokine expression in the diaphragm in response to resistive breathing: the role of oxidative stress. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*. 2011; 300(5):R1152–R1162.

- Spiegelman BM. Transcriptional control of mitochondrial energy metabolism through the PGC1 coactivators. [Review]. *Novartis Found Symp.* 2007; 287:60–63. discussion 63–69. [PubMed: 18074631]
- Spiteller G. Is lipid peroxidation of polyunsaturated acids the only source of free radicals that induce aging and age-related diseases? *Rejuvenation Res.* 2010; 13(1):91–103. [PubMed: 20230283]
- Spurney CF, Rocha CT, Henricson E, Florence J, Mayhew J, Gorni K. CINRG pilot trial of coenzyme Q10 in steroid-treated Duchenne muscular dystrophy. *Muscle Nerve.* 2011; 44(2):174–178. [PubMed: 21698649]
- Squier TC. Oxidative stress and protein aggregation during biological aging. *Exp Gerontol.* 2001; 36(9):1539–1550. doi: S0531-5565(01)00139-5 [pii]. [PubMed: 11525876]
- Stamenovic D, Mijailovich SM, Tolic-Norrelykke IM, Chen J, Wang N. Cell prestress. II. Contribution of microtubules. *Am J Physiol Cell Physiol.* 2002; 282(3):C617–C624. [PubMed: 11832347]
- Stasko SA, Hardin BJ, Smith JD, Moylan JS, Reid MB. TNF Signals via Neuronal-Type Nitric Oxide Synthase and Reactive Oxygen Species to Depress Specific Force of Skeletal Muscle. *J Appl Physiol.* 2013 doi: japplphysiol.00871.2012 [pii]10.1152/japplphysiol.00871.2012.
- Strobel NA, Peake JM, Matsumoto A, Marsh SA, Coombes JS, Wadley GD. Antioxidant supplementation reduces skeletal muscle mitochondrial biogenesis. [Research Support, Non-U.S. Gov't]. *Med Sci Sports Exerc.* 2011; 43(6):1017–1024. [PubMed: 21085043]
- Sun J, Xin C, Eu JP, Stamler JS, Meissner G. Cysteine-3635 is responsible for skeletal muscle ryanodine receptor modulation by NO. *Proc Natl Acad Sci U S A.* 2001; 98(20):11158–11162. [PubMed: 11562475]
- Supinski GS, Callahan LA. Free radical-mediated skeletal muscle dysfunction in inflammatory conditions. *J Appl Physiol.* 2007; 102(5):2056–2063. doi: 01138.2006 [pii]10.1152/japplphysiol.01138.2006. [PubMed: 17218425]
- Thomas JA, Mallis RJ. Aging and oxidation of reactive protein sulfhydryls. *Exp Gerontol.* 2001; 36(9):1519–1526. doi: S0531-5565(01)00137-1 [pii]. [PubMed: 11525874]
- Trebak M, Ginnan R, Singer HA, Jourdain D. Interplay between calcium and reactive oxygen/nitrogen species: an essential paradigm for vascular smooth muscle signaling. *Antioxid Redox Signal.* 2010; 12(5):657–674. [PubMed: 19719386]
- Ullrich ND, Fanchaouy M, Gusev K, Shirokova N, Niggli E. Hypersensitivity of excitation-contraction coupling in dystrophic cardiomyocytes. *Am J Physiol Heart Circ Physiol.* 2009; 297(6):H1992–H2003. doi: 00602.2009 [pii] 10.1152/ajpheart.00602.2009. [PubMed: 19783774]
- Vasilaki A, Mansouri A, Remmen H, van der Meulen JH, Larkin L, Richardson AG. Free radical generation by skeletal muscle of adult and old mice: effect of contractile activity. [In Vitro Research Support, N.I.H., Extramural]. *Aging Cell.* 2006; 5(2):109–117. [PubMed: 16626390]
- Vokurkova M, Xu S, Touyz RM. Reactive oxygen species, cell growth, cell cycle progression and vascular remodeling in hypertension. *Future Cardiol.* 2007; 3(1):53–63. [PubMed: 19804207]
- Wang HJ, Pan YX, Wang WZ, Zucker IH, Wang W. NADPH oxidase-derived reactive oxygen species in skeletal muscle modulates the exercise pressor reflex. *J Appl Physiol.* 2009; 107(2):450–459. [PubMed: 19498097]
- Wang N, Butler JP, Ingber DE. Mechanotransduction across the cell surface and through the cytoskeleton. *Science.* 1993; 260(5111):1124–1127. [PubMed: 7684161]
- Wang N, Naruse K, Stamenovic D, Fredberg JJ, Mijailovich SM, Tolic-Norrelykke IM. Mechanical behavior in living cells consistent with the tensegrity model. *Proc Natl Acad Sci U S A.* 2001; 98(14):7765–7770. [PubMed: 11438729]
- Whitehead NP, Streamer M, Lusambili LI, Sachs F, Allen DG. Streptomycin reduces stretch-induced membrane permeability in muscles from mdx mice. *Neuromuscul Disord.* 2006; 16(12):845–854. [PubMed: 17005404]
- Whitehead NP, Yeung EW, Allen DG. Muscle damage in mdx (dystrophic) mice: role of calcium and reactive oxygen species. *Clin Exp Pharmacol Physiol.* 2006; 33(7):657–662. [PubMed: 16789936]
- Xu S, Touyz RM. Reactive oxygen species and vascular remodelling in hypertension: still alive. *Can J Cardiol.* 2006; 22(11):947–951. [PubMed: 16971980]

Figure 1. Model depicting the effects of the cellular oxidation state on muscle force production
A: basal state from unfatigued muscle. B: unfatigued muscle exposed to reducing agent or antioxidant. C: unfatigued muscle exposed to low-level of ROS. D: Muscle exposed to excess ROS as seen in fatiguing exercise or disease. The dashed vertical lines denote basal redox state (without an additional ROS stimulus) and the optimal redox state (i.e., maximal force). Used with permission (Reid, 2001)

Figure 2. Potential sites for the production of superoxide and nitric oxide in skeletal muscle
 Used with permission (Powers & Jackson, 2008)

Figure 3. Model of signaling events that mediate inflammation induced skeletal muscle weakness in heart failure

Model shows hypothetical mechanism by which chronic heart failure could depress specific force of skeletal muscle. Boxes below the sarcolemma (horizontal band) depict intracellular events that regulate TNFR1/oxidant signaling (right edge, red dashed bar) and oxidative inhibition of myofilament function (green hatched bar). Boxes above sarcolemma represent extracellular events that modulate the process (blue bar). TNF, tumor necrosis factor; TNFR1, TNF receptor subtype 1; SMase, sphingomyelinase; PKC, phosphokinase C; ERK,

extracellular regulatory kinase; PLA2, phospholipase A2; NO, nitric oxide. Reproduced with permission (Reid & Moylan, 2011)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

ROS/RNS

ROS	
Species	Effector
$O_2^{\cdot -}$	JNK, base radical for H_2O_2 , $OH^{\cdot}$, $ONOO^-$
H_2O_2	nf- $\kappa\beta$, c-Src, I $\kappa\beta$, TGF- β , RyR1, PKC- α
$OH^{\cdot}$	<i>c-jun</i>
RNS	
$NO^{\cdot}$	RyR1, guanylate cyclase, albumin, hemoglobin
$ONOO^-$	mitochondrial enzymes (aconitase), RyR1, COX