REVIEW

Journal of Cachexia, Sarcopenia and Muscle (2017)
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12223

Redox homeostasis and age-related deficits in neuromuscular integrity and function

Giorgos K. Sakellariou[1]*, Adam P. Lightfoot[2], Kate E. Earl[3], Martin Stofanko[4] & Brian McDonagh[3,5]

[1]*GeneFirst Ltd, Culham Science Centre, Abingdon, Oxfordshire, OX14 3DB, UK;* [2]*School of Healthcare Science, Manchester Metropolitan University, Manchester, M1 5GD, UK;* [3]*MRC-Arthritis Research UK Centre for Integrated Research into Musculoskeletal Ageing, Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, L7 8TX, UK;* [4]*Microvisk Technologies Ltd, The Quorum, 7600 Oxford Business Park, Oxford, OX4 2JZ, UK;* [5]*Department of Physiology, School of Medicine, National University of Ireland, Galway, Ireland*

Abstract

Skeletal muscle is a major site of metabolic activity and is the most abundant tissue in the human body. Age-related muscle atrophy (sarcopenia) and weakness, characterized by progressive loss of lean muscle mass and function, is a major contributor to morbidity and has a profound effect on the quality of life of older people. With a continuously growing older population (estimated 2 billion of people aged >60 by 2050), demand for medical and social care due to functional deficits, associated with neuromuscular ageing, will inevitably increase. Despite the importance of this 'epidemic' problem, the primary biochemical and molecular mechanisms underlying age-related deficits in neuromuscular integrity and function have not been fully determined. Skeletal muscle generates reactive oxygen and nitrogen species (RONS) from a variety of subcellular sources, and age-associated oxidative damage has been suggested to be a major factor contributing to the initiation and progression of muscle atrophy inherent with ageing. RONS can modulate a variety of intracellular signal transduction processes, and disruption of these events over time due to altered redox control has been proposed as an underlying mechanism of ageing. The role of oxidants in ageing has been extensively examined in different model organisms that have undergone genetic manipulations with inconsistent findings. Transgenic and knockout rodent studies have provided insight into the function of RONS regulatory systems in neuromuscular ageing. This review summarizes almost 30 years of research in the field of redox homeostasis and muscle ageing, providing a detailed discussion of the experimental approaches that have been undertaken in murine models to examine the role of redox regulation in age-related muscle atrophy and weakness.

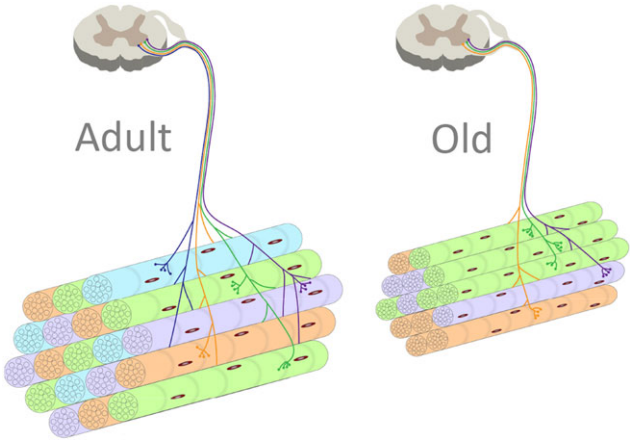
Keywords Frailty; Superoxide dismutase; Neuromuscular junction; Mitochondria; Redox signalling; Motor neurons

Received: 12 October 2016; Revised: 6 April 2017; Accepted: 22 May 2017
**Correspondence to: George K. Sakellariou, GeneFirst Ltd, Culham Science Centre, Abingdon, Oxfordshire OX14 3DB, UK. Tel: +44 (0) 1865 407400; Fax: +44 (0) 1865 407400, Email*: george.sakellariou@hotmail.com

Introduction

Ageing is characterized as the time-dependent functional decline of cells, organs and tissues throughout the body[1] and is the primary risk factor for major human pathologies, including cancer, diabetes, cardiovascular disorders and neurodegenerative/neuromuscular diseases. Loss of skeletal muscle mass and force inherent with ageing has a profound effect on the quality of life of older people. Human investigations have shown that by the age of 70, there is a 25–30% reduction in the cross-sectional area (CSA) of skeletal muscle and a decline in muscle strength by 30–40%,[2] associated with neurological impairments including loss of motor units,[3,4] neuromuscular junction (NMJ) instability,[5] a decline in motor nerve function[6] and increased fibre-type grouping due to continual cycles of denervation and reinnervation[7] (Figure 1). The reduction in muscle strength with age is associated with an increased mortality risk,[8] an increased susceptibility to risk of falls and, subsequently, an increased need for residential care. According to the World Health Organization, the global population of elderly people aged >60 years was 600 million in 2000 and is expected to rise to around 2 billion by 2050[9]; thus, increased demand for medical and social care will inevitably increase, rising

Figure 1 Schematic representation of the morphological neuromuscular alterations/impairments that occur with the advance of age. Ageing skeletal muscle is associated with increased fibre-type grouping due to continual cycles of denervation and reinnervation. Axonal degeneration and motor neuron death, inherent with aging, leads to reduced number of motor axons innervating myofibres. These events inevitably result in loss of motor units and atrophy of the remaining muscle cells.



the financial costs of healthcare systems.[10] Physical activity can undoubtedly delay the progression of ageing muscle affects,[11,12] but even physically active older individuals experience age-associated muscle atrophy and weakness.[13] Age-dependent myofibre atrophy is a life-long process with a complex and multifactorial aetiology that involves both intrinsic and extrinsic factors[7]; despite the importance of this area, elucidation of the primary biochemical and molecular mechanisms underlying the prominent age-associated decline in muscle mass and function has proven to be difficult.

Oxidative damage has been suggested to be among the factors involved in the loss of tissue function that occurs during ageing, and experimental evidence in humans[14–16] and rodents[17–19] has shown that skeletal muscle exhibits age-dependent increases in the products of oxidative damage to biomolecules including proteins, lipids and nucleic acids. Recent reports have attributed the positive correlation between age and oxidative damage to age-related changes in reactive oxygen and nitrogen species (RONS), with skeletal muscle fibres from old rodents exhibiting elevated intracellular RONS levels compared with young/adult rodents.[20,21] The hypothesis that an increased generation of oxidants *in vivo* plays a key role in age-related deficits in muscle mass and function has been examined in several transgenic and knockout studies with inconsistent results.

Many reports[19,22–35] have examined skeletal muscle of rodents lacking and/or overexpressing various key regulatory enzyme systems (in homo/heterozygotic and tissue-specific models) involved in the reduction and/or generation of oxidants to determine whether specific defects in antioxidant protection and the resultant changes in redox homeostasis influence the onset and/or rate of age-related muscle atrophy and functional deficits.

Although the skeletal muscle fibre-type profile differs between murine and human skeletal muscle (myosin heavy chain isoform IIB is not expressed in humans),[36] neuromuscular ageing in both humans and rodents share similar features. These include, but are not limited to, loss of muscle fibres[37] and reduced myofibre CSA[21,38,39] associated with degeneration and structural alterations of the NMJ,[40–42] a decline in functional innervation (partial denervation)[41,43,44] and loss of motor units.[3,44,45] Although it is not justified to extrapolate results from transgenic animal models to human muscles (i.e. to assume that each fibre type exhibits similar age-related phenotypic changes with ageing in different species), the similar characteristics observed during neuromuscular ageing in both rodents and humans suggest that murine models can provide useful experimental models to explore the processes and mechanisms that contribute to skeletal muscle atrophy and weakness.

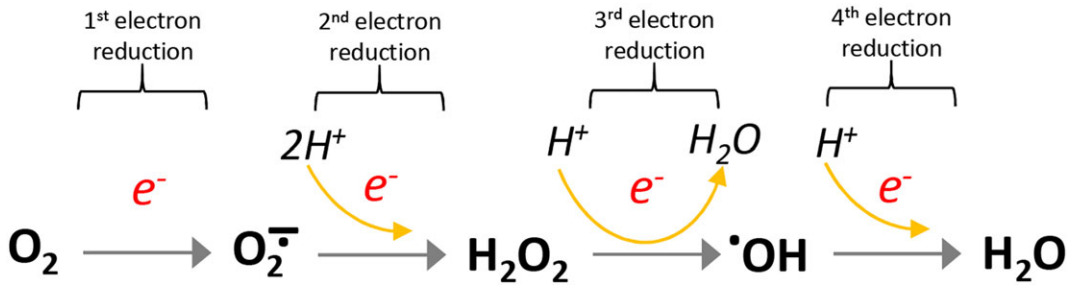
An understanding of the underlying causes of muscle atrophy and functional deficits inherent to ageing is critical for the development of strategies and targeted interventions to preserve the age-related decline in neuromuscular integrity and function. This review summarizes the transgenic approaches that have been undertaken in rodent models to assess whether redox homeostasis is implicated in the processes of sarcopenia, unravel potential mechanisms involved in skeletal muscle ageing and identify areas where further research is warranted. We begin with a brief overview of the chemistry of RONS, sites of production and the antioxidant defence systems expressed in skeletal muscle, followed by a discussion of the redox sensitive pathways and cellular functions controlled by redox homoeostasis. This will be followed by a detailed discussion of the implication of redox homeostasis in neuromuscular ageing and the genetic modifications that have been undertaken to examine the potential link between redox control and age-related deficits in skeletal muscle mass and function. Although we will discuss a broad range of topics related to the muscle redox environment, it is impossible to address all aspects of this expansive field of study in the present review. For topics not covered in detail in this article, we provide references of review articles where necessary.

Skeletal muscle produces reactive oxygen and nitrogen species

Molecular oxygen (O_2) is one of the most abundant elements in the atmosphere (nearly 21% by volume), and its ability to accept electrons makes it vital for a variety of

DOI: 10.1002/jcsm.12223

Figure 2 Reactive oxygen derivatives produced by the sequential reduction of O_2 to H_2O. Superoxide (O_2·⁻), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$).



physiological processes. Aerobic organisms including humans have adapted well to the atmosphere, using atmospheric O_2 by respiration and to obtain energy efficiency.[46] Although O_2 plays a key role in aerobic cellular metabolism, studies in the 1950s showed that O_2 could cause cellular damage[47] by the generation of reactive species (Figure 2), derivatives of O_2.[48] The 'free radical theory of O_2 toxicity' sparked the interest of many research laboratories in the field of redox homeostasis in biological systems and the first studies to report that skeletal muscle produces reactive species appeared in the late 1970s[49] and early 1980s.[50] Over the past three decades, the field of redox biology has expanded rapidly, and with the development of high throughput 'Omics' technologies and sensitive analytical approaches, it is now widely accepted that resting and contracting skeletal muscle produces RONS both *in vivo* and *in vitro*.

Reactive oxygen and nitrogen species generation by myofibres has been detected and quantified by a variety of methods including high-performance liquid chromatography techniques,[30,51] electron-spin resonance spectroscopy (also known as electron paramagnetic resonance),[52,53] fluorescence-based microscopic assays,[54,55] spectrophotometry,[56,57] chemiluminescence[58,59] and transfection methods including *in vivo*[60,61] and *in vitro*.[62] It is widely accepted that superoxide and nitric oxide (NO) are the primary radical species generated by skeletal muscle.[46,63] Table 1 depicts the molecular formulas, half-lives and intracellular concentrations of the major RONS produced by skeletal muscle. A discussion of the primary and 'secondary' RONS follows.

Chemistry of reactive oxygen and nitrogen species produced by skeletal muscle

Superoxide

Superoxide is one of the main radical species produced by skeletal muscle and is derived either from incomplete reduction of O_2 in electron transport systems or as a specific product of enzymatic systems.[75] Resting and contracting skeletal muscle produces superoxide via different pathways, and schematic Figures 3 and 4 depict the various sites and mechanisms that have been proposed for RONS generation in skeletal muscle. Briefly, superoxide is generated by the mitochondrial electron transport chain including complex I, complex III[76,77] and, recently, complex II[78–80]; the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzymes including NOX2, NOX4, DUOX1 and DUOX2[55,56,59,81]; xanthine oxidase[82,83]; and the lipoxygenases (LOXs),[84] which are linked to arachidonic acid released by the phospholipase A_2 enzymes[85,86] (for a detailed review, see Ref. [46]).

Superoxide anion carries a negative charge and cannot diffuse through membranes. However, it can cross membranes through anion channels including the inner membrane anion channel and the voltage-dependent anion channels[55,87,88] (Figure 4). Nonetheless, it has been argued that superoxide can be protonated at physiological pH to

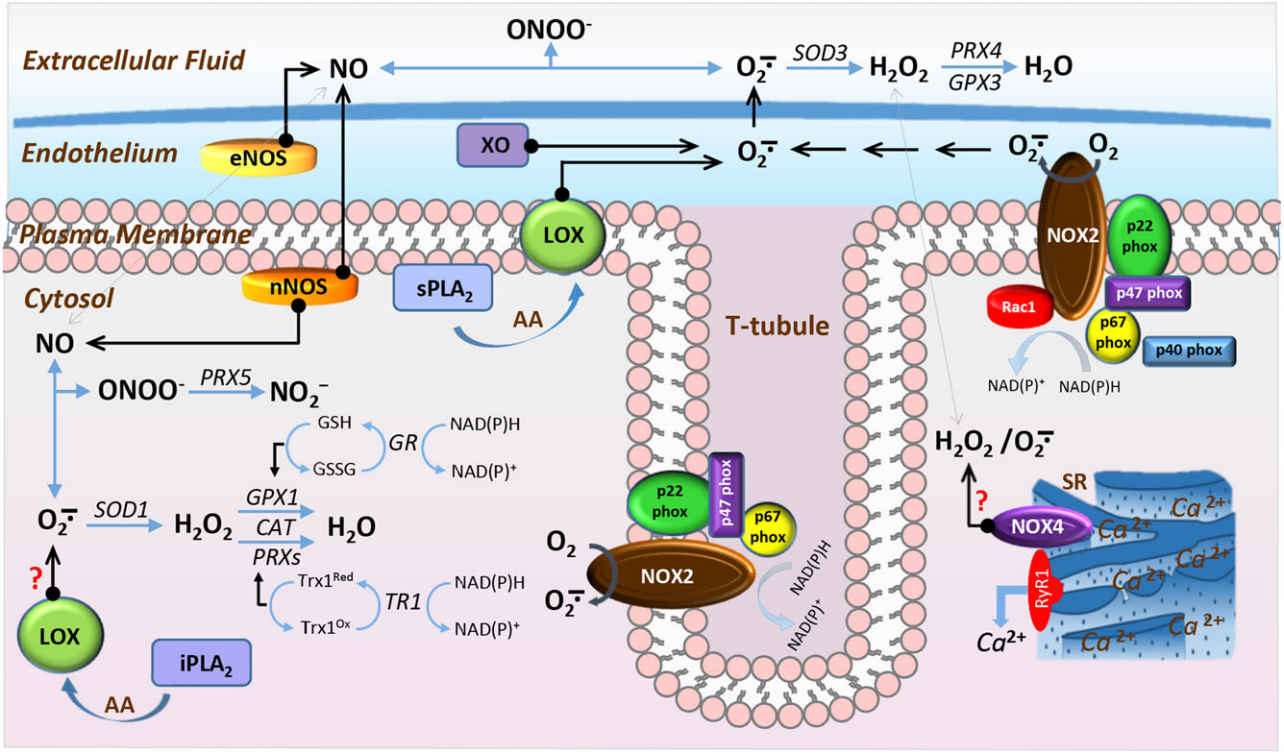
Table 1. Major RONS detected in skeletal muscle, estimates of half-lives and cellular concentrations

Species	Formula	Biological half-life(s)	Estimate cell conc. (M)	References
Superoxide	O_2·⁻	10^{-6}	$1–10^{-12}$	64–66
Hydrogen peroxide	H_2O_2	10^{-5}	$1–10^{-8}$	64,67,68
Hydroxyl radical	$^{\bullet}OH$	10^{-9}	ND	64,69
Nitric oxide	NO	$1–10^{-1}$[a]	$1–10^{-9}$	66,70,71
Peroxynitrite	$ONOO^-$	10^{-2}	2^{-9}	72–74

Not determined (ND).
[a]NO half-life depends on its concentration.

DOI: 10.1002/jcsm.12223

Figure 3 Schematic representation of the non-mitochondrial sites for nitric oxide and superoxide production in skeletal muscle. Superoxide (O_2·⁻) is produced by multicomponent nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 (NOX2), xanthine oxidase 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·⁻ release by the membrane bound LOX. It is uncertain whether the cytosolic LOX enzymes contribute to intracellular O_2·⁻ 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, although the primary ROS product, O_2·⁻, or hydrogen peroxide (H_2O_2) of NOX4 is uncertain. Cytosolic and extracellular O_2·⁻ 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 catalyse the reduction of H_2O_2; GSH is oxidized to glutathione disulfide (GSSG). Reduction of GSSG is catalysed by glutathione reductase (GR), where NADPH 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).



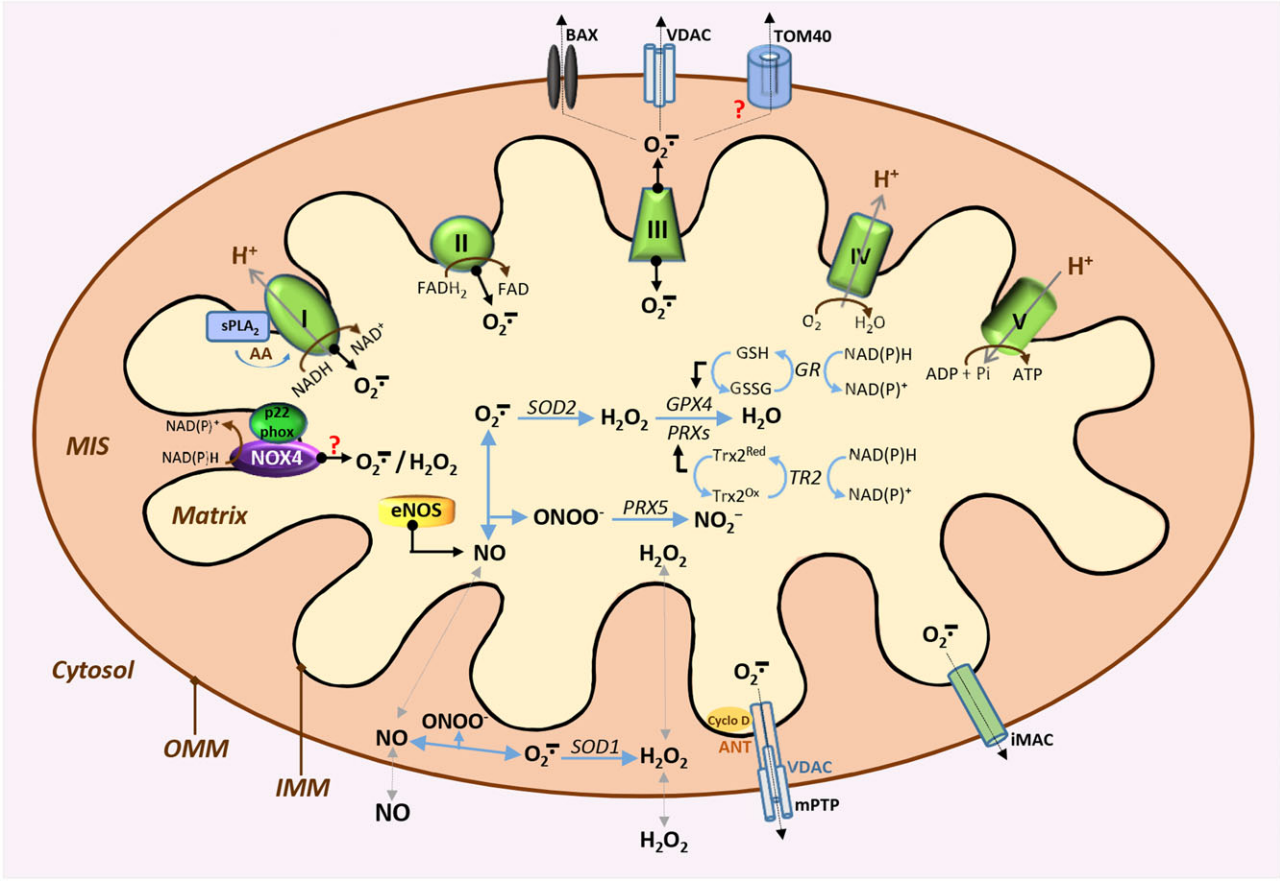
produce the hydroperoxyl radical, enabling the transfer of superoxide across biomembranes.[89] Although superoxide anion has a relatively long half-life, it has a limited oxidizing ability as it does not react directly with polypeptides, sugars or nucleic acids but can interact with other molecules to generate secondary RONS either directly or through enzyme or metal-catalysed processes.[65] In aqueous solutions, dismutation of superoxide into hydrogen peroxide (H_2O_2) can occur spontaneously or catalysed by superoxide dismutases (SODs)[90] with a rate constant ($k = 2 \times 10^9\ M^{-1}\ s^{-1}$),[70] a reaction considered to be very slow as superoxide radicals electrostatically repel each other.[91]

Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is a relatively stable molecule with a long half-life and can diffuse across biomembranes.[92] H_2O_2 has been suggested to be a redox signalling molecule[93] that can interact with redox-sensitive components or pathways, activating various transcription factors in skeletal muscle.[94] In addition to SOD-dependent production of H_2O_2, a number of enzyme systems also generate H_2O_2 including NOX4,[95,96] urate and amino acid oxidases.[97] Moreover, recent evidence supports endoplasmic reticulum (ER) H_2O_2 generation *in vivo*[98] via thiol-disulfide exchange mechanisms.[99] Elevated concentrations of H_2O_2 have shown to alter the catalytic

DOI: 10.1002/jcsm.12223

Figure 4 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 ($O_2^{\cdot-}$) is produced by complex I, complex II and complex III of the mitochondrial electron transport chain of the inner mitochondrial membrane (IMM) and released into the matrix and the mitochondrial intermembrane space (MIS). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4 (NOX4) also contributes to ROS changes, although the primary ROS product, $O_2^{\cdot-}$, 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. $O_2^{\cdot-}$ released into the matrix, and 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 catalyse the reduction of H_2O_2; GSH is oxidized to glutathione disulfide (GSSG). Reduction of GSSG is catalysed by glutathione reductase (GR), where NADPH 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 NADPH. $ONOO^-$ can be reduced predominantly into nitrite ($NO2^-$) by peroxiredoxin V (PRX5). $O_2^{\cdot-}$ is essentially membrane impermeant, while H_2O_2 is readily diffusible. Matrix $O_2^{\cdot-}$ 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) composed 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 $O_2^{\cdot-}$ from the MIS to the cytosol.



activity of enzymes by oxidizing thiol groups of essential amino acids[100]; cytotoxicity of H_2O_2 in skeletal muscle occurs through the generation of hydroxyl radicals via metal-catalysed reactions.[101]

Hydroxyl radical

Hydroxyl radicals have a strong oxidizing potential with a half-life in aqueous solution of less than 1 ns[69] and can react rapidly with almost any biomolecule close to their site of production. Hydroxyl radicals occur in skeletal muscle fibres from the reductive decomposition of H_2O_2 with reduced transition metal ions, iron (Fe) or copper (Cu), through a reaction called the Fenton reaction.[102] There is some controversy over the Fenton reactions particularly *in vivo* due to the concentration of reactive transition metal ions being very low[103] and its small rate constant (k = 10^9–10^{10} M^{-1} s^{-1}).[104] There is, however, evidence that disrupted redox homeostasis can lead to oxidation of Fe cluster-

DOI: 10.1002/jcsm.12223

containing enzymes, thus releasing 'free Fe' enabling hydroxyl radical formation.[105] Hydroxyl radical generation is also facilitated by the Haber–Weiss reaction that makes use of Fenton chemistry, in which Fe or Cu is maintained in a reduced form by superoxide, thus capable of catalysing the generation of hydroxyl radicals from H_2O_2.[106] Hydroxyl radicals are membrane impermeant, and evidence has shown enhanced generation of hydroxyl radicals *in vivo* during muscle contractile activity.[107] Enhanced generation of hydroxyl radical formation can affect calcium (Ca^{2+}) sensitivity and maximum force of skeletal myofibres[102]; further reports have identified increased generation of hydroxyl radicals in neuromuscular disorders including glucocorticoid-induced myopathy[101] and immobilization-induced skeletal muscle atrophy.[108]

Nitric oxide

Nitric oxide, also known as nitrogen monoxide, is a primary radical and arises through the conversion of L-arginine to citrulline by the NO synthases (NOS), utilizing NADPH as a cofactor.[109] NO is a weak reducing agent, with a relatively long half-life[70] and reacts with O_2 to form nitric dioxide and superoxide to produce peroxynitrite.[110] There are three different isoforms of NOS expressed in skeletal muscle; the neuronal NOS (nNOS or type I), the inducible NOS (iNOS or type II) and the endothelial NOS isoenzyme (eNOS or type III).[46,111] nNOS, originally discovered in neuronal tissue, is expressed along the sarcolemma of skeletal muscle fibres and interacts with the dystrophin–glycoprotein complex via a linkage to α1-syntrophin.[112] Type III NOS isoenzyme, originally described in the endothelium through association with caveolin-1, is localized in the muscle mitochondria and is activated through association with heat shock protein 90.[113] Inducible NOS isoenzyme is involved in the immune response and is primarily expressed in skeletal muscle in response to inflammatory conditions or a septic challenge.[114,115] NO has shown to regulate cytoskeletal proteins,[116] and nNOS isoform, strongly expressed in glycolytic muscle fibres,[117] has been reported as the prime source of NO release from skeletal muscle.[118] The importance of NO signalling in muscle physiology is highlighted in mdx mice[119] and humans with Duchenne muscular dystrophy.[112,120] NO responses are largely mediated via cysteine (Cys) *S*-nitrosylation or by coordinated interactions with heme or non-heme Fe and Cu.[121]

Peroxynitrite

Peroxynitrite, a powerful oxidant with a relatively long half-life, is produced through the reaction of NO with superoxide.[122] Evidence in skeletal muscle has shown *in vivo* intracellular generation of peroxynitrite in myofibres of transgenic murine models in which the levels of superoxide and/or NO were up-regulated.[30] The chemical reaction of superoxide with NO to generate peroxynitrite has a reaction rate ($k = 7 \times 10^9$ M^{-1} s^{-1}),[70] which is approximately three-fold higher than the SOD catalysed conversion of superoxide to H_2O_2 ($k = 2 \times 10^9$ M^{-1} s^{-1}) as previously discussed. Peroxynitrite can react with thiol compounds to form disulfides[123] and, along with its protonated form, peroxynitrous acid, can deplete thiol groups and induce protein, phospholipid oxidation and DNA damage.[92,122] Peroxynitrite leads to nitration of tyrosine residues,[124] and *S*-nitrosylation of Cys residues,[125] the list of proteins being nitrated and nitrosylated in skeletal muscle, is continuously growing. Under circumstances where peroxynitrite is generated at high concentrations, it can not only cause oxidative damage to cellular compartments of myofibres[18,30] but also alter the structure and function of proteins resulting in altered catalytic activity of enzymes, altered cytoskeletal organization and impaired cell signal transduction.[122]

Redox regulation in skeletal muscle

Over the last three decades, it has become clear that RONS can act as mediators of contraction-induced damage to skeletal muscle.[68] Muscle cells contain a network of antioxidant defence mechanisms to control the cellular production of RONS and maintain the redox environment. The antioxidant network includes enzymatic and non-enzymatic systems, and the potential role of redox homeostasis as the underlying factor implicated in neuromuscular ageing has been the subject of intensive research in a variety of model organisms. The important technological advances that have occurred in the last few decades have allowed research groups to utilize genetic engineering techniques to alter specific genes or crucial redox components of the antioxidant network and assess whether age-related deficits in neuromuscular integrity and function are mediated by defective redox signalling. Figures 3 and 4 depict the subcellular RONS protective systems expressed in skeletal muscle. Description of the antioxidant mechanisms follows.

Regulatory reactive oxygen and nitrogen species enzymes expressed in skeletal muscle

Superoxide dismutase

Superoxide dismutase found in all O_2-utilizing organisms catalyses the dismutation of superoxide to H_2O_2 and O_2.[126] Three isoforms of SOD that exist are mammalian skeletal

DOI: 10.1002/jcsm.12223

muscle depending on cellular location and the redox active transition metal bound to its active site to accomplish the catalytic breakdown of superoxide[30,46]; copper–zinc SOD (SOD1 or CuZnSOD), localized within the mitochondrial intermembrane space (MIS) and cytosol, requires Cu–Zn as a cofactor; and manganese (Mn) SOD (SOD2 or MnSOD) requires Mn as a cofactor and is expressed in the mitochondrial matrix.[92] Extracellular SOD isoenzyme incorporates Cu–Zn as a cofactor and is present in extracellular fluids and interstitial spaces of tissues.[127] Evidence has shown that exercise can induce an increase in both SOD1 and SOD2 activities in skeletal myofibres.[128] SOD1 protein has a half-life of 6–10 min, whereas SOD2, 5–6 h.[129] Fifteen to thirty-five per cent of the total SOD activity resides within the mitochondria of skeletal muscle, with the SOD2 isoenzyme accounting for 15–20%,[130] and the remaining 65–85% remains within the cytosolic compartment of muscle cells.[131] SOD1 and SOD2 protein expression and activity are higher in oxidative muscle fibres compared with those of fast glycolytic fibres.[103]

Glutathione peroxidase

Glutathione peroxidase (GPX) catalyses the reduction of H_2O_2 or organic hydroperoxide to H_2O and alcohol, respectively, using reduced glutathione (GSH) or in some cases glutaredoxin (GRX) or thioredoxin (TRX) as an electron donor.[92] Five GPX isoforms are reported in mammals, which differ in cellular localization and substrate specificity with GPX1 localized predominantly in the cytosol and a small proportion in the mitochondrial matrix. Seleno-protein GPX4, a membrane-associated enzyme, is partly localized to the MIS, while GPX3 is present in the extracellular space.[132] Although the GPX antioxidant system has not been as extensively described as other antioxidant systems (e.g. SOD redox network), GPX gene expression is controlled by a range of mechanisms including toxins, O_2 tension, metabolic rate and growth and development.[65] The relative amounts of GPX expressed in skeletal muscle are higher in oxidative fibres compared with fibres with low oxidative capacity,[75] and exercise has shown to up-regulate the protein expression and activity of both cytosolic and mitochondrial GPX in skeletal muscle fibres.[131]

Catalase

Catalase (CAT) is distributed within the cytosolic compartment of myofibres and catalyses the breakdown of H_2O_2 into H_2O and O_2.[133] Fe is a required co-factor, bound at the enzyme's active site for its catalytic function.[134] Although GPX is also an H_2O_2 regulator in skeletal muscle and they share common substrates, CAT has a lower affinity for H_2O_2 at low concentrations (K_m = 1 mM) compared with GPX (K_m = 1 μM).[135] In situations where H_2O_2 is significantly elevated, CAT becomes an important H_2O_2 reducing system, and its enzymatic activity prevails because there is no apparent V_{max} and cannot be saturated by H_2O_2 at any concentration.[136] CAT enzymatic activity is higher in oxidative myofibres compared with fast glycolytic fibres[137] and does not require reducing equivalents to function as a H_2O_2 reducer; thus, CAT is considered an energy-efficient enzyme.[138]

Peroxiredoxins

The family of peroxiredoxins (PRXs) initially known as thiol-specific antioxidants[139] are Cys-dependent TRX peroxidases that are capable of reducing both H_2O_2 and organic hydroperoxide.[140] In two Cys peroxiredoxins (2-Cys PRXs), on reaction with H_2O_2, the redox-sensitive Cys residue of each subunit of the PRX homodimer is oxidized to Cys-SOH, which then reacts with a neighbouring Cys-SH to form an intermolecular disulfide.[141] It is noteworthy that PRXVI possesses only one Cys residue in the active site, while other PRXs contain 2-Cys PRXs.[142] The intramolecular disulfide of 2-Cys PRXs is reduced specifically by electrons provided by TRXs, which are then regenerated by TRX reductase at the expense of NADPH,[143] thus restoring the catalytic activity.

Six isoforms of PRX are expressed in skeletal muscle; PRX I, II and VI are localized in the cytosolic compartment, PRXIII exclusively in skeletal muscle mitochondria, PRXIV in the extracellular space and ER and atypical 2-Cys PRXV in the cytosol, mitochondria, nuclei and peroxisomes.[46,142] All of the six mammalian PRX proteins act to degrade H_2O_2. PRXV has also reported to have peroxynitrite reductase activity,[144] and PRXVI has shown to facilitate NOX1[145] and NOX2[146] optimal activities. PRX isoforms are highly abundant in skeletal muscle and have a high catalytic efficiency as peroxidases.[147] However, a number of these isoforms can be inactivated, mediated by the oxidation of the catalytic site Cys to Cys-sulfinic acid (SO_2H) by high levels of H_2O_2. PRXs that have formed SO_2H acids can be reduced by sulfiredoxin, and it has been proposed that this initial inactivation by its substrate plays a cellular signalling role by a 'floodgate mechanism'.[148] It is particularly interesting that there are a number of protein families and isoforms within those families that have either peroxidase activity (GPXs, PRXs and CAT) or regulatory proteins that are sensitive to oxidation (Keap1, aconitase and PTP1B). It is important to recognize that the rate constants within these protein families and isoforms can vary by up to 10^6 M^{-1} s^{-1},[149] suggesting that the concentration of H_2O_2 and the enzymes that regulate it play a significant role in redox signalling. PRX proteins have been shown to play a role in transmitting redox signals into a dynamic biological response and to have subtle changes in both abundance and oxidative state with age.[52,150,151] PRXII has recently been

DOI: 10.1002/jcsm.12223

found to form an interdisulfide with STAT3 in response to cytokines, suggesting that it plays an important regulatory role.[152]

Thioredoxins

Thioredoxins are ubiquitous antioxidant enzymes that contain a canonical dithiol-disulfide active site (Cys-Gly-Pro-Cys),[153] originally discovered in 1964 in *Escherichia coli* as an electron donor for ribonucleotide reductase.[154] It has become clear that TRXs play multivalent cellular roles by serving as electron donors for enzymes such as ribonucleotide reductases, PRXs and methionine sulfoxide reductases (MSRAs),[155] protecting proteins from oxidative aggregation and inactivation.[156] Skeletal muscle expresses TRX1 (expressed in cytosol and nucleus) and TRX2 located within the mitochondrial compartment.[157] The Cys residues of the Cys-Gly-Pro-Cys motif are the key players used by TRXs to reduce disulfide bonds in oxidized substrate proteins and upon completion of a catalytic cycle; these two Cys residues are oxidized and form a disulfide.[156] Oxidized Cys residues are converted back to the reduced state by TRs with TR1 isoform present in the cytosol and nuclei and TR2 in the mitochondria, at the expense of NADPH. TRXs have also been implicated in various cellular processes including protein structure/folding energy utilization, transcription factor regulation and immune/inflammatory response.[156]

Glutaredoxins

Glutaredoxins are a family of thiol-disulfide oxidoreductases that utilize the reducing power of GSH to catalyse disulfide reductions in the presence of NADPH and glutathione reductase (GR).[158] Depending on the number of active site Cys residues, GRXs are divided into dithiol (Cys-X-X-Cys) and monothiol (Cys-X-X-Ser) GRXs.[159] Dithiol GPXs conduct similar functions to the TRX system; they can participate in the regulation of H_2O_2 via PRX pathways,[160] transcription regulation via modulating the activity of nuclear factor κB (NFκB),[161] proliferation and differentiation[162] and apoptosis.[163] Monothiol GRXs function primarily both in the biosynthesis of FeS proteins and Fe homeostasis.[164] GRX1 is mainly localized in the cytosol but can be found in the MIS; it can be translocated into the nucleus and exported from the cell.[159] GRX2 is expressed in the mitochondria,[165] GRX3 in the cytosolic and nuclear compartment, and monothiol GRX5 has a mitochondrial translocation signal and shares the active site motif of GRX3.[166] Evidence has also shown that the GRX system can also catalyse reversible protein glutathionylation,[167] which is an important redox regulatory mechanism, and control the redox state of thiol groups[168] in situations where the redox environment is being compromised.

Additional enzymes expressed in skeletal muscle including isocitrate dehydrogenase and glucose-6-phosphate dehydrogenase are also involved in the antioxidant defence system by providing reducing power in the form of NADPH to the antioxidant enzymes.[169] Although these enzymes do not directly scavenge RONS, their contribution to maintain the redox environment in myofibres is significant.

Non-enzymatic key antioxidants that contribute to the maintenance of muscle cellular redox state

A variety of non-enzymatic antioxidants, endogenous and exogenous (through diet), are found in skeletal muscle and have shown to contribute to the maintenance of muscle redox status. These include not only GSH, bilirubin, uric acid and coenzyme Q_{10} that endogenously produced antioxidants but also dietary antioxidants including carotenoids, vitamin C and vitamin E. A detailed description of the non-enzymatic defence mechanisms in skeletal muscle goes beyond the scope of this review; for a detailed description, see Refs [170,171]. However, we provide a short overview of the main endogenously produced antioxidant, GSH, which plays an important role in maintaining the redox environment in skeletal muscle cells by directly reacting with RONS through a hydrogen atom donation or indirectly during GSH-dependent peroxidase-catalysed reactions,[172] as previously discussed.

Glutathione

The tripeptide GSH (L-γ-glutamyl-L-cysteinyl-glycine) is synthesized in a two-step process catalysed by glutamate-Cys ligase (L-glutamate:L-Cys γ-ligase) and glutathione synthetase (γ-L-glutamyl-L-Cys:glycine ligase).[65] GSH is consumed in various ways, such as by oxidation, conjugation and hydrolysis. GSH can be directly oxidized by RONS, act as a substrate for GSH-dependent enzymatic reactions and conjugate with endogenous and exogenous electrophiles.[172] GSH is distributed to intracellular organelles including the ER, nucleus and mitochondria.[173] GSH can react directly with a variety of radicals by donating a hydrogen atom and has shown to reduce vitamin E and C radicals derived in chain-breaking reactions with lipid peroxyl or alkoxyl radicals.[171]

Mitochondrial organelles lack CAT; the reduction of H_2O_2 is accomplished mainly by GSH, with the participation of either GPX or PRX and by the later conversion of glutathione disulfide (GSSG) back into GSH by GR. Moreover, the GSH system is also associated with the GRX system and the removal of xenobiotics by glutathione *S*-transferases. Under

DOI: 10.1002/jcsm.12223

impaired redox homeostasis, a significant number of proteins can be altered in their function by formation of mixed disulfides and the GSH-dependent disulfide oxidoreductase GRX system catalyses dithiol reactions, reducing GSH-protein mixed disulfides in a coupled system with GR.[173] Oxidative muscle fibres contain a higher GSH content compared with fast glycolytic fibres,[130] although the ratio GSH/GSSG appears to be consistent across various fibre types.[174] Myofibre GSH levels increase in response to exercise,[175,176] and high intracellular levels of GSSG have shown to inactivate enzymes and induce glutathionylation in skeletal muscle.[177]

Skeletal muscle cysteine redox modifications and oxidative damage are regulated by reactive oxygen and nitrogen species

The first human study to report that exercise enhanced oxidative damage appeared in the late 1970s.[49] This study instigated intensive research in the field or redox biology, and the first article to demonstrate that skeletal muscle augmented reactive species in response to contractile activity appeared in 1982.[50] These studies cited the mitochondrial organelle as the major source of reactive species in muscle cells,[50,178] but over the past 35 years, the development of analytical approaches has been instrumental in the discovery of additional redox sites in various subcellular compartments of skeletal myofibres.[46,55] RONS produced by skeletal muscle were initially considered as 'toxic' by-products of metabolic processes inducing cellular damage and since these initial reports, a substantial amount of evidence suggests that redox homeostasis plays an underlying role in various human myopathies, neurodegenerative and metabolic diseases including muscular dystrophies, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease and diabetes; for detailed reviews, see Refs [112,179–181].

The magnitude and species of RONS generated by skeletal muscle have downstream effects on specific protein targets and cellular redox signalling. Recent application of novel redox proteomic approaches has identified and quantified reversible and irreversible modifications of susceptible Cys residues of redox-sensitive proteins expressed in skeletal muscle.[81,151,182] An extended coverage of these goes beyond the scope of this review; for a detailed description, see Refs [183–185]. Briefly, the type of redox modification on Cys residues depends on the concentration and species of RONS as well as the amino acids surrounding the Cys residue. Reversible modifications of Cys residues include glutathionylation, nitrosylation, sulfenylation (–SOH) and inter/intradisulfide bond formation.[183] The largely irreversible modifications include SO_2H and sulfonic (SO_3H) acids.[184]

Contraction-induced RONS by skeletal muscle has shown to contribute to muscle fatigue[186]; induce oxidative damage including lipid peroxidation, protein oxidation and DNA damage[66]; and alter the function of redox-sensitive proteins within myofibres.[151] The sensitivity of a particular target is defined by the intrinsic sensitivity of the molecule to oxidation–reduction and the local redox state,[103] and evidence has shown that RONS produced by skeletal muscle can alter myofilament structure and function.[187] Several myofilament proteins including actin, α-actinin,[151,187] troponin C[188] and myosin heavy chains[189–191] are susceptible to RONS-induced oxidative modifications, thus affecting Ca^{2+} dynamics and Ca^{2+} sensitivity[192] and, inevitably, cross-bridge kinetics,[188] which may result in contractile dysfunction.

Abundant evidence further indicates that altered muscle redox environment due to elevated RONS production by skeletal muscle fibres is implicated in muscle atrophy induced by muscle disuse[193] and disease.[194] The causative links between redox homeostasis and muscle atrophy induced by skeletal muscle inactivity were recently reviewed[195–197] and include reduced anabolic signalling and protein synthesis via inhibition of Akt/mTORC1 signalling, elevated proteolytic pathways including enhanced autophagy, activation of calpains and caspase-3 and increased protein breakdown via the proteasome system.

Skeletal muscle reactive oxygen and nitrogen species are required for multiple intracellular signalling pathways and cellular functions

Although it is widely accepted that RONS produced by skeletal muscle can induce oxidative damage and alter muscle physiology, in situations when the antioxidant system is compromised or when RONS are excessively augmented, abundant evidence indicates that the redox environment plays an important role in modulating multiple signalling pathways and muscle cellular functions.[112,198–203] The advantageous biological effects of RONS in muscle physiology contradict early reports that RONS are by-products of metabolism, inevitably damaging to muscle cells. A detailed discussion of RONS-dependent signal transduction pathways is beyond the scope of this review, but examples of key biochemical pathways and cellular processes that require a particular 'optimal redox state' in skeletal muscle are provided in Table 2. The findings depicted in Table 2 highlight that physiological levels of RONS are essential and play crucial roles in regulating skeletal muscle metabolism and physiology. Deciphering the mechanisms that underlie the divergence between adaptive and maladaptive responses to RONS in skeletal muscle remains an active area of research.[226]

Age-related deficits in skeletal muscle mass and function are associated with altered redox homeostasis

Identification of the mechanisms underlying the structural and functional changes that occur in skeletal muscle during

DOI: 10.1002/jcsm.12223

Table 2. Redox sensitive pathways/processes in skeletal muscle metabolism and physiology

Redox-sensitive cellular functions and biochemical pathways	References
•Regulation of Ca^{2+} release from the sarcoplasmic reticulum (SR) via a ryanodine receptor Ca^{2+} release redox mechanism.	56,81
•Ca^{2+} sensitivity of myofilaments via oxidative modifications of the amino acids in the Ca^{2+} binding sites of cytoskeletal proteins that alter optimum troponin Ca^{2+} binding and actin myosin interactions.	204,205
•Regulation of muscle force production.	202,206,207
•Activation of redox sensitive transcription factors including NFκB, AP-1 (activator protein 1), HSF-1 (heat-shock factor 1), Nrf2 (nuclear factor erythroid 2-related factor) and gene expression.	208–211
•Modulation of contractile activity-dependent increase in RONS regulatory protein expression and HSP content.	212–214
•Activation of key signalling molecules such as PGC1α (peroxisome proliferator-activated receptor α), AMPK (AMP-activated protein kinase) and MAPK (mitogen-activated protein kinase), which regulate cellular mechanisms for muscle adaptation (e.g. oxidative metabolism and mitochondrial biogenesis/function).	198,211,215,216
•Induction of signalling cascades for autophagy or apoptosis under physiological conditions.	198
•Modulation of gene expression of mitochondrial transcription regulators, Sirtuin 1 and mitochondrial biogenesis.	217
•Regulation of ion channels, protein phosphatases and kinases that modulate the activity of various enzymes involved in oxidative phosphorylation, tricarboxylic acid cycle and glycolysis.	121,218,219
•Regulation of contraction-stimulated glucose uptake in skeletal muscle via RONS signalling.	220–223
•Modulation of protein synthesis via the IGF-1 (insulin-like growth factor 1) signalling pathway.	224,225

ageing has stimulated the interest of many laboratories with a goal of identifying pharmaceutical targets to combat physical frailty and mobility impairment that affect up to half the population aged 80 or older.[32] It has been 60 years since Denham Harman proposed the 'free radical theory of ageing'.[227] Although it is now recognized that this theory and its various derivatives do not exclusively explain the ageing process,[228,229] disrupted redox signalling has been suggested to be implicated in the processes of loss of neuromuscular integrity and function that occurs during ageing.[230]

Skeletal muscle decline with advancing age has been linked to an altered oxidative status of redox-responsive proteins,[183] a positive correlation between tissue concentration of oxidized macromolecules and lifespan including an increase in DNA damage,[14,231] accumulation of oxidized proteins[15,16] and increased levels of lipid peroxidation[232,233] in both humans and rodents. Recent quantitative proteomic approaches have further provided evidence that muscle ageing is associated with altered catalytic activity of regulatory enzymes and a reduction in detection of redox-sensitive proteins involved in the generation of precursor metabolites and energy metabolism,[151,183] implying age-related redox changes as an underlying cause of skeletal muscle ageing.

Based on findings in the early 1970s that mitochondria can generate reactive species,[234] a variant of the free radical theory of ageing, the 'mitochondrial free radical theory of ageing' was proposed.[235] Consistent with a role of mitochondria as a contributor to age-related muscle redox changes, reports have shown that isolated[213,236] and intact mitochondria[21] in skeletal muscle fibres exhibit an age-dependent increase in H_2O_2 generation. Considerable evidence has shown that age-related mitochondrial oxidative damage can alter mitochondrial integrity and function in ageing skeletal muscle. Several key features have been observed in ageing skeletal muscle, including a reduction in mitochondrial abundance[237] and oxidative-phosphorylation,[19] accumulation of mutated mtDNA[238] associated with impaired mitophagy,[21,38] increased mitochondrial permeability transition pore sensitivity[54] and increased mitochondrial-mediated apoptosis,[239] which collectively may contribute to age-related loss of neuromuscular integrity and function. Together, these findings may support the conclusion that age-related muscle atrophy and functional deficits are associated with increased oxidative damage and defective redox signalling.

The relationship between redox homeostasis and neuromuscular ageing has been further examined in several mammalian models that have undergone genetic manipulations, to enable the study of aberrant redox homeostasis on the ageing process.

Redox homeostasis and age-related deficits in neuromuscular integrity and function, insights from transgenic animal models

In the last two decades, the field of redox biology has advanced significantly with the development of new analytical approaches and techniques in genetic manipulation. The impact of altered redox homeostasis in loss of neuromuscular integrity and function with ageing has been investigated in several murine models, which have undergone genetic modifications of redox signalling/homeostasis components.[19,23,25,29–34,240–242] Transgenic murine models have provided insight into the importance of RONS regulatory systems in lifespan and neuromuscular ageing, and it has been reported that $SOD2^{-/-}$,[243] $GRX3^{-/-}$,[244] $GPX4^{-/-}$,[245] $TRX1^{-/-}$,[246] $TRX2^{-/-}$,[247] $TR1^{-/-}$[248] and $TR2^{-/-}$[249] murine models are embryonically lethal. Although the embryonic lethal phenotypes

DOI: 10.1002/jcsm.12223

observed in these specific knockout models do not facilitate our understanding on whether defects in redox signalling affect age-dependent deficits in neuromuscular integrity and function, these findings, however, highlight the fundamental importance of the redox systems mentioned in the preceding text during embryonic development.

$SOD1^{-/-}$,[27] $PRX1^{-/-}$,[250] $PRX2^{-/-}$,[251] $TRX2^{+/-}$[252] and $MSRA^{-/-}$[253] rodent models show a reduction in lifespan; in contrast, more recent studies have reported no effect of $MSRA^{-/-}$ on rodent lifespan.[254] $GPX1^{-/-}$,[28,252] $SOD2^{+/-}$,[240] extracellular $SOD^{-/-}$[255,256] and $MSRB^{-/-}$[257] knockout murine models show no effect on lifespan. Similarly, transgenic animal models overexpressing RONS protective enzymes including $SOD1^{Tg}$,[26] $SOD2^{Tg}$,[24] $MSRA^{Tg}$,[258] mice overexpressing human CAT in nuclei ($nCAT^{Tg}$)[259] and peroxisomal targeted CAT ($pCAT^{Tg}$) (the natural site of CAT)[260] have failed to provide evidence of increased lifespan, indicating that RONS are not the fundamental determinants of lifespan. However, $GPX4^{+/-}$,[261] $TRX1^{Tg}$[262] and the mitochondrial CAT overexpressing ($mCAT^{Tg}$) mouse model[263] showed ~7%, ~14% and ~21% increases in lifespan, respectively, which may provide support for the theory of oxidative damage in ageing.

It is noteworthy that the majority of genetic interventions in mice has been undertaken in C57BL/6, the most widely used inbred strain. Recently, it was suggested that this particular strain might not be suitable to study the effect of redox homeostasis in ageing due to a missense mutation in the nicotinamide nucleotide transhydrogenase protein that links the NAD/NADH to NADP/NADPH pool, providing reducing equivalents for TRX reductase and GRX redox enzymes.[264] Table 3 summarizes the genetically engineered rodent models that have been developed to assess the implication of redox homeostasis in age-related deficits in neuromuscular integrity and function.

Genetic modification of mitochondrial redox systems to study the role of mitochondrial redox homeostasis in sarcopenia

Homozygotic mice lacking mitochondrial PRX3 isoform are viable with no signs of muscle atrophy, although this mouse model showed an increase in skeletal muscle mitochondrial ROS, altered mitochondrial morphology and decreased muscle fatigue resistance.[272] These observations indicate that, although lack of PRX3 does not induce atrophy, it plays a crucial role in the contractile function of skeletal muscle by regulating the mitochondrial redox environment.[272] Additional recent studies undertaken in the field of metabolomics have shown that, although homozygotic mice lacking either mitochondrial TRX2[247] or TRX1[246] have embryonic lethal phenotypes, specific deletion of TRX-interacting protein in muscle specific knockout mice induces a reduction in exercise tolerance[279] by maintaining the redox balance during exercise and preserving mitochondrial capacity to switch substrates during glucose deprivation.

Targeted overexpression of the human CAT gene to mitochondria in the $mCAT^{tg}$ model has shown to protect against age-induced deficits in muscle mitochondrial function, improve skeletal muscle respiratory function with age,[19,242] improve voluntary exercise and decrease the intracellular Ca^{2+} leak and the level of oxidized ryanodine receptor 1[32]. This occurs likely due to the attenuation of mito-H_2O_2 which potentially reduces the reliance on antioxidant coupled NADPH-driven reduction of oxidants in the mitochondria, thereby maintaining a higher availability of NADPH for exogenous antioxidant reduction.[280]

Increased oxidative damage and mitochondrial dysfunction have been proposed to contribute to the sarcopenic phenotype that occurs with ageing, and the findings in the preceding texts may suggest that scavenging of H_2O_2 specifically within skeletal muscle mitochondria may potentially rescue age-related myofibre atrophy. However, studies have reported that the $mCAT^{tg}$ model exhibits similar fibrosis levels and loss of muscle fibre size to age-matched old wild-type (WT) mice,[32] indicating that reduced mitochondrial oxidative damage and improved mitochondrial function failed to rescue age-associated muscle wasting. Similarly, heterozygous knockout of MnSOD[241,266–269] and conditional knockout of MnSOD targeted to type IIB skeletal muscle fibres[25,270] showed no major effect on age-related loss of muscle mass and structural changes. However, both these models showed mitochondrial functional deficits associated with elevated mitochondrial oxidative damage[25,266,269] and reduced skeletal muscle aerobic capacity,[265,270] which support a role for MnSOD in regulating mitochondrial function and, subsequently, the aerobic capacity of skeletal muscle.

Moreover, recent studies using not only mice overexpressing the mitochondrial matrix SOD isoform ($SOD2^{Tg}$)[24] but also a double transgenic mouse model, $SOD2^{Tg}$ combined with $mCAT^{Tg}$,[35] failed to preserve skeletal muscle mass with ageing [24] and showed no further improvements in insulin resistance in skeletal muscle of mice when fed on a high fat diet, indicating that increased mitochondrial superoxide scavenging does not improve muscle insulin action in mice fed on high fat diet alone or when coupled to increased H_2O_2 scavenging.[35] Targeted disruption of the mitochondrial GPX4 isoform caused infertility in male mice, yet mitochondrial GPX4 isoform mouse model was fully viable, healthy in appearance, normal in behaviour and showed no difference in body size compared with WT siblings.[271]

In summary, data obtained from the available knockout and transgenic rodent studies, with a focus on mitochondrial redox systems, appear to support that the mitochondrial redox environment is critically important for embryonic development and plays an important role in regulating age-related mitochondrial dysfunction, impaired mitophagy and aspects of skeletal muscle function. However, based on the

DOI: 10.1002/jcsm.12223

Table 3. List of studies that have manipulated RONS regulatory systems to investigate the effect of redox homeostasis in age-related deficits in neuromuscular integrity and function

Model	Neuromuscular phenotype and function	References
Mitochondrial redox systems		
$SOD2^{+/-}$	•No effect on age-related neuromuscular ageing •increased RONS generation in skeletal muscle and elevated mitochondrial oxidative damage •defective signalling in the PI3-Akt pathway •impaired phosphorylation of Akt at Ser473 and Thr308 and decreased differentiation potential •reduced treadmill endurance capacity.	24,265–269
TnIFastCre $SOD2^{fl/fl}$	•No effect on age-related neuromuscular ageing •increased mitochondrial RONS and oxidative damage •complex II-linked mitochondrial dysfunction •reduced contractile muscle function and aerobic exercise capacity.	25,270
$SOD2^{Tg}$	•No effect on age-related muscle atrophy •preserved mitochondrial mass and function •preserved the differentiation potential • no changes in RONS production in resting skeletal muscle myotubes.	24,268
mGPX4-KO	•No effect on age-related neuromuscular ageing.	271
$PRX3^{-/-}$	•No effect on muscle atrophy or skeletal muscle isometric force •increased mitochondrial RONS and altered mitochondrial membrane potential and network •decreased mitochondrial DNA, ATP production, mitofusin 1 and 2 protein levels • increased muscle fatigue resistance.	272
$mCAT^{Tg}$	•No effect on age-related muscle atrophy or fibrosis •reduced mitochondrial oxidative damage and insulin resistance •preserved mitochondrial respiration and ATP synthesis •prevented age-related reduction in AMP-activated protein kinase •improved complex I respiratory dysfunction •improved voluntary exercise and increased skeletal muscle specific force and tetanic Ca^{2+} transients •decreased intracellular Ca^{2+} leak and increased sarcoplasmic reticulum Ca^{2+} load.	19,32,242
Other redox systems		
$nNOS^{Tg}$	•No effect on age-related muscle atrophy or muscle weakness •prevented muscle membrane injury and reduced muscle inflammation following a hindlimb muscle unloading and reloading protocol •increased protein nitration.	30,273
$GPX1^{-/-}$	•No effect on age-related neuromuscular ageing •increased RONS generation in skeletal muscle.	268
$5LOX^{-/-}$	•No effect on surgical denervation-induced muscle atrophy.	22
$12/15LOX^{-/-}$	•Protected against surgical denervation-induced muscle atrophy •prevented NADPH oxidase activity, protein ubiquitination and ubiquitin-proteasome-mediated proteolytic degradation.	22
$TgSOD1^{+/o}$	•No effect on age-related neuromuscular ageing •increased resistant to H_2O_2 cytotoxicity.	26
$TgCAT^{+/o}$	•No effect on age-related neuromuscular ageing •increased resistant to H_2O_2 cytotoxicity.	26
$TgSOD1/CAT^{+/o}$	•No effect on age-related neuromuscular ageing •increased resistant to H_2O_2 cytotoxicity.	26
$SOD1^{-/-}$	•Accelerated neuromuscular ageing phenotype •loss of muscle fibres and CSA and increased number of centronucleated fibres •partial degeneration of NMJs, loss of innervation and motor function •impaired neurotransmitter release, reduced occupancy of the motor endplates by axons, fragmented postsynaptic endplates, terminal sprouting and axon thinning and irregular swelling •sciatic nerve demyelination and changes in neuron structure •reduced contractile force and grip strength •increased levels of oxidative damage and a constitutive activation of redox-sensitive transcription factors •loss of mitochondrial integrity and function •elevated mitochondrial mediated apoptosis and caspase-3 activity.	27,29,30,33,34,274–278
mitoSOD1 $SOD1^{-/-}$	•Prevented the biochemical and morphological defects in the $SOD1^{-/-}$ model •rescued axon outgrowth and normalized mitochondrial density in primary motor neurons *in vitro* •prevented motor neuropathy and preserved NMJ integrity and grip strength.	23
mSOD1KO	•No effect on age-related muscle atrophy •increased GTN skeletal muscle mass •increased degenerative-regenerative phenotype and number of centronucleated fibres •reduced maximum isometric specific force.	34
$SynTgSOD1^{-/-}$	•Prevented the neuromuscular ageing phenotype in the $SOD1^{-/-}$ model •rescued age-related muscle atrophy and muscle weakness •prevented degeneration of NMJ structure and function •no evidence of oxidative damage and adaptations in stress responses •no evidence of up-regulated NFκB signalling.	29
nSOD1KO	•No effect on age-related muscle atrophy of GTN, AT and EDL muscles •quadriceps and soleus showed a reduction in muscle mass •reduced maximum isometric specific force in GTN and EDL muscle •no effect on oxidative damage and adaptations in stress responses •altered NMJ morphology and increased expression of genes associated with denervation.	31

Knockout mice heterozygous for the MnSOD gene ($SOD2^{+/-}$), mice with conditional knockout of MnSOD targeted to type IIB skeletal muscle fibres (TnIFastCre$Sod2^{fl/fl}$), mice overexpressing MnSOD ($SOD2^{Tg}$), mice deficient in mitochondrial GPX4 (mGPX4-KO), mice deficient in PRX3 ($PRX3^{-/-}$), transgenic mice with targeted overexpression of the human CAT gene to mitochondria ($mCAT^{Tg}$), transgenic mice with muscle specific over-expression of rat nNOS ($nNOS^{Tg}$), mice deficient in GPX1 ($GPX1^{-/-}$), mice deficient in 5LOX ($5LOX^{-/-}$), mice deficient in 12/15LOX ($12/15LOX^{-/-}$), hemizygous transgenic mice that overexpress CuZnSOD ($TgSOD1^{+/o}$), CAT ($TgCAT^{+/o}$) and combined CuZnSOD and CAT ($TgSOD1/CAT^{+/o}$), mice deficient in CuZnSOD ($SOD1^{-/-}$), transgenic $SOD1^{-/-}$ mice that exclusively expressed human SOD1 within the MIS (mitoSOD1,$SOD1^{-/-}$), muscle-specific CuZnSOD knockout mice (mSOD1KO), transgenic $SOD1^{-/-}$ mice with neuron-specific expression of CuZnSOD ($SynTgSOD1^{-/-}$), neuron-specific CuZnSOD knockout mice (nSOD1KO), gastrocnemius (GTN), anterior tibialis (AT), extensor digitorum longus (EDL), Akt–mammalian target of rapamycin (mTOR), neuromuscular junction (NMJ), mitochondrial intermembrane space (MIS).

DOI: 10.1002/jcsm.12223

available literature, there is limited evidence to suggest that the age-related changes in mitochondrial redox potential contribute to the loss of muscle mass inherent with ageing. In support of this, recent ageing studies with use of mitochondria-targeted antioxidants failed to provide evidence that defective mitochondrial redox signalling inherent with ageing is the key regulator of age-related myofibre atrophy and weakness.[21,39]

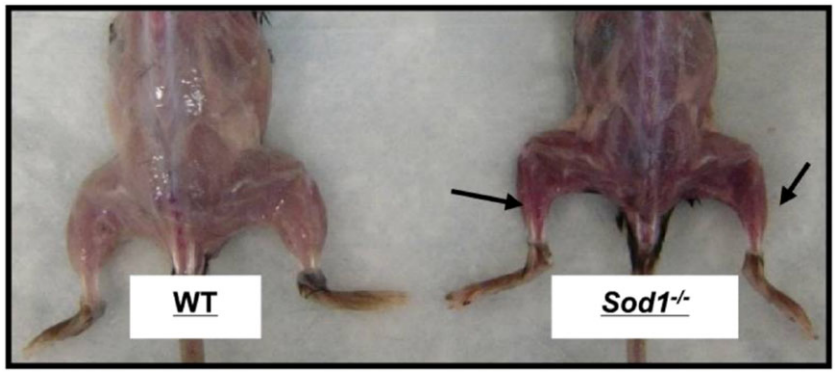
Deletion of CuZnSOD in SOD1$^{-/-}$ mice leads to accelerated neuromuscular ageing and functional deficits

Reduced lifespan observed in SOD1$^{-/-}$,[27] PRX1$^{-/-}$,[250] PRX2$^{-/-}$[251] and TRX2$^{+/-}$[252] models is much more prominent in the SOD1$^{-/-}$ rodent model,[281] indicating that specific key RONS regulatory systems and redox signalling pathways are implicated in the processes of ageing. Moreover, although indistinguishable from WT mice at birth, by 5–8 months of age, SOD1$^{-/-}$ mice show an accelerated neuromuscular ageing phenotype associated with myofibre atrophy (Figure 5), neurological impairments (Figure 6) and functional deficits.[275] The features of the SOD1$^{-/-}$ mouse model mimic those observed in 30 month old WT mice[27,277] and in older humans.[6,277] In addition, in common with old WT mice, skeletal muscle from SOD1$^{-/-}$ rodents exhibits increased levels of oxidative damage[27,29–31,33,34,276,277] and a constitutive activation of redox-sensitive transcription factors[33]; hence, it has been suggested that this knockout murine model represents a useful model for the study of chronic oxidative damage in the context of neuromuscular ageing in an effort to identify potential mechanisms and pathways that underlie sarcopenia in humans. It is noteworthy that hemizygous transgenic mouse models that overexpress CuZnSOD (TgSOD1$^{+/o}$), CAT (TgCAT$^{+/o}$) and combined (TgSOD1/CAT$^{+/o}$) show no increase in lifespan and fail to rescue age-related muscle wasting and functional deficits.[26]

The exacerbated neuromuscular ageing phenotype observed in the SOD1$^{-/-}$ model implies that failure of redox homeostasis in specific subcellular compartments and/or tissues plays an important role in skeletal muscle ageing. As previously discussed, SOD1 is localized within the MIS and cytosol and catalyses the dismutation of superoxide to H_2O_2 and O_2. Thus, the neuromuscular ageing phenotype observed in the SOD1$^{-/-}$ model is likely associated with disrupted redox signalling within both the cytosolic and mitochondrial subcellular compartments. Elevated levels of oxidative damage and atrophy shown in skeletal muscle of SOD1$^{-/-}$ are accompanied by increased mitochondrial generation of reactive species, impaired mitochondrial bioenergetic function and mitochondrial release of proapoptotic factors, which ultimately lead to apoptotic loss of myonuclei.[276] These findings imply that the mitochondrial redox environment plays a central role in regulating skeletal muscle mitochondrial function. In support of this, the physiological and functional importance of maintaining redox homeostasis within the MIS was recently highlighted in a transgenic model that exclusively expressed SOD1 within the MIS (mitoSOD1, Sod1$^{-/-}$) from SOD1$^{-/-}$ mice.[23] The transgenic approach used in the mitoSOD1,Sod1$^{-/-}$ model prevented the morphological and biochemical defects associated with progressive motor axonopathy in skeletal muscle of the SOD1$^{-/-}$ rodents,[23] highlighting the importance of SOD1 redox regulatory enzyme expression in the MIS, and implicated oxidative damage initiated at mitochondrial sites in the pathogenesis of motor axon degeneration.

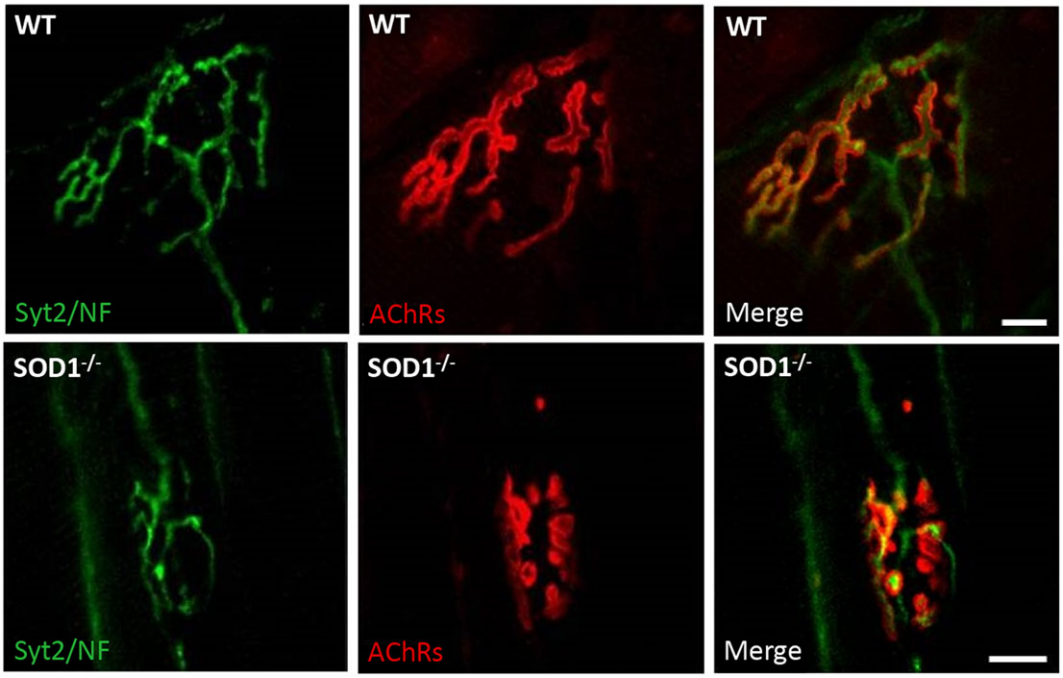
The accelerated muscle ageing phenotype observed in SOD1$^{-/-}$ rodents may imply that excess levels of superoxide within the muscle cells are the underlying reactive species, responsible for the initiation and progression of muscle atrophy that occurs in this model. However, it is plausible to speculate that alternative RONS may play important roles in degeneration of neuromuscular integrity in the SOD1$^{-/-}$ model, such as peroxynitrite or a change in NO bioavailability reacting with excess levels of superoxide. Studies using a

Figure 5 Gross morphology of skinned hindlimb muscles of SOD1$^{-/-}$ and WT mice at 20 months of age. Redrawn from Jang *et al*. 2010.[276]



DOI: 10.1002/jcsm.12223

Figure 6 NMJ immunofluorescence images from AT muscle of SOD1$^{-/-}$ and WT mice at 10 months of age. Left panels: morphology of presynaptic motor neurons stained with antibodies to synaptotagmin-2 and neurofilaments (*green staining*). Middle panels: morphology of postsynaptic AChRs labelled with bungarotoxin (*red staining*). Right panels: merged images of presynaptic motor neurons and AChRs. Redrawn from Sakellariou *et al.*[29] Original magnification: 60X (scale bar = 10 μm).



combination of immunoblotting, high-performance liquid chromatography and real-time fluorescence microscopy methods have monitored specific intracellular RONS in single myofibres isolated from skeletal muscle of SOD1$^{-/-}$ rodents. These studies carried out in resting and contracting skeletal muscle have demonstrated that genetic ablation of SOD1 does not induce the anticipated increase in cytosolic superoxide availability, but instead induced a substantial increase in peroxynitrite formation.[30] These findings may provide important information of the RONS that are implicated in the processes of skeletal muscle ageing and highlight peroxynitrite formation as the important RONS mediator of the exacerbated neuromuscular ageing phenotype observed in the SOD1$^{-/-}$ model. In support of these findings, ageing studies have provided evidence for increased peroxynitrite generation in skeletal muscle of old mice compared with adult mice, indicated by an increase in 3-nitrotyrosine content of muscle proteins, suggesting that peroxynitrite might play an important role in the processes of neuromuscular ageing.[282]

Transgenic mice overexpressing nNOS isoform (nNOS^{Tg}) also exhibit increased peroxynitrite formation in skeletal muscle.[30] However, this model is not associated with changes in skeletal muscle morphology and function, in contrast to the SOD1$^{-/-}$ murine model.[30] A potential explanation might be due to the extent and the subcellular sites of peroxynitrite generation; nNOS is expressed along the sarcolemma of skeletal muscle fibres, whereas SOD1 is expressed in the cytosol and MIS. In support of this, skeletal muscle from SOD1$^{-/-}$ mice (but not in nNOS^{Tg} mice) is associated with increased PRXV protein expression,[30] an enzyme with a high peroxynitrite reductase activity,[144,283] predominantly localized in mitochondria.[147] Hence, the increase in PRXV expression seen in muscle of SOD1$^{-/-}$ mice supports a substantial increase in peroxynitrite in the mitochondrial organelles and highlights that specific RONS formed in specific subcellular compartments and/or tissues are implicated in the processes of neuromuscular ageing in the SOD1$^{-/-}$ model. Additional research based on scavenging of the apparent age-related increase in peroxynitrite generation is warranted to assess the role of peroxynitrite in the loss of neuromuscular integrity and function that occurs with the advance of age.

CuZnSOD gene deletion targeted to skeletal muscle alone does not cause myofibre atrophy

Deciphering the key pathways and mechanisms underlying neuromuscular ageing has been challenging, in part because of the difficulty in unravelling the association between loss

DOI: 10.1002/jcsm.12223

of motor units and loss of muscle mass, both of which occur with the advance of age.[284] Motor nerves and muscles are well known to play a symbiotic role in maintenance of the neuromuscular system; specifically, the viability of motor neurons is recognized to be dependent upon continued exposure to neurotrophic factors released by myofibres.[94] The prominent muscle ageing phenotype described in the SOD1$^{-/-}$ model is associated with a number of neurological impairments, including gross alterations in NMJ morphology, reduced occupancy of the motor endplates by axons, fragmented postsynaptic endplates, terminal sprouting and axon thinning and irregular swelling, loss of motor function and contractility,[276] impaired neurotransmitter release[278] and loss of contractile force.[27] In addition, induction of contraction by using direct muscle stimulation of muscle tissue, circumventing the NMJ, partially rescues the deficit in force, which indicates a loss of functional innervation in the SOD1$^{-/-}$ model.[277] Collectively, these observations may suggest that the age-related deficits in muscle mass and force might be initiated by disrupted motor neuron redox signalling. However, whether the degenerative changes are initiated by altered redox homeostasis proximal and/or distal to the neuromuscular synapses remained inconclusive in these studies. In relation to this, reports have shown that age-related changes in NMJ integrity[285] and reduced muscle strength[286] precede myofibre atrophy, highlighting the importance of the motor neuron system in neuromuscular ageing.

'Conditional knockout models', genetically engineered to lack or inactivate a gene of interest in a specific tissue or cell type, provide a valuable tool to examine the site-specific importance of the function of that particular gene. Recent work has used Cre-Lox targeted approaches to examine whether specific SOD1 gene deletion targeted to skeletal muscle (mSOD1KO) is sufficient to initiate the SOD1$^{-/-}$-associated sarcopenic phenotype.[34] Surprisingly, mSOD1KO mice maintained muscle masses at or above those of WT control mice. Moreover, no detectable increases in global measures of oxidative damage or fibre RONS changes, no reduction in mitochondrial ATP production or adaptive stress responses were observed in muscle from mSOD1KO model.[34] However, specific lack of SOD1 in skeletal muscle of mSOD1KO lead to a reduction of maximum isometric specific force and potentiated muscle regenerative pathways as shown by elevated Akt-mTOR signalling and the presence of extensive central nucleation of muscle fibres.[34] Collectively, these data reveal that, although SOD1 gene deletion targeted specifically to skeletal muscle induced specific functional deficits, loss of SOD1 protein expression restricted to skeletal muscle alone was not sufficient to cause muscle atrophy.[34] These findings suggest that the altered muscle redox environment observed in SOD1$^{-/-}$ is likely not the driving factor for the degeneration of NMJs and loss of muscle mass observed during ageing of the SOD1$^{-/-}$ model.

Neuron-specific expression of CuZnSOD prevents the loss of muscle mass and function that occurs in SOD1$^{-/-}$ mice

To unravel whether the muscle decline and weakness shown in the SOD1$^{-/-}$ model is initiated by defective redox signalling within motor neurons, a recent study generated a transgenic SOD1$^{-/-}$ model in which human SOD1 was expressed under the control of the synapsin 1 promoter (SynTgSOD1$^{-/-}$), termed 'nerve rescue' mice.[29] The experimental work undertaken in this study revealed that sciatic nerve SOD1 content in SynTgSOD1$^{-/-}$ was 20% of control WT mice. Partial rescue of SOD1 expression in motor neurons of the nerve rescue mice reversed all aspects of the accelerated neuromuscular ageing phenotype observed in the SOD1$^{-/-}$ model including the multiple biochemical and physiological changes associated with the exacerbated ageing phenotype.[29] Increased oxidative damage and compensatory up-regulation of redox regulatory enzymes, stress responses and adaptive signalling pathways observed in muscle from SOD1$^{-/-}$ mice[30,33] were not present in the neuron-specific transgenic SynTgSOD1$^{-/-}$ model. Moreover, the accelerated degeneration in NMJ structure, including both presynaptic and postsynaptic NMJ features,[23,276] failure of neuromuscular transmission[29,278] and impaired *in situ* muscle-force generation[34,277] that occur in the whole body SOD1$^{-/-}$ model were completely rescued in the nerve rescue model.[29]

Expression of CuZnSOD in tissues that contain synapses, including brain, spinal cord, sensory and motor nerves in the SynTgSOD1$^{-/-}$ model, excluded any role for other tissues and cell types, which may be anticipated to play an essential role in maintenance of NMJs (e.g. Schwann cells) or muscles (e.g. satellite cells). These findings highlight that failure of redox homeostasis in motor nerves alone is sufficient to generate an ageing phenotype in skeletal muscle and NMJs. This model provided a powerful approach to help elucidate the roles of tissue-specific defects in redox status and highlights that redox homoeostasis in motor neurons plays a key role in neuromuscular ageing.

Neuron-specific reduction of CuZnSOD is not sufficient to initiate a full sarcopenia phenotype

The neuromuscular changes observed in the SOD1$^{-/-}$ model of ageing have been further assessed in a model with targeted deletion of CuZnSOD specifically to neurons (nSOD1KO) by using Sod1-floxed mice crossed to transgenic mice expressing Cre recombinase driven by the nestin promoter.[31] The significant neuronal loss of CuZnSOD activity and protein expression in the nSOD1KO model was not sufficient to replicate the muscle atrophy and weakness observed in the SOD1$^{-/-}$ model. Muscle mass from nSOD1KO mice was not altered in the gastrocnemius (GTN),

DOI: 10.1002/jcsm.12223

anterior tibialis (AT) or extensor digitorum longus (EDL) muscles as opposed to the 30–45% reduction observed in adult SOD1$^{-/-}$[27,277,278] and 30–33 month old WT mice.[287] Despite no change in mass, EDL and GTN showed a small but significant reduction in maximum isometric specific force (8–10% vs. ~30–40% in the SOD1$^{-/-}$ model). Interestingly, quadriceps (~14%) and soleus (<10%) muscle of nSOD1KO mice showed a small but significant reduction in mass, associated with a trend for a reduction in myofibre size.[31] Muscle mitochondrial reactive species generation and altered redox homeostasis and changes in protein expression on RONS regulatory enzymes were not increased in muscle from the nSOD1KO model. Moreover, although there was no evidence of denervation in the nSOD1KO model, NMJ morphology was altered (reduced endplate area) and the expression of genes associated with denervation acetylcholine receptor subunit alpha (AChRα), the transcription factor, Runx1 and GADD45α was increased, supporting a role for neuronal loss of CuZnSOD initiating alterations at the NMJ.[31] The observed changes in NMJ structure/function were much less severe in the nSOD1KO compared with the SOD1$^{-/-}$ model, with no evidence of NMJ fragmentation or denervation, which explains why the nSOD1KO model did not exhibit a similar neuromuscular ageing phenotype shown in the whole body SOD1$^{-/-}$ model.

Collectively, based on the available data with use of conditional knockout and transgenic models, it appears that CuZnSOD deficits in either the motor neuron or muscle 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. The current evidence further suggests that alterations in NMJ morphology and function due to compromised redox homeostasis in motor neurons appear to be the prime event that potentiates muscle mitochondrial dysfunction and oxidative damage that triggers a retrograde response leading to further NMJ damage and dysfunction. Overall, these changes ultimately result in NMJ degeneration, failure of neuromuscular transmission, denervation, loss of muscle fibres, fibre atrophy and, eventually, sarcopenia.

Genetic removal of 12/15-lipoxygenase in 12/15-LOX$^{-/-}$ mice protects against denervation-induced muscle atrophy

Denervation-induced muscle atrophy, previously shown to not only stimulate the autophagy-lysosome pathway[288] but also up-regulate several atrogenes that function as ubiquitin ligases to identify proteins for degradation by the proteasome,[289,290] has been further assessed in the 12/15LOX$^{-/-}$ mouse model.[22] Previous reports have shown that denervation-induced muscle atrophy is associated with activation of cytosolic PLA_2,[291] an enzyme that regulates AA release from membrane phospholipids that act as a substrate for lipid metabolic pathways catalysed by LOXs, cyclooxygenase and cytochrome P450.[46] Genetic ablation of 12/15-LOX but not 5-LOX showed protection against surgical denervation-induced muscle atrophy,[22] implying a selective role for the 12/15-LOX pathway in neurogenic muscle atrophy. Removal of 12/15-LOX (but not 5-LOX) reduced NADPH oxidase activity, protein ubiquitination and ubiquitin-proteasome-mediated proteolytic degradation that were associated with neurogenic-induced muscle atrophy.[22] The findings from this study reveal a novel pathway for neurogenic muscle atrophy and suggest that 12/15-LOX system may have important implications for neuromuscular diseases and neuromuscular deficits inherent with ageing.

Further murine models have been recently developed that resemble many key aspects of neurological impairment in muscle ageing and are also associated with muscle atrophy and contractile dysfunction. These models explore different mechanisms and have been described in recent reviews.[7,292]

Potential therapies to combat the age-related deficits in skeletal muscle function

There is significant academic and commercial interest in the development of therapies, of both pharmacological and non-pharmacological origin, to combat the loss of skeletal muscle mass and function, in the context of neuromuscular ageing and a wide range of myopathies.[293] Physical activity is one of the most effective interventions known to delay the progression of several aspects of muscle ageing. Similar to rodent models,[41,294] human studies have shown that physical activity is beneficial in promoting survival of motor units,[11] facilitating reinnervation of muscle fibres that become denervated secondary to impaired NMJ stability,[12] in attenuating age-related genotoxic stress[14] and preserving redox regulated adaptive responses.[15] However, there is also evidence that the plasticity of the NMJ to physical activity is attenuated with ageing and denervation may become exacerbated by exercise training in very old age.[7] Specifically, aged rats subjected to long-term exercise training exhibited greater muscle atrophy and myocyte oxidative damage compared with aged-matched sedentary controls.[295,296] The potential for physical activity to induce adverse effects when initiated in old age has not been addressed in humans. Further studies (including also adjunct nutritional interventions) are needed to assess the plasticity of the neuromuscular human system in response to physical activity in advanced age, where the remodelled surviving motor units may be further compromised by increased muscle activation.

Antioxidants appear to be a logical intervention in combating the age-related loss of muscle mass and function; however, to date, there have been no robust, longitudinal human studies carried out to address this. The use of

DOI: 10.1002/jcsm.12223

broad-spectrum antioxidants (i.e. vitamins C and E) initially seems a rather attractive proposition, as their mode of action is well established, their efficacy as antioxidants well described and are overall generally well tolerated. However, several studies, primarily from an exercise perspective, have investigated the impact of broad-spectrum antioxidants on skeletal muscle, with the prevailing finding that RONS are in fact crucial components of the adaptive mechanisms within muscle—suggesting that antioxidant intervention in this context may have adverse effects.[297] In addition, despite the causal role of aberrant redox homeostasis in the development of muscular dystrophy,[112] early clinical trials using antioxidants such as vitamins B and E and penicillamine did not show any statistically significant clinical benefits.[298] Similarly, use of pentoxifylline, a phosphodiesterase inhibitor with potent antioxidant and anti-inflammatory activity, failed to provide any improvements on muscle strength and function in Duchenne muscular dystrophy patients,[299] despite showing significant muscle strength restoration on mdx mice.[300] The use of antioxidant therapies in muscular dystrophy has been described in a recent review.[293]

Calorie or dietary restriction has shown to promote survival in mammals and delay the onset of numerous age-related phenotypes including sarcopenia.[301,302] At a biochemical level, calorie restriction interventions have shown to increase sirtuin 1 (a member of the sirtuin family linked to lifespan extension and enhanced mitochondrial biogenesis), the expression of peroxisome proliferator-activated receptor α (PGC1α) (a master regulator of mitochondrial biogenesis and RONS defence system), thus reducing oxidative damage and preserving mitochondrial structural and functional integrity in metabolically active tissues of rodents and humans.[303–305] A direct link among mitochondrial dysfunction, oxidative damage and neuromuscular innervation was recently established in which calorie restriction reversed or attenuated impaired muscle function, loss of innervation and the profound muscle atrophy exhibited in the $SOD1^{-/-}$ mouse model.[287] Specifically, dietary reduction improved mitochondrial function as evidenced by enhanced Ca^{2+} regulation, attenuated mitochondrial oxidative damage, reduced mitochondrial ROS production, increased MnSOD content and sirtuin 3 protein expression.[287]

Similarly, the use of branched-chain amino acids (BCAAs) has also been shown to extend chronological life of rodents and promote muscle efficiency in mammals.[217] Evidence has revealed that BCAA supplementation is coupled to sirtuin 1 expression, increased mitochondrial biogenesis and enhanced RONS protective pathways in middle-aged mice, which ultimately improve the functional capacity of skeletal muscle including physical endurance and motor coordination.[217] It is important to mention that the BCAA supplementation effects were attenuated in eNOS null mutant mice, indicating that BCAA-mediated responses appear to be regulated by redox signalling pathways.[217] Further longitudinal cohort investigations are needed to assess the potential effect of both calorie restriction and BCAA interventions on aspects of neuromuscular ageing in humans.

Recent development of novel antioxidant compounds, with a more specific mode of action (e.g. SS-31 and MitoQ), has allowed researchers to assess specific mechanisms that may potentially alter the age-related loss of muscle mass and function. Treatment of aged mice with SS-31 peptide, a mitochondria-targeted antioxidant, resulted in an overall decrease in markers of oxidative damage and improved specific aspects of skeletal muscle mitochondrial function, mitophagic potential and organelle integrity.[21] However, SS-31 drug treatment showed no impact on the features of sarcopenia including age-related loss of myofibre CSA and muscle function.[21] Another study in aged mice treated with SS-31 reported improved mitochondrial energetics and increased resistance to fatigue.[306] Collectively, these findings provide evidence that mitochondria-derived ROS play a role in some of the aspects of musculoskeletal ageing.

The novel mitochondrial-targeted antioxidant MitoQ has been a compound of significant interest that has undergone phase 1 and 2 clinical trials,[307] to target the RONS-mediated aspects of several pathologies (Parkinson's/multiple sclerosis) and for its potential impact on skeletal muscle. Although there is a large amount of evidence to suggest that MitoQ administration provides beneficial effects, recent studies in the field of muscle metabolism have shown that MitoQ was found to have no effect on exercise-induced adaptations in muscle oxidative capacity in humans.[308] Similarly, in the context of musculoskeletal ageing, MitoQ intervention in old mice failed to rescue the loss of muscle mass and function associated with ageing of skeletal muscle.[39] Overall, targeted antioxidant compounds such as SS-31 and MitoQ are clearly useful from a mechanistic perspective; however, the ability to translate these findings in a human context remains less clear. Additional research is warranted to facilitate our understanding on key areas of defective redox homeostasis and maintenance of neuromuscular integrity in humans. Longitudinal studies of ageing models and humans will help clarify the cause and effect relationships and thus identify relevant therapeutic targets to combat the age-related deficits in skeletal muscle mass and function.

Perspectives and future directions

Multiple theories have been proposed to explain the ageing process,[309] but none has yet received wide acceptance. Nevertheless, the free radical theory of ageing seems to be the theory receiving the widest acceptance as a plausible explanation of the primary biochemical reactions at the basis of the ageing process, and during the last four decades, there has been an enormous increase of information on the effects

DOI: 10.1002/jcsm.12223

of oxidants with age. There is considerable evidence in support of the free radical theory of ageing that comes from a series of studies with invertebrates. The recent technological advantages in the field of molecular genetics have enabled investigators to utilize genetic engineering techniques to alter specific redox genes or processes and examine whether redox homeostatic regulation plays a key role in mammalian ageing (including maximum lifespan, median lifespan and tissue/organ ageing).

Age-related muscle atrophy and weakness, characterized by loss of lean muscle mass and reduced neuromuscular function, is a major contributor to frailty and loss of independence in the elderly, which has a major economic burden on the healthcare systems. Age-dependent loss of muscle mass and strength is a multifactorial process involving a complex interaction of a variety of metabolic processes, and the primary biochemical and molecular mechanisms underlying this process have not been fully determined. Considerable evidence in both humans and various organisms has shown that skeletal muscle decline with advancing age is linked to an altered oxidative status of redox-responsive proteins and increased oxidative modifications of macromolecules. Age-related changes in redox homeostasis have been proposed to play-a key role in sarcopenia as it underlies many age-related human diseases including neurodegenerative disorders, neuromuscular diseases, skeletal muscle pathologies, ischemia-reperfusion injury and diabetes. Over the past two decades, a series of knockout (whole body and tissue specific) and transgenic models have been generated to study whether the redox environment is linked to age-related deficits in neuromuscular integrity and function. In the present review, we have outlined the genetic approaches that have been undertaken in rodent models and provide insights on the role of redox homeostasis in age-related atrophy and weakness.

The majority of knockout and overexpressing mouse models failed to alter the neuromuscular ageing processes, which argue for a role of defective redox signalling in age-related skeletal muscle loss and function implying that the free radical theory of ageing is not as simple and straight forward. Mice deficient in CuZnSOD show a reduction in lifespan and an accelerated neuromuscular ageing phenotype that resembles the biochemical and physiological changes observed in old WT mice and humans indicating that specific RONS regulatory enzymes and/or reactive species are implicated in the processes of muscle ageing. The striking alterations in NMJ integrity/function and loss of innervation observed in the $SOD1^{-/-}$ mouse model highlight the implication of motor neuron integrity in myofibre atrophy and functional deficits. Compromised redox homeostasis of motor neurons as a potential mechanism of sarcopenia in CuZnSOD deficient mice has recently been underlined in a model with specific loss of CuZnSOD targeted to skeletal muscle alone but also in a 'nerve rescue' $SOD1^{-/-}$ mouse model with neuron-specific expression of CuZnSOD, suggesting that failure of redox homeostasis in motor neurons appears to be the prime event initiating sarcopenia during ageing. These studies have shed light on understanding (i) the redox mediated cross-talk between skeletal muscle and motor neurons and (ii) the defective redox signalling events that underlie neuromuscular ageing.

To fully understand the key mechanisms through which redox homeostasis regulates age-related neuromuscular integrity and function, further conditional knockout and transgenic models but also targeted interventions are warranted. Additional research will facilitate our understanding on key areas of defective redox homeostasis and maintenance of neuromuscular integrity. Collectively, this work highlights the important role of the redox environment in maintenance of neuromuscular integrity and function and suggests that defective redox signalling in motor neurons may contribute to age-related deficits in skeletal muscle mass and function. Understanding fully the mechanisms through which the redox environment regulates neuromuscular integrity, muscle mass and function may uncover potential targets/sites for intervention for preventing sarcopenia in humans with the aim to improve the quality of life in the elderly.

Acknowledgements

BMcD was funded by Wellcome Trust ISSF, grant number 097826/Z/11/Z. All authors certify compliance with the Ethical guidelines for authorship and publishing established by the Journal of Cachexia, Sarcopenia, and Muscle.[310]

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013;**153**: 1194–1217.
2. Porter MM, Vandervoort AA, Lexell J. Aging of human muscle: structure, function and adaptability. *Scand J Med Sci Sports* 1995;**5**:129–142.
3. Campbell MJ, McComas AJ, Petito F. Physiological changes in ageing muscles. *J Neurol Neurosurg Psychiatry* 1973;**36**:174–182.

4. McNeil CJ, Rice CL. Fatigability is increased with age during velocity-dependent contractions of the dorsiflexors. *J Gerontol A Biol Sci Med Sci* 2007;**62**:624–629.
5. Hourigan ML, McKinnon NB, Johnson M, Rice CL, Stashuk DW, Doherty TJ. Increased motor unit potential shape variability across consecutive motor unit discharges in the tibialis anterior and vastus medialis muscles of healthy older subjects. *Clin Neurophysiol* 2015;**126**:2381–2389.
6. Ward RE, Boudreau RM, Caserotti P, Harris TB, Zivkovic S, Goodpaster BH, et al. Sensory and motor peripheral nerve function and longitudinal changes in quadriceps strength. *J Gerontol A Biol Sci Med Sci* 2015;**70**:464–470.
7. Hepple RT, Rice CL. Innervation and neuromuscular control in ageing skeletal muscle. *J Physiol* 2016;**594**:1965–1978.
8. Newman AB, Kupelian V, Visser M, Simonsick EM, Goodpaster BH, Kritchevsky SB, et al. Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. *J Gerontol A Biol Sci Med Sci* 2006;**61**:72–77.
9. Buckinx F, Rolland Y, Reginster JY, Ricour C, Petermans J, Bruyere O. Burden of frailty in the elderly population: perspectives for a public health challenge. *Arch Public Health* 2015;**73**:19.
10. Comans TA, Peel NM, Hubbard RE, Mulligan AD, Gray LC, Scuffham PA. The increase in healthcare costs associated with frailty in older people discharged to a post-acute transition care program. *Age Ageing* 2016;**45**:317–320.
11. Power GA, Dalton BH, Behm DG, Vandervoort AA, Doherty TJ, Rice CL. Motor unit number estimates in masters runners: use it or lose it? *Med Sci Sports Exerc* 2010;**42**:1644–1650.
12. Mosole S, Carraro U, Kern H, Loefler S, Fruhmann H, Vogelauer M, et al. Long-term high-level exercise promotes muscle reinnervation with age. *J Neuropathol Exp Neurol* 2014;**73**:284–294.
13. Wiswell RA, Hawkins SA, Jaque SV, Hyslop D, Constantino N, Tarpenning K, et al. Relationship between physiological loss, performance decrement, and age in master athletes. *J Gerontol A Biol Sci Med Sci* 2001;**56**:M618–M626.
14. Cobley JN, Sakellariou GK, Murray S, Waldron S, Gregson W, Burniston JG, et al. Lifelong endurance training attenuates age-related genotoxic stress in human skeletal muscle. *Longevity & healthspan* 2013;**2**:11.
15. Cobley JN, Sakellariou GK, Owens DJ, Murray S, Waldron S, Gregson W, et al. Lifelong training preserves some redox-regulated adaptive responses after an acute exercise stimulus in aged human skeletal muscle. *Free Radic Biol Med* 2014;**70**:23–32.
16. Mecocci P, Fano G, Fulle S, MacGarvey U, Shinobu L, Polidori MC, et al. Age-dependent increases in oxidative damage to DNA, lipids, and proteins in human skeletal muscle. *Free Radic Biol Med* 1999;**26**:303–308.
17. Broome CS, Kayani AC, Palomero J, Dillmann WH, Mestril R, Jackson MJ, et al. Effect of lifelong overexpression of HSP70 in skeletal muscle on age-related oxidative stress and adaptation after nondamaging contractile activity. *FASEB J* 2006;**20**:1549–1551.
18. Vasilaki A, Simpson D, McArdle F, McLean L, Beynon RJ, Van Remmen H, et al. Formation of 3-nitrotyrosines in carbonic anhydrase III is a sensitive marker of oxidative stress in skeletal muscle. *Proteomics Clin Appl* 2007;**1**:362–372.
19. Lee HY, Choi CS, Birkenfeld AL, Alves TC, Jornayvaz FR, Jurczak MJ, et al. Targeted expression of catalase to mitochondria prevents age-associated reductions in mitochondrial function and insulin resistance. *Cell Metab* 2010;**12**:668–674.
20. Palomero J, Vasilaki A, Pye D, McArdle A, Jackson MJ. Aging increases the oxidation of dichlorohydrofluorescein in single isolated skeletal muscle fibers at rest, but not during contractions. *Am J Physiol Regul Integr Comp Physiol* 2013;https://doi.org/10.1152/ajpregu.00530.2012.
21. Sakellariou GK, Pearson T, Lightfoot AP, Nye GA, Wells N, Giakoumaki II, et al. Mitochondrial ROS regulate oxidative damage and mitophagy but not age-related muscle fiber atrophy. *Sci Rep* 2016;**6**:33 944.
22. Bhattacharya A, Hamilton R, Jernigan A, Zhang Y, Sabia M, Rahman MM, et al. Genetic ablation of 12/15-lipoxygenase but not 5-lipoxygenase protects against denervation-induced muscle atrophy. *Free Radic Biol Med* 2014;**67**:30–40.
23. Fischer LR, Igoudjil A, Magrane J, Li Y, Hansen JM, Manfredi G, et al. SOD1 targeted to the mitochondrial intermembrane space prevents motor neuropathy in the Sod1 knockout mouse. *Brain* 2011;**134**:196–209.
24. Lee S, Van Remmen H, Csete M. Sod2 overexpression preserves myoblast mitochondrial mass and function, but not muscle mass with aging. *Aging Cell* 2009;**8**:296–310.
25. Lustgarten MS, Jang YC, Liu Y, Qi W, Qin Y, Dahia PL, et al. MnSOD deficiency results in elevated oxidative stress and decreased mitochondrial function but does not lead to muscle atrophy during aging. *Aging Cell* 2011;**10**:493–505.
26. Mele J, Van Remmen H, Vijg J, Richardson A. Characterization of transgenic mice that overexpress both copper zinc superoxide dismutase and catalase. *Antioxid Redox Signal* 2006;**8**:628–638.
27. Muller FL, Song W, Liu Y, Chaudhuri A, Pieke-Dahl S, Strong R, et al. Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy. *Free Radic Biol Med* 2006;**40**:1993–2004.
28. Perez VI, Van Remmen H, Bokov A, Epstein CJ, Vijg J, Richardson A. The overexpression of major antioxidant enzymes does not extend the lifespan of mice. *Aging Cell* 2009;**8**:73–75.
29. Sakellariou GK, Davis CS, Shi Y, Ivannikov MV, Zhang Y, Vasilaki A, et al. Neuron-specific expression of CuZnSOD prevents the loss of muscle mass and function that occurs in homozygous CuZnSOD-knockout mice. *FASEB J* 2014;**28**:1666–1681.
30. Sakellariou GK, Pye D, Vasilaki A, Zibrik L, Palomero J, Kabayo T, et al. Role of superoxide-nitric oxide interactions in the accelerated age-related loss of muscle mass in mice lacking Cu,Zn superoxide dismutase. *Aging Cell* 2011;**10**:749–760.
31. Sataranatarajan K, Qaisar R, Davis C, Sakellariou GK, Vasilaki A, Zhang Y, et al. Neuron specific reduction in CuZnSOD is not sufficient to initiate a full sarcopenia phenotype. *Redox Biol* 2015;**5**:140–148.
32. Umanskaya A, Santulli G, Xie W, Andersson DC, Reiken SR, Marks AR. Genetically enhancing mitochondrial antioxidant activity improves muscle function in aging. *Proc Natl Acad Sci U S A* 2014;**111**:15250–15255.
33. Vasilaki A, van der Meulen JH, Larkin L, Harrison DC, Pearson T, Van Remmen H, et al. The age-related failure of adaptive responses to contractile activity in skeletal muscle is mimicked in young mice by deletion of Cu,Zn superoxide dismutase. *Aging Cell* 2010;**9**:979–990.
34. Zhang Y, Davis C, Sakellariou GK, Shi Y, Kayani AC, Pulliam D, et al. CuZnSOD gene deletion targeted to skeletal muscle leads to loss of contractile force but does not cause muscle atrophy in adult mice. *FASEB J* 2013;https://doi.org/10.1096/fj.13-228130.
35. Lark DS, Kang L, Lustig ME, Bonner JS, James FD, Neufer PD, et al. Enhanced mitochondrial superoxide scavenging does not improve muscle insulin action in the high fat-fed mouse. *PLoS One* 2015;**10**: e0126732. https://doi.org/10.1371/journal.pone.0126732.
36. Schiaffino S, Reggiani C. Fiber types in mammalian skeletal muscles. *Physiol Rev* 2011;**91**:1447–1531.
37. Lexell J, Taylor CC, Sjostrom M. What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. *J Neurol Sci* 1988;**84**:275–294.
38. Gouspillou G, Sgarioto N, Kapchinsky S, Purves-Smith F, Norris B, Pion CH, et al. Increased sensitivity to mitochondrial permeability transition and myonuclear translocation of endonuclease G in atrophied muscle of physically active older humans. *FASEB J* 2014;**28**:1621–1633.
39. Sakellariou GK, Pearson T, Lightfoot AP, Nye GA, Wells N, Giakoumaki II, et al. Long-term administration of the mitochondria-targeted antioxidant mitoquinone mesylate fails to attenuate age-related oxidative damage or rescue the loss of muscle mass and function associated with aging of skeletal muscle.

FASEB J 2016;https://doi.org/10.1096/fj.201600450R.
40. Oda K. Age changes of motor innervation and acetylcholine receptor distribution on human skeletal muscle fibres. *J Neurol Sci* 1984;**66**:327–338.
41. Valdez G, Tapia JC, Kang H, Clemenson GD Jr, Gage FH, Lichtman JW, et al. Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise. *Proc Natl Acad Sci U S A* 2010;**107**:14863–14868.
42. Wokke JH, Jennekens FG, van den Oord CJ, Veldman H, Smit LM, Leppink GJ. Morphological changes in the human end plate with age. *J Neurol Sci* 1990;**95**:291–310.
43. Krantic S, Mechawar N, Reix S, Quirion R. Molecular basis of programmed cell death involved in neurodegeneration. *Trends Neurosci* 2005;**28**:670–676.
44. Jang YC, Van Remmen H. Age-associated alterations of the neuromuscular junction. *Exp Gerontol* 2011;**46**:193–198.
45. Einsiedel LJ, Luff AR. Alterations in the contractile properties of motor units within the ageing rat medial gastrocnemius. *J Neurol Sci* 1992;**112**:170–177.
46. Sakellariou GK, Jackson MJ, Vasilaki A. Redefining the major contributors to superoxide production in contracting skeletal muscle. The role of NAD(P)H oxidases. *Free Radic Res* 2014;**48**:12–29.
47. Fenn WO, Gerschman R, Gilbert DL, Terwilliger DE, Cothran FV. Mutagenic effects of high oxygen tensions on *Escherichia coli*. *Proc Natl Acad Sci U S A* 1957;**43**:1027–1032.
48. Gerschman R, Gilbert DL, Nye SW, Dwyer P, Fenn WO. Oxygen poisoning and x-irradiation: a mechanism in common. *Science* 1954;**119**:623–626.
49. Dillard CJ, Litov RE, Savin WM, Dumelin EE, Tappel AL. Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. *J Appl Physiol* 1978;**45**:927–932.
50. Davies KJ, Quintanilha AT, Brooks GA, Packer L. Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun* 1982;**107**:1198–1205, doi: S0006-291X(82)80124-1 [pii].
51. Kalyanaraman B, Darley-Usmar V, Davies KJ, Dennery PA, Forman HJ, Grisham MB, et al. Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. *Free Radic Biol Med* 2012;**52**:1–6.
52. McDonagh B, Scullion SM, Vasilaki A, Pollock N, McArdle A, Jackson MJ. Ageing-induced changes in the redox status of peripheral motor nerves imply an effect on redox signalling rather than oxidative damage. *Free Radic Biol Med* 2016;**94**:27–35.
53. Pattwell D, Ashton T, McArdle A, Griffiths RD, Jackson MJ. Ischemia and reperfusion of skeletal muscle lead to the appearance of a stable lipid free radical in the circulation. *Am J Physiol Heart Circ Physiol* 2003;**284**:H2400–H2404.
54. Picard M, Ritchie D, Wright KJ, Romestaing C, Thomas MM, Rowan SL, et al. Mitochondrial functional impairment with aging is exaggerated in isolated mitochondria compared to permeabilized myofibers. *Aging Cell* 2010;**9**:1032–1046.
55. Sakellariou GK, Vasilaki A, Palomero J, Kayani A, Zibrik L, McArdle A, et al. Studies of mitochondrial and nonmitochondrial sources implicate nicotinamide adenine dinucleotide phosphate oxidase(s) in the increased skeletal muscle superoxide generation that occurs during contractile activity. *Antioxid Redox Signal* 2013;**18**:603–621.
56. 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**:26473–26482.
57. Xia R, Webb JA, Gnall LL, Cutler K, Abramson JJ. Skeletal muscle sarcoplasmic reticulum contains a NADH-dependent oxidase that generates superoxide. *Am J Physiol Cell Physiol* 2003;**285**:C215–C221.
58. Mofarrahi M, Brandes RP, Gorlach A, Hanze J, Terada LS, Quinn MT, et al. Regulation of proliferation of skeletal muscle precursor cells by NADPH oxidase. *Antioxid Redox Signal* 2008;**10**:559–574.
59. Whitehead NP, Yeung EW, Froehner SC, Allen DG. Skeletal muscle NADPH oxidase is increased and triggers stretch-induced damage in the mdx mouse. *PLoS One* 2010;**5**: e15354. https://doi.org/10.1371/journal.pone.0015354.
60. Sartoretto JL, Kalwa H, Pluth MD, Lippard SJ, Michel T. Hydrogen peroxide differentially modulates cardiac myocyte nitric oxide synthesis. *Proc Natl Acad Sci U S A* 2011;**108**:15792–15797.
61. Loehr JA, Abo-Zahrah R, Pal R, Rodney GG. Sphingomyelinase promotes oxidant production and skeletal muscle contractile dysfunction through activation of NADPH oxidase. *Front Physiol.* 2014;**5**:530.
62. Espinosa A, Garcia A, Hartel S, Hidalgo C, Jaimovich E. NADPH oxidase and hydrogen peroxide mediate insulin-induced calcium increase in skeletal muscle cells. *J Biol Chem* 2009;**284**:2568–2575.
63. Jackson MJ. Redox regulation of muscle adaptations to contractile activity and aging. *J Appl Physiol (1985)* 2015;**119**:163–171.
64. D'Autreaux B, Toledano MB. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol* 2007;**8**:813–824.
65. Halliwell B, Gutteridge J. *Free Radicals in Biology and Medicine*. Fifth Edition ed: Oxford University Press; 2015.
66. Jackson MJ. Interactions between reactive oxygen species generated by contractile activity and aging in skeletal muscle. *Antioxid Redox Signal* 2013; https://doi.org/10.1089/ars.2013.5383.
67. Giorgio M, Trinei M, Migliaccio E, Pelicci PG. Hydrogen peroxide: a metabolic by-product or a common mediator of ageing signals? *Nat Rev Mol Cell Biol* 2007;**8**:722–728.
68. Jackson MJ. Control of reactive oxygen species production in contracting skeletal muscle. *Antioxid Redox Signal* 2011;**15**:2477–2486.
69. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 2006;**160**:1–40.
70. Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* 1996;**271**:C1424–C1437.
71. Kelm M. Nitric oxide metabolism and breakdown. *Biochim Biophys Acta* 1999;**1411**:273–289.
72. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A* 1990;**87**:1620–1624.
73. Szabo C, Ischiropoulos H, Radi R. Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. *Nat Rev Drug Discov* 2007;**6**:662–680.
74. Nalwaya N, Deen WM. Analysis of cellular exposure to peroxynitrite in suspension cultures. *Chem Res Toxicol* 2003;**16**:920–932.
75. Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev* 2008;**88**:1243–1276.
76. Muller FL, Liu Y, Van Remmen H. Complex III releases superoxide to both sides of the inner mitochondrial membrane. *J Biol Chem* 2004;**279**:49064–49073.
77. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J* 2009;**417**:1–13.
78. Goncalves RL, Quinlan CL, Perevoshchikova IV, Hey-Mogensen M, Brand MD. Sites of superoxide and hydrogen peroxide production by muscle mitochondria assessed ex vivo under conditions mimicking rest and exercise. *J Biol Chem* 2015;**290**:209–227.
79. Quinlan CL, Orr AL, Perevoshchikova IV, Treberg JR, Ackrell BA, Brand MD. Mitochondrial complex II can generate reactive oxygen species at high rates in both the forward and reverse reactions. *J Biol Chem* 2012;**287**:27255–27264.
80. Brand MD. Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. *Free Radic Biol Med* 2016; https://doi.org/10.1016/j.freeradbiomed.2016.04.001.
81. Sun QA, Hess DT, Nogueira L, Yong S, Bowles DE, Eu J, et al. Oxygen-coupled redox regulation of the skeletal muscle ryanodine receptor-Ca2+ release channel

by NADPH oxidase 4. *Proc Natl Acad Sci U S A* 2011;**108**:16098–16103.
82. Gomez-Cabrera MC, Close GL, Kayani A, McArdle A, Vina J, Jackson MJ. Effect of xanthine oxidase-generated extracellular superoxide on skeletal muscle force generation. *Am J Physiol Regul Integr Comp Physiol* 2010;**298**:R2–R8.
83. Hellsten Y, Frandsen U, Orthenblad N, Sjodin B, Richter EA. Xanthine oxidase in human skeletal muscle following eccentric exercise: a role in inflammation. *J Physiol* 1997;**498**:239–248.
84. Zuo L, Christofi FL, Wright VP, Bao S, Clanton TL. Lipoxygenase-dependent superoxide release in skeletal muscle. *J Appl Physiol* 2004;**97**:661–668.
85. Gong MC, Arbogast S, Guo Z, Mathenia J, Su W, Reid MB. Calcium-independent phospholipase A2 modulates cytosolic oxidant activity and contractile function in murine skeletal muscle cells. *J Appl Physiol* 2006;**100**:399–405.
86. 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**:792–800.
87. Han D, Antunes F, Canali R, Rettori D, Cadenas E. Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol. *J Biol Chem* 2003;**278**: 5557–5563.
88. Lustgarten MS, Bhattacharya A, Muller FL, Jang YC, Shimizu T, Shirasawa T, et al. Complex I generated, mitochondrial matrix-directed superoxide is released from the mitochondria through voltage dependent anion channels. *Biochem Biophys Res Commun* 2012;**422**:515–521.
89. Salvador A, Sousa J, Pinto RE. Hydroperoxyl, superoxide and pH gradients in the mitochondrial matrix: a theoretical assessment. *Free Radic Biol Med* 2001;**31**:1208–1215.
90. Fukai T, Ushio-Fukai M. Superoxide dismutases: role in redox signaling, vascular function, and diseases. *Antioxid Redox Signal* 2011;**15**:1583–1606.
91. Sheng Y, Abreu IA, Cabelli DE, Maroney MJ, Miller AF, Teixeira M, et al. Superoxide dismutases and superoxide reductases. *Chem Rev* 2014;**114**:3854–3918.
92. Powers SK, Ji LL, Kavazis AN, Jackson MJ. Reactive oxygen species: impact on skeletal muscle. *Compr Physiol* 2011;**1**:941–969.
93. Marinho HS, Real C, Cyrne L, Soares H, Antunes F. Hydrogen peroxide sensing, signaling and regulation of transcription factors. *Redox Biol* 2014;**2**:535–562.
94. Jackson MJ, McArdle A. Role of reactive oxygen species in age-related neuromuscular deficits. *J Physiol* 2016; https://doi.org/10.1113/JP270564.
95. Takac I, Schroder K, Zhang L, Lardy B, Anilkumar N, Lambeth JD, et al. The E-loop is involved in hydrogen peroxide formation by the NADPH oxidase Nox4. *J Biol Chem* 2011;**286**:13304–13313.
96. Koziel R, Pircher H, Kratochwil M, Lener B, Hermann M, Dencher NA, et al. Mitochondrial respiratory chain complex I is inactivated by NADPH oxidase Nox4. *Biochem J* 2013;**452**:231–239.
97. Halliwell B, Clement MV, Long LH. Hydrogen peroxide in the human body. *FEBS Lett* 2000;**486**:10–13.
98. Mehmeti I, Lortz S, Lenzen S. The H2O2-sensitive HyPer protein targeted to the endoplasmic reticulum as a mirror of the oxidizing thiol-disulfide milieu. *Free Radic Biol Med* 2012;**53**:1451–1458.
99. Ramming T, Okumura M, Kanemura S, Baday S, Birk J, Moes S, et al. A PDI-catalyzed thiol-disulfide switch regulates the production of hydrogen peroxide by human Ero1. *Free Radic Biol Med* 2015;**83**:361–372.
100. Sigel A, Sigel H, Sigel RKO. *Interrelations Between Essential Metal Ions and Human Diseases*. Springer; 2013.
101. Konno S. Hydroxyl radical formation in skeletal muscle of rats with glucocorticoid-induced myopathy. *Neurochem Res* 2005;**30**:669–675.
102. Murphy RM, Dutka TL, Lamb GD. Hydroxyl radical and glutathione interactions alter calcium sensitivity and maximum force of the contractile apparatus in rat skeletal muscle fibres. *J Physiol* 2008;**586**:2203–2216.
103. Radak Z. *Free Radicals in Exercise and Aging*. Human Kinetics; 2000.
104. Halliwell B. How to characterize an antioxidant: an update. *Biochem Soc Symp* 1995;**61**:73–101.
105. Imlay JA. The mismetallation of enzymes during oxidative stress. *J Biol Chem* 2014;**289**:28121–28128.
106. Kehrer JP. The Haber-Weiss reaction and mechanisms of toxicity. *Toxicology* 2000;**149**:43–50.
107. O'Neill CA, Stebbins CL, Bonigut S, Halliwell B, Longhurst JC. Production of hydroxyl radicals in contracting skeletal muscle of cats. *J Appl Physiol (1985)* 1996;**81**:1197–1206.
108. Kondo H, Nishino K, Itokawa Y. Hydroxyl radical generation in skeletal muscle atrophied by immobilization. *FEBS Lett* 1994;**349**:169–172.
109. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991;**43**:109–142.
110. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002;**82**:47–95.
111. Reid MB. Role of nitric oxide in skeletal muscle: synthesis, distribution and functional importance. *Acta Physiol Scand* 1998;**162**:401–409.
112. Allen DG, Whitehead NP, Froehner SC. Absence of dystrophin disrupts skeletal muscle signaling: roles of Ca2+, reactive oxygen species, and nitric oxide in the development of muscular dystrophy. *Physiol Rev* 2016;**96**:253–305.
113. Garcia-Cardena G, Fan R, Shah V, Sorrentino R, Cirino G, Papapetropoulos A, et al. Dynamic activation of endothelial nitric oxide synthase by Hsp90. *Nature* 1998;**392**:821–824.
114. Rigamonti E, Touvier T, Clementi E, Manfredi AA, Brunelli S, Rovere-Querini P. Requirement of inducible nitric oxide synthase for skeletal muscle regeneration after acute damage. *J Immunol* 2013;**190**:1767–1777.
115. Adams V, Nehrhoff B, Spate U, Linke A, Schulze PC, Baur A, et al. Induction of iNOS expression in skeletal muscle by IL-1beta and NFkappaB activation: an in vitro and in vivo study. *Cardiovasc Res* 2002;**54**:95–104.
116. Tidball JG, Spencer MJ, Wehling M, Lavergne E. Nitric-oxide synthase is a mechanical signal transducer that modulates talin and vinculin expression. *J Biol Chem* 1999;**274**:33155–33160.
117. Hirschfield W, Moody MR, O'Brien WE, Gregg AR, Bryan RM Jr, Reid MB. Nitric oxide release and contractile properties of skeletal muscles from mice deficient in type III NOS. *Am J Physiol Regul Integr Comp Physiol* 2000;**278**: R95–R100.
118. Pye D, Palomero J, Kabayo T, Jackson MJ. Real-time measurement of nitric oxide in single mature mouse skeletal muscle fibres during contractions. *J Physiol* 2007;**581**:309–318.
119. Tidball JG, Wehling-Henricks M. Expression of a NOS transgene in dystrophin-deficient muscle reduces muscle membrane damage without increasing the expression of membrane-associated cytoskeletal proteins. *Mol Genet Metab* 2004;**82**:312–320.
120. Brenman JE, Chao DS, Xia H, Aldape K, Bredt DS. Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy. *Cell* 1995;**82**:743–752.
121. Stamler JS, Meissner G. Physiology of nitric oxide in skeletal muscle. *Physiol Rev* 2001;**81**:209–237.
122. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 2007;**87**:315–424.
123. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *J Biol Chem* 1991;**266**:4244–4250.
124. Greenacre SA, Ischiropoulos H. Tyrosine nitration: localisation, quantification, consequences for protein function and signal transduction. *Free Radic Res* 2001;**34**:541–581.
125. Montagna C, Di Giacomo G, Rizza S, Cardaci S, Ferraro E, Grumati P, et al. S-nitrosoglutathione reductase deficiency-induced S-nitrosylation results in neuromuscular dysfunction. *Antioxid Redox Signal* 2014;**21**:570–587.
126. McCord JM, Fridovich I. The utility of superoxide dismutase in studying free radical reactions. I. Radicals generated by the interaction of sulfite, dimethyl sulfoxide, and oxygen. *J Biol Chem* 1969;**244**:6056–6063.

127. Culotta VC, Yang M, O'Halloran TV. Activation of superoxide dismutases: putting the metal to the pedal. *Biochim Biophys Acta* 2006;**1763**:747–758.
128. Oh-ishi S, Kizaki T, Nagasawa J, Izawa T, Komabayashi T, Nagata N, et al. Effects of endurance training on superoxide dismutase activity, content and mRNA expression in rat muscle. *Clin Exp Pharmacol Physiol* 1997;**24**:326–332.
129. Gorecki M, Beck Y, Hartman JR, Fischer M, Weiss L, Tochner Z, et al. Recombinant human superoxide dismutases: production and potential therapeutical uses. *Free Radic Res Commun* 1991;**12-13**:401–410.
130. Leeuwenburgh C, Hollander J, Leichtweis S, Griffiths M, Gore M, Ji LL. Adaptations of glutathione antioxidant system to endurance training are tissue and muscle fiber specific. *Am J Physiol* 1997;**272**: R363–R369.
131. Ji LL, Stratman FW, Lardy HA. Antioxidant enzyme systems in rat liver and skeletal muscle. Influences of selenium deficiency, chronic training, and acute exercise. *Arch Biochem Biophys* 1988;**263**:150–160.
132. Brigelius-Flohe R. Glutathione peroxidases and redox-regulated transcription factors. *Biol Chem* 2006;**387**:1329–1335.
133. Ji LL, Dillon D, Wu E. Alteration of antioxidant enzymes with aging in rat skeletal muscle and liver. *Am J Physiol* 1990;**258**:R918–R923.
134. Kirkman HN, Gaetani GF. Mammalian catalase: a venerable enzyme with new mysteries. *Trends Biochem Sci* 2007;**32**:44–50.
135. Mates JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology* 2000;**153**:83–104.
136. Lledias F, Rangel P, Hansberg W. Oxidation of catalase by singlet oxygen. *J Biol Chem* 1998;**273**:10630–10637.
137. Pereira B, Costa Rosa LF, Safi DA, Medeiros MH, Curi R, Bechara EJ. Superoxide dismutase, catalase, and glutathione peroxidase activities in muscle and lymphoid organs of sedentary and exercise-trained rats. *Physiol Behav* 1994;**56**:1095–1099.
138. Fuchs J, Podda M, Packer L. *Redox-Genome Interactions in Health and Disease*. Taylor & Francis; 2003.
139. Chae HZ, Kim IH, Kim K, Rhee SG. Cloning, sequencing, and mutation of thiol-specific antioxidant gene of *Saccharomyces cerevisiae*. *J Biol Chem* 1993;**268**:16815–16821.
140. Hofmann B, Hecht HJ, Flohe L. Peroxiredoxins. *Biol Chem* 2002;**383**:347–364.
141. Seo MS, Kang SW, Kim K, Baines IC, Lee TH, Rhee SG. Identification of a new type of mammalian peroxiredoxin that forms an intramolecular disulfide as a reaction intermediate. *J Biol Chem* 2000;**275**:20346–20354.
142. Rhee SG, Woo HA. Multiple functions of peroxiredoxins: peroxidases, sensors and regulators of the intracellular messenger H(2)O(2), and protein chaperones. *Antioxid Redox Signal* 2011;**15**:781–794.
143. Chang TS, Cho CS, Park S, Yu S, Kang SW, Rhee SG. Peroxiredoxin III, a mitochondrion-specific peroxidase, regulates apoptotic signaling by mitochondria. *J Biol Chem* 2004;**279**:41975–41984.
144. Dubuisson M, Vander Stricht D, Clippe A, Etienne F, Nauser T, Kissner R, et al. Human peroxiredoxin 5 is a peroxynitrite reductase. *FEBS Lett* 2004;**571**:161–165.
145. Kwon J, Wang A, Burke DJ, Boudreau HE, Lekstrom KJ, Korzeniowska A, et al. Peroxiredoxin 6 (Prdx6) supports NADPH oxidase1 (Nox1)-based superoxide generation and cell migration. *Free Radic Biol Med* 2016;**96**:99–115.
146. Chatterjee S, Feinstein SI, Dodia C, Sorokina E, Lien YC, Nguyen S, et al. Peroxiredoxin 6 phosphorylation and subsequent phospholipase A2 activity are required for agonist-mediated activation of NADPH oxidase in mouse pulmonary microvascular endothelium and alveolar macrophages. *J Biol Chem* 2011;**286**:11696–11706.
147. Wood ZA, Schroder E, Robin Harris J, Poole LB. Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem Sci* 2003;**28**:32–40.
148. Day AM, Brown JD, Taylor SR, Rand JD, Morgan BA, Veal EA. Inactivation of a peroxiredoxin by hydrogen peroxide is critical for thioredoxin-mediated repair of oxidized proteins and cell survival. *Mol Cell* 2012;**45**:398–408.
149. Winterbourn CC. Are free radicals involved in thiol-based redox signaling? *Free Radic Biol Med* 2015;**80**:164–170.
150. Wadley AJ, Aldred S, Coles SJ. An unexplored role for Peroxiredoxin in exercise-induced redox signalling? *Redox Biol* 2015;**8**:51–58.
151. McDonagh B, Sakellariou GK, Smith NT, Brownridge P, Jackson MJ. Differential cysteine labeling and global label-free proteomics reveals an altered metabolic state in skeletal muscle aging. *J Proteome Res* 2014;**13**:5008–5021.
152. Sobotta MC, Liou W, Stocker S, Talwar D, Oehler M, Ruppert T, et al. Peroxiredoxin-2 and STAT3 form a redox relay for H2O2 signaling. *Nat Chem Biol* 2015;**11**:64–70.
153. Aydin J, Andersson DC, Hanninen SL, Wredenberg A, Tavi P, Park CB, et al. Increased mitochondrial Ca2+ and decreased sarcoplasmic reticulum Ca2+ in mitochondrial myopathy. *Hum Mol Genet* 2009;**18**:278–288.
154. Laurent TC, Moore EC, Reichard P. Enzymatic synthesis of deoxyribonucleotides. Iv. Isolation and characterization of thioredoxin, the hydrogen donor from *Escherichia coli* B. *J Biol Chem* 1964;**239**:3436–3444.
155. Arner ES, Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. *Eur J Biochem* 2000;**267**:6102–6109, doi:ejb1701 [pii].
156. Collet JF, Messens J. Structure, function, and mechanism of thioredoxin proteins. *Antioxid Redox Signal* 2010;**13**:1205–1216.
157. Dimauro I, Pearson T, Caporossi D, Jackson MJ. In vitro susceptibility of thioredoxins and glutathione to redox modification and aging-related changes in skeletal muscle. *Free Radic Biol Med* 2012;**53**:2017–2027.
158. Fernandes AP, Holmgren A. Glutaredoxins: glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system. *Antioxid Redox Signal* 2004;**6**:63–74.
159. Hanschmann EM, Godoy JR, Berndt C, Hudemann C, Lillig CH. Thioredoxins, glutaredoxins, and peroxiredoxins—molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling. *Antioxid Redox Signal* 2013;**19**:1539–1605.
160. Hanschmann EM, Lonn ME, Schutte LD, Funke M, Godoy JR, Eitner S, et al. Both thioredoxin 2 and glutaredoxin 2 contribute to the reduction of the mitochondrial 2-Cys peroxiredoxin Prx3. *J Biol Chem* 2010;**285**:40699–40705.
161. Daily D, Vlamis-Gardikas A, Offen D, Mittelman L, Melamed E, Holmgren A, et al. Glutaredoxin protects cerebellar granule neurons from dopamine-induced apoptosis by activating NF-kappa B via Ref-1. *J Biol Chem* 2001;**276**:1335–1344.
162. Murata H, Ihara Y, Nakamura H, Yodoi J, Sumikawa K, Kondo T. Glutaredoxin exerts an antiapoptotic effect by regulating the redox state of Akt. *J Biol Chem* 2003;**278**:50226–50233.
163. Pan S, Berk BC. Glutathiolation regulates tumor necrosis factor-alpha-induced caspase-3 cleavage and apoptosis: key role for glutaredoxin in the death pathway. *Circ Res* 2007;**100**:213–219.
164. Rodriguez-Manzaneque MT, Tamarit J, Belli G, Ros J, Herrero E. Grx5 is a mitochondrial glutaredoxin required for the activity of iron/sulfur enzymes. *Mol Biol Cell* 2002;**13**:1109–1121.
165. Lonn ME, Hudemann C, Berndt C, Cherkasov V, Capani F, Holmgren A, et al. Expression pattern of human glutaredoxin 2 isoforms: identification and characterization of two testis/cancer cell-specific isoforms. *Antioxid Redox Signal* 2008;**10**:547–557.
166. Johansson C, Roos AK, Montano SJ, Sengupta R, Filippakopoulos P, Guo K, et al. The crystal structure of human GLRX5: iron-sulfur cluster co-ordination, tetrameric assembly and monomer activity. *Biochem J* 2011;**433**:303–311.
167. Beer SM, Taylor ER, Brown SE, Dahm CC, Costa NJ, Runswick MJ, et al. Glutaredoxin 2 catalyzes the reversible oxidation and glutathionylation of mitochondrial membrane thiol proteins: implications for mitochondrial redox regulation and antioxidant DEFENSE. *J Biol Chem* 2004;**279**:47939–47951.

168. Kozakowska M, Pietraszek-Gremplewicz K, Jozkowicz A, Dulak J. The role of oxidative stress in skeletal muscle injury and regeneration: focus on antioxidant enzymes. *J Muscle Res Cell Motil* 2015;**36**:377–393.
169. Theodorou AA, Nikolaidis MG, Paschalis V, Sakellariou GK, Fatouros IG, Koutedakis Y, et al. Comparison between glucose-6-phosphate dehydrogenase-deficient and normal individuals after eccentric exercise. *Med Sci Sports Exerc* 2010;**42**:1113–1121.
170. Gomes EC, Silva AN, de Oliveira MR. Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. *Oxid Med Cell Longev* 2012;**2012**:756132.
171. Sen C, Packer L, Hänninen O. *Handbook of Oxidants and Antioxidants in Exercise*. Elsevier Science; 2000.
172. Lushchak VI. Glutathione homeostasis and functions: potential targets for medical interventions. *J Amino Acids* 2012;**2012**:736837.
173. Mari M, Morales A, Colell A, Garcia-Ruiz C, Fernandez-Checa JC. Mitochondrial glutathione, a key survival antioxidant. *Antioxid Redox Signal* 2009;**11**:2685–2700.
174. Ji LL. Exercise and oxidative stress: role of the cellular antioxidant systems. *Exerc Sport Sci Rev* 1995;**23**:135–166.
175. Ji LL, Fu R, Mitchell EW. Glutathione and antioxidant enzymes in skeletal muscle: effects of fiber type and exercise intensity. *J Appl Physiol (1985)* 1992;**73**:1854–1859.
176. Leeuwenburgh C, Fiebig R, Chandwaney R, Ji LL. Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems. *Am J Physiol* 1994;**267**: R439–R445.
177. Juel C. Oxidative stress (glutathionylation) and Na,K-ATPase activity in rat skeletal muscle. *PLoS One* 2014;**9**: e110514. https://doi.org/10.1371/journal.pone.0110514.
178. Davies KJ, Maguire JJ, Brooks GA, Dallman PR, Packer L. Muscle mitochondrial bioenergetics, oxygen supply, and work capacity during dietary iron deficiency and repletion. *Am J Physiol* 1982;**242**: E418–E427.
179. Lightfoot AP, McArdle A, Jackson MJ, Cooper RG. In the idiopathic inflammatory myopathies (IIM), do reactive oxygen species (ROS) contribute to muscle weakness? *Ann Rheum Dis* 2015;**74**:1340–1346.
180. Pal R, Palmieri M, Loehr JA, Li S, Abo-Zahrah R, Monroe TO, et al. Src-dependent impairment of autophagy by oxidative stress in a mouse model of Duchenne muscular dystrophy. *Nat Commun* 2014;**5**:4425.
181. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuropharmacol* 2009;**7**:65–74.
182. McDonagh B, Sakellariou GK, Smith NT, Brownridge P, Jackson MJ. Redox proteomic analysis of the gastrocnemius muscle from adult and old mice. *Data Brief* 2015;**4**:344–348.
183. McDonagh B, Sakellariou GK, Jackson MJ. Application of redox proteomics to skeletal muscle aging and exercise. *Biochem Soc Trans* 2014;**42**:965–970.
184. Kramer PA, Duan J, Qian WJ, Marcinek DJ. The measurement of reversible redox dependent post-translational modifications and their regulation of mitochondrial and skeletal muscle function. *Front Physiol.* 2015;**6**:347.
185. Go YM, Chandler JD, Jones DP. The cysteine proteome. *Free Radic Biol Med* 2015;**84**:227–245.
186. Reid MB. Free radicals and muscle fatigue: of ROS, canaries, and the IOC. *Free Radic Biol Med* 2008;**44**:169–179.
187. Smuder AJ, Kavazis AN, Hudson MB, Nelson WB, Powers SK. Oxidation enhances myofibrillar protein degradation via calpain and caspase-3. *Free Radic Biol Med* 2010;**49**:1152–1160.
188. 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. *Biochim Biophys Acta* 2011;**1810**:391–397.
189. Coirault C, Guellich A, Barbry T, Samuel JL, Riou B, Lecarpentier Y. Oxidative stress of myosin contributes to skeletal muscle dysfunction in rats with chronic heart failure. *Am J Physiol Heart Circ Physiol* 2007;**292**:H1009–H1017.
190. Li M, Ogilvie H, Ochala J, Artemenko K, Iwamoto H, Yagi N, et al. Aberrant post-translational modifications compromise human myosin motor function in old age. *Aging Cell* 2015;**14**:228–235.
191. Prochniewicz E, Spakowicz D, Thomas DD. Changes in actin structural transitions associated with oxidative inhibition of muscle contraction. *Biochemistry* 2008;**47**:11811–11817.
192. Andrade FH, Reid MB, Allen DG, Westerblad H. Effect of hydrogen peroxide and dithiothreitol on contractile function of single skeletal muscle fibres from the mouse. *J Physiol* 1998;**509**:565–575.
193. Tisdale MJ. Mechanisms of cancer cachexia. *Physiol Rev* 2009;**89**:381–410.
194. Maltais F, Decramer M, Casaburi R, Barreiro E, Burelle Y, Debigare R, et al. An official American Thoracic Society/European Respiratory Society statement: update on limb muscle dysfunction in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2014;**189**:e15–e62.
195. Powers SK, Morton AB, Ahn B, Smuder AJ. Redox control of skeletal muscle atrophy. *Free Radic Biol Med* 2016;https://doi.org/10.1016/j.freeradbiomed.2016.02.021.
196. Rodney GG, Pal R, Abo-Zahrah R. Redox regulation of autophagy in skeletal muscle. *Free Radic Biol Med* 2016;**98**:103–112.
197. Romanello V, Sandri M. Mitochondrial quality control and muscle mass maintenance. *Front Physiol.* 2015;**6**:422.
198. Barbieri E, Sestili P. Reactive oxygen species in skeletal muscle signaling. *J Signal Transduct* 2012;**2012**:982794.
199. Espinosa A, Henriquez-Olguin C, Jaimovich E. Reactive oxygen species and calcium signals in skeletal muscle: a crosstalk involved in both normal signaling and disease. *Cell Calcium* 2016;https://doi.org/10.1016/j.ceca.2016.02.010.
200. Eisner V, Csordas G, Hajnoczky G. Interactions between sarco-endoplasmic reticulum and mitochondria in cardiac and skeletal muscle—pivotal roles in Ca(2)(+) and reactive oxygen species signaling. *J Cell Sci* 2013;**126**: 2965–2978.
201. Powers SK, Duarte J, Kavazis AN, Talbert EE. Reactive oxygen species are signalling molecules for skeletal muscle adaptation. *Exp Physiol* 2010;**95**:1–9.
202. Reid MB. Redox interventions to increase exercise performance. *J Physiol* 2015; https://doi.org/10.1113/JP270653.
203. Jackson MJ. Free radicals generated by contracting muscle: by-products of metabolism or key regulators of muscle function? *Free Radic Biol Med* 2008;**44**: 132–41. doi:S0891-5849(07)00388-7 [pii], 10.1016/j.freeradbiomed.2007.06.003.
204. Smith MA, Reid MB. Redox modulation of contractile function in respiratory and limb skeletal muscle. *Respir Physiol Neurobiol* 2006;**151**:229–241.
205. Debold EP. Potential molecular mechanisms underlying muscle fatigue mediated by reactive oxygen and nitrogen species. *Front Physiol.* 2015;**6**:239.
206. Reid MB, Haack KE, Franchek KM, Valberg PA, Kobzik L, West MS. Reactive oxygen in skeletal muscle. I. Intracellular oxidant kinetics and fatigue in vitro. *J Appl Physiol* 1992;**73**:1797–1804.
207. Reid MB, Khawli FA, Moody MR. Reactive oxygen in skeletal muscle. III. Contractility of unfatigued muscle. *J Appl Physiol* 1993;**75**:1081–1087.
208. Vasilaki A, McArdle F, Iwanejko LM, McArdle A. Adaptive responses of mouse skeletal muscle to contractile activity: the effect of age. *Mech Ageing Dev* 2006;**127**:830–839, S0047-6374(06) 00193-X [pii].
209. Lightfoot AP, Sakellariou GK, Nye GA, McArdle F, Jackson MJ, Griffiths RD, et al. SS-31 attenuates TNF-alpha induced cytokine release from C2C12 myotubes. *Redox Biol* 2015;**6**:253–259.
210. Kombairaju P, Kerr JP, Roche JA, Pratt SJ, Lovering RM, Sussan TE, et al. Genetic silencing of Nrf2 enhances X-ROS in dysferlin-deficient muscle. *Front Physiol* 2014;**5**:57.
211. Irrcher I, Ljubicic V, Hood DA. Interactions between ROS and AMP kinase activity in the regulation of PGC-1alpha transcription in skeletal muscle cells. *Am J Physiol Cell Physiol* 2009;**296**: C116–C123.

212. Khassaf M, McArdle A, Esanu C, Vasilaki A, McArdle F, Griffiths RD, et al. Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. *J Physiol* 2003;**549**:645–652.
213. Vasilaki A, Mansouri A, Remmen H, van der Meulen JH, Larkin L, Richardson AG, et al. Free radical generation by skeletal muscle of adult and old mice: effect of contractile activity. *Aging Cell* 2006;**5**:109–117.
214. McArdle A, Pattwell D, Vasilaki A, Griffiths RD, Jackson MJ. Contractile activity-induced oxidative stress: cellular origin and adaptive responses. *Am J Physiol Cell Physiol* 2001;**280**:C621–C627.
215. Kang C, O'Moore KM, Dickman JR, Ji LL. Exercise activation of muscle peroxisome proliferator-activated receptor-gamma coactivator-1alpha signaling is redox sensitive. *Free Radic Biol Med* 2009;**47**:1394–1400.
216. Deval C, Mordier S, Obled C, Bechet D, Combaret L, Attaix D, et al. Identification of cathepsin L as a differentially expressed message associated with skeletal muscle wasting. *Biochem J* 2001;**360**:143–150.
217. D'Antona G, Ragni M, Cardile A, Tedesco L, Dossena M, Bruttini F, et al. Branched-chain amino acid supplementation promotes survival and supports cardiac and skeletal muscle mitochondrial biogenesis in middle-aged mice. *Cell Metab* 2010;**12**:362–372.
218. Corcoran A, Cotter TG. Redox regulation of protein kinases. *FEBS J* 2013;**280**:1944–1965.
219. Wright VP, Reiser PJ, Clanton TL. Redox modulation of global phosphatase activity and protein phosphorylation in intact skeletal muscle. *J Physiol* 2009;**587**:5767–5781.
220. Bradley SJ, Kingwell BA, McConell GK. Nitric oxide synthase inhibition reduces leg glucose uptake but not blood flow during dynamic exercise in humans. *Diabetes* 1999;**48**:1815–1821.
221. 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. *J Physiol* 2009;**587**:3363–3373.
222. Etgen GJ Jr, Fryburg DA, Gibbs EM. Nitric oxide stimulates skeletal muscle glucose transport through a calcium/contraction- and phosphatidylinositol-3-kinase-independent pathway. *Diabetes* 1997;**46**:1915–1919.
223. Sylow L, Moller LL, Kleinert M, Richter EA, Jensen TE. Stretch-stimulated glucose transport in skeletal muscle is regulated by Rac1. *J Physiol* 2015;**593**:645–656.
224. Papaconstantinou J. Insulin/IGF-1 and ROS signaling pathway cross-talk in aging and longevity determination. *Mol Cell Endocrinol* 2009;**299**:89–100.
225. Handayaningsih AE, Iguchi G, Fukuoka H, Nishizawa H, Takahashi M, Yamamoto M, et al. Reactive oxygen species play an essential role in IGF-I signaling and IGF-I-induced myocyte hypertrophy in C2C12 myocytes. *Endocrinology* 2011;**152**:912–921.
226. Alleman RJ, Katunga LA, Nelson MA, Brown DA, Anderson EJ. The "Goldilocks Zone" from a redox perspective—adaptive vs. deleterious responses to oxidative stress in striated muscle. *Front Physiol.* 2014;**5**:358.
227. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 1956;**11**:298–300.
228. Pulliam DA, Bhattacharya A, Van Remmen H. Mitochondrial dysfunction in aging and longevity: a causal or protective role? *Antioxid Redox Signal* 2013;**19**:1373–1387.
229. Edrey YH, Salmon AB. Revisiting an age-old question regarding oxidative stress. *Free Radic Biol Med* 2014;**71**:368–378.
230. Muller FL, Lustgarten MS, Jang Y, Richardson A, Van Remmen H. Trends in oxidative aging theories. *Free Radic Biol Med* 2007;**43**:477–503.
231. Wang AL, Lukas TJ, Yuan M, Neufeld AH. Age-related increase in mitochondrial DNA damage and loss of DNA repair capacity in the neural retina. *Neurobiol Aging* 2010;**31**:2002–2010.
232. Miro O, Casademont J, Casals E, Perea M, Urbano-Marquez A, Rustin P, et al. Aging is associated with increased lipid peroxidation in human hearts, but not with mitochondrial respiratory chain enzyme defects. *Cardiovasc Res* 2000;**47**:624–631.
233. Rosa EF, Silva AC, Ihara SS, Mora OA, Aboulafia J, Nouailhetas VL. Habitual exercise program protects murine intestinal, skeletal, and cardiac muscles against aging. *J Appl Physiol (1985)* 2005;**99**:1569–1575.
234. Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *Biochem J* 1973;**134**:707–716.
235. Harman D. The biologic clock: the mitochondria? *J Am Geriatr Soc* 1972;**20**:145–147.
236. Chabi B, Ljubicic V, Menzies KJ, Huang JH, Saleem A, Hood DA. Mitochondrial function and apoptotic susceptibility in aging skeletal muscle. *Aging Cell* 2008;**7**:2–12.
237. Gouspillou G, Bourdel-Marchasson I, Rouland R, Calmettes G, Biran M, Deschodt-Arsac V, et al. Mitochondrial energetics is impaired in vivo in aged skeletal muscle. *Aging Cell* 2014;**13**:39–48.
238. Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, et al. Decline in skeletal muscle mitochondrial function with aging in humans. *Proc Natl Acad Sci U S A* 2005;**102**:5618–5623.
239. Pollack M, Leeuwenburgh C. Apoptosis and aging: role of the mitochondria. *J Gerontol A Biol Sci Med Sci* 2001;**56**: B475–B482.
240. Van Remmen H, Ikeno Y, Hamilton M, Pahlavani M, Wolf N, Thorpe SR, et al. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol Genomics* 2003;**16**:29–37.
241. McArdle A, van der Meulen J, Close GL, Pattwell D, Van Remmen H, Huang TT, et al. Role of mitochondrial superoxide dismutase in contraction-induced generation of reactive oxygen species in skeletal muscle extracellular space. *Am J Physiol Cell Physiol* 2004;**286**: C1152–C1158.
242. Kruse SE, Karunadharma PP, Basisty N, Johnson R, Beyer RP, MacCoss MJ, et al. Age modifies respiratory complex I and protein homeostasis in a muscle type-specific manner. *Aging Cell* 2016;**15**:89–99.
243. Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, et al. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet* 1995;**11**:376–381.
244. Cha H, Kim JM, Oh JG, Jeong MH, Park CS, Park J, et al. PICOT is a critical regulator of cardiac hypertrophy and cardiomyocyte contractility. *J Mol Cell Cardiol* 2008;**45**:796–803.
245. Yant LJ, Ran Q, Rao L, Van Remmen H, Shibatani T, Belter JG, et al. The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. *Free Radic Biol Med* 2003;**34**:496–502.
246. Matsui M, Oshima M, Oshima H, Takaku K, Maruyama T, Yodoi J, et al. Early embryonic lethality caused by targeted disruption of the mouse thioredoxin gene. *Dev Biol* 1996;**178**:179–185.
247. Nonn L, Williams RR, Erickson RP, Powis G. The absence of mitochondrial thioredoxin 2 causes massive apoptosis, exencephaly, and early embryonic lethality in homozygous mice. *Mol Cell Biol* 2003;**23**:916–922.
248. Jakupoglu C, Przemeck GK, Schneider M, Moreno SG, Mayr N, Hatzopoulos AK, et al. Cytoplasmic thioredoxin reductase is essential for embryogenesis but dispensable for cardiac development. *Mol Cell Biol* 2005;**25**:1980–1988.
249. Conrad M, Jakupoglu C, Moreno SG, Lippl S, Banjac A, Schneider M, et al. Essential role for mitochondrial thioredoxin reductase in hematopoiesis, heart development, and heart function. *Mol Cell Biol* 2004;**24**:9414–9423.
250. Neumann CA, Krause DS, Carman CV, Das S, Dubey DP, Abraham JL, et al. Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. *Nature* 2003;**424**:561–565.
251. Lee TH, Kim SU, Yu SL, Kim SH, Park DS, Moon HB, et al. Peroxiredoxin II is essential for sustaining life span of erythrocytes in mice. *Blood* 2003;**101**:5033–5038.
252. Perez VI, Bokov A, Van Remmen H, Mele J, Ran Q, Ikeno Y, et al. Is the oxidative

stress theory of aging dead? *Biochim Biophys Acta* 2009;**1790**:628–638.
253. Moskovitz J, Bar-Noy S, Williams WM, Requena J, Berlett BS, Stadtman ER. Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. *Proc Natl Acad Sci U S A* 2001;**98**:12920–12925.
254. Salmon AB, Perez VI, Bokov A, Jernigan A, Kim G, Zhao H, et al. Lack of methionine sulfoxide reductase A in mice increases sensitivity to oxidative stress but does not diminish life span. *FASEB J* 2009;**23**:3601–3608.
255. Carlsson LM, Jonsson J, Edlund T, Marklund SL. Mice lacking extracellular superoxide dismutase are more sensitive to hyperoxia. *Proc Natl Acad Sci U S A* 1995;**92**:6264–6268.
256. Sentman ML, Granstrom M, Jakobson H, Reaume A, Basu S, Marklund SL. Phenotypes of mice lacking extracellular superoxide dismutase and copper- and zinc-containing superoxide dismutase. *J Biol Chem* 2006;**281**:6904–6909.
257. Fomenko DE, Novoselov SV, Natarajan SK, Lee BC, Koc A, Carlson BA, et al. MsrB1 (methionine-R-sulfoxide reductase 1) knock-out mice: roles of MsrB1 in redox regulation and identification of a novel selenoprotein form. *J Biol Chem* 2009;**284**:5986–5993.
258. Zhao H, Sun J, Deschamps AM, Kim G, Liu C, Murphy E, et al. Myristoylated methionine sulfoxide reductase A protects the heart from ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 2011;**301**:H1513–H1518.
259. Schriner SE, Ogburn CE, Smith AC, Newcomb TG, Ladiges WC, Dolle ME, et al. Levels of DNA damage are unaltered in mice overexpressing human catalase in nuclei. *Free Radic Biol Med* 2000;**29**:664–673.
260. Ye G, Metreveli NS, Donthi RV, Xia S, Xu M, Carlson EC, et al. Catalase protects cardiomyocyte function in models of type 1 and type 2 diabetes. *Diabetes* 2004;**53**:1336–1343.
261. Ran Q, Liang H, Ikeno Y, Qi W, Prolla TA, Roberts LJ 2nd, et al. Reduction in glutathione peroxidase 4 increases life span through increased sensitivity to apoptosis. *J Gerontol A Biol Sci Med Sci* 2007;**62**:932–942.
262. Mitsui A, Hamuro J, Nakamura H, Kondo N, Hirabayashi Y, Ishizaki-Koizumi S, et al. Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span. *Antioxid Redox Signal* 2002;**4**:693–696.
263. Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, et al. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 2005;**308**:1909–1911.
264. Nickel AG, von Hardenberg A, Hohl M, Loffler JR, Kohlhaas M, Becker J, et al. Reversal of mitochondrial transhydrogenase causes oxidative stress in heart failure. *Cell Metab* 2015;**22**:472–484.
265. Kinugawa S, Wang Z, Kaminski PM, Wolin MS, Edwards JG, Kaley G, et al. Limited exercise capacity in heterozygous manganese superoxide dismutase gene-knockout mice: roles of superoxide anion and nitric oxide. *Circulation* 2005;**111**:1480–1486.
266. Mansouri A, Muller FL, Liu Y, Ng R, Faulkner J, Hamilton M, et al. Alterations in mitochondrial function, hydrogen peroxide release and oxidative damage in mouse hind-limb skeletal muscle during aging. *Mech Ageing Dev* 2006;**127**:298–306.
267. Van Remmen H, Salvador C, Yang H, Huang TT, Epstein CJ, Richardson A. Characterization of the antioxidant status of the heterozygous manganese superoxide dismutase knockout mouse. *Arch Biochem Biophys* 1999;**363**:91–97.
268. Vasilaki A, Csete M, Pye D, Lee S, Palomero J, McArdle F, et al. Genetic modification of the manganese superoxide dismutase/glutathione peroxidase 1 pathway influences intracellular ROS generation in quiescent, but not contracting, skeletal muscle cells. *Free Radic Biol Med* 2006;**41**:1719–1725.
269. Williams MD, Van Remmen H, Conrad CC, Huang TT, Epstein CJ, Richardson A. Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice. *J Biol Chem* 1998;**273**:28510–28515.
270. Lustgarten MS, Jang YC, Liu Y, Muller FL, Qi W, Steinhelper M, et al. Conditional knockout of Mn-SOD targeted to type IIB skeletal muscle fibers increases oxidative stress and is sufficient to alter aerobic exercise capacity. *Am J Physiol Cell Physiol* 2009;**297**:C1520–C1532.
271. Schneider M, Forster H, Boersma A, Seiler A, Wehnes H, Sinowatz F, et al. Mitochondrial glutathione peroxidase 4 disruption causes male infertility. *FASEB J* 2009;**23**:3233–3242.
272. Lee KP, Shin YJ, Cho SC, Lee SM, Bahn YJ, Kim JY, et al. Peroxiredoxin 3 has a crucial role in the contractile function of skeletal muscle by regulating mitochondrial homeostasis. *Free Radic Biol Med* 2014;**77**:298–306.
273. Nguyen HX, Tidball JG. Expression of a muscle-specific, nitric oxide synthase transgene prevents muscle membrane injury and reduces muscle inflammation during modified muscle use in mice. *J Physiol* 2003;**550**:347–356.
274. Hamilton RT, Bhattacharya A, Walsh ME, Shi Y, Wei R, Zhang Y, et al. Elevated protein carbonylation, and misfolding in sciatic nerve from db/db and Sod1(−/−) mice: plausible link between oxidative stress and demyelination. *PLoS One* 2013;**8**: e65725. https://doi.org/10.1371/journal.pone.0065725.
275. Ivannikov MV, Van Remmen H. Sod1 gene ablation in adult mice leads to physiological changes at the neuromuscular junction similar to changes that occur in old wild-type mice. *Free Radic Biol Med* 2015;**84**:254–262.
276. Jang YC, Lustgarten MS, Liu Y, Muller FL, Bhattacharya A, Liang H, et al. Increased superoxide in vivo accelerates age-associated muscle atrophy through mitochondrial dysfunction and neuromuscular junction degeneration. *FASEB J* 2010;**24**:1376–1390.
277. Larkin LM, Davis CS, Sims-Robinson C, Kostrominova TY, Remmen HV, Richardson A, et al. Skeletal muscle weakness due to deficiency of CuZn-superoxide dismutase is associated with loss of functional innervation. *Am J Physiol Regul Integr Comp Physiol* 2011;**301**:R1400–R1407.
278. Shi Y, Ivannikov MV, Walsh ME, Liu Y, Zhang Y, Jaramillo CA, et al. The lack of CuZnSOD leads to impaired neurotransmitter release, neuromuscular junction destabilization and reduced muscle strength in mice. *PLoS One* 2014;**9**: e100834. https://doi.org/10.1371/journal.pone.0100834.
279. DeBalsi KL, Wong KE, Koves TR, Slentz DH, Seiler SE, Wittmann AH, et al. Targeted metabolomics connects thioredoxin-interacting protein (TXNIP) to mitochondrial fuel selection and regulation of specific oxidoreductase enzymes in skeletal muscle. *J Biol Chem* 2014;**289**:8106–8120.
280. Hamilton RT, Walsh ME, Van Remmen H. Mouse models of oxidative stress indicate a role for modulating healthy aging. *J Clin Exp Pathol* 2012;https://doi.org/10.4172/2161-0681.S4-005.
281. Elchuri S, Oberley TD, Qi W, Eisenstein RS, Jackson Roberts L, Van Remmen H, et al. CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. *Oncogene* 2005;**24**:367–380.
282. Pearson T, McArdle A, Jackson MJ. Nitric oxide availability is increased in contracting skeletal muscle from aged mice, but does not differentially decrease muscle superoxide. *Free Radic Biol Med* 2015;**78**:82–88.
283. Trujillo M, Ferrer-Sueta G, Radi R. Peroxynitrite detoxification and its biologic implications. *Antioxid Redox Signal* 2008;**10**:1607–1620.
284. Larsson JE, Wahlstrom G. The influence of age and administration rate on the brain sensitivity to propofol in rats. *Acta Anaesthesiol Scand* 1998;**42**:987–994.
285. Deschenes MR, Roby MA, Eason MK, Harris MB. Remodeling of the neuromuscular junction precedes sarcopenia related alterations in myofibers. *Exp Gerontol* 2010;**45**:389–393.
286. Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, et al. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. *J Gerontol A Biol Sci Med Sci* 2006;**61**:1059–1064.
287. Jang YC, Liu Y, Hayworth CR, Bhattacharya A, Lustgarten MS, Muller FL, et al. Dietary restriction attenuates age-associated

muscle atrophy by lowering oxidative stress in mice even in complete absence of CuZnSOD. *Aging Cell* 2012;**11**:770–782.
288. Schiaffino S, Dyar KA, Ciciliot S, Blaauw B, Sandri M. Mechanisms regulating skeletal muscle growth and atrophy. *FEBS J* 2013;**280**:4294–4314.
289. Sartori R, Schirwis E, Blaauw B, Bortolanza S, Zhao J, Enzo E, et al. BMP signaling controls muscle mass. *Nat Genet* 2013;**45**:1309–1318.
290. Milan G, Romanello V, Pescatore F, Armani A, Paik JH, Frasson L, et al. Regulation of autophagy and the ubiquitin-proteasome system by the FoxO transcriptional network during muscle atrophy. *Nat Commun* 2015;**6**:6670.
291. Bhattacharya A, Muller FL, Liu Y, Sabia M, Liang H, Song W, et al. Denervation induces cytosolic phospholipase A2-mediated fatty acid hydroperoxide generation by muscle mitochondria. *J Biol Chem* 2009;**284**:46–55.
292. Rudolf R, Deschenes MR, Sandri M. Neuromuscular junction degeneration in muscle wasting. *Curr Opin Clin Nutr Metab Care* 2016;**19**:177–181.
293. Choi MH, Ow JR, Yang ND, Taneja R. Oxidative stress-mediated skeletal muscle degeneration: molecules, mechanisms, and therapies. *Oxid Med Cell Longev* 2016;**2016**: 6842568. https://doi.org/10.1155/2016/6842568.
294. Deschenes MR, Roby MA, Glass EK. Aging influences adaptations of the neuromuscular junction to endurance training. *Neuroscience* 2011;**190**:56–66.
295. Betik AC, Thomas MM, Wright KJ, Riel CD, Hepple RT. Exercise training from late middle age until senescence does not attenuate the declines in skeletal muscle aerobic function. *Am J Physiol Regul Integr Comp Physiol* 2009;**297**: R744–R755.
296. Thomas MM, Khan W, Betik AC, Wright KJ, Hepple RT. Initiating exercise training in late middle age minimally protects muscle contractile function and increases myocyte oxidative damage in senescent rats. *Exp Gerontol* 2010;**45**:856–867.
297. Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, et al. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci U S A* 2009;**106**:8665–8670.
298. Rando TA. Oxidative stress and the pathogenesis of muscular dystrophies. *Am J Phys Med Rehabil* 2002;**81**: S175–S186.
299. Escolar DM, Zimmerman A, Bertorini T, Clemens PR, Connolly AM, Mesa L, et al. Pentoxifylline as a rescue treatment for DMD: a randomized double-blind clinical trial. *Neurology* 2012;**78**:904–913.
300. Burdi R, Rolland JF, Fraysse B, Litvinova K, Cozzoli A, Giannuzzi V, et al. Multiple pathological events in exercised dystrophic mdx mice are targeted by pentoxifylline: outcome of a large array of in vivo and ex vivo tests. *J Appl Physiol (1985)* 2009;**106**:1311–1324.
301. Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, et al. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* 2009;**325**:201–204.
302. Marzetti E, Lees HA, Wohlgemuth SE, Leeuwenburgh C. Sarcopenia of aging: underlying cellular mechanisms and protection by calorie restriction. *Biofactors* 2009;**35**:28–35.
303. Anderson RM, Weindruch R. Metabolic reprogramming, caloric restriction and aging. *Trends Endocrinol Metab* 2010;**21**:134–141.
304. Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C, et al. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science* 2003;**299**:896–899.
305. Civitarese AE, Carling S, Heilbronn LK, Hulver MH, Ukropcova B, Deutsch WA, et al. Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. *PLoS Med* 2007;**4**:e76.
306. Siegel MP, Kruse SE, Percival JM, Goh J, White CC, Hopkins HC, et al. Mitochondrial-targeted peptide rapidly improves mitochondrial energetics and skeletal muscle performance in aged mice. *Aging Cell* 2013;**12**:763–771.
307. Rodriguez-Cuenca S, Cocheme HM, Logan A, Abakumova I, Prime TA, Rose C, et al. Consequences of long-term oral administration of the mitochondria-targeted antioxidant MitoQ to wild-type mice. *Free Radic Biol Med* 2010;**48**:161–172.
308. Shill DD, Southern WM, Willingham TB, Lansford KA, McCully KK, Jenkins NT. Mitochondria-specific antioxidant supplementation does not influence endurance exercise training-induced adaptations in circulating angiogenic cells, skeletal muscle oxidative capacity or maximal oxygen uptake. *J Physiol* 2016;https://doi.org/10.1113/JP272491.
309. Medvedev ZA. An attempt at a rational classification of theories of ageing. *Biol Rev Camb Philos Soc* 1990;**65**:375–398.
310. von Haehling S, Morley JE, Coats AJ, Anker SD. Ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2015. *J Cachexia Sarcopenia Muscle* 2015;**6**:315–316.