

Symposium Review article

Role of Nerve-Muscle Interactions and ROS in Regulation of Muscle Proteostasis with Aging.

Aphrodite Vasilaki¹, Arlan Richardson^{2,3}, Holly Van Remmen^{3,4}, Susan V Brooks⁵, Lisa Larkin^{5,6}, Anne McArdle¹ and Malcolm J Jackson¹.

¹Department of Musculoskeletal Biology, MRC Arthritis Research UK Centre for Integrated Research into Musculoskeletal Ageing (CIMA), Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK; ²Department of Geriatric Medicine and the Reynolds Oklahoma Center on Aging, Oklahoma University Health Science Center, Oklahoma City, OK, USA; ³Oklahoma City VA Medical Center, Oklahoma City, OK, USA; ⁴Aging and Metabolism Division, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; ⁵Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, USA; ⁶Department of Biomedical Engineering, The University of Michigan, Ann Arbor, MI, USA.

Keywords: neuromuscular homeostasis, Cu/Zn superoxide dismutase; Frailty; Oxidative stress; Sarcopenia.

Address correspondence to:

Dr. A. Vasilaki

Institute of Ageing and Chronic Disease

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1113/JP274336](https://doi.org/10.1113/JP274336).

This article is protected by copyright. All rights reserved.

University of Liverpool

Liverpool, L7 8TX, U.K.

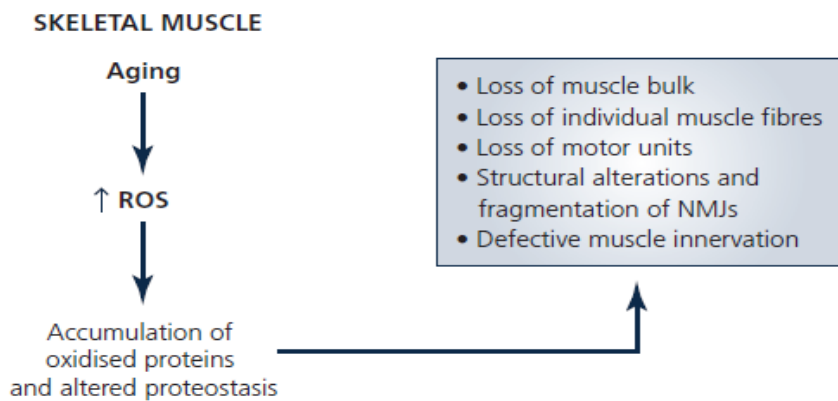
Tel: (44)151 7949069

E-mail: vasilaki@liverpool.ac.uk

Abstract

Skeletal muscle aging is characterised by atrophy, a deficit in specific force generation, increased susceptibility to injury, and incomplete recovery after severe damage. The hypothesis that increased generation of Reactive Oxygen Species (ROS) *in vivo* plays a key role in the aging process has been extensively studied, but remains controversial. Skeletal muscle generates ROS at rest and during exercise. ROS can cause oxidative damage particularly to proteins. Indeed, products of oxidative damage accumulate in skeletal muscle during aging and the ability of muscle cells to respond to increased ROS becomes defective. The aim of this review is to examine the evidence that ROS manipulation in peripheral nerves and/or muscle modifies proteostasis mechanisms in skeletal muscle and plays a key role in initiating sarcopenia.

Author N



The Journal of
Physiology

Abbreviations

AP-1, activator protein-1; CMA, chaperone-mediated autophagy; Cu/ZnSOD, copper, zinc superoxide dismutase; EMG, electromyography; GPx, glutathione peroxidase; HO-1, heme oxygenase-1; HSPs, heat shock proteins; LAMP-2A, lysosome-associated membrane protein type 2A; MA, macroautophagy; MnSOD, manganese superoxide dismutase; mtROS, mitochondrial ROS; NFκB, nuclear transcription factor kappa B; NMJs, neuromuscular junctions; ROS, reactive oxygen species; SOD, superoxide dismutase; SOD1, copper, zinc superoxide dismutase; WT, wild type.

Introduction

A 30-50% loss of muscle mass occurs between the ages of 50 and 80 years (Fried *et al.*, 2001; Bortz, 2002; Espinoza & Walston, 2005; Cesari *et al.*, 2006) that impacts profoundly on the *quality of life* of

older people resulting in a reduced ability to carry out everyday tasks and increased likelihood of falling. All individuals lose muscle mass and develop age-related muscle weakness (termed sarcopenia when it reaches clinically relevant severity), although some individuals are more prone to sarcopenia. The underlying mechanisms are unclear, however an individual's initial muscle mass, appears a critical factor influencing the risk of developing sarcopenia. For example, veteran athletes lose muscle mass at the same rate as sedentary individuals, but have a high peak muscle mass in earlier life and reach the threshold for poor function at a later age than sedentary individuals (Pearson *et al.*, 2002). Muscle mass is dictated by the number of muscle fibres and the size of the fibres. The decline in muscle mass and strength in people after the age of ~50 appears primarily due to loss of muscle fibres with weakening of the remaining fibres (Marzetti *et al.*, 2009). For example, in humans, Lexell *et al.* (1988) reported 40% fewer fibres in the vastus lateralis quadriceps muscles of older individuals (Lexell *et al.*, 1988).

Data clearly indicate that in aging man and rodents, loss of motor neurons accompanies the loss of muscle fibres (Campbell *et al.*, 1973; Brown *et al.*, 1988; Einsiedel & Luff, 1992; Larsson & Ansved, 1995), but whether motor neuron loss is cause or a consequence of muscle fibre deficits has not been definitively established due largely to the lack of available techniques to directly count motor units (a motor unit consists of a single α -motor neuron and all of the muscle fibres it innervates) in humans. Measurements have been limited to post-mortem anatomical estimates or estimates based on electromyography (EMG). Post-mortem anatomical studies have shown that people aged 75 years have 30% fewer motor neurons supplying the muscles of the lower limbs compared with young adults (Kawamura *et al.*, 1977; Tomlinson & Irving, 1977; Mittal & Logmani, 1987). One of the first studies to demonstrate motor unit loss during healthy aging using EMG was by Campbell *et al.* (1973), who recorded evoked potentials and compared these with maximum M-waves to estimate that there were 50% fewer motor units in the extensor digitorum brevis in people above 60 years. Brown *et al.* (1988) also demonstrated that subjects over 60 years of age have approximately half the numbers of motor units of subjects less than 60 years of age (Brown *et al.*, 1988) and more recently, using intramuscular and surface EMG signals from the vastus lateralis during voluntary contractions, Piasecki *et al.* demonstrated that the total number of motor units in muscles from older individuals (above 65 years old) was between 50% and 60% lower compared to those in young (Piasecki *et al.*,

2016b). The range of techniques available to estimate motor unit numbers in humans and their limitations have been reviewed elsewhere (Piasecki *et al.*, 2016a).

Several studies have also reported swollen, segmental demyelinated and remyelinated axons in peripheral nerve of old animals and humans (Sharma *et al.*, 1980; Grover-Johnson & Spencer, 1981; Adinolfi *et al.*, 1991; Verdu *et al.*, 2000) and such neuronal changes have been proposed to play a major role in the age-related loss of muscle mass and function (Delbono, 2003). Neuromuscular junctions (NMJs) in muscle fibres of old mice show a variety of alterations, including axonal swellings, sprouting, synaptic detachment, withdrawal of axons from postsynaptic sites, and fragmentation of the acetylcholine receptors (AChRs) (Valdez *et al.*, 2010; Chai *et al.*, 2011; Vasilaki *et al.*, 2016).

Reactive oxygen species (ROS) and their role in the loss of skeletal muscle mass during aging.

Regulated changes in ROS formation are important in signalling to maintain normal physiological processes in all cells including muscles and neurons. The main physiological mechanism by which cells regulate ROS activities (and hence protect against oxidative damage) is by modification of the expression and activities of regulatory enzymes such as manganese superoxide dismutase (MnSOD), copper-zinc superoxide dismutase (Cu/ZnSOD), catalase, glutathione peroxidases (GPx) and heme oxygenase-1 (HO-1) (Powers & Jackson, 2008). Acute increases in ROS generation in skeletal muscle lead to activation of a number of redox-sensitive transcription factors, including NFκB and AP-1 (Vasilaki *et al.*, 2006b; Gomez-Cabrera *et al.*, 2008) with the subsequent increased expression of antioxidant defence enzymes such as SOD and catalase and cytoprotective proteins such as heat shock proteins (McArdle *et al.*, 2001; Vasilaki *et al.*, 2006b). Motor neurons also have the capacity to upregulate some of these cytoprotective proteins in response to exogenous reactive oxygen and nitrogen species (Bishop *et al.*, 1999).

ROS activities in many tissues increase with age and there is evidence that increased ROS generation may be involved in several age-related pathologies. In skeletal muscle, *in vitro* and *in vivo* studies have provided evidence of an age-related increase in ROS production (Vasilaki *et al.*, 2006a;

Palomero *et al.*, 2013). Increased ROS production can lead to changes in the redox state of muscle cells with potentially serious effects on muscle such as cumulative damage to cellular macromolecules including lipids in cell membranes, DNA, and sub-cellular membranes and structures. A major effect of aberrant ROS generation is oxidative damage to proteins (Levine & Stadtman, 2001; Dalle-Donne *et al.*, 2003; Ghezzi & Bonetto, 2003). There is a large body of evidence supporting the accumulation of ROS-induced damaged proteins in senescent cells and tissues from old animals and our previous work has shown that muscles of old wild-type mice have an elevated content of a marker of oxidative damage, 3-nitrotyrosine residues in the major cytosolic protein, carbonic anhydrase III (Vasilaki *et al.*, 2007) and increased protein carbonyl content (Broome *et al.*, 2006) in comparison with muscles from adult wild type mice. Accumulation of oxidised proteins can lead to formation of insoluble protein aggregates, therefore, carbonylated proteins and other irreversibly modified proteins must be degraded in order to prevent them from forming aggregates.

The main players in proteostasis maintenance influencing skeletal muscle are the chaperones, the calcium-dependent calpains, the ubiquitin-proteasome and the lysosome-autophagy systems. These components are responsible for the fate of unfolded, misfolded or oxidised proteins i.e. whether they will refold into their original conformation or whether they will be removed from the cell (Kaushik & Cuervo, 2015; Anthony, 2016; Hohn *et al.*, 2017). When a protein is targeted for degradation, chaperones often dictate which proteolytic pathway that these unfolded, misfolded or oxidised proteins will follow. Many chaperones are heat shock proteins (HSPs) which are named according to their molecular weight e.g. the HSP70 family (which consists of the constitutively expressed HSC70 and highly inducible HSP70) and HSP90 and interventions that maintain overexpression of HSPs, such as HSP70, prevent the accumulation of oxidative damage and preserve some aspects of age-related muscle dysfunction (McArdle *et al.*, 2004; Broome *et al.*, 2006).

During chaperone-mediated autophagy (CMA), substrate proteins are first recognised and bind to heat shock cognate, HSC70. The resulting complex is then targeted to the lysosomes by binding to the lysosomal CMA receptor known as lysosome-associated membrane protein type 2A (LAMP-2A), at which point the target protein is unfolded and translocated into the lysosomal lumen for degradation (Cuervo & Wong, 2014; Zhou *et al.*, 2017). CMA operates at basal conditions in most mammalian cells, but it is mostly activated in response to stressors, such as oxidative stress (Xilouri

& Stefanis, 2016). The activity of CMA has been shown to decline with age in some tissues such as the central nervous system and this decline, which associates with the accumulation of damaged/oxidised/aggregated proteins has been proposed to contribute to tissue dysfunction and possibly to some common age-related human disorders, such as Parkinson's and Alzheimer's disease (Xilouri & Stefanis, 2016). However, little is known about CMA in other tissues such as skeletal muscle during aging. Our previous work has shown that oxidative damage to proteins in muscle during aging is associated with an increase in HSC70 content in quiescent muscles of old mice (Vasilaki *et al.*, 2006b) suggesting that muscles of old mice are trying to adapt in order to prevent the accumulation of oxidised proteins. This is in contrast to the findings from a recent study by Zhou and colleagues who demonstrated decreased protein levels of HSC70 and LAMP-2A in muscle from old mice but an increase in ubiquitinated proteins (Zhou *et al.*, 2017). Currently, the function of CMA in skeletal muscle is not well understood and since we did not measure LAMP-2A or ubiquitinated proteins in our study, we can only speculate that the increased content of HSC70 in muscles of old mice does not result in functional CMA as it may be the case that the formation of damaged proteins overwhelms their degradation and instead contributes to protein aggregation and subsequent loss in proteostasis mechanisms. It is also worth noting that once misfolded proteins organise into oligomers or insoluble aggregates, the only option for their elimination is by degradation in lysosomes via macroautophagy (MA) or expulsion outside the cell by exosomes (Kaushik & Cuervo, 2015). Therefore further studies are required in order to identify the exact mechanisms involved.

A Novel Mouse Model of Frailty: The Cu/Zn Superoxide Dismutase Knockout Mouse. A clear link between age-related muscle loss and increased ROS production has been indicated by studies of mice lacking Cu/ZnSOD (*Sod1*^{-/-} mice). These mice have a significantly shortened lifespan (~30%) compared to WT mice and have an accelerated decline in muscle mass and function (Muller *et al.*, 2006). In addition, adult *Sod1*^{-/-} mice show loss of motor function and contractility, declines in nerve conduction, decline in the number of motor units, partial denervation, degeneration of NMJs and increased muscle mitochondrial ROS (mtROS) generation and mitochondrial dysfunction (Flood *et al.*, 1999; Shefner *et al.*, 1999; Jang *et al.*, 2010; Vasilaki *et al.*, 2010; Larkin *et al.*, 2011; Sims-Robinson *et al.*, 2013; Deepa *et al.*, 2017). In contrast to adult WT mice, but in common with old WT mice, adult mice lacking Cu/ZnSOD have an elevated content of 3-nitrotyrosine residues (Vasilaki *et*

al., 2007) and also demonstrate a constitutive activation of NFκB and a constitutive increase in the content of HSPs in muscle at rest (Vasilaki *et al.*, 2010).

The great extent to which changes in *Sod1*^{-/-} mice mimic normal aging indicates that *Sod1*^{-/-} mice provide a model in which to study mechanistic links between oxidative stress and sarcopenia and to gain insight into mechanisms of age-associated atrophy and weakness. While strong associations exist between degeneration of NMJs and declines in mass and force both in *Sod1*^{-/-} mice and with normal aging (Jang & Van Remmen, 2011), knowledge of whether age-associated muscle wasting and weakness are due to changes proximal or distal to neuromuscular synapses is a major gap in our understanding of sarcopenia. To address questions of the relative importance of pre- vs. post-synaptic changes, we developed unique mouse models with tissue-specific targeting of Cu/ZnSOD. These models have generated several key findings: 1) Partial restoration of Cu/ZnSOD only in neurons of *Sod1*^{-/-} mice prevented the increases in muscle mtROS production, premature muscle atrophy, and weakness observed in *Sod1*^{-/-} mice (Sakellariou *et al.*, 2014). In contrast to *Sod1*^{-/-} mice, the level of protein nitration and the protein content of a peroxynitrite reductase, peroxiredoxin 5 (PRXV), as well as the contents of key HSPs in skeletal muscle from these mice were not different from wild type levels, indicating no change in the overall redox status. 2) Mice with *Sod1* deficiency in neurons alone (*nSod1*KO mice) do not show atrophy in gastrocnemius (GTN) muscles, showed only mild weakness and limited evidence of NMJ disruption, and show no significant changes in either mtROS generation or oxidative damage measured by 3-nitrotyrosine residues suggesting that Cu/ZnSOD deficit in the motor neuron alone is not sufficient to initiate a full sarcopenic phenotype (Sataranatarajan *et al.*, 2015). 3) Finally, mice lacking Cu/ZnSOD only in muscle fibres do not show NMJ degeneration or muscle atrophy, no changes in the HSP content and oxidative damage is not elevated; but do show weakness and increased susceptibility to injury (Zhang *et al.*, 2013). Collectively, these data suggest that redox homeostasis in motor neurons is a critical factor in initiating sarcopenia, but that the progression of sarcopenia is determined by complex interactions between both pre- and post-synaptic factors (Figure 1).

Because adult mice lacking Cu/ZnSOD reproduce many of the main features seen in old WT mice they may indicate important mechanisms that lead to loss of muscle fibres and function that are relevant to the aging of WT mice. The *Sod1*^{-/-} mice as well as our other novel mouse models with tissue specific modulation of Cu/ZnSOD have also demonstrated the importance of nerve–muscle

interactions in the maintenance of neuromuscular function where ROS homeostasis is altered. Because our data indicate that motor neuron deficits arising from an oxidised redox status are critical in sarcopenia, our future work will focus on determining the impact of oxidative stress in motor neurons on NMJ formation and maintenance and the impact of directly disrupting NMJs on key post-synaptic muscle functions.

In summary, current findings support the hypothesis that increased generation of ROS is an important component of the ageing process, providing a link between accumulation of oxidative damage and muscle dysfunction.

Additional information

Competing Interests

None

Author contributions

AV has produced the manuscript and is responsible for the content. All authors approved the final version of the manuscript and all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

The authors would like to thank National Institutes of Health AG051442 for funding. AR and HVR are supported by a Senior Research Career Scientist award from the US Department of Veterans Affairs.

Acknowledgements

The authors would like to thank their present and previous collaborators and colleagues.

References

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

- Anthony TG. (2016). Mechanisms of protein balance in skeletal muscle. *Domestic animal endocrinology* **56 Suppl**, S23-32.
- Bishop A, Marquis JC, Cashman NR & Demple B. (1999). Adaptive resistance to nitric oxide in motor neurons. *Free radical biology & medicine* **26**, 978-986.
- Bortz WM, 2nd. (2002). A conceptual framework of frailty: a review. *The journals of gerontology Series A, Biological sciences and medical sciences* **57**, M283-288.
- Broome CS, Kayani AC, Palomero J, Dillmann WH, Mestril R, Jackson MJ & McArdle A. (2006). Effect of lifelong overexpression of HSP70 in skeletal muscle on age-related oxidative stress and adaptation after nondamaging contractile activity. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **20**, 1549-1551.
- 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. *Journal of neurology, neurosurgery, and psychiatry* **36**, 174-182.
- Cesari M, Leeuwenburgh C, Lauretani F, Onder G, Bandinelli S, Maraldi C, Guralnik JM, Pahor M & Ferrucci L. (2006). Frailty syndrome and skeletal muscle: results from the Invecchiare in Chianti study. *The American journal of clinical nutrition* **83**, 1142-1148.
- 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.

Cuervo AM & Wong E. (2014). Chaperone-mediated autophagy: roles in disease and aging. *Cell research* **24**, 92-104.

Dalle-Donne I, Rossi R, Giustarini D, Milzani A & Colombo R. (2003). Protein carbonyl groups as biomarkers of oxidative stress. *Clinica chimica acta; international journal of clinical chemistry* **329**, 23-38.

Deepa SS, Bhaskaran S, Espinoza S, Brooks SV, McArdle A, Jackson MJ, Van Remmen H & Richardson A. (2017). A new mouse model of frailty: the Cu/Zn superoxide dismutase knockout mouse. *GeroScience* **39**, 187-198.

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

Einsiedel LJ & Luff AR. (1992). Alterations in the contractile properties of motor units within the ageing rat medial gastrocnemius. *Journal of the neurological sciences* **112**, 170-177.

Espinoza S & Walston JD. (2005). Frailty in older adults: insights and interventions. *Cleveland Clinic journal of medicine* **72**, 1105-1112.

Flood DG, Reaume AG, Gruner JA, Hoffman EK, Hirsch JD, Lin YG, Dorfman KS & Scott RW. (1999). Hindlimb motor neurons require Cu/Zn superoxide dismutase for maintenance of neuromuscular junctions. *The American journal of pathology* **155**, 663-672.

Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G & McBurnie MA. (2001). Frailty in older adults: evidence for a phenotype. *The journals of gerontology Series A, Biological sciences and medical sciences* **56**, M146-156.

Ghezzi P & Bonetto V. (2003). Redox proteomics: identification of oxidatively modified proteins. *Proteomics* **3**, 1145-1153.

- Gomez-Cabrera MC, Domenech E & Vina J. (2008). Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. *Free radical biology & medicine* **44**, 126-131.
- Grover-Johnson N & Spencer PS. (1981). Peripheral nerve abnormalities in aging rats. *Journal of neuropathology and experimental neurology* **40**, 155-165.
- Hohn A, Weber D, Jung T, Ott C, Hugo M, Kochlik B, Kehm R, Konig J, Grune T & Castro JP. (2017). Happily (n)ever after: Aging in the context of oxidative stress, proteostasis loss and cellular senescence. *Redox biology* **11**, 482-501.
- 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 journal : official publication of the Federation of American Societies for Experimental Biology* **24**, 1376-1390.
- Jang YC & Van Remmen H. (2011). Age-associated alterations of the neuromuscular junction. *Experimental gerontology* **46**, 193-198.
- Kaushik S & Cuervo AM. (2015). Proteostasis and aging. *Nature medicine* **21**, 1406-1415.
- Kawamura Y, Okazaki H, O'Brien PC & Dych PJ. (1977). Lumbar motoneurons of man: I) number and diameter histogram of alpha and gamma axons of ventral root. *Journal of neuropathology and experimental neurology* **36**, 853-860.
- Larkin LM, Davis CS, Sims-Robinson C, Kostrominova TY, Remmen HV, Richardson A, Feldman EL & Brooks SV. (2011). Skeletal muscle weakness due to deficiency of CuZn-superoxide dismutase is associated with loss of functional innervation. *American journal of physiology Regulatory, integrative and comparative physiology* **301**, R1400-1407.

- Larsson L & Ansved T. (1995). Effects of ageing on the motor unit. *Progress in neurobiology* **45**, 397-458.
- Levine RL & Stadtman ER. (2001). Oxidative modification of proteins during aging. *Experimental gerontology* **36**, 1495-1502.
- Lexell J, Taylor CC & Sjostrom 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. *Journal of the neurological sciences* **84**, 275-294.
- Marzetti E, Lees HA, Wohlgemuth SE & Leeuwenburgh C. (2009). Sarcopenia of aging: underlying cellular mechanisms and protection by calorie restriction. *Biofactors* **35**, 28-35.
- McArdle A, Dillmann WH, Mestral R, Faulkner JA & Jackson MJ. (2004). Overexpression of HSP70 in mouse skeletal muscle protects against muscle damage and age-related muscle dysfunction. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **18**, 355-357.
- McArdle A, Pattwell D, Vasilaki A, Griffiths RD & Jackson MJ. (2001). Contractile activity-induced oxidative stress: cellular origin and adaptive responses. *American journal of physiology Cell physiology* **280**, C621-627.
- Mittal KR & Logmani FH. (1987). Age-related reduction in 8th cervical ventral nerve root myelinated fiber diameters and numbers in man. *Journal of gerontology* **42**, 8-10.
- Muller FL, Song W, Liu Y, Chaudhuri A, Pieke-Dahl S, Strong R, Huang TT, Epstein CJ, Roberts LJ, 2nd, Csete M, Faulkner JA & Van Remmen H. (2006). Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy. *Free radical biology & medicine* **40**, 1993-2004.

- Palomero J, Vasilaki A, Pye D, McArdle A & Jackson MJ. (2013). Aging increases the oxidation of dichlorohydrofluorescein in single isolated skeletal muscle fibers at rest, but not during contractions. *American journal of physiology Regulatory, integrative and comparative physiology*.
- Pearson SJ, Young A, Macaluso A, Devito G, Nimmo MA, Cobbold M & Harridge SD. (2002). Muscle function in elite master weightlifters. *Medicine and science in sports and exercise* **34**, 1199-1206.
- Piasecki M, Ireland A, Jones DA & McPhee JS. (2016a). Age-dependent motor unit remodelling in human limb muscles. *Biogerontology* **17**, 485-496.
- Piasecki M, Ireland A, Stashuk D, Hamilton-Wright A, Jones DA & McPhee JS. (2016b). Age-related neuromuscular changes affecting human vastus lateralis. *The Journal of physiology* **594**, 4525-4536.
- Powers SK & Jackson MJ. (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev* **88**, 1243-1276.
- 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). Neuron-specific expression of CuZnSOD prevents the loss of muscle mass and function that occurs in homozygous CuZnSOD-knockout mice. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **28**, 1666-1681.
- 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 biology* **5**, 140-148.

Sharma AK, Bajada S & Thomas PK. (1980). Age changes in the tibial and plantar nerves of the rat. *J Anat* **130**, 417-428.

Shefner JM, Reaume AG, Flood DG, Scott RW, Kowall NW, Ferrante RJ, Siwek DF, Upton-Rice M & Brown RH, Jr. (1999). Mice lacking cytosolic copper/zinc superoxide dismutase display a distinctive motor axonopathy. *Neurology* **53**, 1239-1246.

Sims-Robinson C, Hur J, Hayes JM, Dauch JR, Keller PJ, Brooks SV & Feldman EL. (2013). The role of oxidative stress in nervous system aging. *PloS one* **8**, e68011.

Tomlinson BE & Irving D. (1977). The numbers of limb motor neurons in the human lumbosacral cord throughout life. *Journal of the neurological sciences* **34**, 213-219.

Valdez G, Tapia JC, Kang H, Clemenson GD, Jr., Gage FH, Lichtman JW & Sanes JR. (2010). Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 14863-14868.

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

Vasilaki A, McArdle F, Iwanejko LM & McArdle A. (2006b). Adaptive responses of mouse skeletal muscle to contractile activity: The effect of age. *Mechanisms of ageing and development* **127**, 830-839.

Vasilaki A, Pollock N, Giakoumaki I, Goljanek-Whysall K, Sakellariou GK, Pearson T, Kayani A, Jackson MJ & McArdle A. (2016). The effect of lengthening contractions on neuromuscular junction structure in adult and old mice. *Age (Dordr)* **38**, 259-272.

Vasilaki A, Simpson D, McArdle F, McLean L, Beynon RJ, Van Remmen H, Richardson AG, McArdle A, Faulkner JA & Jackson MJ. (2007). Formation of 3-nitrotyrosines in carbonic anhydrase III is a sensitive marker of oxidative stress in skeletal muscle. *Proteomics Clin Appl* **1**, 362-372.

Vasilaki A, van der Meulen JH, Larkin L, Harrison DC, Pearson T, Van Remmen H, Richardson A, Brooks SV, Jackson MJ & McArdle A. (2010). 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* **9**, 979-990.

Verdu E, Ceballos D, Vilches JJ & Navarro X. (2000). Influence of aging on peripheral nerve function and regeneration. *J Peripher Nerv Syst* **5**, 191-208.

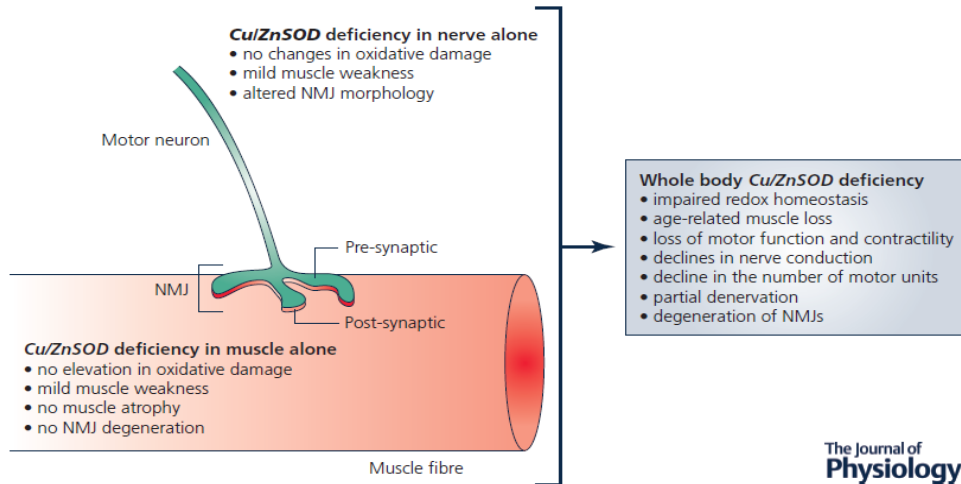
Xilouri M & Stefanis L. (2016). Chaperone mediated autophagy in aging: Starve to prosper. *Ageing research reviews* **32**, 13-21.

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 journal : official publication of the Federation of American Societies for Experimental Biology* **27**, 3536-3548.

Zhou J, Chong SY, Lim A, Singh BK, Sinha RA, Salmon AB & Yen PM. (2017). Changes in macroautophagy, chaperone-mediated autophagy, and mitochondrial metabolism in murine skeletal and cardiac muscle during aging. *Aging* **9**, 583-599.

List of Figures

Figure 1. Cu/ZnSOD deficits in either the motor neuron or muscle alone are not sufficient to initiate a full sarcopenic phenotype as seen in *Sod1*^{-/-} mice.



3



The authors Aphrodite Vasilaki (Liverpool), Arlan Richardson (Oklahoma), Holly Van Remmen (Oklahoma), Susan Brooks (Michigan), Lisa Larkin (Michigan), Anne McArdle (Liverpool) and Malcolm Jackson (Liverpool) (shown above left to right) are long term collaborators. This collaborative work was initiated in 2002 by Professor John Faulkner from the Muscle Mechanics Laboratory at the University of Michigan. The major funding for the collaborative work comes from a Program-project supported by the US National Institute on Aging (NIH). The overall aim of our work is to understand the role of ROS in normal muscle physiology and skeletal muscle aging.

Auth

This article is protected by copyright. All rights reserved.