Published in final edited form as:

Free Radic Biol Med. 2019 February 20; 132: 50–57. doi:10.1016/j.freeradbiomed.2018.11.038.

Aberrant redox signalling and stress response in age-related muscle decline: Role in inter- and intra-cellular signaling

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Abstract

Age-associated frailty is predominantly due to loss of muscle mass and function. The loss of muscle mass is also associated with a greater loss of muscle strength, suggesting that the remaining muscle fibres are weaker than those of adults. The mechanisms by which muscle is lost with age are unclear, but in this review we aim to pull together various strands of evidence to explain how muscle contractions support proteostasis in non-muscle tissues, particularly focussed on the production and potential transfer of Heat Shock Proteins (HSPs) and how this may fail during ageing. Furthermore we will identify logical approaches, based on this hypothesis, by which muscle loss in ageing may be reduced. Skeletal muscle generates superoxide and nitric oxide at rest and this generation is increased by contractile activity. In adults, this increased generation of reactive oxygen and nitrogen species (RONS) activate redox-sensitive transcription factors such as nuclear factor κ B (NF κ B), activator protein-1 (AP1) and heat shock factor 1 (HSF1), resulting in increases in cytoprotective proteins such as the superoxide dismutases, catalase and heat shock proteins that prevent oxidative damage to tissues and facilitate remodelling and proteostasis in both an intra- and inter-cellular manner. During ageing, the ability of skeletal muscle from aged organisms to respond to an increase in ROS generation by increased expression of cytoprotective proteins through activation of redox-sensitive transcription factors is severely attenuated. This age-related lack of physiological adaptations to the ROS induced by contractile activity appears to contribute to a loss of ROS homeostasis, increased oxidative damage and age-related dysfunction in skeletal muscle and potentially other tissues.

Keywords

Skeletal muscle; Aging; ROS; HSPs; Exosomes; NMJ

1. Ageing is associated with a decline in skeletal muscle structure and function

Age-associated frailty [1] is predominantly due to loss of muscle mass and function, increased susceptibility of muscle to injury and impaired ability for muscles to recover from damage [2,3]. In humans, a 30–50% loss of muscle mass occurs between the ages of 50 and

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80 years [4–7]. The loss of muscle mass is also associated with a greater loss of muscle strength, whereby muscle loss occurs at a rate of 0.6–0.7% per year with a greater loss of strength of between 2.5% and 4% per year [8], suggesting that the remaining muscle fibres are weaker than those of adults [9–13]. However, others have suggested that remaining muscle fibres are not weaker [14,15], possibly due, at least in part to the fact that this loss is not consistent across the whole body. Thus, whether a defect occurs at the level of the single fibre remains unclear, with studies showing conflicting findings (see [16] for review).

This functional deficit impacts profoundly on the quality of life of older people, since many are at, or near to strength thresholds that limit the ability to carry out everyday tasks with increased disability and loss of independence. Despite this, only modest progress has been made in understanding the mechanisms responsible for age-associated muscle atrophy and weakness. Lack of understanding of such mechanisms represents a critical barrier to progress toward developing optimal therapies to lessen the severity of frailty and the devastating effects of this on older people.

Rodents are commonly used as models of human muscle ageing. Most of the intrinsic and extrinsic changes regulating muscle ageing in humans have also been observed in rodents, indicating that mice and rats are models of human sarcopenia [17]. Advantages of using rodent models for investigation of human ageing include access to a mammalian system with a relatively short life span and facility for genetic manipulation. Ageing humans show loss of muscle fibres and rats and mice also show reduced muscle fibres with ageing, particularly in the gastrocnemius, tibialis anterior (TA) and extensor digitorum longus (EDL). The maximum isometric force decreases more than muscle mass during ageing in mice in a similar manner to humans, even when expressed relative to the cross-sectional area of the muscle [18,19]. Thus, the age-related loss of muscle strength cannot be solely explained by the loss of muscle mass: both muscle ‘quantity’ and ‘quality’ decline in aged animals [17]. The time course and characteristics of contraction-induced muscle injury and recovery are similar for mice [20,21], rats [22,23], and humans [24,25]. Studies in mice have demonstrated that recovery of muscle function following contraction-induced damage was severely blunted in old mice [26,27]. The mechanisms by which this occurs are unclear although it suggested that this failure in regeneration plays a role in age-related loss of muscle function. It has been proposed that the decreased activity of resident satellite cells is responsible both for the poor regeneration in old mammals and so the development of sarcopenia, but a recent study examining the effect of inducible depletion of PAX7-positive satellite cells on the development of sarcopenia did not support this proposal since removal of these cells neither accelerated nor exacerbated sarcopenia [28].

2. Loss of motor neurons accompanies the loss of muscle fibres in old age

Data clearly indicate that, in both man and rodents, loss of motor neurons accompanies the loss of muscle fibres [29–37] with a 25–50% reduction in the number of α -motor neurons has been reported with ageing although it has been suggested that denervated muscle fibres can persist for some time in both rodents and humans [38]. Small motor neurons (which tend

to innervate type I fibres) are preserved relative to large motor neurons. Over time, the loss of large motor neurons is partially compensated by a sprouting phenomenon through which small motor neurons innervate temporarily denervated type II fibres and hence these fibres acquire a slower phenotype. Power et al. [39] report a gradual reduction in motor unit numbers as early as the third decade of life with a more rapid decline into very old age and support the hypothesis that muscle mass is maintained by reinnervation, particularly from neighbouring motor neurons. Eventually the “giant” motor units are lost [40] and work by Piasecki et al. [41] has suggested that a failure of the sprouting to expand the motor unit size distinguishes sarcopenic from pre-sarcopenic muscles. Hepple [42] also suggests that it is at the point where motor unit expansion fails that muscle atrophies. Hunter et al. [43] proposed that brain cortex changes contribute to the changes in structure and function of the neuromuscular system with reductions in both grey and white matter.

A significant proportion of remaining NMJs are disrupted in ageing rodents [44–48] and humans [49,50] with reduced overlap of pre and post-synaptic terminals together with larger more dispersed post-synaptic end plates (Fig. 1). Decreased vesicle density and neurotransmitter release is also reported although this release is far in excess of that needed to initiate contraction as part of a presumed ‘safety factor’ mechanism [43]. In contrast, Jones et al. [51] suggested that NMJs were stable across the entire adult lifespan although Hepple [42] raised some concerns with the nature of the sample in this study. The functional and biochemical effect of the evident NMJ disruption remains unclear, with the suggestion that a relatively small overlap of pre- and post-synaptic junctions was necessary for successful neuronal transmission and muscle fibre activation [52–54]. Deschenes [55] proposed that anatomical remodelling of the pre-synaptic nerve terminal branching and altered post-synaptic distribution of the receptor sites occurs before muscle atrophy is seen [56,57] and is associated with neurophysiological alterations including a more rapid loss of endplate potential strength during continued activation of the pre-synaptic neuron. In contrast, Hunter and colleagues reported that NMJ changes are associated with changes in the timing and magnitude of synaptic inputs and that the changes in EMG of single motor units of humans indicate a decrease in transmission stability [43]. There is also some evidence to indicate that the NMJ of older mammals lose the ability to adapt to changes in demand [55].

Whether the loss of motor neurons drives the loss of muscle fibres or is secondary to it is unknown. The situation is further complicated since studies in rodents have shown that changes in the structure and function of motor neurons occur at a different rates in hindlimb compared with forelimb muscles [58]. Hunter et al. [43] highlight the variable age-related changes in motor function among older adults and suggest that this variability is related to other factors such as inflammation and physical activity and may result in difficulties for interpretation of cross-sectional studies, particularly in humans [8].

3. Does increased activity preserve muscle or motor unit structure and function?

Data on the effect of exercise as an intervention to preserve motor units is unclear. A number of studies showed that increased activity provides protection against the loss of motor units.

Thus, a study of masters runners demonstrated a greater number of motor units and improved neuromuscular stability in this cohort compared with age-matched controls although this seemed to be muscle dependent, whereby preservation was only evident in the muscles undergoing long-term activity [59]. Data from Mosole et al. [60] demonstrated preservation of muscle strength in physically active seniors compared with sedentary controls and particularly demonstrated a reduction in the number of small angulated fibres, potentially representing changes in denervation-innervation process [61]. However, this preservation of motor units was not evident in other studies. Thus, Piasecki et al. [37] demonstrated no effect of lifelong exercise in masters athletes on the motor unit number decline with age. Lazarus and Harridge [62] suggested that the discrepancies between studies may arise due to the compounded effects of inactivity whereby a threshold of activity is needed for optimal effects and any additional activity is unlikely to have any additional benefit. This is supported by a study which suggests that NMJ structure and function may be relatively preserved in more healthy older people [52].

Evidence for a beneficial effect of exercise on motor neurons and NMJ in rodents is also unclear and further complicated by the relatively sedentary nature of caged rodents. Kanda et al. [63] demonstrated some small improvements in motor neuron and peripheral nerves following a moderate swimming intervention from middle to old age in rats. Additional studies by this group [64] demonstrated that overloading of muscle by unilateral surgical ablation of synergistic muscles in middle aged rats resulted in muscle hypertrophy but had no effect on the age-related loss of motor neurons in the hypertrophied muscle. Studies by Krishnan et al. [65] examined the effect of resistance wheel running on the development of sarcopenia in middle to old age rats and demonstrated little effect on ageing nerves despite evidence of protection against sarcopenia [66].

Thus, it is unclear whether the most obvious practical means of intervention (e.g. exercise) can prevent the loss of motor units or influence the compensatory sprouting that occurs in older age [33,66].

Modified ROS signalling by muscle fibres has been implicated in the mechanisms by which muscle mass and function are reduced with age, and denervation has been proposed to be a major factor driving this modified signalling.

4. Increased generation of reactive oxygen species (ROS) by muscle during contractile activity stimulates key adaptive responses to exercise through redox-signalling pathways

Increased activities of ROS and oxidative damage to lipids, proteins and DNA have been linked to a myriad of pathologies [67,68] including muscle loss in ageing [69] and other disorders affecting the integrity or function of NMJ such as amyotrophic lateral sclerosis (ALS) [70]. However, in normal physiology, ROS are signalling molecules that initiate adaptations to physiological stresses through modification of specific residues in proteins and subsequent changes in gene expression [71–76]. Contractile activity increases the generation of superoxide and nitric oxide (NO) by skeletal muscle fibres with the formation

of secondary ROS and reactive nitrogen species [77–80]. Contractile activity in muscles of young rodents leads to activation of an NADPH oxidase (NOX2) which is proposed to be the major source of superoxide and hydrogen peroxide (H₂O₂) in contracting muscle [81–83]. The mechanisms controlling NADPH oxidase activation in response to contractions are unclear, although Espinosa et al. [84] proposed that activation was dependent upon depolarisation of the muscle membrane. Electrical stimulation of muscle contractions leads to extracellular release of ATP and activation of purinergic (P2Y₁) receptors with subsequent activation of protein kinase C (PKC) leading to assembly and activation of the NADPH oxidase complex [85]. ATP and other purines are released into the NMJ synaptic cleft during muscle activation and play a crucial feedback role in regulating acetylcholine (ACh) release from the axon terminal [86]. These effects of ATP appear mediated by ROS generated by muscle NADPH oxidase located at the NMJ [87].

H₂O₂ generated during contractions activates a number of redox-regulated transcription factors, including NF- κ B, AP-1, HSF-1 and nrf2 [76,88–90] with a subsequent adaptation of the muscle through increased expression of regulatory enzymes and cytoprotective proteins including Heat Shock Proteins (HSPs) [27,91] necessary for remodelling of muscle tissue (Fig. 2). Inhibition of NADPH oxidase impairs these responses to exercise in vivo [93] suggesting a direct role for ROS generated by NOX2 in the activation of these adaptive processes. The full extent of the adaptations to exercise mediated by redox-regulated processes has not been established although ROS may also stimulate increased expression of genes associated with mitochondrial biogenesis and insulin sensitivity [89,94,95].

5. Denervation modifies activation of intercellular signalling by increased H₂O₂ generation by muscle mitochondria

Disruption of motor neurons and denervation of muscle leads to aberrant mitochondrial function in denervated and adjacent non-denervated muscle fibres. Data indicate that the defective muscle mitochondrial function and increase in muscle mitochondrial H₂O₂ generation that occurs with ageing, may result from functional denervation of individual fibres [96,97]. Recent data from our group have indicated a key role for motor neuron integrity in the regulation of skeletal muscle mitochondria with the potentiation of the effect of denervation on individual muscle fibres. Sectioning of motor nerves (or nerve crush) leads to increased mitochondrial generation of reactive oxygen species and activation of potentially degenerative pathways in both denervated muscle and in neighbouring innervated muscle fibres [97,98].

6. Mouse models with modified ROS signalling demonstrate accelerated loss of muscle mass and function

Adult whole body superoxide dismutase 1 null (Sod1KO) mice show an accelerated skeletal muscle ageing phenotype [99–101] that is accompanied by degeneration of NMJs [99]. Surprisingly, mice with a muscle specific deletion of Sod1 (mSod1KO mice) show no evidence of NMJ degeneration or loss of muscle fibres [102,103]. To determine the role of motor neurons in the loss of muscle mass and function, we subsequently established a

transgenic Sod1KO mouse in which human SOD1 was expressed in neurons of the whole body SOD1 null mice under control of a synapsin 1 promoter (nSOD1-Tg-Sod1KO mice). These “nerve rescue” mice expressed SOD1 in central and peripheral neurons but not in other tissues. These mice showed no loss of muscle mass or function at 8–12 months of age, when significant reductions were seen in whole body Sod1KO mice [104]. These data implicate a lack of Sod1, and hence modified ROS generation, specifically in motor neurons in the pathogenesis of the accelerated muscle ageing phenotype seen in the Sod1KO mice.

Evidence of the accumulation of oxidation in peripheral nerves of older rodents includes increased lipofuscin and lipid peroxidation (MDA adducts) as well as increases in polyubiquitinated proteins and pro-inflammatory mediators such as NFkB and TNF- α [105], supporting a role of modified ROS generation in motor neurons in sarcopenia. Increased local activities of ROS have been shown to suppress neuromuscular transmission in NMJ reducing the efficiency of stimulation of contractions [106,107] and chronic exposure to excess ROS has been shown to disrupt NMJ structure and function [108]. Further work by our laboratory proposed that impaired redox signalling, rather than oxidative damage, in peripheral nerve plays a key role in muscle loss in *Sod1*^{-/-} mice and potentially sarcopenia during ageing [106].

7. Redox-regulated adaptations to contractions are attenuated during ageing

ROS-stimulated transcriptional responses to contractile activity are attenuated in mice during ageing including HSP production [90]. Our studies indicate that these age-related attenuations in responses to exercise are substantially replicated in man [109]. Data from transgenic mice show the importance of maintaining the ability to activate adaptations in response to increased muscle ROS generation during contractions, since overexpression of HSPs prevented age-related losses of muscle mass and function in old animals [95,110]. To determine potential mechanisms underlying the attenuation in adaptive responses to contractile activity in ageing, Palomero et al. [111] examined contracting muscle fibres and reported that, in contrast to fibres from adult mice, those from old mice had an increased activity of ROS at rest and showed no further increase with contractions, suggesting that activation of the NADPH oxidase may be impaired or masked in muscles of old mice. We have also observed this attenuated response in adult Sod1KO mice [101] and in both aged wild type and SOD1KO mice the attenuated responses appeared to be due to a chronic increase in muscle mitochondrial H₂O₂ production [96,112].

8. Release of HSPs by muscles is proposed to modulate proteostasis in other tissues

Intercellular communication from skeletal muscle to other tissues has been demonstrated for some time. For example, muscle releases cytokines (termed myokines) which can exert autocrine, paracrine and long-distance endocrine effects (eg, [113]). Work from our laboratory has demonstrated that changes in muscle malonaldehyde occur rapidly following a non-damaging contraction protocol and this is rapidly followed by transient increases in

malonaldehyde content on serum and liver [114]. Similarly, the transient decrease in protein thiol content of muscle following non-damaging contractions was mirrored in the liver following muscle contractions [114].

The HSP response occurs in a robust manner in some cell types, particularly skeletal muscle, but this response is not ubiquitously demonstrated by all cells [115,116] and those cells with a weaker response can demonstrate an increased susceptibility to cell damage and death [117,118]. Mature neurons in particular do not produce HSPs [119]. Evidence suggests that HSPs can be actively secreted and have important extracellular functions [120] (Fig. 2) and that this inter-cellular communication is critical to ensure that proteostasis is maintained across different tissues of the whole organism [121]. HSP transfer from glia to axon was described in the 1980s [122] and HSP70 release from mammalian cells by a non-golgi-mediated process suggested that HSP release and uptake may be selective [123]. A number of cell types secrete HSPs, including HSP27, HSP70 and HSP90 and the levels of these HSPs secreted increases significantly following physiological stresses [123,124], including from muscle following exercise [125].

Healthy cells can communicate via secretion of membrane vesicles (collectively called extracellular vesicles) [126–128]. Cells release extracellular vesicles that can deliver their molecules to adjacent or distant cells to modify gene expression, signalling, and overall function of the target cell. Exosomes are often called microparticles, microvesicles, or ectosomes and range in size from nanometers to micrometers. They form from internal vesicles generated within multivesicular endosomal compartments, secreted into the extracellular milieu when these compartments fuse with the plasma membrane. Exosomes are classically defined by their size and are nano-sized vesicles that act as antigen-presenting vesicles and as shuttles for molecules such as proteins and RNA to target other cells and tissues [129].

Exosomes are highly enriched with membrane spanning proteins, such as the tetraspanins CD9, CD63 and CD81 [130]. Exosomes contain a range of proteins, either common to exosomes generated by a range of cell types or proteins specifically synthesised by the cell of origin [131]. The mRNA and microRNA content of exosomes has been well documented and microRNAs have been shown to be transferred to recipient cells where they can have biological function [132]. Interestingly, denervation results in changes in microRNA content of muscle fibres and in the exosomes released by denervated muscle fibres and further work has demonstrated functional effects of these microRNAs [133]. The presence of HSPs, in particularly HSC70 in the lumen of exosomes is widely described, such that these proteins are routinely used as markers of exosomes [134–136]. Exosomes have been proposed to be the primary vehicle for intercellular macromolecular transfer [137]. Inter-cellular HSP transmission can occur via exosome transfer. HSP family members generally lack a classical signalling peptide and therefore the ability to be classically-secreted via the Golgi apparatus. Studies have demonstrated that HSPs can be released actively from viable cells, by non-classical means [138] and it is suggested that a large proportion of HSP60 and HSP70 released into the extracellular space is through the exosomal pathway [139]. Lancaster and Febbraio [125] demonstrated that exosomes contribute significantly to the cellular export of HSP70 from human peripheral blood mononuclear cells (PBMCs). Protein radiolabelling

approaches have demonstrated that newly synthesised proteins can be transferred between cell types via exosomes [137] and proteostasis is non-cell autonomously maintained in some cells by HSPs expressed in remote cells [140,141] whereby intercellular transmission of HSPs via exosomes was responsible for this maintenance of proteostasis. These authors suggest that such intercellular transmission via exosomes compensates for the imbalance in HSP levels following stress amongst different cells and tissues [141]. Thus, the potential for cell-to-cell communication by exosomes and the effects of failure of this process provides a potential mechanism by which one tissue can have dramatic effects on another tissue, for example, during ageing.

9. HSPs are released from muscle in exosomes and this release is modified during exercise and ageing

Frühbeis et al. [142] and Whitham et al. [143] observed an increased release of small vesicles/exosomes into the circulation after acute exercise in physically active healthy men. Whitham et al. [143] demonstrated that the exosomes had increased content of HSPs post-exercise and that liver cells were capable of taking up the muscle-derived exosomes. Others have reported altered plasma exosomal profiles, including microRNA and HSP content, following exercise and during ageing [144–146]. There is therefore evidence that a reduced or altered HSP profile of exosomes is produced by skeletal muscle during ageing and that this altered muscle HSP production may influence ageing of other tissues. Of particular interest is the possibility that HSPs released by skeletal muscle are crucial for maintenance of motor neurons. Neurons may have axons that are thousands of times greater than the diameter of the soma of origin and lack the normal stress response seen in other cells [137]. Despite this, neurons can become stress resistant if HSP70 is present in the extracellular fluid and several authors propose that protection is due to cell-to-cell transfer of HSPs [137]. Differentiated neurons do not produce HSPs [119] and are dependent on the shuttle of HSPs from surrounding cells. Data from *Drosophila* support the possibility that muscle helps maintain stress resistance in neurons since muscle specific overexpression of HSP23 provided protection to muscle cells but importantly, neurons and glia were also protected, suggesting a cell non-autonomous protection of the neurons by muscle [147].

In the light of these data it is tempting to speculate that muscle-derived exosomes may support proteostasis in motor neurons and that when muscle release of HSPs is modified with ageing, the lack of maintenance of proteostasis in motor neurons during ageing eventually leads to a failure of neuronal integrity. Thus, interventions targeted to maintain muscle mitochondrial function during ageing could potentially preserve neuromuscular (and potentially whole body) integrity, a process that is under active investigation.

In summary, ageing is associated with a decline in skeletal muscle structure and function and this is accompanied by a loss of motor neurons. Increased generation of ROS by muscle during contractile activity stimulates key adaptive responses to exercise through redoxsignalling pathways but these responses are attenuated during ageing. Studies have shown that denervation modifies activation of inter-cellular signalling by increased H₂O₂ generation by muscle mitochondria. With ageing, altered ROS signalling, due at least in part

to increased mitochondrial ROS production results in failure of muscle to adapt, with a failure in the generation HSPs following contractile activity, suggesting that denervation may be at least in part responsible for this failure in adaptive responses. The poor adaptive responses in muscles of old mammals results in a lack of cytoprotection. It is tempting to speculate that muscle-derived exosomes may support proteostasis in motor neurons and that when muscle release of HSPs is modified with ageing, the lack of maintenance of proteostasis in motor neurons during ageing eventually leads to a failure of neuronal integrity. Thus, interventions targeted to maintain muscle mitochondrial function during ageing will preserve neuromuscular (and potentially whole body) integrity.

Acknowledgments

The authors would like to thank their many present and previous collaborators and colleagues and to acknowledge generous financial support from the BBSRC, MRC, Wellcome Trust and United States National Institute on Aging (Grant no. AG051442).

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Fig. 1.

High power image of a single innervated neuromuscular junction (NMJ) from the Thy1-YFP mouse (A). NMJ appearance from muscles of adult Thy1-YFP (B) and CFP (C) overexpressing mice and old Thy1-YFP (D) and CFP (E) overexpressing mice and lower power image (F) showing example of heterogeneous distribution of denervated NMJs on surface fibres of the TA muscle of a 26 month old Thy1-YFP mouse. Acetyl choline receptors are stained in B–F with α -bungarotoxin (red) and the neural tissue appears green due to the YFP or blue due to the cyan transgene. In this old mouse (F) a small proportion of apparently denervated NMJ can be seen (red coloured) which show lack of any pre-synaptic input, although the majority of NMJ show good overlap of pre-synaptic and post-synaptic structures.

Fig. 2.

Schematic representation of the relationship between contractile activity and exosomal release of HSPs and other proteins from muscle fibres. In the hypothesised mechanism, contractile activity leads to the activation of a NADPH oxidase and the generation of H_2O_2 at the muscle membrane. This ROS stimulates the activation of a number of transcription factors and increased expression of HSPs and other proteins which are released from the muscle fibre to provide potential support for non-muscle tissues. During ageing, muscle responses to exercise through this pathway are diminished leading to a relative failure of HSP release and diminished support for nerves and other non-muscle tissues – see text for details. Figure modified from [92]. Transcription factors (TFs); Nuclear Factor Kappa B (NF κ B), Activator protein 1 (AP1), Heat shock Factor 1 (HSF1), nuclear factor erythroid 2-related factor 2 (Nrf2).