Review
article

The process of aging and neuromuscular junction morphology of limb muscles: a systematic review

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Abstract

Sarcopenia is a multi-factorial process that can be characterized by morphological changes. Mounting of evidence suggests that loss of motor neurons and structural changes on the neuromuscular junction (NMJ) may contribute to the progression of skeletal muscle aging. Other studies revealed that the age-related changes in NMJ morphology vary among different skeletal muscle types and could potentially be related to muscle activity levels. Thus, the aim of the present study was to do a critical and systematic review about the impact of aging on the NMJ morphology of limb muscles. We used Pubmed database and the following keywords: "neuromuscular junction" AND aging AND morphology. A systematic review was done and eight papers were retrieved. Several mechanisms are implicated at the etiology of sarcopenia. Oxidative stress, growth factors and stabilization proteins are involved in the maintenance of the integrity of the neuromuscular junction morphology and any disturbance might trigger denervation predominance.

Keywords: morphology, mechanisms, aging, skeletal muscle, neuromuscular junction.

1 Introduction

A gradual loss of muscle mass occurs during aging and morphological changes can appear. Overall research suggested that decrease in the motor neurons number, neuromuscular junction structural changes, increase pre-synaptic denervation, and total loss of motor units contribute to the progression of muscle aging (JANG and VAN REMMEN, 2011; VALDEZ, TAPIA, LICHTMAN et al., 2012). In muscle fibers, the denervation and re-innervation cycles, lead to a preferential denervation of the fast-twitch fibers, resulting in the conversion of type II (fast fibers) to type I (slow fibers) muscle fibers (JANG and VAN REMMEN, 2011; CHIU, WEBER, ADAMSKI et al., 2011; VALDEZ, TAPIA, LICHTMAN et al., 2012). Moreover, denervation outplaces re-innervation and the loss of muscle mass shall ultimately compromises skeletal muscle function (NARICI and MAFFULLI, 2010; JANG and VAN REMMEN, 2011; CHIU, WEBER, ADAMSKI et al., 2011).

Age-related changes in Neuromuscular Junction (NMJ) morphology can vary among different skeletal muscle types and shall potentially be related to muscle activity levels (DESCHENES and WILSON, 2003; VALDEZ, TAPIA, LICHTMAN et al., 2012). In general, NMJs of rostral muscles show less age related changes than caudal muscles, whereas hind limb and neck muscles are similar affect by aging (VALDEZ, TAPIA, LICHTMAN et al., 2012).

The NMJ adaptability is vastly demonstrate in models of denervation/re-innervation process, electrical stimulation, muscle unloading, peripheral nervous system post born development, and others. Recently, image technical advances (such as confocal microscopy analysis) have identified significant changes in development of age-associated alterations in the neuromuscular structure and development of sarcopenia. Also, aging studies use a large variability of animal strain and this fact might turn difficulty to choose the best animal model to study this important process. The Brown Norway rat is the most frequent animal used in aging studies, however others animals such as mice C57BJ6, Sprague-Dawley rats and others, have also been frequently used (ALWAY, DEGENS, KRISHNAMURTHY et al., 2003; RICE, LINDERMAN, KINNARD et al., 2005; RICE and BLOUGH, 2006; WOLDEN-HANSON, 2010; CHIU, WEBER, ADAMSKI et al., 2011; SHEARD and ANDERSON, 2012).

According to Chiu, Weber, Adamski et al. (2011), clinical tests for skeletal muscle function in human sarcopenic patients are routinely performed in the lower extremities since it shows the most significant muscle degeneration during aging. In the past ten years, other reviews were published, but none has focused only in the limb muscles (LEXELL, 1997; JANG and VAN REMMEN, 2011). To the knowledge, there is no published systematic review in the issue until now. Thus, the aim of the present study was to do a critical and systematic review on animal studies about the effect of aging on the NMJ morphology of limb muscles.

2 Materials and Methods

A systematic review was conducted in April/May 2013 using the PubMed database. The search was conducted using the key words "neuromuscular junction", aging and morphology that appear in all fields. We selected the papers published in the last 10 years because many publications regarding the best methodologies were done recently. We included animal studies published in English language. Reviews and meta-analysis, as well as interventions such as surgical denervation/re-innervation, electrical stimulation

and unloading or ablation surgery were disregarded. A secondary exclusion criteria was applied, and papers which analyzed the administration of nutritional supplements or drugs, and also had metabolic or neurodegenerative diseases were also excluded.

3 Results and Discussion

Through the intersection of the keywords, we obtained eight publications (VALDEZ, TAPIA, LICHTMAN et al., 2012; CHAI, VUKOVIC, DUNLOP et al. 2011; KULAKOWSKI, PARKER and PERSONIUS, 2011; WANG, HEBERT, RICH et al., 2011; DESCHENES, ROBY, EASON et al., 2010; JANG, LUSTGARTEN, LIU et al., 2010; DESCHENES and WILSON, 2003; MESSI and DELBONO, 2003) which met the inclusion criteria. In Table 1, we can identify other details about the selected papers.

The genetically modified animal models were used in five of the eight studies, with predominant of mice C57BJ6 strain. The Soleus muscle (SL) was analyzed in six of eight publications, followed by Tibialis anterior, Extensor Digitorum Longus (EDL) and Gastrocnemius, which were studied in four, three and two publications, respectively. A plausible explanation for this fact is that the Soleus muscle is vital to posture and commonly use in everyday tasks. In Table 2, it is possible to verify the methodology used in these publications.

The NMJ is a specialized synapse from peripheral nervous system formed by the alpha motor neuron axon terminals (pre-synaptic), the synaptic cleft, the membrane of skeletal muscle (post-synaptic) and Acetylcholine receptors (AChRs). The changes that occur on NMJ morphology during late aging are well known and documented, however mostly researchers usually incorporate every muscle type in their analysis (JANG and VAN REMMEN, 2011). In Table 3, we describe the main results published by each paper. According to Valdez, Tapia, Lichtman et al. (2012) the NMJs in rostral muscles show less age related changes than in caudal muscles, whereas hind limb and neck muscles are similarly affected by aging. Chai, Vukovic, Dunlop et al. (2011) analyzed the EDL confocal microscopy images, of 3 months-old mice, and found that both pre-synaptic nerve terminals and the post-synaptic motor endplates were well organized and precisely compact. However, at late age, both compartments suffered serious remodeling. The confocal study showed disorganized and expanded pre-synaptic nerve terminals in older NMJ compared to the younger ones. Also, the postsynaptic endplates appeared diffuse and

Table 1. General description of the selected publications.

Study	Institution	Country	Journal
Valdez, Tapia, Lichtman et al. (2012)	Harvard	USA	PLoS One
Chai, Vukovic, Dunlop et al. (2011)	Western Australia	Australia	PLoS One
Kulakowski, Parker and Personius (2011)	Buffalo	USA	Journal of Applied Physiology
Wang, Hebert, Rich et al. (2011)	Wright State	USA	Neuroreport
Deschenes, Roby, Eason et al. (2010)	College of William & Mary	USA	Experimental. Gerontology
Jang, Lustgarten, Liu et al. (2010)	Texas	USA	FASEB Journal
Deschenes and Wilson (2003)	College of William & Mary	USA	Journal of Neurobiology
Messi and Delbono (2003)	Wake Forest	USA	Journal of Neuroscience

Table 2. Methodological description of each aging study, according to the animal strain, age and muscle studied.

Study	Animal Strain	Age	Muscle(s) Analysed
Valdez, Tapia, Lichtman et al. (2012)	Thyl-XFP and SOD-G93A transgenic mice and wild-type C57BL/6 male mice	Young (4-5 months-old) and old (22-28 months-old)	EDL, Gastrocnemius, Gracillis, Soleus, Tibialis Anterior
Chai, Vukovic, Dunlop et al. (2011)	C57BL/6J female mice	Young (3 months-old) and old (29 months-old)	EDL/Soleus
Kulakowski, Parker and Personius (2011)	Heterozygous B6.129S2-Ntrk2^{tm1Bbd}/J mice or wild-type littermates (TrkB+/+) and C57BL/6 mice	Young (6,9±0,3 [TrkB+/-] and 6,4±0,3 [TrkB+/+] months-old) and old (24 months-old) TrkB and old wild-type C57BL/6 (24 months-old)	Soleus
Wang, Hebert, Rich et al. (2011)	MRF4-null and wild-type mice	Control (no age described) and old (19-20 months-old)	Tibialis anterior and Soleus
Deschenes, Roby, Eason et al. (2010)	Male Fisher 344 rats	Young (10 months-old) and old (21 months-old)	Plantaris and Soleus
Jang, Lustgarten, Liu et al. (2010)	Sod 1-/- and wild-type mice	Young (14 months-old) and old (18 to 22 months-old)	Gastrocnemius/ Plantaris/ Soleus
Deschenes and Wilson (2003)	Male Fisher 344 rats	Young (8 months-old) and old (22 months-old)	Soleus
Messi and Delbono (2003)	FVB or S1S2 IGF-1 transgenic mice	Young (2-6 months-old) and Old (22-24 months-old)	EDL

Table 3. Primary results found in the publications on regard to the aging NMJs morphology.

Study	Primary Results
Valdez, Tapia, Lichtman et al. (2012)	↑ fragmentation of AChR-rich post-synaptic membrane; ↓ AChR density in the post-synaptic membrane; ↑ denervation; ↑ sprouts from nerve terminals that extended beyond the post-synaptic apparatus; ↑ thinning of preterminal and/or terminal portions of the axon; ↑ multiple innervation.
Chai, Vukovic, Dunlop et al. (2011)	EDL: ↑ disorganization and sprouting of pre-synaptic nerve terminals; ↑ diffuse, irregular and fragmented post-synaptic endplates; ↑ diameter NMJs; ↑ NMJs located close to disorganized and partially or fully denervated ones; ↑ denervation of NMJs; Soleus: Quantitation revealed no significant change in the number of totally denervated NMJs.
Kulakowski, Parker and Personius (2011)	↓ TrkB = ↑ dissociating AChRs from one or a few contiguous regions (clusters) into multiple less clearly delineated regions; ↑ expanding overall AChR end-plate area, perimeter and number of denervated fibers.
Wang, Hebert, Rich et al. (2011)	↓ synaptic vesicles of pre-synaptic terminals; ↑ denervation in NMJ;
Deschenes, Roby, Eason et al. (2010)	Plantaris: ↑ synaptic remodeling, total branch length, average branch length, and branching complexity in fast-twitch NMJs; ↑ total branch length and branching complexity in slow-twitch NMJs; ↑ post-synaptic dimensions. ↑ total and stained perimeter lengths around endplates and larger total and stained areas in fast- and slow-twitch endplates; Soleus: ↑ both total and stained area measurements in slow-twitch NMJs.
Jang, Lustgarten, Liu et al. (2010)	↓ synaptic cleft and synaptic vesicles; ↑ pre terminal thinning, distension and sprouting; ↑ fragmentation of the post-synaptic end plate; ↑ denervation.
Deschenes and Wilson (2003)	↓ area and perimeter length of Ach vesicle and receptor regions;
Messi and Delbono (2003)	↑ number of nerve terminals and nerve branching; ↑ extra terminal sprouts; ↓ thickness nerve terminals with senescent; ↑ nerve thickness in both young and old IGF mice; ↓ pre-synaptic terminal and post-synaptic area; ↑ simplification, shrinkage, and fragmentation of the post terminal;

irregular, whereas quantification showed a 2.5 fold increase in denervation process. Moreover, the degree of structural irregularities had barely changed in the third year (VALDEZ, TAPIA, LICHTMAN et al., 2012). However, Soleus aged NMJ was not found to have the same effect as EDL muscle. According to Valdez, Tapia, Lichtman et al. (2012), nearly 90% of NMJs from aged EDL showed one or more of the following characteristics: fragmentation of AChR into small islands, decreased AChR density in mostly post-synaptic membrane, partial or complete denervation of post-synaptic apparatus, sprouts arising from nerve terminals, swelling or distension in pre terminal axons, thinning of pre terminal and/or portions of the axon terminal, and convergence of 2 or more axons on a single postsynaptic site, leading to multiple innervation. These anatomical changes are similar to those reported previously for Tibialis anterior, Gastrocnemius and Gracilis muscles (VALDEZ, TAPIA, KANG et al., 2010). Corroborating these findings, Deschenes, Roby and Glass (2011) demonstrated a tendency to increase the number of pre-synaptic branches in both fast and slow NMJs of Plantaris muscle in aged rats. Valdez, Tapia, Lichtman et al. (2012) concluded that the changes were less impacting in Soleus than EDL muscle however it was seen only at the slow NMJs.

Several mechanisms may explain the denervation seen at those previous studies. In this review, four of the eight selected publications studied transgenic models and possible explanations for the process of sarcopenia will be discussed.

Kulakowski, Parker and Personius (2011) studied B6.129S2-^{Ntrk2tmlBbd}/J transgenic mice, and compared it to young and aged C57BJ6 wild-type models. This animal model showed 50% reduction of tyrosine kinase receptor (TrkB). This receptor works with the AChRs, mediating the stabilization of intracellular signaling in the NMJ. Thus, AChRs has been seen to expand, and cluster during normal aging, whereas expression of TrkB receptor appears to undergo reduction in NMJ at this same period (KULAKOWSKI, PARKER and PERSONIUS, 2011). Post

synaptic AChR expansion, fragmentation and denervation was observed in TrkB animals similar to that found in ancient wild-type animals. Kulakowski, Parker and Personius (2011) also demonstrated that TrkB animals show transmission failure, which might indicate that these morphological changes is sufficient to alter synaptic function. Personius and Parker (2013) also quoted that TrkB protein expression was decreased in senescent (24-month-old) compared to 3-12 month-old mice and loss of TrkB expression was concurrent with age-related changes in AChR morphology. According to Balice-Gordon (1997), relatively early in the aging process, AChR area expands, fragments, and the area of pre and post-synaptic overlap becomes progressively smaller as the number of presynaptic vesicles decreases (JACOB and ROBBINS, 1990). Corroborating, Frey, Schneider, Xu et al. (2000) showed a denervation activity in Soleus and Gastrocnemius muscles of aged mice.

The age related changes in growth factors may be critical for maintenance of the NMJ structure (JANG and VAN REMMEN, 2011; MESSI and DELBONO, 2003; WANG, HEBERT, RICH et al., 2011). Wang, Hebert, Rich et al. (2011) studied the Tibialis anterior (TA) and Soleus muscle, using mouse models that did not express the MRF4 transcription factor. It was showed that MRF4 is enriched in sub-synaptic nuclei of adult animals however it is reduced at aged animals (WANG, HEBERT, RICH et al., 2011; McGEACHIE, KOISHI, ANDREWS et al. 2005; THOMPSON, FILATOV, CHEN et al., 2005). The TA muscles from aged control and aged MRF4-null mice were examined in the region of their pre- and post-synaptic areas. Some of the NMJs from the aged MRF4-null mice showed a loss of synaptic vesicle marker, SV2B, either or almost fully to a severe degree (WANG, HEBERT, RICH et al., 2011). When the authors analyzed superimposed views of NMJs, a loss of the vesicular marker from the presynaptic terminal was seen in aged group. To determine the loss of muscle-type specificity, the Soleus muscle was examined, and the loss of the vesicular marker and vesicles were similar to TA muscle in the MRF4 -null mice compared to controls. This data indicates that MRF4 contributes to influence the stabilization of the pre-synaptic components in aged animals and may have a potential role in maintenance of post-synaptic specialization (WANG, HEBERT, RICH et al., 2011; McGEACHIE, KOISHI, ANDREWS et al., 2005).

Another muscle growth factor that appeared to be vital for the protection and preservation of NMJ against aging is the insulin-like growth factor 1 (IGF-1) (JANG and VAN REMMEN, 2011; MESSI and DELBONO, 2003). Messi and Delbono (2003) studied the overexpression of IGF-1 in transgenic mice and compared them to old and young wild-type animals. The analysis of the nerve terminal in EDL muscles from senescent mice showed a decrease in the percentage of cholinesterase-stained zones (CSZ). Furthermore, the number of nerve terminal branching was partially or entirely reversed by persistent overexpression of IGF-1. IGF-1 also prevents the decrease in the pre and post terminal area, reduction in the number, length and density of post-synaptic folds of the aging muscles (MESSI and DELBONO, 2003; PESTRONK, DRACHMAN and GRIFFIN, 1980). The augment of nerve terminals sprouting with increased aging and IGF-1 injection is in agreement with other works (ROBBINS, 1992; KIMPINSKI and MEAROW, 2001). Payne, Messi, Zheng et al. (2007) showed signs that the injections of IGF-1-TTC fusion protein prevented age-related alterations to the nerve terminals at the neuromuscular junctions. Messi and Delbono (2003) also reported that nerve terminal thickness was increased in young and old transgenic mice. IGF-I deficiency is associated with decreased axonal diameter (GAO, SHINSKY, INGLE et al., 1999). Apel, Callahan, Northam et al. (2010) showed that in both young and aged animals, IGF-1 significantly improved Tibial nerve axon number, diameter, density, myelination and Schwann cell activity and preserved the morphology of the postsynaptic NMJ in Gastrocnemius muscle. However, the authors suggested that neural regeneration induced by IGF-1 was only considered secondary in NMJ. Pre terminal area reduction may also be prevented by IGF-1 in old rodents (PESTRONK, DRACHMAN and GRIFFIN, 1980). The decrease in the post terminal surface might be related to age-dependent decreases in acetylcholine receptor binding in EDL muscle (BANKER, KELLY and ROBBINS, 1983). The motor endplate decreases in number, length, area and post synaptic density during aging (MESSI and DELBONO, 2003). According to Fahim and Robbins (1982), endplate remodeling seems to occur with reduction of the junctional area or redistribution of terminal volume. Evidence from other studies suggests that IGF-1 injections prevented age-related alterations to the nerve terminals at the neuromuscular junctions of TA muscles (PAYNE, ZHENG, MESSI et al., 2006). Messi and Delbono (2003) concluded that overexpressing IGF-1 in skeletal muscle prevents morphological changes that shall occur during aging.

According to Milton and Sweeney (2012), elevated levels of reactive oxidative species (ROS) may accelerate the process of aging. Directly to examine the role of chronic oxidative stress in vivo, Jang, Lustgarten, Liu et al. (2010) studied the mouse model which lacks the antioxidant enzyme CuZnSOD (SOD1) (JANG, LUSTGARTEN, LIU et al., 2010). This model is characterized by high levels of oxidative damage and an acceleration of muscle loss. Aged SOD1 mice showed an increase in muscle mitochondrial content near the NMJ, but despite this increase, it could not prevent the denervation of NMJs and fragmentation of acetylcholine receptors. As regard to structural changes, muscles fibers from Gastrocnemius muscle showed fragmented post synaptic endplates compared to wild type mice. More shocking was the 80% fragmentation of NMJs from old SOD1 mice compared with only 3% of wild types. In this manner, superoxide-induced NMJ degeneration is also a potential mechanism involved in the process of sarcopenia.

Other molecules have also been implicated in NMJ maintenance. According to Punga and Ruegg (2012), the NMJ stability is a complex process that requires the coordination of actions including pre and postsynaptic factors, receptors and proteins. Agrin is a neurotrypsin substrate involved in specific mechanisms in NMJ region. Butikofer, Zurlinden, Bolinger et al. (2011) showed that removing Agrin from the synaptic basal lamina causes fragmentation and sarcopenia. This protein also activates muscle specific kinase (MuSK), a type I receptor tyrosine kinase (RTK), and a key organizer of NMJ formation secreted by motoneurons to direct NMJ formation (DECHIARA, BOWEN, VALENZUELA et al., 1996). Moreover, the low-density lipoprotein receptor (LDLR), a surface receptor,

has been implicated in the stability of NMJ (NYKJAER and WILLNOW, 2002). One member of this family, LRP4 (LDLR-related protein 4) interacts with MuSK in a manner that is enhanced by neural agrin. According to Zong, Zhang, Gu et al. (2012) LRP4 is a co-receptor of neural agrin that is required and sufficient to activate MuSK, and initiate downstream signaling cascades for AChR clustering. Despite these observations, little is known about the mechanism by which LRP4 transduces signals from agrin to MuSK and several mechanisms are now under investigation to explain the complexity of aging sarcopenia.

4 Conclusion

This review allowed us to conclude that aged limb muscles undergo dramatic changes in their NMJ organization and several mechanisms might be involved such as increased oxidative stress, reduced growth factor levels and impairment of the cellular communication.

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