



Review

Episodic denervation as a driver of loss of skeletal muscle redox homeostasis and muscle weakness in sarcopenia: Possible amelioration by exercise

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ABSTRACT

Substantial reductions in muscle motor unit numbers accompany ageing and occur in parallel the age-related changes in skeletal muscle mass and fibre number. These motor unit changes are reflected in reduced motor neuron numbers and size, axonal integrity and disrupted pre- and post-synaptic neuromuscular junctions (NMJ). Conversely, data indicate that the effects of ageing on neuromuscular transmission are relatively minor. Some authors have therefore argued that structural degeneration of motor axons and NMJ are unimportant in the pathogenesis of sarcopenia and for a non-neurogenic origin for ageing-induced muscle loss. Increased Reactive Oxygen Species (ROS) activities and changes in redox status are a feature of ageing and may play a key role in muscle loss through increased mitochondrial peroxide generation. This article will review the changes in motor units and NMJ seen during ageing and develop the argument that the changes in muscle mitochondrial peroxide generation and redox status may be caused by age-related changes in neuromuscular structure, but are not directly related to neuromuscular transmission. This provides an alternative explanation on how age-related changes in neural tissue might drive skeletal muscle fibre loss and weakness. Exercise interventions are known to reduce muscle loss and weakness in the elderly, but studies of such interventions on age-related changes in motor units, motor neurons or NMJ structure and function provide conflicting data. A further aim is therefore to identify areas where there is a need for novel research to understand whether, and how, targeted or long-term exercise might influence neuromuscular changes in ageing.

1. Introduction

Ageing of skeletal muscle is characterised by loss of mass and contractile force and has a profound impact on the quality of life of older people. Loss of skeletal muscle begins in middle age and continues until the end of life.¹ In older people, declining muscle mass and function causes instability and increased risk of falls with a loss of independence.² The cross-sectional area of skeletal muscle is reduced by 25%–30% and muscle strength by 30%–40% by age 70 years.³ A decrease in the number of muscle fibres, and atrophy and weakening of those fibres remaining^{4–6} contribute to the reduction in muscle mass and function with age in humans and rodents. This is termed sarcopenia and the intrinsic and extrinsic changes regulating muscle ageing in humans occur in rodents, indicating that ageing mice and rats are relevant models of human sarcopenia.^{7,8} While there is undoubtedly a major effect of the ageing process on the loss of muscle mass and weakness seen in elderly populations, multiple other factors play a role in individuals, including lack of exercise, increased sedentary behaviour, sub-optimal nutrition, social

isolation and sub-optimal health care.⁹

The loss of skeletal muscle that occurs with ageing appears to occur in parallel with loss of motor units in both humans and rodents.^{10,11} A motor unit is the functional unit for contraction and comprises the motor neuron, neuromuscular junctions (NMJ) and skeletal muscle fibres innervated by a single motor neuron as shown schematically in Fig. 1.¹² Some data indicate that ageing is associated with a 25%–50% reduction in the number of motor neurons^{13–15} due to selective loss of large fast α -motor neurons leading to an apparent increase in the proportion of type I (slow twitch) or type IIa muscle fibers that is particularly apparent in humans.^{16,17} The relationship between ageing-induced changes in the structure and function of peripheral nerves and age-related loss of muscle mass and weakness is complex and there also appears to be a time delay between the changes in nerve structure and function and the apparent muscle loss seen with ageing.¹⁸ The likely explanation is that appearance of any overt muscle phenotype is delayed due to expansion of the size of motor units with collateral re-innervation of denervated NMJ. The time delay in loss of muscle fibres therefore reflects the period of time until the ability to expand motor unit size is exceeded. While motor units have a

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Abbreviation list

AChR	Acetyl choline receptor
AP-1	Activator protein 1 (AP-1) transcription factor
BDNF	Brain-derived neurotrophic factor
EDL	Extensor digitorum longus
GDNF	Glial cell line-derived neurotrophic factor
HSF-1	Heat shock factor 1 transcription factor
H ₂ O ₂	Hydrogen peroxide
IGF-1	Insulin-like growth factor 1
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NAV1.5	Voltage gated sodium channel
NCAM	Neural cell adhesion molecule
NF-κB	Nuclear factor kappa B transcription factor
NMJ	Neuromuscular junction
PGC1α	Peroxisome proliferator-activated receptor-gamma

	coactivator-1alpha
ROS	Reactive oxygen species
Sod1	Cu, Zn superoxide dismutase
Sod1KO mice	Mice with whole body deletion of Cu, Zn superoxide dismutase
SynTgSod1KO mice	Mice with whole body deletion of Sod1 in which Sod1 is transgenically expressed only in neurons
i-mn-Sod1KO mice	Mice in which deletion of Sod1 in motor neurons can be induced by tamoxifen injections
TA	Tibialis anterior
Thy1-CFP mice	Mice expressing cyan fluorescent protein in tissues under the Thy1 promoter (nerve specific)
Thy1-YFP mice	Mice expressing yellow fluorescent protein in tissues under the Thy1 promoter (nerve specific)
WT mice	Wild type mice (non-genetically modified)
YFP	Yellow fluorescent protein

capacity for expansion to compensate for motor neuron loss, there appears to be a maximum ability to increase the size of an individual motor unit.¹⁹ In humans, Piasecki et al. showed that motor unit loss occurred early in the ageing process and that failure to expand was characteristic of sarcopenia.²⁰

In this review the changes in neural structures associated with age-related muscle loss will be described to demonstrate the substantial degeneration and reorganisation that does not appear to be associated with a significant failure of neuromuscular transmission. Examples from

our own work will be used to argue that, despite the lack of effect on transmission, transient denervation events lead to significant increased muscle mitochondrial peroxide generation and changes in muscle redox homeostasis in the denervated and neighbouring innervated muscle fibres. This provides an alternative explanation of how motor unit degeneration can lead to degeneration of muscle during ageing. Additionally, the potential of exercise to ameliorate age-related decline in the neuromuscular system will be discussed and clear areas where further studies are required will be described.

2. Age-related changes in neuromuscular structure

Age-related changes have been reported to occur in all parts of the neural structures innervating skeletal muscles. While human studies have focused on measurements of motor unit numbers and nerve function because of limited access and the availability of relevant techniques, rodent models have provided a great deal of information on the structural changes that occur in axons, motor neurons and NMJ with ageing and on neuronal and NMJ function.²¹ It is important to note that while the functions of the components of the neuromuscular system may be identical across species, NMJ differ structurally between humans and animals and examples for different species are shown schematically in Fig. 2.²² The classic images of “pretzel” shaped NMJ that are reproduced in many text books are most obviously found in rodents. Human NMJ are amongst the smallest known and release the smallest number of transmitter quanta, but age-related changes are seen in NMJ from all vertebrates.²³

The key structures that occur in the motor unit have been studied extensively in rodent models and particularly in mouse experimental

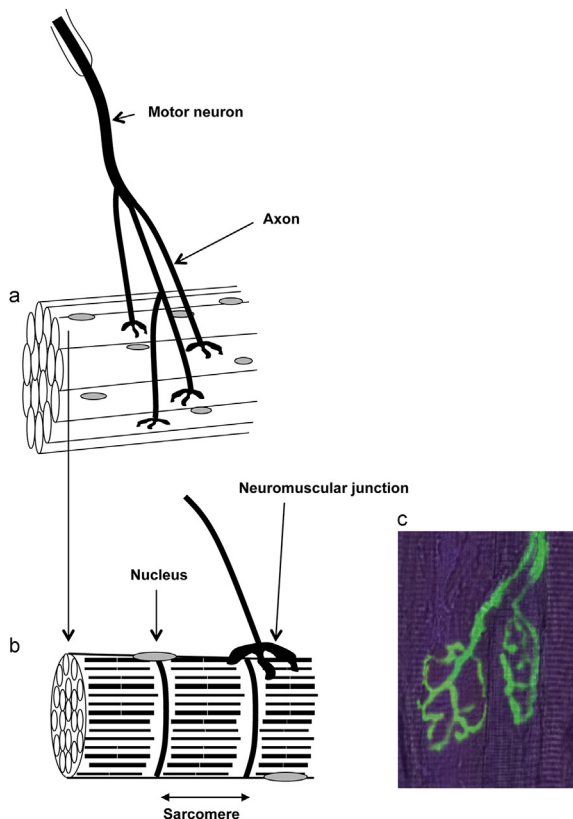


Fig. 1. Schematic representation of the organisation of a motor unit (a) bundle of muscle fibres innervated by a motor neuron, (b) neuromuscular junction on a single muscle fibre, (c) Fluorescent image of neuromuscular junctions and peripheral axons from the Tibialis Anterior (TA) muscle of a young *thy1-YFP* mouse. The underlying muscle fibres are stained with phalloidin. Reproduced from.¹²

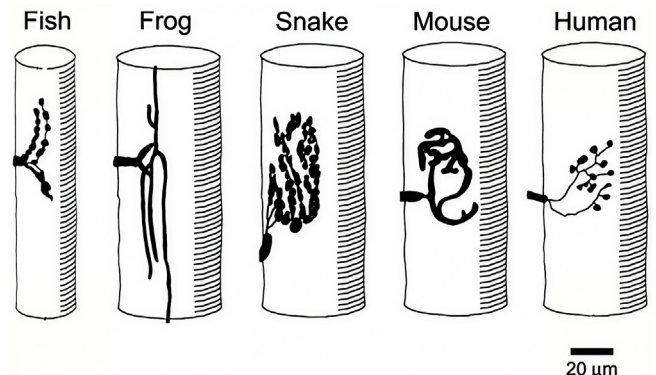


Fig. 2. Pattern of vertebrate muscle innervation. Note the variety of sizes and conformations with human terminals among the smallest. Reproduced from.²²

models. Our studies have focussed on mouse models to attempt to understand how changes in motor unit composition and function influence the redox status of the innervated skeletal muscle.

2.1. Age-related changes in axons and motor neurons

Previous data indicate the occurrence of denervation in muscle fibres from elderly human subjects. This has been primarily inferred from the occurrence of muscle fibre type grouping, accumulation of severely atrophic angular fibres, and increased expression of proteins associated with denervation such as neural cell adhesion molecule (NCAM) and the voltage gated sodium channel isoform (Nav1.5) by the atrophic fibres.²⁴ We directly examined axonal structure in the sciatic nerves of adult (6–8 month) and old (26–28 month) mice. Example cross sections of the sciatic nerves are shown in Fig. 3.²⁵ Quantitative data derived from the cross sections showed significant reductions in axon diameter, axon area and myelin area in axons from old mice compared with adult illustrating the major structural changes which subsequent studies suggest is mainly due to selective loss of large axons.²⁶

The structural changes seen in axons from ageing rodents, such as those illustrated in Fig. 3, likely reflect age-related changes in both motor and sensory neurons and more specific approaches are required to directly measure the effect of ageing on motor neurons. Various approaches have been used to study motor neurons. In rodents direct counting of motor neuron numbers and measurements of motor neuron areas in cross-sections of the lumbar spinal cord is undertaken^{15,27} or retrograde labelling of transected nerves with suitable fluorescent proteins.^{26,28} An example of labelling of motor neurons by retrograde transport of label from the transected sciatic nerve and the tibial nerve branches in the mouse gastrocnemius muscle is shown in Fig. 4.²⁶ This latter elegant approach demonstrated an ~30% decrease in motor neuron numbers in 24–29 month aged mice in comparison with 6–9 month adult mice.²⁶ This figure also shows comparative data obtained from adult mice lacking Sod1.²⁶ This is an *in vivo* mouse model of “rapid ageing” which will be discussed in detail later in this review.

2.2. Age-related changes in NMJ in mice

Numerous changes have been reported to occur in both the pre- and postsynaptic NMJ structures in ageing rodents. The pattern of changes is both heterogeneous and patchy across different muscles of mice but areas with considerable evidence of loss of innervation, increased fragmentation of the NMJ, increased axonal sprouting and loss of NMJ complexity have been reported.^{23,26,29} Fig. 5 shows examples of these changes and illustrates the patchy distribution of changes in NMJ during ageing. Transient, episodic loss of integrity of peripheral motor neurons

occurs repeatedly throughout life. This is usually followed by rapid reinnervation, but the reinnervation appears to eventually become less efficient or fail with ageing and lead to the sustained structural changes in the NMJ shown in Fig. 5.³⁰ The site of the failure is unclear although axonal regeneration becomes severely impaired during ageing, potentially secondarily to Schwann cell senescence.³¹

3. Do structural changes in axons, motor neurons and NMJ correlate with loss of function?

The previous sections illustrated the substantial changes in neuromuscular structure that occur with ageing and suggest that major changes in neuromuscular function are likely to occur and contribute to muscle loss in ageing. However, some authors have argued that this is not the case and that despite the extensive structural changes particularly in the NMJ there is little correlation with impairment of function.²³ Although the data are not entirely clear cut it does not appear there is any consistent failure in NMJ transmission in aged rodents in which there is substantial disruption of NMJ structure.³² The question therefore remains as to whether the neuromuscular changes contribute to sarcopenia in the elderly or may potentially be a consequence of it.

Various experimental mouse models have been developed which show degradation of the NMJ and provide insight into the role of NMJ disruption in muscle loss. One example in a mouse model is over-expression of neurotrypsin in motor neurons which destabilises NMJs by increasing the cleavage of agrin.³³ This genetic modification led to a reduced number of muscle fibres, increased heterogeneity of fibres, more centralized nuclei, fibre-type grouping, and an increased proportion of type I fibres which the authors described as a sarcopenia.³⁴ These data therefore suggest that specific changes in the NMJ can lead to a sarcopenia-like phenotype and support the possibility that changes seen at the NMJ in ageing may be important drivers of sarcopenia.

Supportive data for a primary role for neuronal deficits in sarcopenia have also been obtained from our studies of mice lacking superoxide dismutase 1 (*Sod1KO* mice). Muller and co-workers initially described an accelerated skeletal muscle ageing phenotype in whole body *Sod1KO* mice³⁵ and skeletal muscles of *Sod1KO* mice were found to exhibit mitochondrial abnormalities, degeneration of NMJs and loss of innervation, and loss of contractile force during adulthood which are similar to the hind limb muscle phenotype seen in old wild-type mice.^{4,36–39} To try and determine the tissue sites most crucial to the effects of lack of Sod1 in causing early skeletal muscle loss, mice with a genetic deletion of Sod1 specifically targeted to skeletal muscle were examined. These studies found no effect of Sod1 deletion targeted to skeletal muscle on muscle mass even in older adult mice (up to 16–17 months) and contractile function was only marginally reduced.⁴⁰ Conversely, in a mouse model in

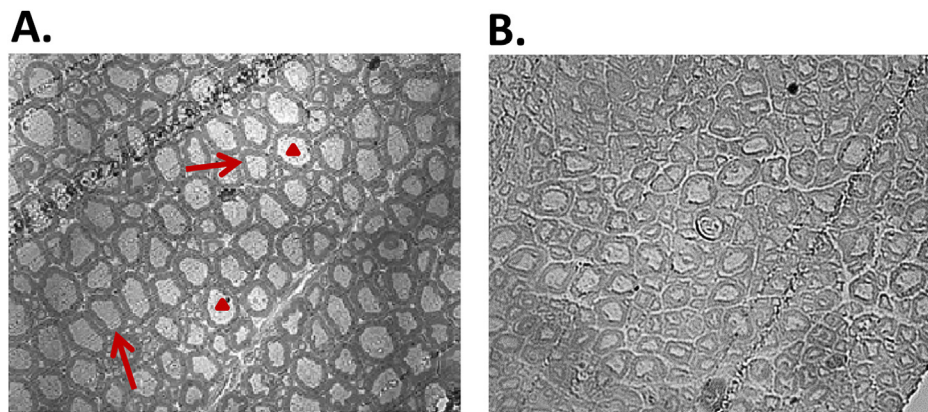


Fig. 3. Example semi-thin cross-sections from transverse sections of the sciatic nerves of adult (A) and old (B) mice ($n = 7$). Myelin sheaths are indicated by arrows and axons by arrowheads. Reproduced from.²⁵

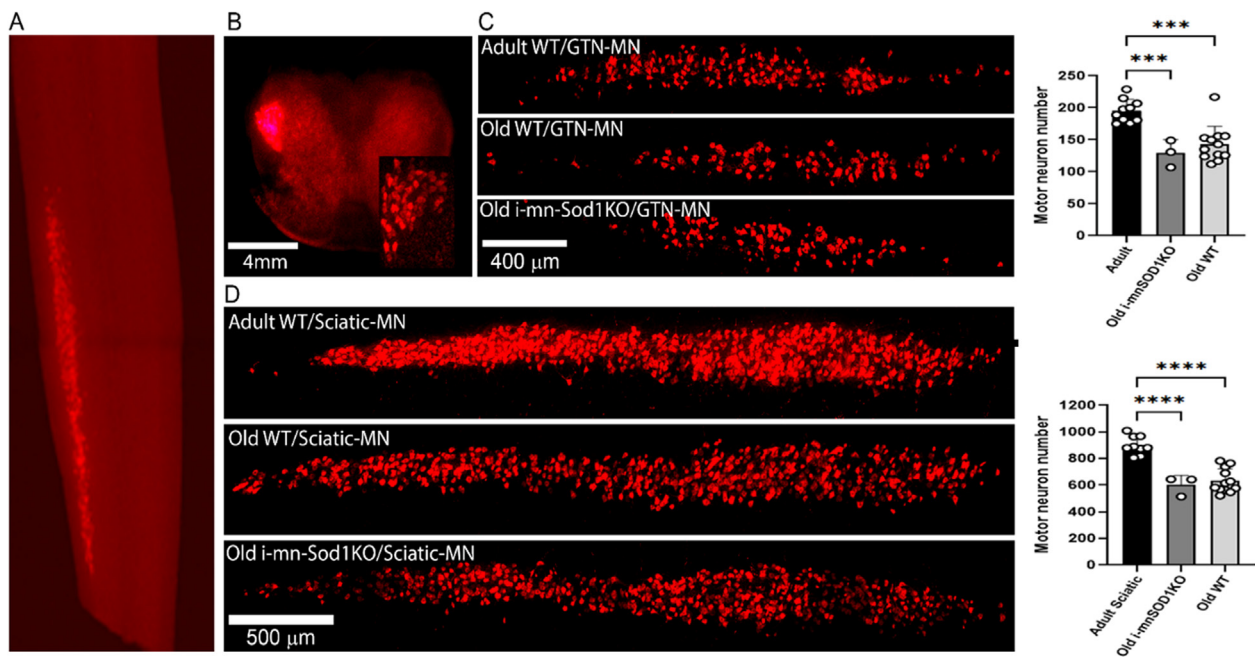


Fig. 4. Quantification of motor neurons identified by retrograde labelling via transection of whole sciatic and the tibial nerve branches to the gastrocnemius. Representative images to show specific localisation of the retrograde label to the lumbar spinal cord (A) and the lateral ventral horn (B) after sciatic nerve transection (inset displays labelled individual motor neurons). Typical pattern of labelling of motor neurones in adult and old WT and old i-mnSOD1KO mice and quantification of numbers of labelled motor neurons following: transection of the medial and lateral heads of the gastrocnemius (C), transection of the whole sciatic nerve (D). Data are presented as mean \pm SD, *** p < 0.001 from two-way ANOVA. Reproduced from.²⁶

which the human SOD1 gene was specifically targeted to neurons on a whole body SOD1KO background (*SynTgSodKO* mice) the neuron-specific expression of Sod1 prevented the muscle atrophy, NMJ degeneration, and muscle weakness phenotypes that occur in the *Sod1KO* mice suggesting that the effects of lack of SOD1 in motor neurons was a critical mediator of muscle innervation and atrophy.⁴¹ As shown in Fig. 4, adult *Sod1KO* mice also show a comparable loss of motor neurons to that seen in ageing wild type mice emphasising the role of Sod1 in motor neuron integrity.

The potential role of a motor neuron lack of Sod1 in the muscle phenotype, was initially investigated in mice with a constitutive embryonic neuron-specific deletion of Sod1,⁴¹ but these mice did not show significant atrophy of muscles even at 20 months of age, although mild contractile dysfunction and signs of denervation were evident. These data appeared to contradict the conclusions from studies of the nerve rescue (*SynTgSodKO*) mice⁴¹ and it was hypothesized that the specific approach used to delete neuronal Sod1, using the nestin-cre, may not have been sufficient to induce a phenotype, or that the deletion during embryonic development may induce compensatory effects that altered the effect of motor neuron deletion in the mice at later ages.³⁹ A mouse model with inducible deletion of Sod1 (*i-mn-Sod1KO*) targeted by a neuron-specific cre recombinase driven by the Thy1 promoter was generated. The findings obtained showed that neuronal deletion of Sod1 in adult mice resulted in accelerated atrophy and contractile dysfunction of skeletal muscle as well as a disruption of NMJ morphology in older mice.³⁹ Thus, in both of these divergent mouse models an initial defect in motor nerves appears sufficient to induce a sarcopenic phenotype in mice.

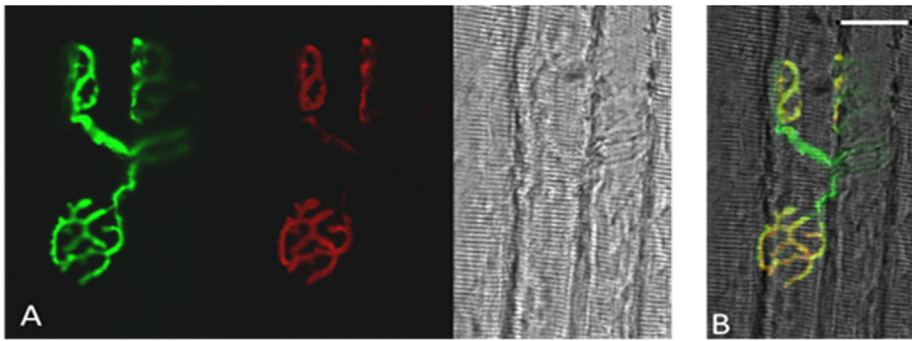
Thus, many studies have reported severe disruption of neuromuscular structures with loss of motor neurons and axons during ageing associated with multiple changes at the NMJ. These changes are associated with substantial adaptation of neuromuscular structures such as reinnervation of denervated fibres resulting from axonal sprouting and potentially leading to increased fibre type grouping and motor unit expansion.⁴² Such changes may substantially affect the contractile performance of

specific muscles leading to slower response times and hence less ability to undertake detailed tasks due to the decrease in the number of motor units and an increase in their size. It is also argued that this motor unit remodelling will only be partially successful and large motor units will eventually be lost.²⁰ When large motor units are lost this will lead to loss of muscle fibres providing an explanation for, at least part, of the sarcopenia phenotype. It has been argued that the muscle weakness in ageing is due to loss of fibres, atrophy of remaining fibres and intrinsic weakness in remaining fibres. The age-related fibre loss has been attributed to denervation and motor unit remodelling whereas the gradual degradation with fibre atrophy has been attributed to impaired muscle protein synthesis.^{43,44}

This demarcation between denervation as a cause of fibre loss with an alternative origin for the coincident activation of atrophic processes in inducing sarcopenia appears to be based on the lack of any direct correlation between the major structural changes seen in axons, motor neurons and NMJ and any associated functional deficits in the relevant muscles.²³ Thus, for example, in one study of ageing mice, we found ~15% of all muscle fibres in the extensor digitorum longus muscle appeared fully denervated while ~80% of NMJs showed disruption, but changes in NMJ structure showed no correlation with muscle force generation.²⁹ Willadt et al.,²³ also reviewed this area and concluded: "On balance, there is little evidence to support the view that muscle loss of strength in elderly humans in general and sarcopenia in particular are caused by impaired neuromuscular transmission".

The possibility that the age-related changes in neuronal structure leads to degenerative changes in muscle fibres through alternative mechanisms, unrelated to failure of neuromuscular transmission, appears to have received little attention. Our work examining the *Sod1KO* mouse and other experimental models has prompted us to examine the possibility that the innervation status of muscle fibres influences redox homeostasis in muscle, since loss of redox homeostasis has also been claimed to play a major role in muscle loss and weakness leading to sarcopenia.⁴⁵

Adult



Old

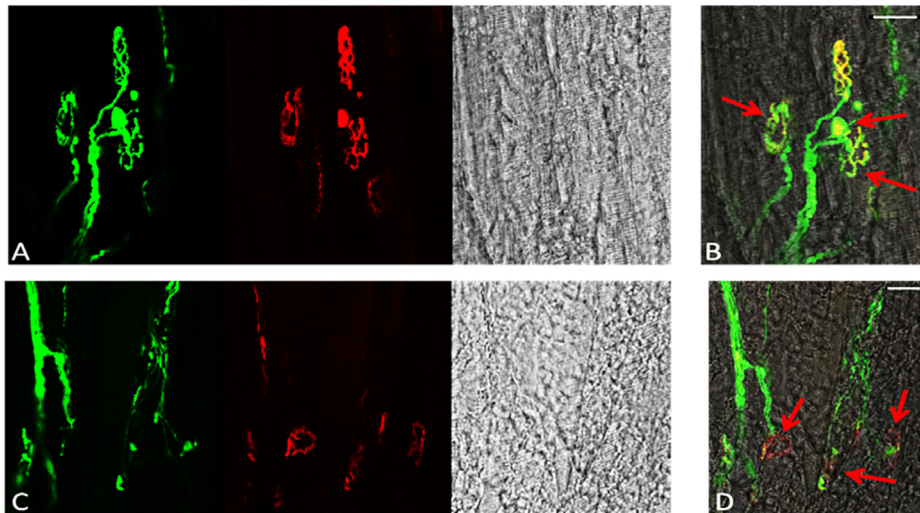


Fig. 5. Upper panel - Adult mice. (A) Representative longitudinal section of a quiescent EDL muscle from adult mice showing yellow fluorescent protein (YFP) in motor neurons (green), AChRs labelled with Alexa-594- α -bungarotoxin (red) and image of the fibres under bright field. (B) Merged image from A. **Lower panels - Old mice.** (A & C) Representative longitudinal section of a quiescent EDL muscles from old mice showing YFP in motor neurons (green), Acetylcholine receptors (AChRs) labelled with Alexa-594- α -bungarotoxin (red) and image of the fibres under bright field. (B & D) Merged images from A & C. Red arrows show age-related structural changes including nerve terminal fragmentation with some nerve terminals forming spherical and partially or fully denervated NMJs. Reproduced and modified from.²⁹

4. Reactive oxygen species (ROS), redox homeostasis and age-related loss of muscle mass and function

There have been dramatic advances in understanding the fundamental mechanisms underlying the ageing process in non-mammalian and mammalian models^{46,47} and these studies are informing investigations of the mechanisms underlying age-related degeneration of muscle tissue.⁴⁸ One of these fundamental mechanisms is redox regulation and the role of ROS as a driver of age-related changes. A factor clearly associated with loss of function during ageing in numerous tissues is oxidative damage, and experimental evidence from humans and rodents indicates that skeletal muscles and other musculoskeletal tissues show age-dependent increases in the products of oxidative damage to biomolecules including proteins, lipids and nucleic acids.^{49–52} Various reports have attributed the positive correlation between age and oxidative damage to age-related changes in ROS production, with skeletal muscles from old mice exhibiting a higher intracellular ROS generation in comparison to muscles from young mice.^{53,54}

Although it now seems clear that the level of cellular ROS generation and oxidative damage is not a fundamental determinant of lifespan, some authors have argued that the age-related changes in ROS activities and oxidative damage are important mediators of age-related disorders.⁵⁵ Mitochondrial peroxide generation has also been repeatedly reported to be increased in skeletal muscle during ageing.^{56,57}

ROS play a role in skeletal muscle physiology and skeletal muscle fibres respond to contractions by an increase in the intracellular generation of superoxide and nitric oxide (NO) with the formation of

secondary ROS and reactive nitrogen species.^{58–60} In normal physiology, these species act as signalling molecules by modifying targeted specific residues in proteins,^{61,62} including activation of a number of transcription factors, such as NF- κ B, AP-1 and HSF-1^{63–66} and an increased expression of regulatory enzymes and cytoprotective proteins.^{67,68} Key processes involved in muscle adaptations to exercise have been intensively studied for a number of years and among these, multiple pathways have been identified where redox regulation appears important including the key pathways leading to multiple adaptations to exercise.⁶⁹ Thus, age-related changes in ROS generation or accumulation of oxidised products may lead to dysregulation of signalling in addition to oxidative damage in tissues of the neuromuscular system.

5. Innervation as a regulator of muscle redox status

Mice lacking *Sod1* were discussed earlier as a model primarily affecting motor neuron integrity which leads to a sarcopenic phenotype. *Sod1* (Cu, Zn superoxide dismutase) is a key regulatory enzyme for conversion of superoxide to hydrogen peroxide and mice with whole body deletion of the enzyme showed an increase in tissue oxidative damage associated with neuromuscular changes with ageing. This was described by the discoverer as “accelerated age-related loss of muscle mass”.^{35,70} Adult *Sod1KO* 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 at 8 months old.^{71,37,38} The fibre loss in *Sod1KO* mice is accompanied by degeneration of NMJs.³⁷ These changes are also seen in

old control wild type (WT) mice, but not until after 22 months of age. Hence *Sod1KO* mice were proposed as a useful model to examine the potential role of ROS in skeletal muscle ageing.⁷² Studies of this mouse model as a model of accelerated skeletal muscle ageing^{27,40,41,73,74} highlighted to us the potential role of disruption of neuromuscular integrity in regulation of muscle mitochondrial ROS generation. Previous studies had also indicated that transection of the innervating nerve of mouse muscle caused a large increase in muscle mitochondrial peroxide generation⁷⁵ and together these different approaches identified a key role for motor neuron and NMJ integrity in regulation of muscle mitochondrial ROS generation in old mice.

5.1. Effects of denervation or nerve crush on muscle mitochondrial peroxide generation

Our group examined the effects of experimental surgical denervation of muscles on the response of mitochondria in the denervated muscle. Initial studies demonstrated the time course of changes induced by surgical transection of the peroneal nerve in mice. These experiments showed that mitochondria responded rapidly to denervation by significantly increased peroxide generation by 3 days post-denervation which preceded any significant effect on muscle fibre atrophy (Fig. 6). Peak generation of peroxides by mitochondria occurred at 7 days post-denervation when pre-synaptic neural structure was absent from the muscle, but post-synaptic structures remained intact.⁷⁵ We also examined the effect of partial denervation of the mouse tibialis anterior (TA) muscle to determine the extent to which denervation of a specific group

of muscle fibres would influence neighbouring innervated fibres and muscle function. This study revealed a substantial increase in mitochondrial peroxide generation in the denervated fibres and also in neighbouring innervated fibres⁷⁵ suggesting a propagation of the effects of denervation between neighbouring fibres that influences mitochondrial peroxide generation.

In other studies, transient nerve insult (crush injury) was used to examine mitochondrial peroxide generation in a reversible model (which might better reflect the situation *in vivo* during ageing) and also examine whether any changes in mitochondrial peroxide generation would be reflected in cytosolic peroxide content.⁷⁶ Different sub-cellular compartments have very different abilities to generate H_2O_2 and ability to scavenge H_2O_2 through different enzyme systems and it is this latter property that appears to underlie the intracellular compartmentalisation of H_2O_2 .⁷⁷ The thioredoxin-based antioxidant system is crucial for maintenance of the low cytosolic H_2O_2 content in comparison with both mitochondria and extracellular H_2O_2 levels.^{77,78} This system primarily acts through peroxiredoxins which scavenge H_2O_2 at the low concentrations found intracellularly. Peroxiredoxins convert H_2O_2 to water and they themselves are reduced by thioredoxins which in turn are reduced by NADPH. Deletion or inhibition of components of this system lead to loss of the mitochondria to cytosol H_2O_2 gradient.⁷⁸

Fig. 7 shows the relationship between neuromuscular junction structure, cytosolic H_2O_2 content (measured *in vivo* using the H_2O_2 specific probe *HyPer2*) and mitochondrial peroxide production in muscles of adult and old mice and illustrates the effect of loss of neuromuscular integrity on mitochondrial peroxide generation was relatively

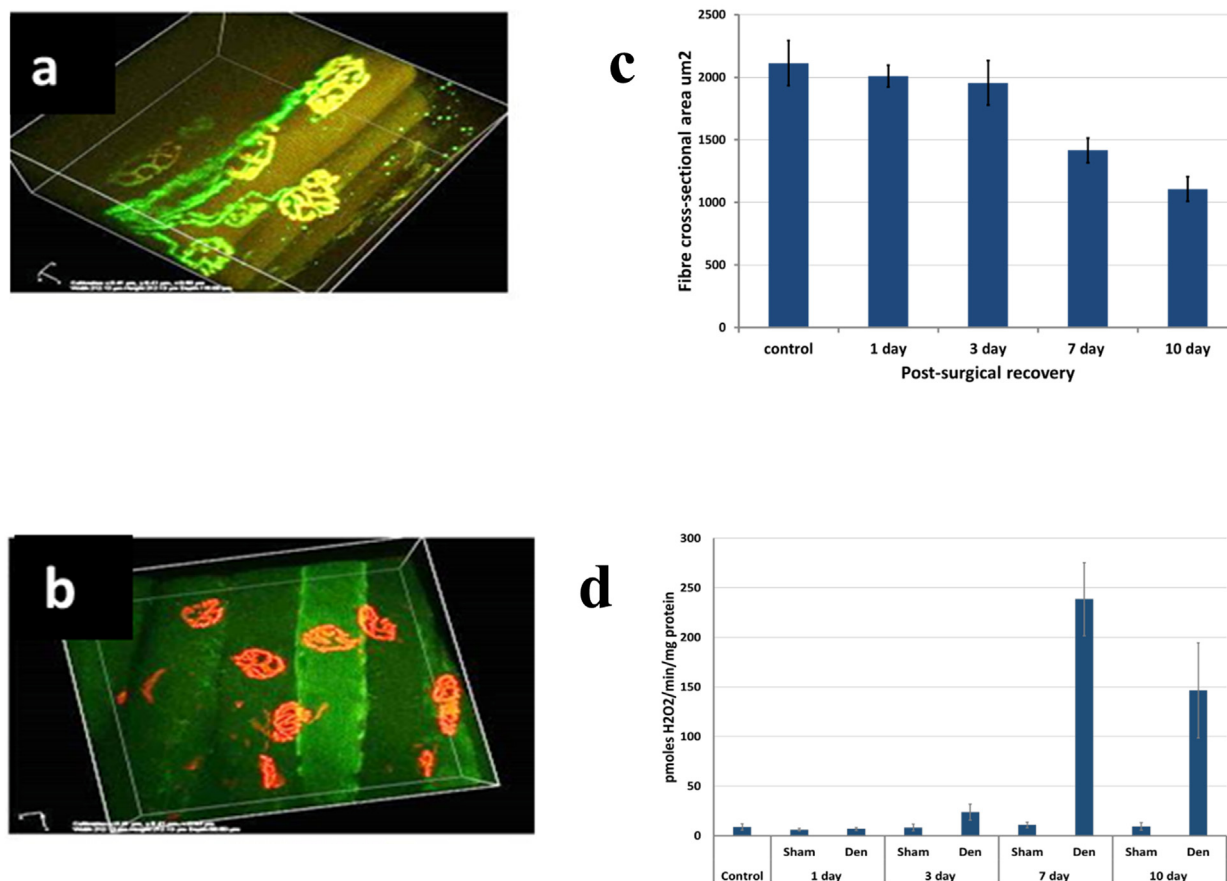


Fig. 6. Effect of peroneal nerve transection on structure of tibialis anterior (TA) muscle. (a) Confocal images of NMJ and peripheral axons in control muscle of *thy1-YFP* mice, showing YFP in motor neurons (green) and AChRs labelled with Alexa-594- α -bungarotoxin (red), (b) muscle at 10 days post nerve transection with loss of pre-synaptic structures with retention of AChRs. (c) The temporal effect of denervation on the cross-sectional area of fibres in the TA muscle. (d) Rate of mitochondrial H_2O_2 production by permeabilised bundles of fibres from the TA assessed using the amplex red assay. Following surgical transection of the peroneal nerve there was a gradual increase in the rate of production observed in state 1, which was significant by 3 days and further increased at 7 and 10 days post-surgery. Reproduced and modified from.⁷⁵

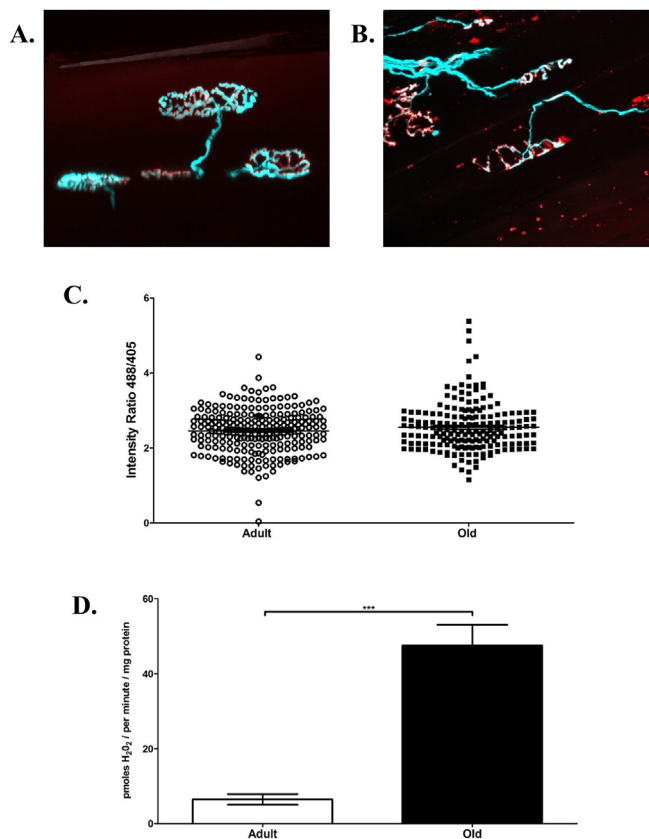


Fig. 7. Neuromuscular junction, *HyPer2* *intra vital* hydrogen peroxide assessment and mitochondrial peroxide generation; comparison between adult and aged mice. Representative fluorescent images of NMJ's from adult (6–8 months) (A) and old (26 months) (B) *Thy1-CFP* mice showing the pre-synaptic terminal motor nerves (blue) and motor endplates stained with α -bungarotoxin (red) (scale bar = 50 μ m). No significant difference was observed between *HyPer2* transfected TA muscles from adult and old mice when assessing the ratio of emissions at 516 nm after 488 and 405 excitation (488/405 ratio) (C) from individual fiber ratios. (D) Rate of amplex red oxidation (expressed as H_2O_2 generation) during state 1 respiration (i.e. in the absence of added substrates) from permeabilised TA fibers from adult and old mice (** $p < 0.001$, Mann Whitney, $n = 11$ for adult, $n = 7$ for old). Reproduced from.⁷⁶

specific and not reflected in cytosolic peroxide content.⁷⁶ Further studies have examined the effect of denervation on regulatory pathways influencing redox homeostasis in muscle. These data showed that the increase in denervation-induced mitochondrial peroxide production stimulates adaptations to protect the muscle fibres, but that these are inefficient and that over the longer term the changes in mitochondrial peroxide generation may activate degenerative and atrophic processes in the fibres.⁷⁹ Overall these data suggest that loss of innervation in fibres contributes to increased mitochondrial ROS generation⁷⁵ and associated mitochondrial degeneration⁷⁹ in denervated and neighbouring innervated fibres. This may provide an additional or alternative explanation as to how serial, episodic denervation of a small number of fibres leads to more widespread degeneration and atrophy of fibres in muscles during ageing. A schematic illustrating how this potential process might occur over the lifespan is presented in Fig. 8.

6. Possible amelioration of age-related changes in the neuromuscular system by exercise

There is general evidence that a sedentary lifestyle or chronic disuse of muscle is relatively deleterious to neuromuscular morphology and function in comparison with active individuals.^{16,18,32,80–82} Furthermore,

Piasecki and colleagues have claimed that failure to expand motor unit size to compensate for declining motor unit numbers distinguishes sarcopenic from non-sarcopenia on older men.²⁰ These observational studies in humans appear to indicate a strong link between activity levels and maintenance of a healthy neuromuscular system, and studies of extended bed rest inducing muscle atrophy in human subjects also provide some support in indication for disruption of NMJ function and an increase in the number of denervated (NCAM positive) muscle fibres in muscle biopsies of subjects.^{83–86} However, interventional data examining the effect of different exercise protocols on motor units, motor neurons, axons or NMJ are relatively rare and less convincing in demonstrating beneficial effects. While resistance exercise training is acknowledged to be the optimum approach to prevention or reversal of muscle loss and weakness in elderly subjects,⁸⁷ Soendenbroe et al.,²⁴ cite three studies which have examined the effect of exercise interventions on numbers of apparent denervated fibres in human muscle biopsies,^{88,89} two of which found that resistance exercise reduced the number of apparent denervated fibres^{88,89} while a study of treadmill walking found no difference.⁹⁰ Also relevant in this area is the potential effect of neuromuscular electrical stimulation which has been claimed to have a positive effect on neuroplasticity, increasing specific neurotrophic factors that mediate axonal sprouting and formation of NMJ.⁴³

Pre-clinical mouse-based studies have provided further information on the potential of exercise to improve neuromuscular structure and function in ageing. In detailed studies, Valdez and colleagues found that one month of wheel running exercise in 22-month-old mice reduced age-related synaptic changes but had no effect on motor neuron number or muscle fibre turnover,⁹¹ whereas other workers have shown morphological and functional improvements in NMJ of 25 month old mice with exercise. Additional studies have shown beneficial effects of voluntary wheel running on NMJ transmission but not loss of motor units in aged mice.⁹² A comprehensive systematic review of the effects of physical exercise on NMJ degeneration during ageing was recently published by Wang et al.⁹³ and includes both human and animal studies. Only 20 papers in total were found suitable for inclusion in the systematic review, but they indicate a great heterogeneity of responses to different types of exercise. Nevertheless, the authors concluded that “endurance training, compared with resistance and voluntary exercise regimens was found to have a more pronounced effect on NMJ structural remodelling, particularly in fast twitch muscle fibers”. They also reported that physical exercise was observed to promote the formation of acetylcholine receptor clusters and concluded that “research on exercise-related therapies could potentially attenuate the progression of neuromuscular degeneration”.⁹³

There are good mechanistic arguments why exercise should benefit neuromuscular ageing as proposed by Taetzsch and Valdez.⁹⁴ PGC1 α is a key molecule that regulates mitochondria and is increased by exercise.⁹⁵ This molecule is thought to act in relaying the beneficial effects of exercise to skeletal muscle and promoting mitochondrial function.⁹⁴ Overexpression of PGC1 α in muscle increases the number of mitochondria in skeletal muscle and improves the expression of key genes involved in NMJ turnover in old mice⁹⁶ and mouse disease models.⁹⁷ Additionally others have suggested that exercise has systemic effects to increase various growth factors that benefit the neuromuscular system, such as insulin-like growth factor-1 (IGF1), brain derived neurotrophic factor (BDNF) and glial cell line derived neurotrophic factor (GDNF).^{98,99}

The difficulties of designing and executing definitive studies in this area even in rodents should not be underestimated since there are many potential variables which have not been examined. These include choice of type of exercise (e.g. resistance, or endurance), duration of exercise training and age at start (e.g. short term practical study covering weeks, or lifelong to prevent age-related changes), choice of endpoint (e.g. structural: motor neuron numbers, NMJ structure, axonal structure, motor unit numbers, muscle fibre types, muscle fibre numbers, fibre atrophy; or functional: neuron function, NMJ function, muscle force generation). Hence it is unsurprising that from the relatively small number of intervention studies undertaken to date it is difficult to draw firm conclusions.

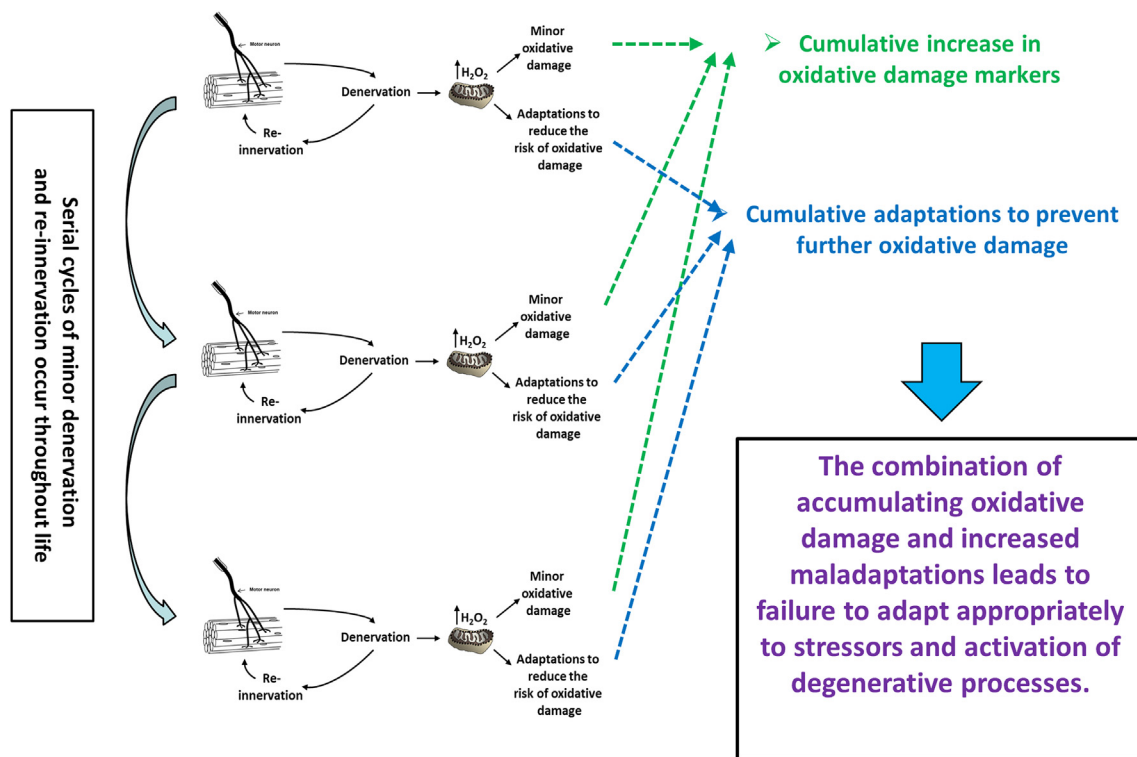


Fig. 8. It is envisaged that small episodes of denervation, such as loss of single terminal axons occur frequently throughout life and that this is rapidly repaired by axonal sprouting and outgrowth. Each cycle of denervation and re-innervation leads to a transient increase in mitochondrial peroxide generation in the denervated and neighbouring muscle fibers which cause minor oxidative damage and induction of adaptive responses to attempt to prevent further oxidative damage. The repetition of this process many times over a lifetime eventually leads to the cumulative increase in oxidative damage seen in muscle tissue of older animals and man, to a cumulative adaptive increase in regulatory proteins designed to prevent oxidative damage, failure to adapt appropriately to stressors and activation of degenerative processes. Modified and redrawn from.⁶⁹

7. Conclusions

Human and animal studies indicate that substantial changes in motor neuron numbers and size, axonal integrity and NMJ structure at pre- and post-synaptic levels occur with ageing. These are associated with significant changes in axonal sprouting and NMJ turnover, but data indicate the effect on neuromuscular transmission is relatively minor. This mismatch between structural changes and NMJ transmission has been attributed to a substantial reserve capacity to ensure successful activation of muscle contraction even in the presence of minor amounts of residual pre- to post-synaptic contact and has been used by some authors to argue that structural degeneration of motor axons and NMJ are unimportant in the pathogenesis of sarcopenia. Increased ROS activities and changes in muscle redox status are a feature of muscle from older humans and animals and have been claimed to play a key role in muscle loss in ageing and we argue that these changes may be caused by age-related changes in neural input to muscle, but not directly related to the success or failure of neuromuscular transmission. These studies provide an alternative explanation on how age-related changes in neural tissue might drive muscle fibre loss and weakness in skeletal muscle.

Exercise is the only intervention known to reliably reduce muscle loss and weakness in the elderly, but despite significant amounts of research there is a clear need for further research to understand how targeted or long-term exercise programmes might ameliorate the neuromuscular changes in ageing and consequently changes in muscle redox homeostasis. Current data are sparse and isolated, but provide sufficient encouragement to support well designed and definitive studies in human and animal models.

Declaration of competing interest

I, Malcolm J Jackson, am an Editorial Board Member for *Sports Medicine and Health Science* and was not involved in the editorial review or the decision to publish this article.

I confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

I confirm that I have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing I confirm that we have followed the regulations of my institution concerning intellectual property.

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