Chapter 13
Redox Homeostasis in Age-Related Muscle Atrophy

Giorgos K. Sakellariou and Brian McDonagh

Abstract Muscle atrophy and weakness, characterized by loss of lean muscle mass and function, has a significant effect on the independence and quality of life of older people. The cellular mechanisms that drive the age-related decline in neuromuscular integrity and function are multifactorial. Quiescent and contracting skeletal muscle can endogenously generate reactive oxygen and nitrogen species (RONS) from various cellular sites. Excessive RONS can potentially cause oxidative damage and disruption of cellular signaling pathways contributing to the initiation and progression of age-related muscle atrophy. Altered redox homeostasis and modulation of intracellular signal transduction processes have been proposed as an underlying mechanism of sarcopenia. This chapter summarizes the current evidence that has associated disrupted redox homeostasis and muscle atrophy as a result of skeletal muscle inactivity and aging.

Keywords Sarcopenia · Redox signaling · Antioxidants · Nerve · Superoxide

13.1 Background

Loss of skeletal muscle mass and function is among the most consistent and striking change associated with the advance of age [1]. Age-related muscle atrophy (sarcopenia) is described as a progressive loss of lean muscle mass and muscle function, which has a significant effect on the quality of life of older people and overall morbidity. A reduction in overall muscle function with age is linked to an increased mortality risk [2], which leads to instability, a subsequent increased risk of falls and consequently an increased demand for medical and social care. Deficits in skeletal muscle begin at a relatively young age and continue until the end of life [3]; human studies have reported that by the age of 70, there is a 25–30% reduction in the fiber

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cross-sectional area of skeletal muscle and a subsequent reduction in muscle strength by 30–40% [4].

Reduced muscle mass and contractile force inherent with aging have been extensively studied in both murine models and humans and are associated with various neurological impairments including loss of motor units [5, 6], structural alterations and degeneration of neuromuscular junctions (NMJ) [7–10], a decline in motor nerve function (partial denervation) [9, 11–13], impaired nerve redox signaling [14], and changes in fiber type related to continual cycles of denervation and reinnervation [15]. While physical activity can inhibit the decline of muscle functional deficits [16], even physically active older adults exhibit age-related deficits in muscle mass and function [17]. Age-related muscle atrophy and weakness is a lifelong process with a multifactorial and complex etiology that involves both extrinsic and intrinsic factors [15]. However, elucidation of the primary molecular and biochemical mechanisms underlying the age-related decline in neuromuscular integrity and function has yet to be determined.

13.2 Reactive Oxygen and Nitrogen Species (RONS) Produced by Skeletal Muscle

The cellular damage induced by O_2 toxicity was first reported more than 50 years ago and related to the increased generation of reactive species [18, 19], as a result of derivatives of O_2 (Fig. 13.1). Studies in the 1980s reported that reactive species are endogenously generated in skeletal muscle [20–22]. It has since been determined that both resting and contracting myofibers can generate reactive oxygen and nitrogen species (RONS). Reactive oxygen species (ROS) refer to O_2-derived molecules that are reactive species including O_2-centered radicals but also non-radical species which are reactive derivatives of O_2 [23]. Similarly, the term reactive nitrogen species (RNS) refers to both nitrogen radicals along with other reactive molecules where the reactive center is nitrogen [24–26]. RONS generation by skeletal muscle has been detected and quantified by a variety of methods including fluorescence-based

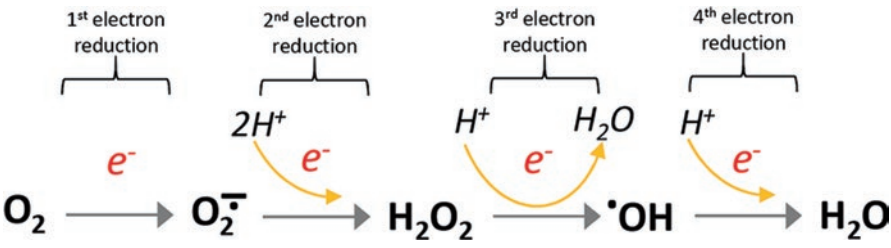

Fig. 13.1 Reactive oxygen derivatives produced by the sequential reduction of $O_2$to H_2O. Superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$). (Redrawn from Sakellariou et al. [88])

microscopic assays [27, 28], spectrophotometry [29, 30], chemiluminescence [31, 32], HPLC techniques [33, 34], electron spin resonance spectroscopy (also known as electron paramagnetic resonance, EPR) [35, 36], and transfection methods including in vivo [37, 38] and in vitro [39]. Using a combination of the above techniques, it has been determined that the primary radical species generated by skeletal muscle include superoxide and nitric oxide (NO) [26, 40, 41].

13.2.1 Superoxide

Superoxide is derived either from the incomplete reduction of O_2 during metabolism in the electron transport chain (ETC) or as a specific product of dedicated enzymatic systems [42]. The subcellular location of superoxide generation in skeletal muscle is dependent on whether the muscle is quiescent or contracting, as different pathways are involved. Figures 13.2 and 13.3 depict the different sites within skeletal muscle and proposed reactions for RONS generation. Superoxide generation is associated with electron leakage and incomplete O_2 metabolism by mitochondrial ETC including complex I and complex III [43, 44] but also more recently complex II [45–47]. However, dedicated enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzymes including NOX2, NOX4, DUOX1, and DUOX2 [28, 29, 32, 48], xanthine oxidase (XO) [49, 50], and the lipoxygenases (LOXs) [51] which are linked to arachidonic acid (AA) release by the phospholipase A_2 enzymes (PLA_2) [52, 53] are also sources of superoxide; for a detailed review, see Ref. [54].

13.2.2 Nitric Oxide

Nitric oxide (NO) is endogenously generated within cells by the nitric oxide synthases (NOS), through the conversion of L-arginine to citrulline utilizing NADPH as a cofactor [55]. NO is a primary radical, and its concentration has been demonstrated to be regulated by NOS isoenzymes: the neuronal NOS (type I or nNOS), the inducible NOS (type II or iNOS), and the endothelial NOS isoenzyme (type III or eNOS) [54, 56]. nNOS was originally discovered in neuronal tissue but has also been shown to be expressed in the plasma membrane of skeletal muscle fibers where it interacts with the dystrophin-glycoprotein complex via a linkage to α1-syntrophin [57]. The eNOS isoenzyme was originally described in the endothelium where it is associated with caveolin-1; in skeletal muscle it is localized in the mitochondria and has been reported to be activated by heat shock protein 90 (HSP90) [58]. The expression of iNOS in skeletal muscle is increased in response to inflammatory conditions or following a septic challenge [59, 60]. NO has shown to interact with a number of different cytoskeletal proteins mainly through reactive cysteine residues and the formation of S-nitrosated residues [61]. The nNOS isoform is particularly

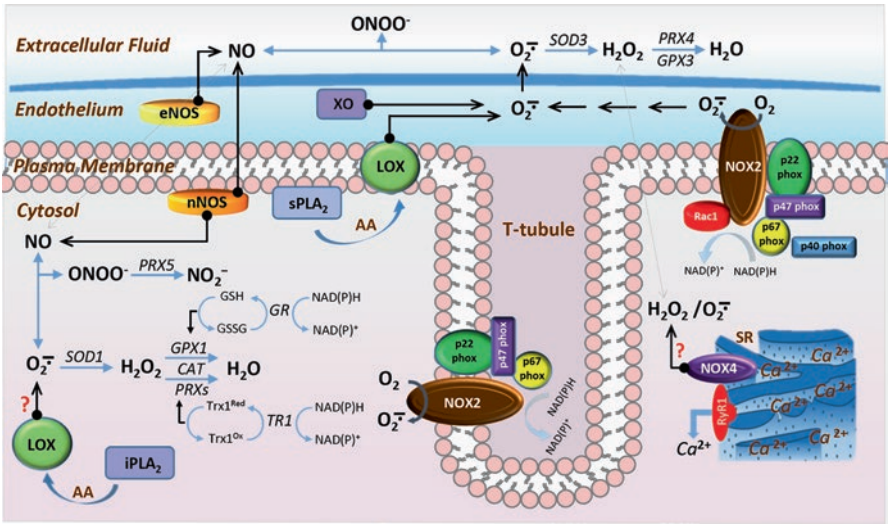

Fig. 13.2 Schematic representation of the non-mitochondrial sites for nitric oxide and superoxide production in skeletal muscle. Superoxide ($O_2^{\bullet-}$) is produced by multicomponent NAD(P)H oxidase 2 (*NOX2*), xanthine oxidase (*XO*), and the lipoxygenases (*LOX*) which activity is regulated by the phospholipase A_2 enzymes (PLA_2). Arachidonic acid (*AA*) release by the membrane bound calcium-dependent PLA_2 ($sPLA_2$) facilitates extracellular $O_2^{\bullet-}$ release by the membrane bound LOX. It is uncertain whether the cytosolic LOX enzymes contribute to intracellular $O_2^{\bullet-}$ changes which substrate availability might be regulated by the cytosolic calcium-independent PLA_2 ($iPLA_2$). NAD(P)H oxidase 4 (*NOX4*) also contributes to ROS changes, though the primary ROS product, $O_2^{\bullet-}$ or hydrogen peroxide (H_2O_2) of NOX4 is uncertain. Cytosolic and extracellular $O_2^{\bullet-}$ is dismuted into H_2O_2 by superoxide dismutase (*SOD*), SOD1 and SOD3, respectively, or reacts rapidly with membrane permeant nitric oxide (*NO*) produced by the endothelial and neuronal nitric oxide synthase (*eNOs* and *nNOS*) to form peroxynitrite ($ONOO^-$). H_2O_2 formed within the extracellular space is reduced into H_2O by the action of glutathione peroxidase 3 (*GPX3*) or peroxiredoxin IV (*PRX4*), while cytosolic H_2O_2 is reduced into H_2O by glutathione peroxidase 1 (*GPX1*), catalase (*CAT*), or peroxiredoxins (*PRXs*). Reduced glutathione (*GSH*) provides the electrons to GPX to catalyze the reduction of H_2O_2; GSH is oxidized to glutathione disulfide (*GSSG*). Reduction of GSSG is catalyzed by glutathione reductase (*GR*), where NAD(P)H is used as the reducing agent. Cytosolic *PRXs* utilize thioredoxin 1 ($Trx1^{Red}$) for their reducing action. Oxidized form of Trx1 ($Trx1^{Ox}$) is reduced by thioredoxin reductase 1 (*TR1*), by utilizing electrons from NAD(P)H. $ONOO^-$ can be reduced predominantly into nitrite ($NO2^-$) by peroxiredoxin V (*PRX5*). Sarcoplasmic reticulum (*SR*). (Redrawn from Sakellariou et al. [88])

expressed in glycolytic or fast muscle fibers [62] and has been suggested to be the primary source of NO release from myocytes [63]. The close proximity of nNOS to the dystrophin-glycoprotein complex has a pivotal role in skeletal muscle physiology as highlighted from studies utilizing the mdx mice [64] but also in humans suffering from muscle dystrophy [57, 65]. It has been suggested that NO has a direct functional signaling role via the formation of S-nitrosylated sites with effects on protein activity or indirectly by interactions with heme or nonheme Fe and Cu [66].

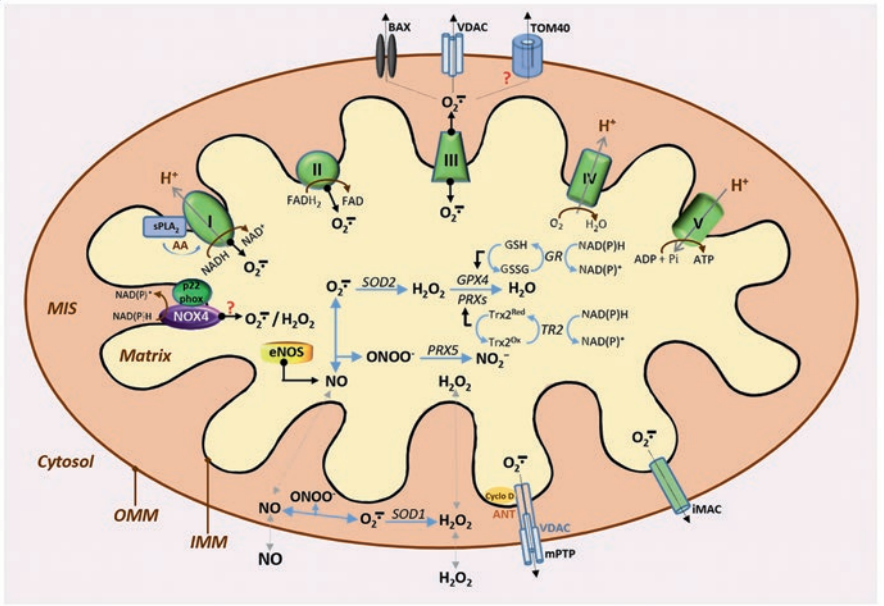

Fig. 13.3 Schematic representation of the mitochondrial sites for nitric oxide and superoxide production and the channels that mediate the release of superoxide to the cytosolic compartment in skeletal muscle. Superoxide ($O_2^{\bullet-}$) is produced by complex I, complex II, and complex III of the mitochondrial electron transport chain (ETC) of the inner mitochondrial membrane (*IMM*) and released into the matrix and the mitochondrial intermembrane space (*MIS*). NAD(P)H oxidase 4 (*NOX4*) also contributes to ROS changes, though the primary ROS product, $O_2^{\bullet-}$ or hydrogen peroxide (H_2O_2) of NOX4 is uncertain. Arachidonic acid (*AA*) release by the calcium-dependent phospholipase A_2 enzymes ($sPLA_2$) interacts with complex I and enhances superoxide generation by this complex. $O_2^{\bullet-}$ released into the matrix, and the MIS is dismuted into H_2O_2 by superoxide dismutase (*SOD*), SOD2 and SOD1, respectively, or reacts rapidly with nitric oxide (*NO*) produced by the endothelial nitric oxide synthase (*eNOS*) to form peroxynitrite ($ONOO^-$). H_2O_2 is reduced into H_2O by the action of glutathione peroxidase 4 (*GPX4*) or peroxiredoxins (*PRXs)*. Reduced glutathione (*GSH*) provides the electrons to GPX4 to catalyze the reduction of H_2O_2; GSH is oxidized to glutathione disulfide (*GSSG*). Reduction of GSSG is catalyzed by glutathione reductase (*GR*), where NAD(P)H is used as the reducing agent. Mitochondrial *PRXs* utilize thioredoxin 2 ($Trx2^{Red}$) for their reducing action. Oxidized form of Trx2 ($Trx2^{Ox}$) is reduced by thioredoxin reductase 2 (*TR2*), by utilizing electrons from NAD(P)H. $ONOO^-$ can be reduced predominantly into nitrite ($NO2^-$) by peroxiredoxin V (*PRX5*). $O_2^{\bullet-}$ is essentially membrane impermeant, while H_2O_2 is readily diffusible. Matrix $O_2^{\bullet-}$ can diffuse to the cytosol through the inner membrane anion channel (*iMAC*) that spans the IMM and the outer mitochondrial membrane (*OMM*) or via the mitochondrial permeability transition pore (*mPTP*) comprised of the voltage-dependent anion channels (*VDAC*) on the OMM, the adenine nucleotide translocator (*ANT*) located on the IMM, and cyclophilin D (*Cyclo D*) located in the matrix. Channels of the OMM including VDAC, BAX, and possibly the translocase of outer membrane 40 (*TOM40*) can also mediate the release of $O_2^{\bullet-}$ from the MIS to the cytosol. (Redrawn from Sakellariou et al. [88])

13.2.3 Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a relatively stable molecule in comparison with the other reactive species with a longer half-life; hence H_2O_2 been suggested as the most likely candidate for redox signaling pathways [67]. H_2O_2 can interact with redox-sensitive components or pathways typically via oxidation of sensitive Cys residues and has been demonstrated to regulate the activity of a variety of transcription factors in skeletal muscle [68]. In aqueous solutions, superoxide can be protonated to produce hydroperoxyl radical or reduced undergoing a dismutation reaction to produce H_2O_2 [69]. In addition, a number of enzyme systems have also been reported to generate H_2O_2 including NOX4 [70, 71], urate, and amino acid oxidases [72]. Moreover, recent evidence supports endoplasmic reticulum (ER) H_2O_2 generation in vivo [73] via thiol-disulfide exchange mechanisms [74]. The catalytic activity of a wide range of metabolic enzymes can be modulated by H_2O_2, typically by oxidation of catalytic Cys residues or residues essential for disulfide bonds [75]. In addition there are a number of different enzymes that use H_2O_2 as a substrate including the peroxiredoxins, glutathione peroxidases, and catalase; isoforms of these enzymes are located in specific cellular locations which would suggest that it plays an important physiological signaling role.

13.2.4 Hydroxyl Radical

The hydroxyl radical is a highly reactive molecule due to its strong oxidizing potential and can rapidly react with biomolecules located close to its site of generation. In skeletal muscle fibers and other biological systems, hydroxyl radicals are typically generated as a result of the Fenton reaction that involves the reductive decomposition of H_2O_2 with reduced transition metal ions, copper (Cu) or iron (Fe) [76]. Oxidation of FeS cluster enzymes can result in an increase of "free iron" within the cell, allowing for the formation of hydroxyl radicals and altered redox homeostasis [77]. Similar to the Fenton reaction, the Haber-Weiss reaction can also generate hydroxyl radicals by Fenton chemistry, Fe or Cu is maintained in a reduced form by superoxide, which can result in the formation of hydroxyls from H_2O_2 [78]. There is some in vivo evidence to suggest that during skeletal muscle contractile activity, there is enhanced hydroxyl radical generation [79]. An increased intracellular concentration of highly reactive hydroxyl radicals can affect calcium dynamics and maximum force of skeletal myofibers [76]. There are a number of neuromuscular disorders such as including glucocorticoid-induced myopathy [80] and immobilization-induced skeletal muscle atrophy [81] that have reported an increase in hydroxyl radical formation.

13.2.5 Peroxynitrite

Peroxynitrite is another endogenously generated reactive species that can act as an intracellular oxidant; it is primarily generated by the reaction between NO and superoxide, often as a result of the close proximity of NOX and NOS enzymes [82]. Further evidence to support endogenous generation of peroxynitrite in skeletal muscle is shown in studies using transgenic animals where the levels of NO and/or superoxide were elevated [34]. Similar to the some of the other reactive species, peroxynitrite can oxidize sensitive Cys residues involved in disulfides or catalytic sites [83]. The protonated form, peroxynitrous acid, is also highly reactive and can oxidize Cys residues resulting in protein oxidation, phospholipid and DNA damage [82, 84]. It has also been reported that peroxynitrite is involved in tyrosine nitration [85] as well as the formation of S-nitrosylated Cys residues [86]; mass spectrometry approaches have identified an increasing number of proteins being nitrosylated and nitrated in skeletal muscle. In conditions where there are high concentrations of peroxynitrite, it can result in reversible and irreversible oxidation of cellular compartments of myofibers [34, 87], affecting overall enzymatic activity through structural modifications, including altered cytoskeletal dynamics and an impair cell signal transduction [82].

13.3 Primary Antioxidant Enzymes Expressed in Skeletal Muscle

Skeletal muscle expresses a sophisticated system to control the production of oxidants and protect the myofibers from oxidative damage. The system that functions to prevent oxidative damage consists of enzymatic and nonenzymatic antioxidants that work in a coordinated fashion to regulate redox disturbances in the muscle cell. An extended coverage of these goes beyond the scope of this chapter (for detailed review, see Ref. [88]. However, we summarize the most important enzymatic systems expressed in skeletal muscle including superoxide dismutases, catalase, glutathione peroxidases, peroxiredoxins, and glutaredoxins.

Superoxide dismutase (SOD) was discovered in 1969 and represents a family of metalloenzymes that catalyze the one electron dismutation of superoxide into O_2 and H_2O_2 [26]. There are three SOD isoenzymes depending on the metal ion bound to the active site. Skeletal muscle expresses copper-zinc SOD (*SOD1* or CuZnSOD), which is a highly stable enzyme present within the cytosol and the mitochondrial intermembrane space (MIS), and manganese-SOD (*SOD2* or MnSOD) which is found in the mitochondrial matrix [89]. There is however an additional isoform of SOD, the extracellular SOD isoenzyme (*SOD3* or EcSOD) [90] which is present in the interstitial spaces of tissues and extracellular fluids of many cell types and tissues and its primary function is to reduce superoxide formed outside the cell membrane [90].

Catalase (CAT), a homotetramer with a molecular mass of 240kDa catalyzes the reduction of H_2O_2 into H_2O and O_2. CAT is mainly found in the cytosolic compartment of the muscle fibers and requires heme (Fe^{3+}) bound at the enzyme's active site for its catalytic function [91]. CAT enzymatic activity increases with increased H_2O_2, and reports have shown that protein expression and activity is higher in highly oxidative myofibers [92]. CAT does not require reducing equivalents to function as a H_2O_2 reducer; thus CAT is considered an energy-efficient antioxidant [93].

Glutathione peroxidase (GPX), a homotetramer with each 22kDa subunit containing a selenium atom in the form of a selenocysteine, also catalyzes the reduction of H_2O_2 to H_2O or organic hydroperoxides (ROOH) to alcohol, using reduced glutathione (GSH) or in some cases thioredoxin (TRX) or glutaredoxin (GRX) as an electron donor [94]. In addition, reports also suggest that GPX is also implicated in the reduction of hydroxyl radical by elimination of H_2O_2 [95]. Mammalian cells express five isoforms of GPX (GPX1-GPX5), which differ in cellular localization and substrate specificity [96] with GPX1 as the cytosolic form [97] and GPX4 as the most widely expressed. GPX4 is a membrane-associated enzyme, partly localized to the MIS. GPX3 also known as plasma or extracellular GPX is present in the extracellular space [98, 99], whereas GPX2 is mainly expressed in the gastrointestinal system [100]. GPX5 is expressed in the epididymis in the mammalian male reproductive tract and is the least studied isoenzyme [100, 101]. The expression of the GPX genes is controlled by different mechanisms including O_2 tension, metabolic rate, toxins, and xenobiotics [23] as well as growth and development [102]. Similarly, to CAT, oxidative muscle fibers express higher amounts of GPX compared with glycolytic myofibers [100]. Though there is an overlap between the function of GPX and CAT, GPX has a higher affinity for H_2O_2 at low concentrations. However, under conditions where H_2O_2 is significantly increased, CAT becomes more significant in protecting biological systems, and its catalytic function prevails since it cannot be saturated under any H_2O_2 concentration since there is no apparent Vmax [103].

Peroxiredoxins (PRXs) initially described as thiol-specific antioxidants [104] were discovered in the late 1980s [105, 106] and are a family of cysteine-dependent thioredoxin peroxidases [107]. PRXs are capable of reducing both ROOH and H_2O_2 [108] with the use of electrons provided by thioredoxins [108]. Skeletal muscles express six isoforms of PRXs, which are present in the cytosolic compartment (PRX I, II, VI), the mitochondrion (PRX III), the extracellular space, and endoplasmic reticulum (PRX IV) [42]. PRXV is expressed in the cytosol, mitochondria, nuclei, and perixosomes [108] and is considered a peroxynitrite reductase [109]. PRX proteins have recently received much attention as they have shown to play a key role in transmitting redox signals into a dynamic biological response and to have subtle changes in both abundance and oxidative state with age [35, 110, 111].

Glutaredoxins (GRXs) are small ubiquitous disulfide oxidoreductases which share many of the functions of TRXs but are reduced by GSH rather than a specific reductase [122]. GRXs are small redox enzymes that exist in either a reduced or oxidized form and are involved in the protection and repair of protein and nonprotein thiols during compromised redox homeostasis [112]. GRXs are divided into

monothiol (Cys-X-X-Ser) and dithiol (Cys-X-X-Cys) GRXs [113]. Dithiol GPXs participate in the regulation of H_2O_2 via PRX pathways [114], proliferation and differentiation [115], transcription regulation via modulating the activity of nuclear factor κB (NFκB) [116], and apoptosis [117]. Monothiol GRXs are implicated in iron sulfur (FeS) cluster biosynthesis and Fe homeostasis [118]. GRX1 prevents oxidative damage and apoptosis and is found in the cytosol, and the MIS. GPX1 has also shown to translocate into the nucleus and exported from the cell [113]. GRX2 is localized in the mitochondria [119] and GRX3 in the nuclear and cytosolic compartment. Monothiol GRX5 has a mitochondrial translocation signal and shares the active-site motif of GRX3 [120]. Reports have also revealed that the GRX system can also catalyze reversible protein glutathionylation [121] and regulate the redox state of thiol groups [122] during aberrant redox control.

In addition to the main antioxidant enzyme defense network, skeletal muscle also expresses glucose-6-phosphate dehydrogenase (G6PD) and isocitrate dehydrogenase (IDH) which do not directly scavenge RONS but play a pivotal role in redox regulation by providing reducing power in the form of NADPH to the antioxidant enzymatic systems [123]. In addition, skeletal muscle also contains nonenzymatic antioxidants, which regulate reactive species and protect muscle cells from oxidative injury. These are H_2O soluble and fat soluble and are classified into two categories: (i) the endogenously produced and (ii) dietary antioxidants which cannot be synthesized or induced and must be taken from the diet. The main nonenzymatic antioxidants found in myofibers include GSH, uric acid, bilirubin, and coenzyme Q_{10} endogenously produced antioxidants but also dietary antioxidants including vitamin C, vitamin E, and carotenoids. An extended coverage of the nonenzymatic defense systems in skeletal muscle goes beyond the scope of this review; for a detailed review, see Refs. [124, 125].

13.4 Age-Related Muscle Atrophy Is Linked to Increased Oxidative Damage

The dual role of RONS to act as signaling molecules at low concentrations but also damage critical cellular compartment when produced at high concentrations is fundamental in skeletal muscle physiology/pathology. Reports in humans [126–128] and rodents [87, 129, 130] have provided evidence that age-related muscle atrophy is linked to an altered oxidative status of redox-responsive proteins [131], elevated concentration of oxidized macromolecules including an increase in DNA damage [126, 132], increased levels of lipid peroxidation [133, 134], and accumulation of oxidized proteins [127, 128]. Increased DNA damage has been shown to alter genetic stability which may induce the expression of genes that regulate cell proliferation and/or block the expression of certain genes, thus permitting damage with increasing age [135]. RONS-induced DNA sequence changes or mutations have been suggested to affect the cellular state of differentiation [23, 136] and

accumulation of mitochondrial DNA damage [132] which may prevent the rejuvenation of the mitochondrial population and lead to bioenergetic decline and cellular death [137]. In addition, aged skeletal muscle exhibits an accumulation of catalytically inactive or less active forms of enzymes and the observed age-related changes in catalytic activity have been suggested to occur due to oxidative modifications induced by RONS [138, 139].

Recent reports have provided evidence that increased oxidative damage inherent with aging is linked to age-associated changes in RONS, with myofibers from old rodents exhibiting increased intracellular RONS levels compared to young/adult rodents [140, 141]. Oxidants can modulate various intracellular signal transduction pathways, and age-related disruption of these processes due to compromised redox homeostasis has been suggested as contributing factor to muscle atrophy inherent with aging. The role of redox homeostasis in age-related muscle atrophy and weakness has been studied in various model organisms (reviewed in [88]) which have undergone genetic manipulations (transgenic and knockout models) and have provided insight into the function of RONS regulatory systems in neuromuscular aging.

13.5 Deletion of Cu-Zn Superoxide Dismutase in *SOD1*$^{-/-}$ Mice Leads to Accelerated Neuromuscular Aging and Functional Deficits

The association between redox regulation and age-related atrophy has been studied in several mammalian models which have undergone genetic manipulations (reviewed in [88]), to enable the study of disrupted redox signaling on the aging process. Deletion of CuZnSOD in mice (*SOD1*$^{-/-}$ mice) leads to a reduction in lifespan and an accelerated aging phenotype associated with myofiber atrophy (Fig. 13.4), neurological impairments (Fig. 13.5), and functional deficits [142]. Elevated oxidative damage has also been observed in skeletal muscles from

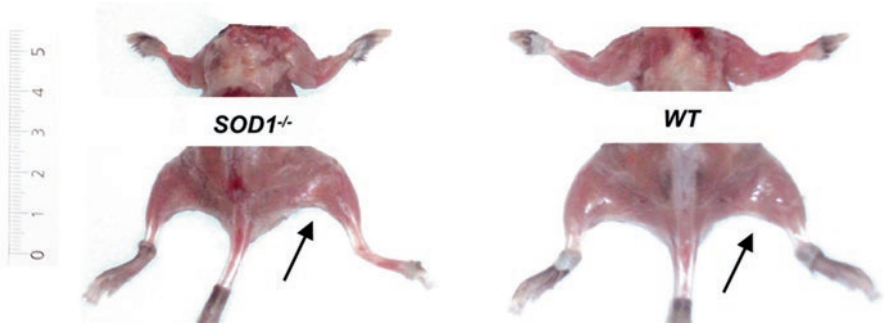

Fig. 13.4 Gross morphology of skinned hindlimb and forelimb muscles of *SOD1*$^{-/-}$ and *WT* mice at 12 months of age. Arrows indicate the phenotypic hindlimb muscle changes observed in *SOD1*$^{-/-}$ compared to *WT* mice. (Redrawn from Sakellariou et al. [14])

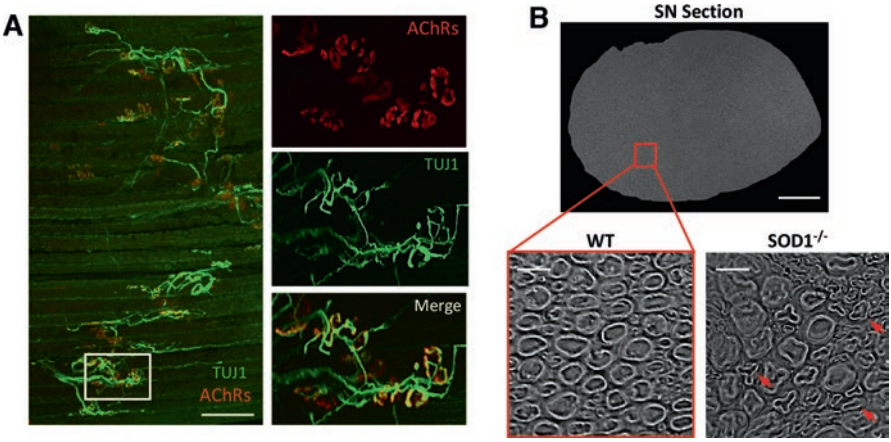

Fig. 13.5 Neuromuscular junction structure and peripheral nerve integrity in *SOD1*$^{-/-}$mice. (**a**) Intravital immunofluorescence imaging of neuromuscular junctions (NMJ) of an *AT* muscle from a *SOD1*$^{-/-}$ mouse. Presynaptic motor neurons immunolabeled with neuronal class III β-tubulin monoclonal antibody (TUJ1), a neuronal marker (green), and postsynaptic motor endplate acetylcholine receptors (AChRs) stained with Alexa Fluor 594-conjugated α-bungarotoxin (red). Right panels show enlarged area marked by white box in the left panel. 10x original magnification (left panel). Scale bar, 150μm. (**b**) Transverse section of a sciatic nerve (*SN*) from a *WT* (*SOD1*$^{+/+}$) mouse (top panel). 20x original magnification. Scale bar, 100μm; Bottom left panel shows enlarged area marked by red box in the top panel to show the morphology and myelin thickness of motor axons of the peripheral nerve. 60x original magnification. Scale bar, 10μm; Transverse section of a *SN* from a *SOD1*$^{-/-}$ mouse (bottom right panel). Note reduced myelin thickness of motor axons from peripheral nerve of the *SOD1*$^{-/-}$ model, indicated by arrowheads. 60x original magnification. Scale bar, 10μm. (Redrawn from Sakellariou et al. [14])

SOD1$^{-/-}$ mice [34, 143–149], and many features of the muscles of *SOD1*$^{-/-}$ mice including loss of fibers, reduction in contractile force, a constitutive activation of redox-sensitive transcription factors [146], degeneration of neuromuscular junctions (NMJ), and of loss of innervation resemble those observed in old wild-type mice [144, 145] and in older humans [13, 144]. Hence, it has been suggested that the *SOD1*$^{-/-}$ model may potentially provide a useful model to study the role of chronic oxidative stress in loss of skeletal muscle and to uncover potential targets for intervention for preventing age-related muscle wasting.

The prominent sarcopenic phenotype observed in the *SOD1*$^{-/-}$ model is associated with a number of neurological impairments (Fig. 13.5), including striking alterations in NMJ and peripheral nerve integrity/function (Fig. 13.5), motor axon degeneration, postsynaptic endplate fragmentation, terminal sprouting and axon thinning and irregular swelling, reduced occupancy of the motor endplates by axons, loss of innervation and motor function [143], impaired neurotransmitter release [150], and reduction in isometric force [145]. Collectively, these findings may suggest that the muscle atrophy phenotype shown in the *SOD1*$^{-/-}$ model might be initiated by disrupted redox signaling in motor neurons.

Disrupted redox signaling in motor neurons as a potential mechanism of sarcopenia in *SOD1*$^{-/-}$ mice has recently been assessed in genetically engineered mouse models including models with targeted deletion of CuZnSOD specifically in skeletal muscle alone [149] or motor neurons [148] but also in a "nerve rescue" *SOD1*$^{-/-}$ mouse model with neuron-specific expression of CuZnSOD [147], using a transgenic SOD1$^{-/-}$ mouse model in which SOD1 was expressed under control of the synapsin 1 promoter. The data from these studies provided evidence that CuZnSOD deficits in either the muscle or motor neuron alone are not sufficient to initiate a full sarcopenic phenotype and that deficits in both tissues are required to recapitulate the loss of muscle and function observed in the SOD1$^{-/-}$ model. Moreover, the data further showed that neuron-specific insertion of SOD1 corrected the skeletal muscle aging phenotype observed in SOD1$^{-/-}$ mice indicating that deficits in redox homeostasis in motor nerves appear to be the underlying factor that initiates mitochondrial dysfunction and oxidative damage which triggers a retrograde response leading to further NMJ degeneration and dysfunction. These reports have provided insight into the understanding of (i) the defective redox signaling events that underlie age-related atrophy and (ii) the redox-mediated cross talk between motor neurons and skeletal muscle.

13.6 Neuromuscular Aging Is Associated with Redox Proteomic Changes

In order to unravel the mechanisms responsible for the structural and functional changes associated with neuromuscular aging, many laboratories have begun to investigate both the proteome and site-specific redox modifications within skeletal muscle, to identify those proteins that change in abundance but also to identify those proteins that are particularly sensitive to redox changes.

Site-specific RONS-induced redox modifications of key regulatory enzymes can alter a wide variety of metabolic pathways related to cellular response to energy and stress. Modulation of the activity of downstream protein targets by redox modifications can also influence a variety of key regulators of distinct posttranslational modifications (PTMs) such as phosphorylation, ubiquitination, and acetylation, including components that control metabolic rate such as AMP-activated protein kinase (AMPK), protein kinase C (PKC), sirtuin 1, and mammalian target of rapamycin (mTOR) [131]. In skeletal muscle a number of redox-sensitive proteins are involved in excitation-contraction coupling; these modifications can specifically affect calcium homeostasis including calcium release, binding, and sequestration through site-specific redox modifications of specific cysteine (Cys), e.g., sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) and ryanodine receptor 1 (Ryr1) [151, 152]. The nature or type of RONS-induced redox modification is dependent on a number of factors including the residues modified (typically Cys), the species and concentration of RONS generated, and the properties of the amino acids

surrounding the modified residue which can influence the sensitivity to modifications. One of the goals of redox proteomic approaches is to identify the RONS modification, the amino acid residue that has been modified, and the relative quantification of the modified amino acid, including both reversible and irreversible modifications which have shown to influence contractile force [48, 111, 153]. The major reversible RONS-induced modifications of Cys residues include sulfenylation (-SOH), glutathionylation (-SSG), nitrosylation (-SNO), and inter-/intradisulfide bond formation (-S-S-) [131]. The largely irreversible modifications include sulfonic ($-SO_3H$) or sulfinic (SO_2H) acid formation [154].

Neuromuscular aging exhibits an altered redox proteome with subsequent biochemical and physiological effects on the cytoskeleton, mitochondria, calcium signaling and sequestration [155–157]. Redox proteomic approaches have demonstrated that skeletal muscle aging is correlated with altered catalytic activity of a number of regulatory enzymes and an overall reduction in the identification of redox-sensitive proteins particularly involved in the generation of precursor metabolites and energy metabolism [111, 131]. These results suggest that age-related redox changes have a significant role in the loss of skeletal muscle mass and function inherent with aging. Reversible redox modifications on specific proteins are essential for correct adaptive response to contractile activity with activation of specific pathways, and skeletal muscle has shown to develop a dysregulated redox response with aging [111, 131]. However, irreversible oxidative modifications as a result of excessive RONS can lead to insoluble protein aggregates and protein degradation, which have been reported to increase in neurodegenerative diseases and aging [158]. Recent reports have demonstrated that reversible and irreversible redox modifications of myofilament proteins can modify both structure and function [159]; several regulatory and cytoskeletal myofilament proteins including troponin C [160], actin, α-actinin [111, 159], and myosin heavy chains [161–163] are susceptible to RONS-induced oxidative modifications, thus affecting Ca^{2+} dynamics and Ca^{2+} sensitivity [164] and as a result cross-bridge cycling [160] which ultimately affects contractile function.

13.7 Causative Links Between Disrupted Redox Signaling and Muscle Atrophy

There are a number of studies that have demonstrated a link between increased intracellular RONS concentrations and an altered redox environment in skeletal muscle atrophy, as a result of either muscle disuse [165] or disease [166]. The causative links between redox homeostasis and skeletal muscle atrophy include signaling pathways that regulate both protein synthesis and protein breakdown [167–169]. Regular exercise can help maintain skeletal muscle mass, yet contracting skeletal muscle generates RONS predominantly from NOX and NOS systems [28], which in turn are thought to acutely activate a variety of redox-regulated transcription factors (Nrf-2, NF-κB) required for adaptation to exercise [170]. In exercise studies it has

been reported that ingesting high doses of vitamin C and E can blunt the beneficial and adaptive responses induced by exercise in skeletal muscle presumably by disrupting the RONS signaling cascade [71]. However, in skeletal muscle from older individuals, there is a higher basal level of RONS, and as a result, chronic activation of many redox-regulated transcription factors may blunt many of the beneficial adaptive responses following an acute RONS-dependent increase during exercise [172].

The IGF1-Akt pathway is one of the key global regulators of protein synthesis; a number of studies have demonstrated that activation of IGF1 receptor can promote muscle hypertrophy, while inactivation is related to an impairment of muscle growth. [173]. The role of oxidative damage in relation to IGF1 signaling is unclear with reports suggesting that it may result in the promotion and inhibition of Akt signaling [174]. Studies using C2C12 myotubes have shown that oxidative damage due to chronic exposure to low levels of H_2O_2 attenuates Akt phosphorylation which would be predicted to result in an overall decrease in protein synthesis, increased proteolysis, and as a result increased muscle atrophy [174]. In support of this finding, a recent report demonstrated that administration of the mitochondrial targeted antioxidant peptide SS-31 resulted in an increase in the phosphorylated form of Akt and mTORC1 indicating that aberrant redox homeostasis can attenuate muscle protein synthesis by inhibiting the Akt/mTORC1 signaling pathway [175].

Growing evidence suggests that disrupted redox signaling due to enhanced RONS generation effects autophagy-mediated protein breakdown, a highly regulated lysosomal pathway used for the degradation of non-myofibril cytosolic proteins and organelles in skeletal muscle [167]. RONS can directly affect this process as oxidative damage induced by H_2O_2 treatment of fibroblasts can result in an increase in the expression of key autophagy components such as LC3, beclin1, and increased formation of autophagosomes [176]. RONS may also alter the activity of the regulators of autophagy; for example, the inactivation of ATG4 can prevent the cleavage of LC3 during the generation of the autophagosome, which is an essential step in the process of autophagy [167, 177].

Furthermore, the regulation of the proteasomal degradation pathway can also be regulated by intracellular RONS. In vivo studies have demonstrated that increased RONS can promote muscle protein breakdown via increased activity of the proteasome system [178], [14] but also through the activation of calpains, specific proteases that are involved in the selective cleavage of target proteins [179].

13.8 Perspectives

Muscle atrophy and weakness, in the context of neuromuscular aging and a wide range of myopathies, has a significant effect on individuals with respect to independence and overall quality of life. There is ongoing research to develop both pharmacological and non-pharmacological therapeutic approaches to inhibit or prevent loss of skeletal muscle mass and function [180]. Age-related skeletal muscle atrophy is

a multifactorial process, involving a variety of metabolic processes and signaling pathways whose disruption ultimately result in skeletal muscle loss and functional deficits. The primary biochemical and molecular mechanisms responsible for muscle atrophy have not been fully identified. Considerable evidence in both humans and various organisms has shown that the myofibrillar redox environment can influence the activity of crucial pathways involved in biogenesis and degradation but also the regulation of excitation contraction coupling, making it an attractive target for interventional approaches. There is a wealth of scientific research from both human and animal studies that have described an altered redox environment within skeletal muscle with age, in particular increased oxidation of redox-sensitive proteins and macromolecules correlated with age-related atrophy. An altered redox environment has also been described in many age-related diseases including neurodegenerative disorders, neuromuscular diseases, and diabetes. However, whether disrupted redox signaling is the initial cause of disease, development or a consequence leading to disease progression has yet to be fully determined. To elucidate the role of redox homeostasis in age-related disease, particularly in neuromuscular integrity and function, the generation of tissue-specific knockout models and the development of sensitive tools for measuring RONS generation and the subsequent redox modifications and signaling roles are warranted. Identification of the precise signaling roles of endogenously generated RONS and the balance between RONS signaling and oxidative damage will increase our understanding of the role of redox homeostasis in skeletal muscle adaptation to exercise and maintaining neuromuscular integrity. Increased understanding of the precise molecular pathways that regulate the balance between adaptation and muscle growth compared with disuse and atrophy may reveal potential therapeutic targets for intervention and ultimately prevent sarcopenia in humans.

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