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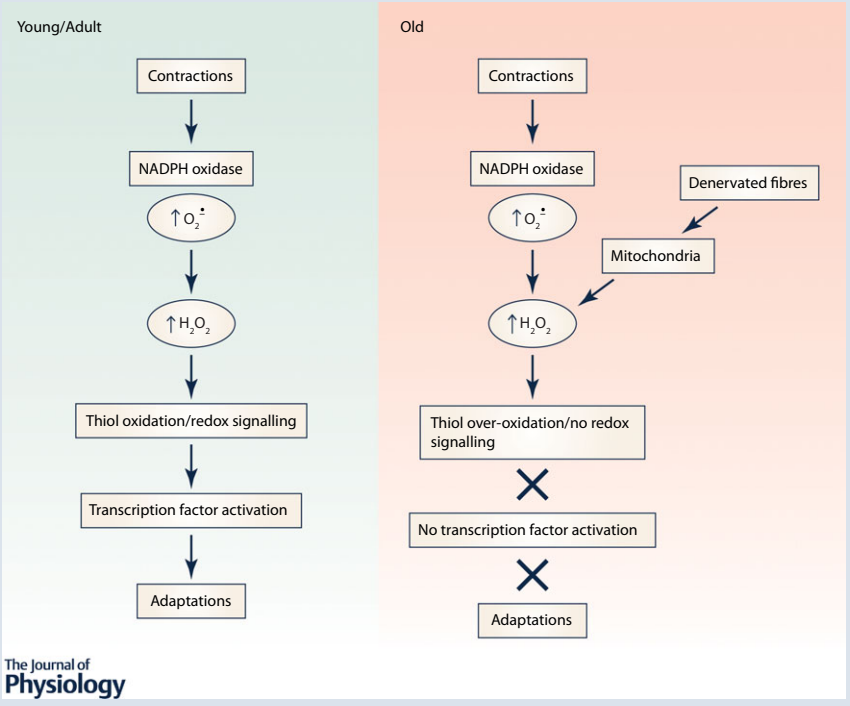
TOPICAL REVIEW

Role of reactive oxygen species in age-related neuromuscular deficits

Malcolm J. Jackson and Anne McArdle

MRC-Arthritis Research UK Centre for Integrated Research into Musculoskeletal Ageing (CIMA), Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool L69 3GA, UK



Abstract Although it is now clear that reactive oxygen species (ROS) are not the key determinants of longevity, a number of studies have highlighted the key role that these species play in age-related diseases and more generally in determining individual health span. Age-related loss of skeletal muscle mass and function is a key contributor to physical frailty in older individuals and our current understanding of the key areas in which ROS contribute to age-related deficits in muscle is through defective redox signalling and key roles in maintenance of neuromuscular integrity. This

Malcolm Jackson is Head of the Institute of Ageing and Chronic Disease at the University of Liverpool and Director of the MRC-Arthritis Research UK Centre for Integrated Research into Musculoskeletal Ageing (CIMA). He trained in Biochemistry and undertook a PhD at University College, London with the late Professor Richard H. T. Edwards. He is a member of the BBSRC DRINC Strategy panel. His major research area is the role of free radicals and reactive oxygen species in skeletal muscle. **Anne McArdle's** PhD was from the University of Liverpool and postdoc studies included training with Dr John Faulkner in the Muscle Mechanics Laboratory at the University of Michigan. She holds a personal Chair at Liverpool and her research interests are in muscle ageing with emphasis on stress responses (particularly heat shock proteins) in muscle and the cross-talk between muscle and inflammatory cells. She is Head of the Department of Musculoskeletal Biology and Athena SWAN lead for the Institute of Ageing and Chronic Disease.

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topical review will describe how ROS stimulate adaptations to contractile activity in muscle that include up-regulation of short-term stress responses, an increase in mitochondrial biogenesis and an increase in some catabolic processes. These adaptations occur through stimulation of redox-regulated processes that lead to the activation of transcription factors such as NF-κB, AP-1 and HSF1 which mediate changes in gene expression. They are attenuated during ageing and this appears to occur through an age-related increase in mitochondrial hydrogen peroxide production. The potential for redox-mediated cross-talk between motor neurons and muscle is also described to illustrate how ROS released from muscle fibres during exercise may help maintain the integrity of axons and how the degenerative changes in neuromuscular structure that occur with ageing may contribute to mitochondrial ROS generation in skeletal muscle fibres.

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Corresponding author M. J. Jackson: Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool L69 3BX, UK. Email: mjj@liverpool.ac.uk

Abstract figure legend Schematic illustration of the process of redox signalling of responses to contractile activity and their modification during ageing. In muscle from young and adult subjects, contractile activity leads to activation of muscle NADPH oxidase(s) with generation of superoxide that is rapidly converted to hydrogen peroxide with local oxidation of redox-active thiols and activation of specific redox-sensitive transcriptional pathways. This mediates multiple adaptations to the contractile activity including stress responses and mitochondrial biogenesis. In old subjects, this process is attenuated by over-production of hydrogen peroxide by mitochondria which at the level of individual fibres may be related to the partially or full fibre denervation.

Introduction

The free radical theory of ageing was originally formulated in the 1950s (Harman, 1956) and has been one of the most resilient and examined of the many subsequent theories that have been proposed. It is now recognised that the free radical theory and its various derivatives cannot exclusively explain the ageing process (Romano *et al.* 2010; Pulliam *et al.* 2013) and in particular there is no invariable direct relationship between the extent of free radical, or reactive oxygen-induced damage (i.e. oxidative damage) and the onset or rate of ageing in tissues (Muller *et al.* 2007*a*). Nevertheless data indicate that some aspects of the ageing phenotype and age-related disorders appear to be mediated by reactive oxygen species (ROS) (Muller *et al.* 2007*a*; Salmon *et al.* 2010). In this topical review we will address two key areas relating to how ROS influence ageing of the neuromuscular system and play a role in age-related deficits in skeletal muscle. These are the disruption of redox signalling in muscle that occurs during ageing and the role of ROS in nerve–muscle interactions that maintain muscle mass and function. The aim is to highlight current developments in these topics and to identify areas where further research is required.

Age-related loss of skeletal muscle mass and function (sarcopenia)

The term 'sarcopenia' was coined over 20 years ago (Evans & Campbell, 1993), and the definition was recently revised as a 'progressive age-related loss of muscle mass and associated muscle weakness' (Lynch, 2011). Between the ages of 50 and 80 years a 30–50% loss of muscle mass and decrease in strength occur that are major contributors to physical frailty which has a major negative effect on the quality of life of older people and contributes to loss of independence in older people (Young & Skelton, 1994). Despite the importance of this area limited progress has been made in understanding the mechanisms responsible for age-associated muscle atrophy and weakness.

Analysis of post-mortem human vastus lateralis muscles have shown a 40% reduction in total muscle area accompanied by ∼50% loss of muscle fibres between 50 and 80 years of age (Lexell *et al.* 1988). Old rodents also show reductions in muscle fibre number with ageing (Larkin *et al.* 2011). The fibre loss is associated with a loss of motor units (Campbell *et al.* 1973; Larson & Ansved, 1995) and the number of motor axons innervating skeletal muscles are decreased in old rodents (Larson & Ansved, 1995) and old humans (Krantic *et al.* 2005). Despite the strong associations between the losses of muscle fibres and motor axons, a cause–effect relationship between the loss of these two tissues has not yet been established.

The surviving motor neurons show axonal sprouting that has been proposed to rescue muscle fibres that have become temporarily denervated, resulting in an increase in average motor unit size (Brown *et al.* 1988). It has been proposed that the ability of motor units to increase their size is limited, and muscle fibres and motor units are eventually lost (Delbono, 2003). Ageing is associated with numerous pre- and postsynaptic structural abnormalities in peripheral nerve endings, including segmental demyelination (Adinolfi *et al.* 1991), demyelinated and remyelinated axons, and denervated Schwann cell columns (Grover-Johnson & Spencer, 1981),

synaptic detachment, partial or complete withdrawal of axons from postsynaptic sites, and fragmentation of postsynaptic motor endplates (Jang *et al.* 2010; Chai *et al.* 2011). Recent data from rodents also indicate that changes in the peripheral regions of motor units are observed prior to any loss in number of motor neuron cell bodies in the lumbar spinal cord (Chai *et al.* 2011), suggesting that degenerative processes in the peripheral regions of motor nerves may play an important role.

Role of oxidative damage in ageing

The effect of ageing on levels of oxidative damage in tissues of many species has been studied extensively and it is apparent that all tissues, including skeletal muscle, of old organisms contain greater oxidative damage to lipids, DNA and proteins in comparison with younger organisms (e.g. Vasilaki *et al.* 2006*b*). In non-mammalian models, initial interventions to reduce the ROS activities throughout life extended lifespan (Orr & Sohal, 2003), but work from Richardson and colleagues (Perez *et al.* 2009) and Gemms and Doonan (2009) has demonstrated a lack of any true correlation between the level of oxidative damage and lifespan in different models and argues strongly against a primary role for oxidative damage in ageing (Gems & Doonan, 2009). In mammals, few genetic manipulations to reduce ROS activities have resulted in increased lifespan (e.g. Schriner *et al.* 2005). It therefore seems clear that levels of ROS generation and oxidative damage are not the fundamental determinants of lifespan.

Although ROS may not be the fundamental determinant of lifespan many studies have indicated that mitochondrial ROS generation is increased in tissues, including skeletal muscle, during ageing and that this is associated with impaired mitochondrial function and oxidative damage (e.g. Vasilaki *et al.* 2006*b*). Furthermore authors have argued that this increased ROS generation with age is important in contributing to age-related diseases (Muller *et al.* 2007*a*) and more generally to individual health span (Salmon *et al.* 2010).

Redox signalling in skeletal muscle and its dysregulation during ageing

Contractile activity increases the generation of superoxide and nitric oxide (NO) by skeletal muscle fibres with the formation of secondary reactive oxygen species (ROS) and reactive nitrogen species (Powers & Jackson, 2008). NO generation is regulated by the nitric oxide synthases, but the sites that generate superoxide during exercise have remained relatively unclear. Initial data suggested that the mitochondrial electron transport chain was the predominant source of superoxide although a number of studies have identified NADPH oxidase enzymes in the plasma membrane, T-tubules and mitochondria (Sakellariou *et al.* 2014*b*). Recent studies have directly compared the generation of superoxide from mitochondrial and cytosolic sources in contracting skeletal muscle (Sakellariou *et al.* 2013; Pearson *et al.* 2014) and these data indicate that NAD(P)H oxidases are the major source during a short period of contractions (Sakellariou *et al.* 2013; Pearson *et al.* 2014). This has significant implications for understanding the role of localised ROS generation in contracting muscle since the only function of NAD(P)H oxidases is to generate superoxide (or hydrogen peroxide) and hence these species are not produced by chance, or as a by-product of metabolism.

In normal physiology ROS mediate some adaptive processes to physiological stresses through changes in gene expression (Droge, 2002). Signalling by these reactive molecules appears to be mainly achieved by targeted modifications of specific residues in proteins (Janssen-Heininger *et al.* 2008). In skeletal muscle the ROS and NO generated during contractile activity appear to mediate the activation of a number of redox-regulated transcription factors, including Nuclear Factor-kappa B (NF-κB), Activator Protein-1 (AP-1), Heat Shock Factor-1 (HSF-1) and nuclear factor erythroid 2 -related factor 2 (Nrf2) (Ji *et al.* 2004; Vasilaki *et al.* 2006*b*; Ristow *et al.* 2009) with a subsequent increased expression of regulatory enzymes and cytoprotective proteins (McArdle *et al.* 2001). The full extent of the adaptive processes in muscle that are regulated through redox-dependent systems is unclear, but appears to also include some catabolic processes and mitochondrial biogenesis (Powers & Jackson, 2008).

The finding that ROS mediate adaptations to contractile activity and other adaptive responses in tissues is based on three lines of evidence: (i) demonstration of an association of increased ROS generation with the response; (ii) the inhibitory effect of suppression or scavenging of ROS on the response; and (iii) data demonstrating that specific ROS can activate the relevant pathway. Hydrogen peroxide (H_2O_2) is widely viewed as the only ROS likely to play a major role in signalling and H_2O_2 has been shown to activate NF-κB (Zhang *et al.* 2001), AP-1 (Aggeli *et al.* 2006) and many other transcription factors (Marinho *et al.* 2014). Thus the concept has arisen that H_2O_2, which is generated at specific sites within muscle but is readily diffusible, can interact with activation pathways for these specific transcription factors leading to their activation. These studies have utilised H_2O_2 concentrations typically in the range 10^{-4}–10^{-3} M and it is relevant to consider whether these concentrations have any *in vivo* relevance. The intracellular H_2O_2 concentration is in the order of 10^{-9}–10^{-8} M (Sies, 2014) and we have calculated that the increase in muscle during contractions appears to be to a maximum of 10^{-7} M (Jackson, 2011). This is therefore a factor of 1000 below the concentrations reported to activate most transcription factors *in vitro*. Thus, the generally held concept of H_2O_2 generated from a specific

enzyme system that is localised at specific sub-cellular sites, then diffusing through the cell and encountering redox-regulated proteins with which it reacts may be relatively naive.

An alternative explanation for the process of redox signalling has evolved to account for this recognition that most so-called redox-sensitive proteins are unlikely to be oxidised by H_2O_2 at physiological concentrations and the possibility of redox signalling through thiol oxidation by H_2O_2 has evolved. This potential mechanism involves the transfer of oxidative equivalents directly from a sensitive thiol peroxidase to a specific target protein through direct protein–protein contact allowing conversion of the oxidising equivalent from H_2O_2 into a disulphide bond that can be subsequently transmitted to other substrates through the formation of intermolecular disulphides. Thus thiol peroxidases transmit oxidising equivalents to a specific target protein to facilitate H_2O_2 signalling (Sobatta *et al.* 2015). This mechanism has been well documented in yeast (Delaunay *et al.* 2002; Gutscher *et al.* 2009), but has only recently been shown to account for activation of a transcription factor by H_2O_2 in animal cells (Sobatta *et al.* 2015). Key components of such signalling pathways are peroxiredoxins (Prx) and thioredoxins (Trx). Prx are a family of antioxidant enzymes which reduce hydroperoxides to water in the presence of electron donors. Prx are classified by the number of cysteine (Cys) residues involved in the peroxidase activity: 2-Cys Prx and 1-Cys Prx. The 2-Cys Prxs form a disulphide bond by reacting with peroxides and the disulphide is reduced by thioredoxin (Trx) which is then reduced by Trx reductase and NAD(P)H (Park *et al.* 2014). Prx are generally considered to be important antioxidant enzymes in the cytosol (Prx1, Prx2, Prx5), mitochondria (Prx3, Prx5) and endoplasmic reticulum (Prx4). Importantly and in contrast to the relatively poor reactivity of the so-called redox-sensitive proteins involved in activating transcription factors discussed previously, Prx are several orders of magnitude more reactive with H_2O_2 (Sobatta *et al.* 2015) and act to scavenge H_2O_2 at the low concentrations found in muscle fibres.

This latter potential signalling system does not appear to have been studied in skeletal muscle, but recent studies in other cell types indicate that Prxs can function as a signal peroxidase to activate specific pathways. Prx1 has been shown to activate the transcription factor ASK1 (Jarvis *et al.* 2012), and Prx2 forms a redox relay with the transcription factor STAT3 such that oxidative equivalents flow from Prx2 to STAT3 generating disulphide-linked STAT3 oligomers with modified transcriptional activity (Sobatta *et al.* 2015). Figure 1 shows examples of how the two potential mechanisms for redox signalling might account for the activation of a transcription factor (TF) such as NF-κB by contraction-induced ROS in skeletal muscle.

It will be important to define which (if either) of these potential redox signalling systems plays a role in adaptations of muscle to contractions since they may provide alternative mechanisms by which aberrant ROS influence the age-related loss of muscle mass and function. Thus for instance, ROS mediate increased expression of heat shock proteins (HSPs) and other cyto-protective proteins in muscle following contractions in adult mice (Vasilaki *et al.* 2006*a*; Jackson & McArdle, 2011) and this response is attenuated in old mice (Vasilaki *et al.* 2006*a*). Furthermore this attenuated response contributes to age-related loss of muscle mass and function (Jackson & McArdle, 2011). Thus transgenic mouse studies have demonstrated that aberrant activation of adaptive responses plays a key role in age-related muscle dysfunction since lifelong overexpression of cytosolic HSP70 or mitochondrial HSP10 normalised NF-κB activation at rest and reduced functional deficits in muscle of old mice (Kayani *et al.* 2010).

We have previously proposed that aberrant hydrogen peroxide generation from mitochondria that occurs during ageing could explain this attenuation of adaptive responses leading to a failure to induce important cyto-protective and other responses (Jackson & McArdle 2011), but this has not been examined experimentally. Understanding of the processes by which the redox-mediated adaptations to contractions occur is therefore a pre-requisite to defining how they are modified by ageing.

Effect of modification of ROS on neuromuscular ageing

There are a small number of studies that indicate that a very specific manipulation of ROS activities can preserve muscle function during ageing (e.g. Schriner *et al.* 2005) and in collaboration with colleagues in the USA our group have undertaken studies to examine the effects of deletion of regulatory enzymes for ROS on neuromuscular ageing in mice. Despite frequent observation of increased oxidative damage in these models, no clear relationship with neuromuscular ageing was generally seen. The exception to this pattern was in mice with a whole body deletion of Cu,Zn superoxide dismutase (Sod1) which show neuromuscular changes with ageing that have been claimed to reflect an accelerated skeletal muscle ageing process (Muller *et al.* 2006). Adult Sod1 knockout (KO) mice show a decline in skeletal muscle mass, loss of muscle fibres and a decline in the number of motor units, loss of motor function and contractility, partial denervation and mitochondrial dysfunction by 8 months of age (Larkin *et al.* 2011). These are all changes that are also seen in old wild-type (WT) mice, but not until after 22–24 months of age.

Sod1 is present in both the cytosol of cells and within the mitochondrial inter-membrane space (Kawamata &

Manfredi, 2010) and hence lack of Sod1 may influence redox homeostasis in the mitochondria and cytosol. Jang *et al.* (2010) showed that this model was associated with a large increase in mitochondrial H_2O_2 production and in our studies we examined the nature of other ROS that are generated in the cytosol of muscle from mice lacking Sod1. We concluded that increased peroxynitrite in muscle may play an important role in the phenotype of Sod1KO mice since muscle fibres from adult Sod1KO mice did not show an increase in cytosolic superoxide availability at rest, but muscles demonstrated evidence for an increase in peroxynitrite. In Sod1KO mice, this was indicated by an increased 3-nitrotyrosine (3-NT) content of muscle proteins and increased expression of the peroxynitrite reductase, peroxiredoxin V (Sakellariou *et al.* 2011).

We also showed that, in common with old WT mice, muscles of Sod1KO mice demonstrated a constitutive activation of NF-κB with increased production of pro-inflammatory cytokines and a constitutive increase in the content of a number of HSPs in muscle at rest and also failed to further activate cytoprotective adaptive responses to contractile activity. This results in diminished acute additional expression of HSPs and other cyto-protective proteins following contractile activity. This failed activation in response to contractile activity could potentially occur through a lack of induction of additional superoxide and/or hydrogen peroxide during contractile activity (Sakellariou *et al.* 2011). Other data suggest that this lack of a contraction-induced generation of ROS in the Sod1KO mice may be due to a failure of activation of muscle NADPH oxidase activity (Sakellariou *et al.* 2013). Thus, a further effect of the lack of Sod1 that mimics that seen in old WT mice is a failure of redox-mediated signalling of adaptive responses to contractile activity.

In subsequent work our group of investigators has examined whether the muscle atrophy in this model is initiated by changes within muscle fibres or motor neurons. Surprisingly, mice with skeletal muscle-specific

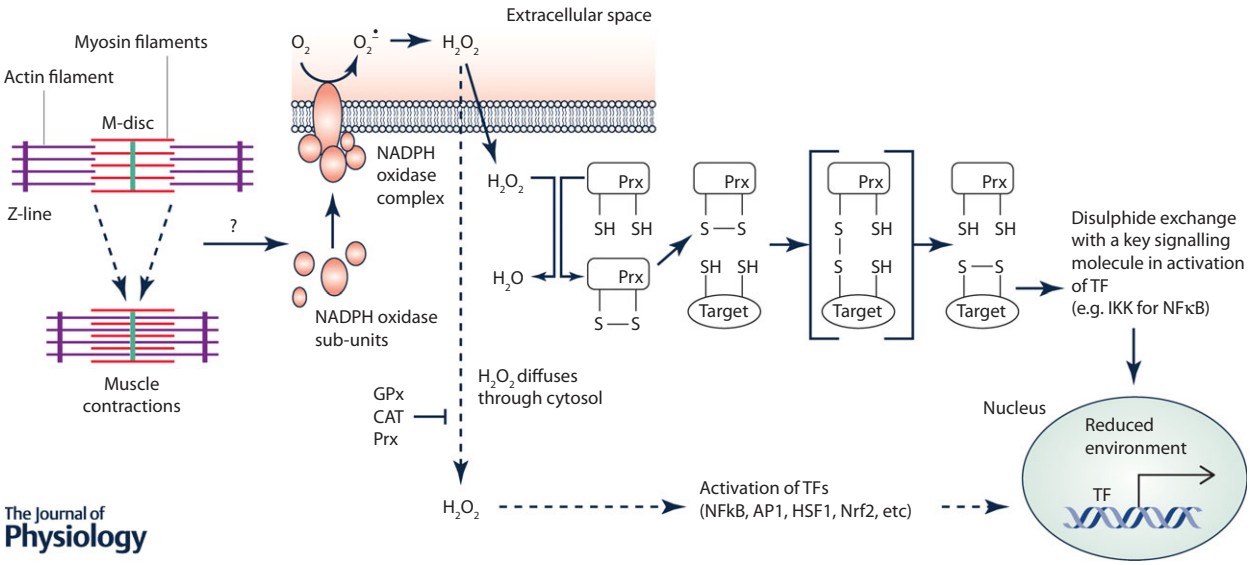

Figure 1. Schematic representation of the two potential pathways of redox signalling to account for activation of key transcription factors following contractile activity in skeletal muscle
Contractions initially lead to activation of NADPH oxidase (probably Nox2) within muscle. This occurs through rapid translocation of the regulatory sub-units of NADPH oxidase to a muscle membrane and assembly of the catalytic enzyme. It is currently unknown how contractile activity leads to activation of this enzyme. The NADPH oxidase generates superoxide that is rapidly converted to H_2O_2. Some evidence suggests that the major NADPH oxidases predominantly generate superoxide on the outside of the muscle fibre with some H_2O_2 generated rapidly diffusing into the fibre although this is not firmly established. The process by which the increased H_2O_2 leads to activation of transcriptional responses is the subject of debate, but the conventional view is that local concentrations of H_2O_2 are sufficiently high for it to diffuse through the cytoplasm and interact with redox-sensitive components of pathways activating various transcription factors (shown as a dashed line in the scheme). Note that the cytoplasm contains various enzymes that can degrade H_2O_2 and compounds (e.g. glutathione) with which it can react. The alternative pathway involves the reaction of low levels of H_2O_2 with a highly reactive protein (e.g. Prx or Trx) that is closely associated with the NADPH oxidase with subsequent formation of disulphides and disulphide exchange with partner proteins thus transferring oxidising equivalents from H_2O_2 to proteins with which it would not react at low concentrations. Subsequent activation of the TF occurring through disulphide exchange with a key signalling protein. This pathway has not yet been shown to occur in skeletal muscle. Ageing appears to influence the overall scheme leading to an inability of contractile activity to further activate these transcription factors, but it is currently unknown how this occurs. GPx, glutathione peroxidase; CAT, catalase; Prx, peroxiredoxin; IKK, I kappa B kinase; TF, transcription factor.

deletion of Sod1 (mSod1KO mice) show no evidence of neuromuscular junction degeneration (NMJ) or loss of muscle fibres and indeed showed some muscle hypertrophy (Zhang *et al.* 2013). Our group also examined whether the changes in ROS generation observed in Sod1KO mice were also seen in mSod1KO mice. The multiple changes seen in Sod1KO mice were not observed in the muscles of mSod1KO mice, including the increases in 3-NT, catalase and peroxiredoxin V previously reported in muscles of Sod1KO mice (Zhang *et al.* 2013). To determine the role of motor neurons in the loss of muscle mass and function in Sod1KO mice, we subsequently established a transgenic Sod1KO mouse in which human SOD1 is expressed in neurons under the control of a synapsin 1 promoter (nSOD1-Tg-Sod1KO mice). These 'nerve rescue' mice expressed SOD1 in central and peripheral neurons but not other tissues. Sciatic nerve CuZnSOD content in nSOD1-Tg-Sod1KO mice was ∼20% of WT control mice, but they showed no loss of muscle mass or maximum isometric specific force production at 8–12 months of age, when significant reductions were seen in Sod1KO mice (Sakellariou *et al.* 2014*a*). Thus these data implicate a lack of Sod1 specifically in motor neurons in the pathogenesis of the accelerated muscle ageing phenotype seen in the Sod1KO mice. We have also recently examined the effect of neuron-specific Sod1 knockout in nSod1KO mice, but this model also does not recapitulate the full sarcopenia phenotype seen in Sod1KO mice and shows only minor changes in muscle mass and function (Sataranatarajan *et al.* 2015). The implication of this work appears to be that both neurons and muscle contribute to maintenance of neuromuscular function in this model and that deletion of Sod1 in both tissues is necessary to generate the full sarcopenic phenotype.

Thus studies of the Sod1KO model have demonstrated the importance of nerve–muscle interactions in the maintenance of neuromuscular function where ROS homeostasis is compromised during ageing. Since adult mice lacking Sod1 replicate many of the features seen in old WT mice they may indicate key mechanisms that lead to loss of muscle fibres and function that are relevant to the ageing of WT mice. Nevertheless we stress that Sod1KO mice provide a model to identify fundamental mechanisms that are highly relevant to understanding muscle ageing, but do not believe that a simple lack of Sod1 contributes to sarcopenia in WT mice or humans.

Redox cross-talk between neurons and muscle

The situation cited above for the nerve rescue Sod1KO mice provides an example of how restoration of neuronal ROS homeostasis can restore defective function in muscle mitochondria that is associated with increased ROS generation. An analogous situation appears to occur in experimental denervation or nerve crush which has been found to lead to activation of a number of degenerative pathways in the denervated muscle, including an increased mitochondrial generation of reactive oxygen species (Muller *et al.* 2006) and increased generation of pro-inflammatory cytokines (Cea *et al.* 2013). Muller *et al.* (2007*b*) reported a remarkably large increase in muscle mitochondrial H_2O_2 generation following denervation and subsequent studies in our laboratory have shown that this increased mitochondrial H_2O_2 release is already apparent within 3 days of nerve transection. The reason for this rapid activation of specific degradatory pathways is unclear. It is possible that initially this may reflect an attempt to restore innervation, since products such as cytokines are released from the muscle fibre and some cytokines have been claimed to stimulate axonal sprouting, but if prolonged must inevitably lead to degradation of the denervated muscle fibres. Further work from this group also showed that other peroxides in addition to H_2O_2 were released from mitochondria from denervated muscle (Bhattacharya *et al.* 2009) and that inhibition of 12/15 lipoxygenase could ameliorate some of the muscle atrophy induced by denervation (Bhattacharya *et al.* 2014) . Thus together these data suggest that muscle mitochondrial ROS generation plays a role in the muscle degeneration seen following denervation.

Motor nerves and muscles are well known to play a symbiotic role in maintenance of the neuromuscular system and in particular the viability of motor neurons is recognised to be dependent upon continued exposure to neurotrophic factors generated by skeletal muscle fibres in addition to Schwann cells and neurons (Luff, 1998). Regular exercise is recognised to induce structural and functional changes in motor neurons (Gardiner *et al.* 2006), but in contrast to the situation with skeletal muscle there appear to be no data indicating that contractile activity or exercise training up-regulate endogenous regulatory proteins for ROS and other cytoprotective proteins in motor neurons. Motor neurons do have the capacity to up-regulate these cytoprotective proteins in response to exogenous reactive oxygen and nitrogen species (Bishop *et al.* 1999) and neurotrophic factors (e.g. Glial cell-derived neurotrophic factor and brain-derived neurotrophic factor) have been reported to promote neuronal survival by increasing defences against oxidative damage (Gabaizadeh *et al.* 1997).

In order to explain this paradox we are currently examining whether, because of proximity to their target muscle fibres, the peripheral axons of motor nerves are exposed to increased extracellular activities of ROS derived from the muscle fibres during contractile activity (Vasilaki *et al.* 2006*b*). This must inevitably occur and preliminary data support this hypothesis. Previous studies of ROS derived from contracting skeletal muscle indicate that they cause transient oxidation in other non-contracting tissues

(Close *et al.* 2007) and we hypothesise that this level of oxidation is unlikely to produce substantial oxidative damage, but acts as a stimulus for the up-regulation of cytoprotective systems.

We therefore postulate that redox cross-talk between muscle and neurons through release of H_2O_2 (and potentially other ROS) plays differing roles depending on the innervation state of the muscle. In normal innervated muscle fibres contractile activity leads to generation of NADPH oxidase-derived H_2O_2 in the extracellular space that interacts with adjacent neurons inducing up-regulation of cytoprotective proteins in the axons. During ageing, studies have shown that skeletal muscle does not release equivalent amounts of ROS to the extracellular space (Vasilaki *et al.* 2006*b*) and hence this cytoprotective cross-talk will not occur, potentially reducing the capacity of the nerve to prevent oxidative damage. In contrast if the fibre becomes denervated (as also occurs to some extent in ageing) the fibre mitochondria release very large amounts of H_2O_2 (and other ROS) that can diffuse out of the fibre to neurons and other adjacent tissues. The effect of this very large and

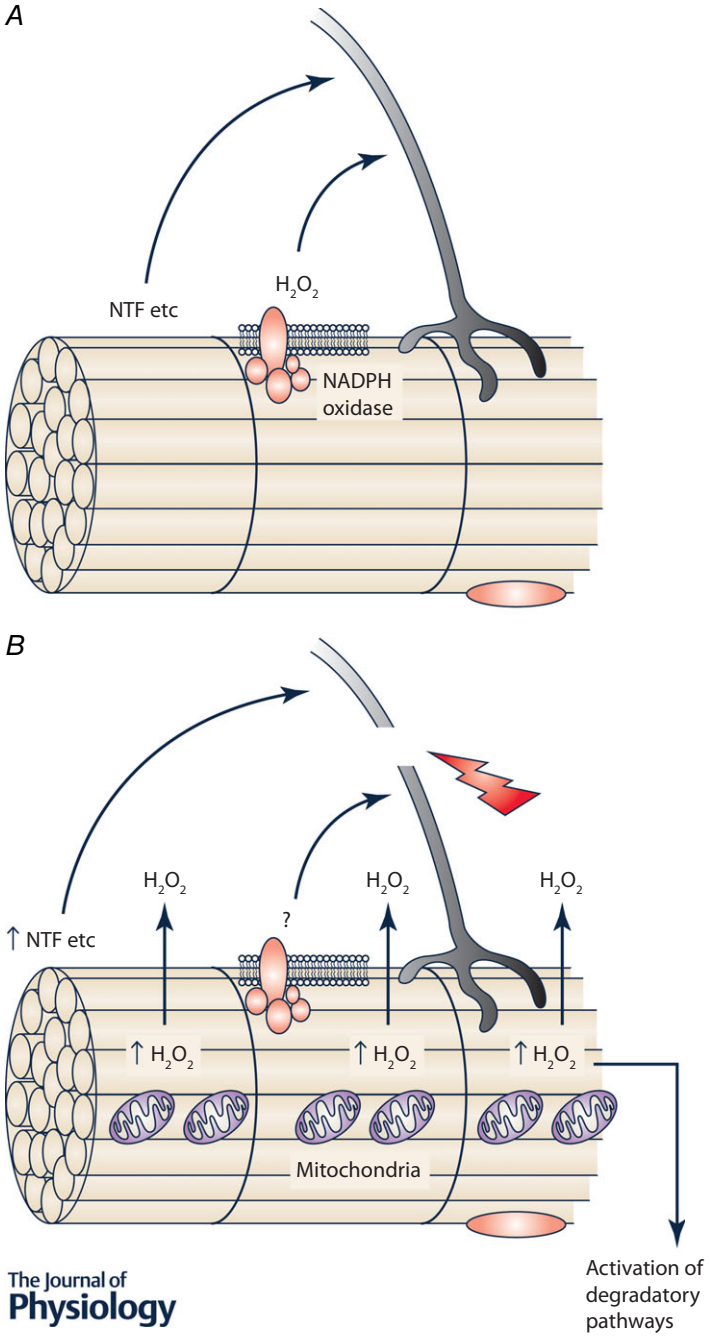

Figure 2. Schematic representation of putative redox cross-talk from muscle to neurons
A shows the situation in innervated muscle fibres from young/adult where contractile activity leads to generation of NADPH oxidase-derived H_2O_2 in the extracellular space that interacts with adjacent neurons inducing up-regulation of cytoprotective proteins in the axons. During ageing, this process is likely to be modified since skeletal muscle from old mice does not release equivalent amounts of ROS to the extracellular space (Vasilaki *et al.* 2006*b*) and hence this cytoprotective cross-talk will not occur. *B* shows the effect of denervation in young/adult organisms and may also reflect the situation in some fibres from old organisms. Denervation of a fibre induces the fibre mitochondria to release very large amounts of H_2O_2 (and other ROS) that can diffuse out of the fibre to interact with neurons and other adjacent fibres or tissues. These changes may initially represent an initial attempt to stimulate adaptations/axonal sprouting, but if sustained must inevitably lead to degeneration of the muscle fibre and potentially other local tissues. NTF, neurotrophic factors.

prolonged increase is unclear. It may initially represent an attempt to stimulate adaptations/axonal sprouting, but if sustained must inevitably lead to degeneration of the muscle fibre and potentially other local tissues. Figure 2 illustrates some aspects of this redox cross-talk between muscle fibres and motor neurons that may influence neuromuscular ageing.

Conclusions

Understanding how we age and ways of ameliorating the negative physical, mental and social effects of ageing is a major global challenge. Physical frailty is driven by loss of muscle mass and function and hence preventing this is key to reduction in frailty. Our current understanding of the key areas in which ROS contribute to age-related deficits in muscle is through defective redox signalling and maintenance of neuromuscular integrity. Both are areas that still require further work to fully understand the mechanisms involved, but also appear amenable to targeted interventions that have the potential to help prevent age-related neuromuscular decline with a consequent improvement in quality of life for older people.

References

Adinolfi AM, Yamuy J, Morales FR & Chase MH (1991). Segmental demyelination in peripheral nerves of old cats. *Neurobiol Aging* **12**, 175–179.

Aggeli IK, Gaitanaki C & Beis I (2006). Involvement of JNKs and p38-MAPK/MSK1 pathways in H_2O_2-induced upregulation of heme oxygenase-1 mRNA in H9c2 cells. *Cell Signal* **18**, 1801–1812.

Bhattacharya A, Hamilton R, Jernigan A, Zhang Y, Sabia M, Rahman MM, Li Y, Wei R, Chaudhuri A & Van Remmen H (2014). Genetic ablation of 12/15-lipoxygenase but not 5-lipoxygenase protects against denervation-induced muscle atrophy. *Free Radic Biol Med* **67**, 30–40.

Bhattacharya A, Muller FL, Liu Y, Sabia M, Liang H, Song W, Jang YC, Ran Q & Van Remmen H (2009). Denervation induces cytosolic phospholipase A2-mediated fatty acid hydroperoxide generation by muscle mitochondria. *J Biol Chem* **284**, 46–55.

Bishop A, Marquis JC, Cashman NR & Demple B (1999). Adaptive resistance to nitric oxide in motor neurons. *Free Radic Biol Med* **26**, 978–986.

Brown WF, Strong MJ & Snow R (1988). Methods for estimating numbers of motor units in biceps-brachialis muscles and losses of motor units with aging. *Muscle Nerve* **11**, 423–432.

Campbell MJ, McComas AJ & Petito F (1973). Physiological changes in ageing muscles. *J Neurol Neurosurg Psychiatry* **36**, 174–182.

Cea LA, Cisterna BA, Puebla C, Frank M, Figueroa XF, Cardozo C, Willecke K, Latorre R & Sáez JC (2013). De novo expression of connexin hemichannels in denervated fast skeletal muscles leads to atrophy. *Proc Natl Acad Sci USA* **110**, 16229–16234.

Chai RJ, Vukovic J, Dunlop S, Grounds MD & Shavlakadze T (2011). Striking denervation of neuromuscular junctions without lumbar motoneuron loss in geriatric mouse muscle. *PLoS One* **6**, e28090.

Close GL, Kayani AC, Ashton T, McArdle A & Jackson MJ (2007). Release of superoxide from skeletal muscle of adult and old mice: an experimental test of the reductive hotspot hypothesis. *Aging Cell* **6**, 189–195.

Delaunay A, Pflieger D, Barrault MB, Vinh J & Toledano MB (2002). A thiol peroxidase is an H_2O_2 receptor and redox-transducer in gene activation. *Cell* **111**, 471–481.

Delbono O (2003). Neural control of aging skeletal muscle. *Aging Cell* **2**, 21–29.

Dröge W (2002). Free radicals in the physiological control of cell function. *Physiol Rev* **82**, 47–95.

Evans WJ & Campbell WW (1993). Sarcopenia and age-related changes in body composition and functional capacity. *J Nutr* **123**, 465–468.

Gabaizadeh R, Staecker H, Liu W & Van De Water TR (1997). BDNF protection of auditory neurons from cisplatin involves changes in intracellular levels of both reactive oxygen species and glutathione. *Brain Res Mol Brain Res* **50**, 71–78.

Gardiner P, Dai Y & Heckman CJ (2006). Effects of exercise training on α-motoneurons. *J Appl Physiol (1985)* **101**, 1228–1236.

Gems D & Doonan R (2009). Antioxidant defense and aging in *C. elegans*: is the oxidative damage theory of aging wrong? *Cell Cycle* **8**, 1681–1687.

Grover-Johnson N & Spencer PS (1981). Peripheral nerve abnormalities in aging rats. *J Neuropathol Exp Neurol* **40**, 155–165.

Gutscher M, Sobotta MC, Wabnitz GH, Ballikaya S, Meyer AJ, Samstag Y & Dick TP (2009). Proximity-based protein thiol oxidation by H_2O_2-scavenging peroxidases. *J Biol Chem* **284**, 31532–31540.

Harman D (1956). Ageing: a theory based on free radical and radiation chemistry. *J Gerontol* **11**, 298–300.

Jackson MJ (2011). Control of reactive oxygen species production in contracting skeletal muscle. *Antiox Redox Signal* **15**, 2477–2486.

Jackson MJ & McArdle A (2011). Age-related changes in skeletal muscle reactive oxygen species generation and adaptive responses to reactive oxygen species. *J Physiol* **589**, 2139–2145.

Jang YC, Lustgarten MS, Liu Y, Muller FL, Bhattacharya A, Liang H, Salmon AB, Brooks SV, Larkin L, Hayworth CR, Richardson A & Van Remmen H (2010). Increased superoxide in vivo accelerates age-associated muscle atrophy through mitochondrial dysfunction and neuromuscular junction degeneration. *FASEB J* **24**, 1376–1390.

Janssen-Heininger YM, Mossman BT, Heintz NH, Forman HJ, Kalyanaraman B, Finkel T, Stamler JS, Rhee SG & van der Vliet A (2008). Redox-based regulation of signal transduction: principles, pitfalls, and promises. *Free Radic Biol Med* **45**, 1–17.

Jarvis RM, Hughes SM & Ledgerwood EC (2012). Peroxiredoxin 1 functions as a signal peroxidase to receive, transduce, and transmit peroxide signals in mammalian cells. *Free Rad Biol Med* **53**, 1522–1530.

Ji LL, Gomez-Cabrera MC, Steinhafel N & Vina J (2004). Acute exercise activates nuclear factor (NF)- κB signaling pathway in rat skeletal muscle. *FASEB J* **18**, 1499–1506.

Kawamata H & Manfredi G (2010). Import, maturation, and function of SOD1 and its copper chaperone CCS in the mitochondrial intermembrane space. *Antioxid Redox Signal* **13**, 1375–1384.

Kayani AC, Close GL, Dillmann WH, Mestril R, Jackson MJ & McArdle A (2010). Overexpression of HSP10 in skeletal muscle of transgenic mice prevents the age-related fall in maximum tetanic force generation and muscle cross-sectional area. *Am J Physiol Regul Integr Comp Physiol* **299**, R268–R276.

Krantic S, Mechawar N, Reix S & Quirion R (2005). Molecular basis of programmed cell death involved in neurodegeneration. *Trends Neurosci* **28**, 670–676.

Larkin LM, Davis CS, Sims-Robinson C, Kostrominova TY, Van Remmen H, Richardson A, Feldman EL & Brooks SV (2011). Skeletal muscle weakness due to deficiency of CuZn-superoxide dismutase is associated with loss of functional innervation. *Am J Physiol Regul Integr Comp Physiol* **301**, R1400–R1407.

Larsson L & Ansved T (1995). Effects of ageing on the motor unit. *Prog Neurobiol* **45**, 397–458.

Lexell J, Taylor CC & Sjöström M (1988). 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* **84**, 275–294.

Luff AR (1998). Age-associated changes in the innervation of muscle fibers and changes in the mechanical properties of motor units. *Ann N Y Acad Sci* **854**, 92–101.

Lynch GS (ed.) (2011). *Sarcopenia – Age-Related Muscle Wasting and Weakness*. Springer.

McArdle A, Pattwell D, Vasilaki A, Griffiths RD & Jackson MJ (2001). Contractile activity-induced oxidative stress: Cellular origin and adaptive responses. *Am J Physiol Cell Physiol* **280**, C621–C627.

Marinho HS, Real C, Cyrne L, Soares H & Antunes F (2014). Hydrogen peroxide sensing, signaling and regulation of transcription factors. *Redox Biol* **2**, 535–562.

Muller FL, Lustgarten MS, Jang Y, Richardson A & Van Remmen H (2007*a*). Trends in oxidative ageing theories. *Free Radic Biol Med* **43**, 477–503.

Muller FL, Song W, Jang YC, Liu Y, Sabia M, Richardson A & Van Remmen H (2007*b*). Denervation-induced skeletal muscle atrophy is associated with increased mitochondrial ROS production. *Am J Physiol Regul Integr Comp Physiol* **293**, R1159–R1168.

Muller FL, Song W, Liu Y, Chaudhuri A, Pieke-Dahl S, Strong R, Huang TT, Epstein CJ, Roberts LJ 2nd, Csete M, Faulkner J A & Van Remmen H (2006). Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy. *Free Radic Biol Med* **40**, 1993–2004.

Orr WC & Sohal RS (2003). Does overexpression of Cu,Zn-SOD extend life span in *Drosophila melanogaster*? *Exp Gerontol* **38**, 227–230.

Park J, Lee S, Lee S & Kang SW (2014). 2-Cys peroxiredoxins: emerging hubs determining redox dependency of mammalian signaling networks. *Int J Cell Biol* **2014**, e715867.

Pearson T, Kabayo T, Ng R, Chamberlain J, McArdle A & Jackson MJ (2014). Skeletal muscle contractions induce acute changes in cytosolic superoxide, but slower responses in mitochondrial superoxide and cellular hydrogen peroxide. *PLoS One* **9**, e96378.

Pérez VI, Bokov A, Van Remmen H, Mele J, Ran Q, Ikeno Y & Richardson A (2009). Is the oxidative stress theory of aging dead? *Biochim Biophys Acta* **1790**, 1005–1014.

Powers SK & Jackson MJ (2008). Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production. *Physiol Rev* **88**, 1243–1276.

Pulliam DA, Bhattacharya A & Van Remmen H (2013). Mitochondrial dysfunction in aging and longevity: a causal or protective role? *Antioxid Redox Signal* **19**, 1373–1387.

Ristow M, Zarse K, Oberbach A, Klöting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR & Blüher M (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci USA* **106**, 8665–8670.

Romano AD, Serviddio G, de Matthaeis A, Bellanti F & Vendemiale G (2010). Oxidative stress and aging. *J Nephrol* **15**, S29–36.

Sakellariou GK, Davis CS, Shi Y, Ivannikov MV, Zhang Y, Vasilaki A, Macleod GT, Richardson A, Van Remmen H, Jackson MJ, McArdle A & Brooks SV (2014*a*). Neuron-specific expression of CuZnSOD prevents the loss of muscle mass and function that occurs in homozygous CuZnSOD-knockout mice. *FASEB J* **28**, 1666–1681.

Sakellariou GK, Jackson MJ & Vasilaki A (2014*b*). Redefining the major contributors to superoxide production in contracting skeletal muscle. The role of NAD(P)H oxidases. *Free Radic Res* **48**, 12–29.

Sakellariou GK, Pye D, Vasilaki A, Zibrik L, Palomero J, Kabayo T, McArdle F, Van Remmen H, Richardson A, Tidball JG, McArdle A & Jackson MJ (2011). Role of superoxide-nitric oxide interactions in the accelerated age-related loss of muscle mass in mice lacking Cu,Zn superoxide dismutase. *Aging Cell* **10**, 749–760.

Sakellariou GK, Vasilaki A, Palomero J, Kayani A, Zibrik L, McArdle A & Jackson MJ (2013). 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* **18**, 603–621.

Salmon AB, Richardson A & Pérez VI (2010). Update on the oxidative stress theory of aging: does oxidative stress play a role in aging or healthy aging? *Free Radic Biol Med* **48**, 642–655.

Sataranatarajan K, Qaisar R, Davis C, Sakellariou GK, Vasilaki A, Zhang Y, Liu Y, Bhaskaran S, McArdle A, Jackson M, Brooks SV, Richardson A & Van Remmen H (2015). Neuron specific reduction in CuZnSOD is not sufficient to initiate a full sarcopenia phenotype. *Redox Biol* **5**, 140–148.

Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC & Rabinovitch PS (2005). Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* **308**, 1909–1911.

Sies H (2014). Role of metabolic H_2O_2 generation: redox signaling and oxidative stress. *J Biol Chem* **289**, 8735–8741.

Sobotta MC, Liou W, Stöcker S, Talwar D, Oehler M, Ruppert T, Scharf AN & Dick TP (2015). Peroxiredoxin-2 and STAT3 form a redox relay for H_2O_2 signaling. *Nat Chem Biol* **11**, 64–70.

Vasilaki A, McArdle F, Iwanejko LM & McArdle A (2006*a*). Adaptive responses of mouse skeletal muscle to contractile activity: The effect of age. *Mech Ageing Dev* **127**, 830–839.

Vasilaki A, Mansouri A, Remmen H, van der Meulen JH, Larkin L, Richardson AG, McArdle A, Faulkner JA & Jackson MJ (2006*b*). Free radical generation by skeletal muscle of adult and old mice: effect of contractile activity. *Aging Cell* **5**, 109–117.

Young A & Skelton DA (1994). Applied physiology of strength and power in old age. *Int J Sports Med* **15**, 149–151.

Zhang J, Johnston G, Stebler B & Keller ET (2001). Hydrogen peroxide activates NFκB and the interleukin-6 promoter through NFκB-inducing kinase. *Antioxid Redox Signal* **3**, 493–504.

Zhang Y, Davis C, Sakellariou GK, Shi Y, Kayani AC, Pulliam D, Bhattacharya A, Richardson A, Jackson MJ, McArdle A, Brooks SV & Van Remmen H (2013). CuZnSOD gene deletion targeted to skeletal muscle leads to loss of contractile force but does not cause muscle atrophy in adult mice. *FASEB J* **27**, 3536–3548.

Additional information

Competing interests

None declared.

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